Chair Dallas May
Colorado Parks and Wildlife Commission
Hilary Hernandez, CPW Regulations Manager
6060 Broadway
Denver, CO 80216
dallas.may@state.co.us
dnr_cpwcommission@state.co.us
hilary.hernandez@state.co.us

December 18, 2024

**Re: Opposition to September 27, 2024 Citizen Petition for Rulemaking requesting pause in gray wolf reintroduction activities pending completion of management changes**

Dear Chair May, Commissioners, and Ms. Hernandez:

The undersigned groups oppose the citizen petition for rulemaking submitted by the Middle Park Stockgrowers Association and 20 other entities on September 27, 2024. The petition requests that Colorado Parks and Wildlife be required to pause gray wolf reintroductions until the agency has made various changes to its rules and policies—a request that would undermine Colorado law and the voters' will.

Colorado is in the midst of a historic, voter-mandated effort to restore gray wolves, a native carnivore that occupied the state for millennia until extirpated by humans. As part of the restoration process, Colorado established a wolf management program with numerous provisions aimed at minimizing harm to the livestock industry. For example, Colorado compensates the owners of wolf-killed livestock up to $15,000, the most generous compensation program in the country.

Nonetheless, anti-wolf livestock groups and their allies now seek to disrupt Colorado's democratically enacted reintroduction effort, claiming deficiencies in the state's management program. They request an amendment to Chapter W-16 of the Commission's procedural rules, 2 Colo. Code Regs. § 406-16, that would prohibit CPW from reintroducing more wolves until the Commission and CPW have taken seven steps relating to wolf management.

## DISCUSSION

CPW should deny the petition. An agency has "broad discretion to decide whether to engage in a rulemaking," and judicial review of such decisions is "extremely limited" and "highly deferential." *Colo. Oil and Gas Conservation Comm'n v. Martinez*, 433 P.3d 22, 27 (Colo. 2019) (quoting *Massachusetts v. EPA*, 549 U.S. 497, 527–28 (2007)). The petition here should be denied because (1) granting it would violate CPW's legal obligations and (2) the agency is already undertaking the seven measures requested in the petition.

## I. Granting the petition would violate CPW's legal obligations.

Colorado state agencies may—indeed, must—deny a petition for rulemaking if granting it would violate their legal obligations. For example, the Supreme Court upheld the Colorado Oil and Gas Conservation Commission's denial of a rulemaking petition where the action requested was contrary to statute. *Martinez*, 433 P.3d at 25. Here, granting the petition would violate (1) state statute and (2) the agency's own wolf management plan.

### A. Pausing releases would violate state statute

Colorado law directs CPW to "restore" a "self-sustaining population" of gray wolves to Colorado. Colo. Rev. Stat. §§ 33-2-105.8(2)(a), (3)(a)(III). A pause in wolf reintroductions would be directly contrary to this mandate because it would undermine progress toward a self-sustaining population. As discussed in Exhibit 1 to this letter, population size and genetic diversity strongly influence population viability. Without more wolves, Colorado's small, nascent population will remain susceptible to, among other things, genetic drift, population bottlenecks, and Allee effects from difficulty finding mates. Thus, additional releases on an annual basis are essential to fulfilling CPW's legal mandate to restore a self-sustaining population of gray wolves to Colorado.

Pausing releases now would also conflict with the December 31, 2023 statutory deadline. *See* Colo. Rev. Stat. § 33-2-105.8(2)(d). Although the statute does not expressly set out a multi-year timeline for wolf releases, the December 2023 deadline is properly construed as a date by which full restoration efforts must begin. The statute does not provide CPW authority to halt or pause restoration efforts once started, and pausing releases would undermine the statutory deadline by weakening the ecological value of the first year of releases. In other words, the statute plainly requires that wolves be released during the 2023–24 winter, and, for the reasons set out in Exhibit 1, the restoration value of those wolves—and the considerable time and taxpayer dollars spent to release and manage them—diminishes if they are not immediately followed by more wolves.

Indeed, in its response to Club 20's September 3, 2024 letter, CPW explained that pausing wolf releases would be inconsistent with its statutory mandate. Colorado Parks & Wildlife Oct. 31, 2024 Letter to Club 20 re Response to September 3, 2024 Wolf Concerns. CPW acknowledged that Proposition 114 and state statute require the agency to establish a self-sustaining population of wolves in Colorado and stated that it "do[es] not believe pausing wolf releases is consistent with" that mandate. *Id.* at 1, 3. More specifically, and consistent with the science noted above, CPW explained that any pause of restoration efforts would likely result in additional wolf mortalities from the preliminary source before those wolves have had the opportunity to breed and pair. *Id.* In CPW's view, that would "mak[e] it harder to achieve Proposition 114's mandate to establish a self-sustaining population of wolves in Colorado." *Id.* Any such one-year pause would put CPW "back to square one, at considerable cost that will only have to be duplicated as CPW continues to work to meet the mandates of state statute." *Id.*

We agree with CPW's assessment. Pausing wolf releases would waste the first year of release and management efforts, render meaningless the December 31, 2023 deadline, and put the agency back to square one with its restoration mandate. That outcome is inconsistent with law and should be rejected.

### B. Pausing releases would violate CPW's wolf plan.

Pausing releases would also violate the Colorado Wolf Restoration and Management Plan. Colorado agencies are obligated to follow their own rules and guidance documents. *See, e.g.*, *Rags over the Ark. River, Inc. v. Colo. Parks & Wildlife Bd.*, 360 P.3d 186, 191 (Colo. App. 2015) (explaining that an agency is bound by the regulations it enacts). That includes formal management plans. *See, e.g.*, *Utah Env't Congress v. Bosworth*, 372 F.3d 1219, 1221 (10th Cir. 2004) (requiring agency compliance with its own management plan); *see also* Colo. Rev. Stat. § 33-2-105.8(2)(a) (requiring commission to "[d]evelop a plan to restore and manage gray wolves in Colorado, using the best scientific data available")).

Here, CPW's wolf plan calls for annual wolf releases. The plan contemplates releasing "30 to 50 wolves over a 3-to-5 year period," and specifically "10-15 wild wolves *annually* . . . over the course of 3 to 5 years." Wolf Plan 20 (emphasis added). Although the plan provides for some flexibility in that annual schedule, it does so only to protect wolves in the event of unreasonably high mortality rates during the first year of releases. Wolf Plan 22 (allowing suspension of releases in specific situations involving "[u]nexplained [wolf] losses or an unusually large number of losses"). The petition does not seek to pause reintroductions on that basis. Nor could it, as wolf mortality during the first six months after release was not "unreasonably high," and certainly not below the 70 percent threshold that triggers "protocol review." *Id.* Instead, the petition seeks to pause releases because livestock producers take issue with implementation of specific elements of the plan's livestock conflict components—an exemption found nowhere in the plan and contrary to its prescription of annual releases.

In short, the petition asks CPW to violate its legal duties under statute and its own plan. CPW should reject that request.

## II. The petition does not establish a basis for pausing reintroductions.

Even if CPW had the authority to pause reintroductions while it engages in the requested rulemaking, the petition does not establish a valid basis for doing so. The petitioners base their request for relief on assertions that CPW's wolf management plan unlawfully (1) fails to resolve conflicts between wolves and livestock producers and (2) imposes land, water, and resource use restrictions. Petition at 4–6.

Both assertions are false. Regarding conflict resolution, the undersigned groups acknowledge—and thank the agency for—the immense time and effort that CPW staff have expended to prevent and respond to wolf-livestock conflicts in the state. We are aware that countless hours have been spent training CPW staff, livestock producers, and community members on nonlethal control measures, and, as noted, Colorado offers the most generous compensation program in the country. Regarding land, water, and resource restrictions, the petitioners identify only purported "suggest[ions]" made by CPW staff—not actual rules or prohibitions—plus a denial of a lethal take permit when a livestock producer refused to eliminate a dead pit, which is an obvious wolf attractant. Petition at 6. Certainly, CPW should aim to administer its rules in a predictable, consistent manner, but it need not accommodate ill-advised ranching practices—even if they are "common" or "traditional." *Id.* The grievances laid out in the petition show little more than the livestock industry's reticence to change and generic dissatisfaction with the state's wolf management efforts, not violations of a legal duty.

Beyond the petition's flimsy foundation, it should be denied because CPW is already undertaking all seven of the measures the petition requests. *See Martinez*, 433 P.3d at 24, 26, 32–33 (affirming denial of rulemaking petition where agency explained that it was already addressing concerns raised in the rulemaking petition).

- **Chronic depredation**

The petition asks the Commission to "adopt[] a definition of 'chronic depredation' with mandated lethal take requirements of chronically depredating wolves and provide[] notice to the impacted communities and livestock producers of this generally applicable standard." Petition at 2.

In a recent press release, CPW explained that it will publish a "Wolf-Livestock Conflict Minimization Program Guide in December," which will provide livestock producers with a wide set of tools and resources and "describe CPW's depredation investigation process, compensation program and lethal management criteria, including the definition of 'Chronic Depredation'." Colorado Parks & Wildlife, *CPW and CDA Prepare for 2025 Gray Wolf Releases with Improved Livestock Conflict Minimization Program* (Dec. 9, 2024).[1]

- **Testing and evaluating nonlethal measures**

The petition requests that "CPW test[] and evaluate[] alternative forms of non-lethal measures for keeping wolves from attacking livestock and working dogs to identify what measures work in what field conditions and for how long the measures are effective." Petition at 2.

CPW will administer "a grant program to assist with implementing non-lethal wolf conflict reduction measures," which offers grants of up to $20,000 that "can be used to prepare, educate, and coordinate with producers to implement non-lethal conflict reduction measures and directly implement measures such as range riding and carcass disposal." Dec. 9 Press Release. CPW also explained in its October 31, 2024 response to Club 20 that it is developing site vulnerability assessments to help to identify "the most effective nonlethal techniques to avoid and minimize" wolf-livestock conflict. Oct. 31 Letter at 2.

- **Site assessments and education**

The petition requests that "CPW develop[] a program to conduct site assessments of areas where wolves are interacting with livestock and working dogs and educate livestock producers

---

[1] https://cpw.state.co.us/news/12092024/cpw-and-cda-prepare-2025-gray-wolf-releases-improved-livestock-conflict-minimization.

on managing wolf conflicts and implementing site-specific, effective non-lethal measures to minimize livestock losses." Petition at 2.

CPW has "recently updated and formalized the site assessment process for 2025," and "22 producers have signed up to receive site assessments since October." Dec. 9 Press Release. Additionally, at the November 14, 2024 CPW Commission meeting, CPW staff explained that the new assessment process would streamline existing procedures, make the assessments more consistent, and train staff internally to better conduct such assessments. Site assessments are voluntary, free of charge, and specific to individual producers.

- **Range riders**

The petition requests that "CPW develop[] a range rider program for areas where wolves are either currently interacting with livestock and working dogs or can be expected to interact after additional wolves are introduced, acquire[] sufficient funding for this range rider program, and implement[] this program prior to the next introduction of wolves." Petition at 2.

CPW is "partnering with" the Colorado Department of Agriculture to "create the Colorado Range Rider program that will launch in early 2025." Dec. 9 Press Release. Under that program, "CDA and CPW staff members, as well as any future contract range riders, will go through specialized training to create a team of trained range riders with connections to local communities who can be deployed to support producers at short notice." *Id.*

- **Rapid response team**

The petition requests that "CPW hire[], train[], and put[] in place a rapid response to [sic] team to immediately respond to reports of wolves harassing or depredating livestock and working dogs and keep[] that team in the impacted area until the threat is removed." Petition at 2.

CPW and CDA have "significantly increased conflict reduction specialist staff throughout the first year of wolf restoration," bringing on five wildlife damage specialists between April and June and recruiting five more in December. Dec. 9 Press Release. These positions are in addition to a nonlethal conflict reduction program manager who started in summer 2024 and two nonlethal mitigation specialists with CDA who started in December 2024. *Id.*

Furthermore, CPW has "developed criteria for different levels of response as a guide for field staff to use when addressing wolf conflict and depredations." *Id.* These criteria help staff estimate conflict risk and resources allocation on a site-specific basis, with input from the livestock producer and evaluation of a site assessment. *Id.*

- **Carcass management best practices**

The petition requests that "CPW collaborate[] with livestock producers and other state, local and federal agencies to develop best practices for carcass management in rural areas and communicate[] with impacted communities and livestock producers about these best practices." Petition at 2.

CPW conferred with neighboring states and assembled "[a] list of best practice recommendations compiled from lessons learned in other states."[2] Dec. 9 Press Release.

- **Transparency**

The petition requests that "CPW create[] a transparent plan to communicate and consult with local county officials, impacted communities, and livestock producers in advance of any wolf introductions that could affect them." Petition at 2.

CPW announced plans to hold three public meetings in Western Slope counties in December, and it "continues to meet with local communities and elected officials for open discussions about potential release locations and how to prepare to live with wolves." Dec. 9 Press Release. Additionally, CPW "reinforced with [its] staff that they are able to share wolf activity information with landowners in the areas of that activity," and put plans in place to enhance transparency, including a communication plan for new wolf releases to facilitate dialogue with counties and livestock producers. Oct. 31 Letter at 1–2. At the November 14, 2024 Commission meeting, CPW staff also noted that the agency recently met with four counties—Eagle, Pitkin, Rio Blanco, and Garfield—and county officials regarding the status of wolf introduction efforts and criteria used to select release sites.

In sum, CPW has already taken all seven steps that the petition asks it to take. As such, the petition should be denied. *See Martinez*, 433 P.3d at 33.

## CONCLUSION

For the reasons above, and in firm support of Colorado's historic wolf restoration effort, the undersigned groups urge CPW to deny the September 27, 2024 Citizen Petition for Rulemaking.

*/s/ Delaney Rudy*
Delaney Rudy
Colorado Director
Western Watersheds Project
PO Box 1175
Paonia, CO
(970) 648-4241
Delaney@westernwatersheds.org

*/s/Lindsay Larris*
Lindsay Larris
Conservation Director
WildEarth Guardians
3798 Marshall Street, Suite 8

---

[2] https://docs.google.com/presentation/d/17gJlSbnYpjunHvRn1FG-zB_sPggxIhVpQ_H1X8hM2Qs/edit#slide=id.p

Wheat Ridge, CO
(720) 334-7301
LLarris@wildearthguardians.org

*s/Allison N. Henderson*
Allison N. Henderson
Southern Rockies Director
Senior Attorney
Center for Biological Diversity
P.O. Box 3024
Crested Butte, CO 81224
(970) 309-2008
ahenderson@biologicaldivesity.org

/s/ *Nicholas Arrivo*
Managing Attorney
The Humane Society of the United States
1255 23rd St NW
Washington DC, 20037
(202) 961-9446
narrivo@humanesociety.org

*s/Rob Edward*
Rob Edward, President
Rocky Mountain Wolf Project
1309 E 3rd Ave, Suite #5
Durango, CO 81301
(970) 236-1942
rob.edward@rockymountainwolfproject.org

*/s/ Kelly Nokes*
Kelly E. Nokes
Western Environmental Law Center
P.O. Box 218
Buena Vista, CO 81211
(575) 613-8051
nokes@westernlaw.org

*/s/ Thomas Delehanty*
Thomas Delehanty
Jessica Zausmer
EARTHJUSTICE
633 17th Street, Suite 160
Denver, CO 80202
(303) 996-9628
(303) 996-9614

tdelehanty@earthjustice.org
jzausmer@earthjustice.org

cc. Dan Gibbs
Executive Director, Colorado Department of Natural Resources
dan.gibbs@state.co.us

Jeff Davis
Director, Colorado Parks and Wildlife
dnr_cpw_director@state.co.us

# Exhibit 1

## Biological Need for Proceeding with Wolf Reintroductions

The petition to delay the next round of Colorado wolf releases detracts from CPW's ability to use their professional judgment to carry out their legislative obligation. But it is also problematic biologically, as it threatens the viability of the wolf population in Colorado.

C.R.S. 33-2-105.8 directs the restoration and management of gray wolves as "necessary or beneficial for establishing and maintaining a self-sustaining population in Colorado"... "in a manner that fosters the species' capacity to sustain itself successfully." Population viability is the biological concept that underlies this legislative intention for a self-sustaining population, and refers to the ability of a population to persist and to avoid extinction or extirpation.

The Colorado wolf plan uses the value of 150-200 wolves as an estimate of when the Colorado wolf population can sustain itself. The purpose of reintroductions is to provide a source population of 30-50 wolves that can grow to this size range. While reintroductions don't bring the population immediately into a range that can be considered self-sustaining or viable, they create a base population that can proceed in that direction. Reintroductions must happen in a timely manner to bolster this natural population growth. A wealth of scientific literature emphasizes the importance of establishing large, genetically diverse founding populations (Wolf et al. 1998; Forbes and Boyd 1997; Miller et al. 1999; vonHoldt et al. 2008).

Population viability is strongly related to effective population size and genetic diversity, which are themselves linked. Small, fragmented populations are threatened to a greater degree because of demographic stochasticity, which represents fluctuation in effective population size, birth rate, and death rate through time. They are also particularly vulnerable to environmental stochasticity, which is the demographic fluctuation caused by unpredictable events like extreme weather, disease, and human-caused mortality (Pletscher et al. 1997, Darimont et al 2004, Creel and Rotella 2021, Brandell et al. 2021). Small populations with limited genetic diversity are less able to adapt to a changing environment, which threatens the population viability in the face of climate change and development (Funk et al. 2018), and wolves in Colorado are particularly vulnerable to landscape change (Carroll et al. 2006).

These dynamics mean that the viability of small, isolated populations is more threatened by genetic drift, population bottlenecks, and Allee effects from difficulty finding mates (Hedrick et al. 2019, Liberg et al. 2005, Stenglein and Van Deelen 2016). The viability of small populations is limited because of the limited genetic diversity offered by fewer individuals, which necessitates management intervention like the introduction of new individuals (Fritts and Carbyn 1995, Hedrick et al. 2014, Carroll et al. 2003). Because of the gauntlet of unrestricted wolf killing in Wyoming, Colorado's wolves are largely isolated from gene flow, or the natural introduction of new individuals, to increase genetic diversity.

In addition, genetic drift and inbreeding can further reduce genetic diversity over time. Studies have shown that wolves have a number of behavioral mechanisms that limit the occurrence of inbreeding (vonHoldt 2010), as well as a unique social structure that limits the number of reproducing animals in the population. These inbreeding-avoidance behaviors help maintain the genetic diversity of wolf populations, but in a situation with so few wolves on the ground, these behaviors can limit the potential of wolves finding a mate. A study of wolf recovery in the Yukon found that colonization of vacant territories by young wolf pairs was the primary mechanism of early population recovery (Hayes and Harestad 2000), further emphasizing the importance of adding more dispersal-age individuals to the Colorado population.

Studies have also shown that mortality risk to individual wolves is higher when the density of wolves in an area is lower, and that mortality is also negatively correlated with pack membership (Smith et al. 2014). In other words, reintroduced wolves are more likely to survive when they are able to maintain higher density and achieve greater pack formation, both of which continued reintroductions will facilitate in Colorado. Without timely further reintroductions, the findings of this study suggest that the individual wolves on the landscape in Colorado are less likely to survive. Thus, the potential delay threatens the progress of the reintroduction effort so far.

Furthermore, recent analysis of gray wolf populations across North America found that while gray wolves fall above minimum effective population sizes needed to avoid extinction due to inbreeding depression in the short term, they still remain below sizes predicted to be necessary to avoid long-term risk of extinction (vonHoldt et al. 2024). The Colorado reintroduction represents a key piece in improving the viability of the entire continent's gray wolf population, and this peer-reviewed study reveals fragmentation and genetic limitations that necessitate this continued effort. This is exacerbated by the threat of politically-driven gray wolf delisting (Carroll 2021).

Simply put, declining to reintroduce additional wolves in a timely manner threatens the ecological viability of Colorado's small, isolated population. We hope that you will consider this biological framework when you consider the petition to delay the next round of introductions, for the preservation of maximum viability potential and allow for the most prudent, science based wildlife management.

# Literature Cited

Brandell, Ellen E., et al. "Patterns and processes of pathogen exposure in gray wolves across North America." *Scientific reports* 11.1 (2021): 3722.

Carroll, Carlos, et al. "Impacts of landscape change on wolf restoration success: planning a reintroduction program based on static and dynamic spatial models." *Conservation Biology* 17.2 (2003): 536-548.

Carroll, Carlos, et al. "Defining recovery goals and strategies for endangered species: the wolf as a case study." *BioScience* 56.1 (2006): 25-37.

Carroll, Carlos, et al. "Wolf Delisting Challenges Demonstrate Need for an Improved Framework for Conserving Intraspecific Variation under the Endangered Species Act." *Bioscience* 71.1 (2021): 73-84.

Creel, Scott, and Jay J. Rotella. "Meta-analysis of relationships between human offtake, total mortality and population dynamics of gray wolves (Canis lupus)." *PLoS one* 5.9 (2010): e12918.

Darimont, C. T., et al. "Predators in natural fragments: foraging ecology of wolves in British Columbia's central and north coast archipelago." *Journal of Biogeography* 31.11 (2004): 1867-1877.

Forbes, Stephen H., and Diane K. Boyd. "Genetic Structure and Migration in Native and Reintroduced Rocky Mountain Wolf Populations." *Conservation Biology* 11.5 (1997): 1226-1234.

Fritts, Steven H., and Ludwig N. Carbyn. "Population viability, nature reserves, and the outlook for gray wolf conservation in North America." *Restoration Ecology* 3.1 (1995): 26-38.

Funk, W. C., et al. "Improving conservation policy with genomics: a guide to integrating adaptive potential into US Endangered Species Act decisions for conservation practitioners and geneticists." *Conservation Genetics* 20 (2019): 115-134.

Hayes, RD and, and Alton S. Harestad. "Demography of a recovering wolf population in the Yukon." *Canadian Journal of Zoology* 78.1 (2000): 36-48.

Hedrick, Philip W., et al. "Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population." *Conservation genetics* 15 (2014): 1111-1121.

Hedrick, P. W., et al. "Genetics and extinction and the example of Isle Royale wolves." *Animal Conservation* 22.3 (2019): 302-309.

Liberg, Olof, et al. "Severe inbreeding depression in a wild wolf Canis lupus population." *Biology letters* 1.1 (2005): 17-20.

Miller, Brian, et al. "Biological and technical considerations of carnivore translocation: a review." *Animal Conservation Forum*. Vol. 2. No. 1. Cambridge University Press, 1999.

Pletscher, Daniel H., et al. "Population dynamics of a recolonizing wolf population." *The Journal of wildlife management* (1997): 459-465.

Smith, Douglas W., et al. "Survival of colonizing wolves in the northern Rocky Mountains of the United States, 1982–2004." *The Journal of Wildlife Management* 74.4 (2010): 620-634.

Stenglein, Jennifer L., and Timothy R. Van Deelen. "Demographic and component Allee effects in southern Lake Superior gray wolves." *PLoS One* 11.3 (2016): e0150535.

vonHoldt, Bridgett M., et al. "The genealogy and genetic viability of reintroduced Yellowstone grey wolves." *Molecular ecology* 17.1 (2008): 252-274.

vonHoldt, Bridgett M. *Canid Population Dynamics and Evolutionary Genomics*. University of California, Los Angeles, 2010.

vonHoldt, Bridgett M., et al. "Demographic history shapes North American gray wolf genomic diversity and informs species' conservation." *Molecular ecology* 33.3 (2024): e17231.

Wolf, C. Magdalena, Theodore Garland Jr, and Brad Griffith. "Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts." *Biological conservation* 86.2 (1998): 243-255.

**Exhibit 2**

# Biological Studies

scientific reports

OPEN

# Patterns and processes of pathogen exposure in gray wolves across North America

Ellen E. Brandell[1]✉, Paul C. Cross[2], Meggan E. Craft[3], Douglas W. Smith[4], Edward J. Dubovi[5], Marie L. J. Gilbertson[6], Tyler Wheeldon[7], John A. Stephenson[8], Shannon Barber-Meyer[9], Bridget L. Borg[10], Mathew Sorum[11], Daniel R. Stahler[4], Allicia Kelly[12], Morgan Anderson[13], H. Dean Cluff[14], Daniel R. MacNulty[15], Dominique E. Watts[16], Gretchen H. Roffler[17], Helen Schwantje[18], Mark Hebblewhite[19], Kimberlee Beckmen[20], Heather Fenton[14,21] & Peter J. Hudson[1]

**The presence of many pathogens varies in a predictable manner with latitude, with infections decreasing from the equator towards the poles. We investigated the geographic trends of pathogens infecting a widely distributed carnivore: the gray wolf (*Canis lupus*). Specifically, we investigated which variables best explain and predict geographic trends in seroprevalence across North American wolf populations and the implications of the underlying mechanisms. We compiled a large serological dataset of nearly 2000 wolves from 17 study areas, spanning 80° longitude and 50° latitude. Generalized linear mixed models were constructed to predict the probability of seropositivity of four important pathogens: canine adenovirus, herpesvirus, parvovirus, and distemper virus—and two parasites: *Neospora caninum* and *Toxoplasma gondii*. Canine adenovirus and herpesvirus were the most widely distributed pathogens, whereas *N. caninum* was relatively uncommon. Canine parvovirus and distemper had high annual variation, with western populations experiencing more frequent outbreaks than eastern populations. Seroprevalence of all infections increased as wolves aged, and denser wolf populations had a greater risk of exposure. Probability of exposure was positively correlated with human density, suggesting that dogs and synanthropic animals may be important pathogen reservoirs. Pathogen exposure did not appear to follow a latitudinal gradient, with the exception of *N. caninum*. Instead, clustered study areas were more similar: wolves from the Great Lakes region had**

[1]Center for Infectious Disease Dynamics, Department of Biology, Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA 16802, USA. [2]U.S. Geological Survey, Northern Rocky Mountain Science Center, Bozeman, MT 59715, USA. [3]Department of Ecology, Evolution, and Behavior, University of Minnesota, Saint Paul, MN 55108, USA. [4]Yellowstone Center for Resources, Wolf Project, P.O. Box 168, Yellowstone National Park, WY 82190, USA. [5]Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850, USA. [6]Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN 55108, USA. [7]Ontario Ministry of Natural Resources and Forestry, Trent University, 2140 East Bank Drive, Peterborough, ON K9L 1Z8, Canada. [8]Grand Teton National Park, P.O. Drawer 170, Moose, WY 83012, USA. [9]U.S. Geological Survey, Northern Prairie Wildlife Research Center, 8711 37th St. SE, Jamestown, ND 58401, USA. [10]Denali National Park and Preserve, Central Alaska Inventory and Monitoring Network, P.O. Box 9, Denali Park, AK 99755, USA. [11]Yukon-Charley Rivers National Preserve, Central Alaska Inventory and Monitoring Network, 4175 Geist Road, Fairbanks, AK 99709, USA. [12]Department of Environment and Natural Resources, Government of the Northwest Territories, P.O. Box 900, Fort Smith, NT X0E 0P0, Canada. [13]British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2000 South Ospika Blvd., Prince George, BC V2N 4W5, Canada. [14]Environment and Natural Resources, Government of the Northwest Territories, North Slave Region, NT X1A 2P9, Canada. [15]Department of Wildland Resources, Utah State University, Logan, UT 84322, USA. [16]U.S. Fish and Wildlife Service, Kenai National Wildlife Refuge, P.O. 2139, Soldotna, AK 99669, USA. [17]Division of Wildlife Conservation, Alaska Department of Fish and Game, 802 3rd Street, Douglas, AK 99824, USA. [18]Wildlife and Habitat Branch, British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2080 Labieux Road, Nanaimo, BC V9T 6J9, Canada. [19]Wildlife Biology Program, Department of Ecosystem and Conservation Sciences, Franke College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA. [20]Division of Wildlife Conservation, Dept of Fish and Game, 1300 College Road, Fairbanks, AK 99701, USA. [21]Ross University School of Veterinary Medicine, Basseterre, West Indies KN-03, St. Kitts and Nevis. ✉email: ebrandell08@gmail.com

**lower odds of exposure to the viruses, but higher odds of exposure to *N. caninum* and *T. gondii*; the opposite was true for wolves from the central Rocky Mountains. Overall, mechanistic predictors were more informative of seroprevalence trends than latitude and longitude. Individual host characteristics as well as inherent features of ecosystems determined pathogen exposure risk on a large scale. This work emphasizes the importance of biogeographic wildlife surveillance, and we expound upon avenues of future research of cross-species transmission, spillover, and spatial variation in pathogen infection.**

The prevalence and dynamics of infectious diseases can vary spatially across the distribution of their hosts depending on host demographics, host contact patterns, and pathogen survival. For example, Ferrari et al.[1] showed how the cyclic dynamics of measles varies with human birth rate and seasonality. In a similar manner, Hudson et al.[2] showed how the oscillations of red grouse (*Lagopus lagopus scotica*) abundance, driven by a caecal nematode, varied geographically according to the host growth rate and parasite transmission rate, and this drives longer cycle periods with increasing latitude. Pathogens that infect multiple host species may be more common at lower latitudes when this corresponds with increased numbers of host species or individuals. For example, parasites with complex life cycles that depend on the presence of intermediate hosts[3,4] and seasonal aggregations, which vary with climate, can increase transmission and drive outbreaks[5]. In this paper we addressed the question: How does pathogen seroprevalence in gray wolves (*Canis lupus*) vary across North America and does geography provide a suitable proxy?

Generally, human and wildlife pathogen pressures (e.g., parasite burden, richness, prevalence) decline as latitude increases[6–11] (i.e., towards the poles). Latitude is a proxy for other variables that predictably vary over space and affect pathogen transmission, which may be a function of pathogen survival or host density. For example, latitude can be used to describe the climate envelope for chytrid fungus, where higher latitudes (e.g., cooler temperatures, higher rainfall) are more optimal for fungal survival than lower latitudes. Consequently, chytrid infection intensity is significantly higher at higher latitudes[12]. Understanding the mechanisms driving transmission provides a deeper understanding of host–pathogen dynamics but requires detailed datasets that are often challenging to collect. Here, we assess how well geography alone can explain the observed variation in seroprevalence, and contrast this with variables that may confer a mechanistic understanding of pathogen exposure at individual and population levels, such as wolf and human densities, wolf age, sex, coat color, pack size, or habitat quality (Table 2).

In North America, wolves suffer from both enzootic and epizootic pathogens that can result in chronic disease or acute outbreaks, causing morbidity, mortality, and reduced recruitment[13–16]. Patterns of seroprevalence across wolf populations have not been comprehensively explored, but individual studies have shown notable differences in seroprevalence. For instance, *Neospora caninum* antibodies were not detected in any wolves sampled from the Alaska Peninsula[17], while 66% of adult wolves in northeastern Minnesota were seropositive[18]. This has constrained our understanding about what pathogens we can expect wolves to be exposed to and at what frequency. To investigate the drivers of pathogen exposure, we compiled a serological dataset of North American wolves spanning 17 study areas across 80° of longitude, from the Alaska Peninsula in the west to Ontario in the east, and 50° of latitude, from Ellesmere Island in the north to Arizona and New Mexico in the south (Fig. 1). Wolf sera were tested for antibodies to four viruses: canine adenovirus-1 (i.e., adenovirus), canine parvovirus-2 (i.e., parvovirus), canine distemper virus (i.e., distemper), canine herpesvirus (i.e., herpesvirus), and two protozoa: *Neospora caninum*, and *Toxoplasma gondii* (Table 1).

For directly transmitted pathogens (e.g., adenovirus, herpesvirus, parvovirus), contact rate (i.e., population density) determines transmission rates, and consequently pathogen seroprevalence and outbreak size[19]. Population density is also important for pathogens with environmental transmission (e.g., parvovirus) such that environmental reservoirs and contamination may accumulate more quickly at higher host densities[20]. The presence and population densities of sympatric reservoir hosts, including synanthropic animals, is also important for the dynamics of multi-host viruses (e.g., canine distemper, *T. gondii*)[21–26], as well as parasites with intermediate hosts (e.g., *Neospora caninum*)[27]. Our large-scale dataset captures natural variation in human density, wolf density (e.g., population density, pack size, pack density), host presence (i.e., habitat quality), and primary prey, allowing us to examine their importance (Table 2, Fig. 2).

In addition to larger scale processes, individual characteristics also play a role in wolf pathogen dynamics. North American wolves generally display two coat color phenotypes, black and gray, that vary latitudinally[28]. The black genotype is important for mounting immune responses[29], and thus it has been hypothesized that the black color is maintained via selection from pathogen pressure[30,31]. This leads us to predict that black wolves are more likely to survive an exposure and test positive. Other individual traits, such as age and sex, may also influence pathogen exposure and should also be considered. Specifically, males tend to have higher pathogen prevalence than females due to physiology (e.g., sex and stress hormones) and behavior (e.g., contact patterns), and older individuals have had more time to be exposed to infectious diseases, thus tend to have elevated seroprevalence[32–36].

In wildlife diseases literature, there is a lack of broad scale assessments in exposure trends that also include the animal's ecology as mechanisms. We tested how well a suite of variables conferring mechanisms (Table 2) explained and predicted differences in probability of pathogen exposure across North American wolf populations, compared with latitude and longitude alone.

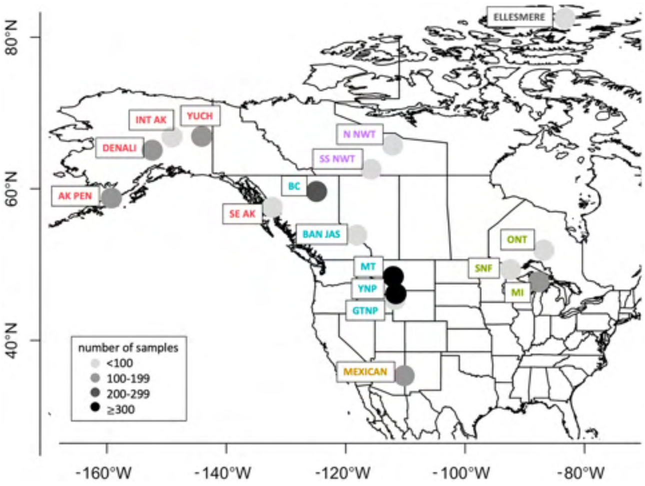


**Figure 1.** A map[38] depicting where wolves were sampled across North America for pathogen and parasite testing, and relative sample size from each study area is shown in shades of gray (increasingly dark gray = increasing sample size)[39]. Each study area is identified as follows: Alaska Peninsula (AK PEN), Denali National Park (DENALI), central-eastern Alaska (INT AK), Yukon-Charley Rivers National Preserve (YUCH), Ellesmere Island (ELLESMERE), North Slave Northwest Territories (N NWT), South Slave Northwest Territories (SS NWT), British Columbia (BC), southeastern Alaska (SE AK), Banff and Jasper National Parks (BAN JAS), Montana (MT), Yellowstone National Park (YNP), Grand Teton National Park (GTNP), Mexican wolves (MEXICAN), Ontario (ONT), Superior National Forest (SNF), and the Upper Peninsula of Michigan (MI). Study area labels were colored by region: High Arctic (gray), Subarctic (purple), Alaska (red), central Rocky Mountains (turquoise), Great Lakes (green), and Mexican (gold), and displayed as a circle with a 200-km radius.

| Pathogen | Transmission route | Alternative Hosts | Symptoms / effects | Population consequences |
|---|---|---|---|---|
| Canine adenovirus | Direct via respiratory secretions; fecal–oral | None | Fever, liver inflammation | Mild |
| Canine distemper virus | Direct via respiratory droplets/secretions; airborne | Carnivores—grizzly (*Ursus arctos*) and black bears (*Ursus americanus*), cougars (*Puma concolor*), lynx (*Lynx canadensis*), coyotes (*Canis latrans*), raccoons (*Procyon lotor*), skunks (*Mephitis mephitis*), domestic dogs (*Canis lupus familiaris*) | Enamel hypoplasia, seizures, death—mostly affects pups or naive, immunocompromised adults | Severe (albeit acute) |
| Canine herpesvirus | Vertical; sexual; direct via respiratory droplets/ secretions | None | Adult females: abortion; pups: lethargy, sudden death | Mild |
| Canine parvovirus | Fecal–oral | Domestic dogs | Pups: diarrhea, lethargy, death | Moderate (but variable) |
| *Neospora caninum* | Ingesting infected tissue (definitive) or oocysts (intermediate); vertical | Intermediate: Ungulates<br>Definitive: canids—coyotes, foxes (*Vulpes vulpes*) | Muscle weakness, tremors, loss of coordination | Mild |
| *Toxoplasma gondii* | Ingesting infected tissue or oocysts; vertical | Intermediate: Warm-blooded animals<br>Definitive: felids—cougars, lynx, bobcat (*Lynx rufus*), domestic cats (*Felis catus*) | Increased aggression and risk-taking | Mild |

**Table 1.** A list of wolf pathogens that were examined for populations sampled across North America (Fig. 1) and their characteristics[37]. 'Alternative hosts' refers to hosts other than wolves that occur within the study areas that we expect to be important in transmitting pathogens to wolves. 'Population consequences' describes the known or expected severity of these pathogen infections on wolf population size or growth rate (minimal, moderate, severe).

## Materials and methods

**Serology dataset.** We aimed to compile a serological dataset from wolf populations across North America. We collaborated with wildlife professionals across the continent and attempted to obtain samples from a variety of ecosystems. While our dataset is expansive, there are gaps for two primary reasons: (1) wolves were not sampled or intensively studied in many areas, and (2) wolves do not occupy much of their historic North American range, especially in the south, thus they are absent from much of the United States. At a broad scale, our dataset is a fair representation of where wolves presently occur and are studied across North America.

All wolf samples analyzed for this study were previously collected by wildlife professionals within each study area. No wolf was captured or handled for the purposes of this study. Samples previously collected from live-captured wolves were handled and sampled according to the American Society of Mammalogists (Sikes et al.

| Variable name | Description | Rationale for inclusion/prediction |
|---|---|---|
| *Latitude*$^{+}$ | Latitude at study area centroid | Latitude may capture geographic variation in pathogen infections; we predicted that seroprevalence decreases as latitude increases. |
| *Longitude*$^{+}$ | Longitude at study area centroid | Longitude may capture geographic variation in pathogen infections. |
| *Age class*$^{*+}$ | Estimate of wolf age class: pup (< 1), subadult (1–2), and adult (≥ 3) | As individuals age, they have more time to be exposed to pathogens, thus older wolves will have higher seroprevalence. Age category is less error-prone than numerical age estimates. |
| *Year*$^{*}$ | Biological year, birth month = first month | Pathogen exposure may be predictable by year (i.e., endemics), or unpredictable (i.e., epidemics). |
| *Study area*$^{*}$ | Study area abbreviation | Study area may describe variation in pathogen exposure, not accounted for by other variables. |
| *Habitat quality*$^{*}$ | Index for habitat quality based on land cover type and topography | A continuous estimate of the habitat quality of the study area, this covariate considers habitat characteristics that carnivores, especially wolves, positively select. This is a proxy for the presence of sympatric carnivore hosts. Prediction: seroprevalence increases with habitat quality. |
| *Human density*$^{*}$ | Number of people/1000-km$^2$ | Provides information about how urban the area is, and thus the potential for contact between unvaccinated dogs or synanthropic species (e.g., rodents, coyotes, raccoons, skunks, cats) and wolves. Prediction: seroprevalence increases with human density. |
| *Wolf density*$^{*}$ | Number of wolves/1000-km$^2$; mean annual density results in one estimate per study area | Population density is related to direct transmission rates and environmental contamination. Prediction: seroprevalence increases with wolf density. |
| *Pack size*$^{*}$ | Mean annual pack size; one estimate per study area | This tells us about the daily contacts of a wolf, which differs from contact rate at the population-level. Prediction: seroprevalence increases with pack size. |
| *Sex*$^{*}$ | Male or Female | There is evidence that males have higher pathogen prevalence than females across many taxa and pathogens—we predict males have higher seroprevalence. |
| *Coat color*$^{*}$ | Gray or Black | The locus that confers black coat color in wolves is linked to beta-defensin genes, which increases the responsiveness of the innate immune system. We assume gray = missing k-locus, black = presence of k-locus. Prediction: black wolves have higher seroprevalence. |
| *Age* | Estimate of wolf age; integer to two decimal places | As individuals age, they have more time to be exposed to pathogens, thus we predicted older wolves have higher seroprevalence. |
| *Social status* | Breeder or non-breeder | Breeders typically have higher stress levels and energetic demands than non-breeders, which we predict increases seroprevalence. |
| *Prey species* | Top two primary prey species | *N. caninum* or *T. gondii* may be more prevalent in different intermediate hosts. Prediction: seroprevalence is higher where white-tailed deer are a primary prey species. |
| *Pack membership* | Name of the pack the wolf was a member of when sampled | There may be heterogeneities in pathogen exposure based on pack membership. |
| *Pack density* | Number of packs/1000-km$^2$; mean annual density results in one estimate per study area | Contact among wolves from different packs is likely influenced by the number of packs in the population. Prediction: seroprevalence increases with pack density. |

**Table 2.** A list of variables considered for inclusion in generalized linear mixed models predicting pathogen and parasite exposure. Variable descriptions and rationales or predictions are provided; a $^{*}$ indicates the variable was included in the final *complete model*, a $^{+}$ indicates the variable was included in the *geographic model.*

2016), or similar guidelines, and approved by the appropriate institutions—see Supplementary Table S5 for specific approval and permits associated with samples included in our database. All samples from the northern Northwest Territories and about half from Ellesmere Island, Nunavut, were air dried, blood-soaked filter paper, and almost all other samples were serum collected from the cephalic or saphenous vein during live capture. We leveraged stored (i.e., frozen at – 20° to – 80° Celsius) samples for this study and, where needed, we coordinated with local wildlife professionals to ship serum to the Animal Health Diagnostic Center at Cornell University (Ithaca, NY, USA) where samples were screened for antibodies to the six pathogens of interest.

We screened wolf samples from 13 of the 17 study areas, and we used previously published serological results for the remaining 4 study areas (Supplementary Table S2). Previously published datasets included in our analyses were: Mexican[40], Banff and Jasper National Parks[41], Alaska Peninsula[17], and a portion of the samples from Superior National Forest[18]. Here we discuss how samples were analyzed at the Animal Health Diagnostic Center at Cornell University, which comprised about 80% of our dataset (see Supplementary Table S3 for other testing information). Virus neutralization assays were performed to detect antibodies to canine adenovirus, distemper virus, and herpesvirus; hemagglutination inhibition assays were used for parvovirus; indirect fluorescent assays were used for *N. caninum*; enzyme-linked immunosorbent assay or monocyte activation tests were used for *T. gondii*. All assays provided titer values except for the indirect fluorescent and enzyme-linked immunosorbent assays, which provided a positive, negative, or suspect/equivocal result. Sample collection and test methods for the previously published samples were identical or equivalent to methods implemented for the other 13 study areas, thus are directly comparable (Supplementary Tables S2, S3).

The response variable in our models was a binary variable representing previous exposure (1), i.e., seropositive result, or not (0), i.e., seronegative result. A result was seropositive when the titer dilution was equal or greater than the standard titer cutoff provided by the assay manufacturer (Supplementary Table S3), or if the assay was

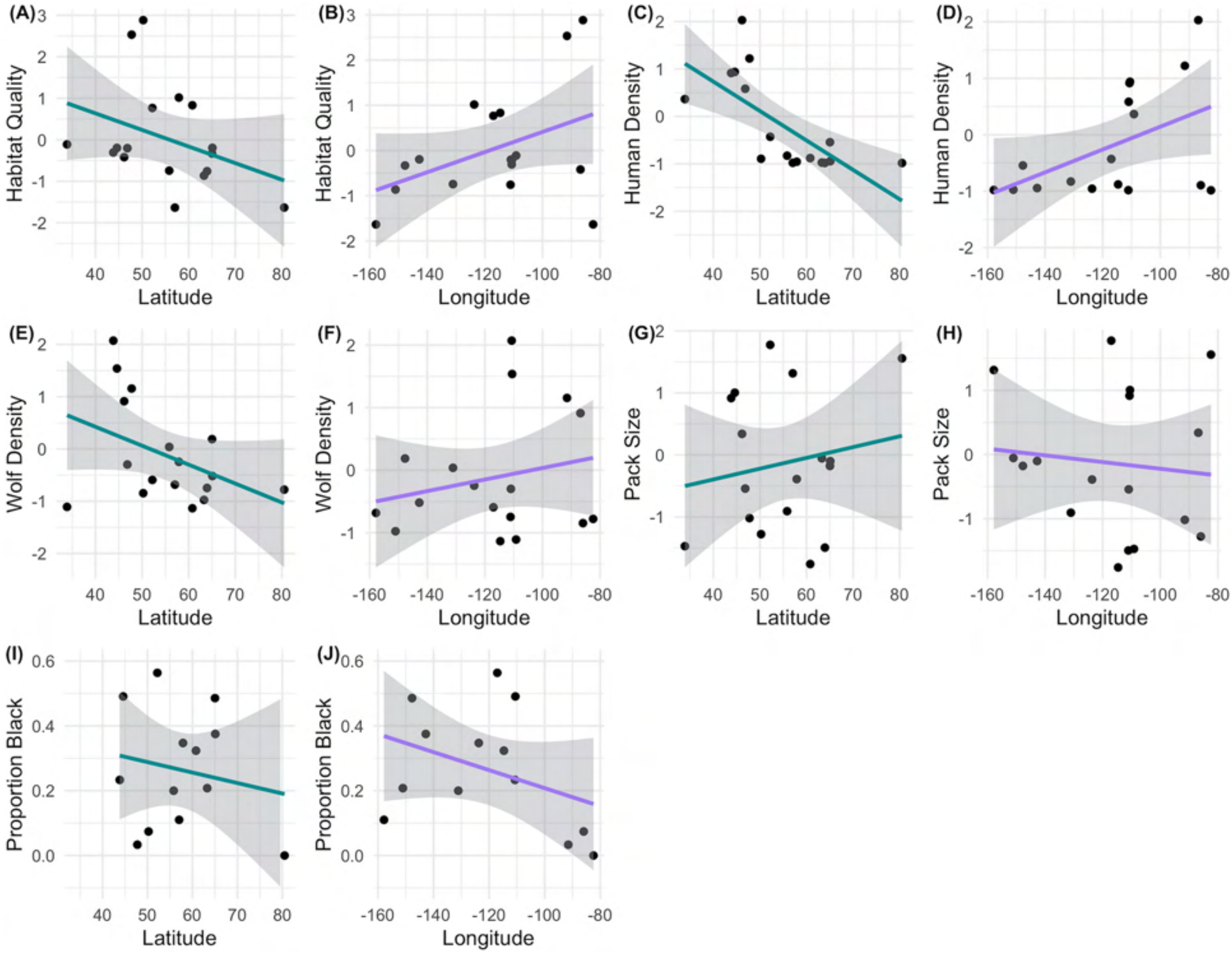


**Figure 2.** The effect of latitude (teal lines) and longitude (purple lines) on selected standardized, continuous predictor variables that were included in the generalized linear mixed models[39]: (**A**,**B**) habitat quality, (**C**,**D**) human density, (**E**,**F**) wolf density, (**G**,**H**) pack size, as well as (**I**,**J**) proportion of black wolves (for **I**,**J**, note that MI, MT, and N NWT were removed due to lack of coat color data; MEXICAN was removed as *Canis lupus baileyi* does not present a black phenotype). Gray polygons are 95% confidence intervals around univariate regression lines.

positive/suspect (suspect comprised ~ 3% of the total dataset). As such, we assumed that serological assays were considered to be perfect, which is unlikely to be true. To address this, we assessed population seroprevalence using standard and conservative titer cutoffs; the standard cutoff is the lab-recommended value (Supplementary Table S3), and the conservative cutoff is one dilution above the standard cutoff. We found that pathogen prevalence was minimally affected by titer cutoff and we do not believe that this affected our results (Supplementary Fig. S1). Therefore, we present results using a standard titer cutoff specific to each assay and sample type. Note too that only individuals that survived an exposure were available to be sampled for serological analyses, thus lack of antibody detection may mean that the pathogen does not exist in that study area, or alternatively, that it caused high mortality locally and was not detected.

**Model construction.** We constructed and analyzed models predicting the probability that a wolf was exposed to a given pathogen using R v3.6.3[39]. We tested how well geography (i.e., latitude and longitude) explained and predicted pathogen exposure compared with mechanistic predictor variables. Two models were constructed for each pathogen: a *complete model* and a *geographic model* (Eq. 1). The *geographic model*, which acted as a null/uninformative model, contained latitude and longitude, and controlled for the effect of age. The *complete model* contained selected predictor variables (Table 2). Both models included random effects (generalized linear mixed model, 'GLMM'). Models were fit with a complementary-log–log link and a Bernoulli error distribution using the function *glmer* in the package *lme4*[42]. In the *complete model*, *year* and *study area* were both considered as random effects, where *year* was nested within *study area* because we posited that the effect of year differed within each study area. Nesting year within study area gave us a random effect for *study area* alone, as well as *study area*year*. *Study area* was the only random effect considered in the *geographic model*. The form of our GLMMs was:

$$Y_{ijk} = \text{Bernoulli}\left(p_{ijk}\right) \tag{1}$$

$$f\left(p_{ijk}\right) = \beta_0 + \beta_1 x_{1ijk} + \cdots + \beta_n x_{nijk} + \alpha_j + \gamma_{jk} + \varepsilon_i$$

$$\alpha_j \sim \text{ Normal}\left(0, \sigma^2\right)$$

$$\gamma_{jk} \sim \text{ Normal}\left(0, \sigma^2\right)$$

where $Y_{ijk}$ is the seropositive result for the $n_{jk}$ trial from the $i$th individual from the $j$th study area in year $k$; $p_{ijk}$ is the probability of exposure from the $i$th individual from the $j$th study area in year $k$; $x_{nijk}$ is the $i$th value of the $j$th study area in the $k$th year for the $n$th predictor; $\beta_n$ are the estimated predictor coefficients; $\alpha_j$ is the study area-specific effect; $\gamma_{jk}$ is the effect of year within that study area; $\varepsilon_i$ is the remaining error in seropositivity. The *year* effects, including $\gamma_{jk}$, did not appear in the *geographic model*. The link function (*f*) applied is the complementary-log–log.

All metadata were collected specifically for this project such that we determined our hypotheses *a priori*[43] (Table 2, Supplementary Table S1). All variables considered were expected to influence pathogen exposure. Table 2 displays variables considered for inclusion in the models, descriptions, and rationales or predictions. Each sample was assumed to be unique, given that < 7% of the data were recollared wolves. If multiple age estimates were given (e.g., 3 or 4 years old), we randomly selected one age estimate. Some variables were removed prior to model building due to lack of sufficient data, including *pack membership*, *social status*, and *pack density* (Supplementary Table S4). *Prey species* was not included because primary prey species were too similar across study areas (e.g., a combination of elk, deer spp., moose, caribou), and after exploratory plotting, did not appear to provide additional information above *study area* and *habitat quality*. Prey species also are likely reflected in wolf density and pack size[44–47]. We included *age class* instead of *age* in our models because *age* was based on tooth wear and body size, and is an error-prone estimate especially for older ages[48]. We used coat color as a proxy for the presence of the K-locus allele, which is supported by Anderson et al.[28] who found that > 98% of wolves from Yellowstone and western Canada classified as 'black' did indeed have the K-locus genotype.

We also considered *wolf density*, *pack size*, *human density*, *habitat quality*, and *sex* as potentially important predictors of pathogen exposure (Table 2). Wolves were counted in all study areas, including annual population counts and pack size estimates. These data were typically collected during aerial or ground tracking surveys in the winter. If more than one estimate was available per year within a study area, which was common for pack sizes, they were averaged to create one annual *wolf density* (number of wolves/1000-km$^2$/year) and one annual mean *pack size* (mean number of wolves/pack/year) value per study area. To estimate *human density* and *habitat quality*, we first had to determine how large of an area should be considered, as most areas did not have clearly defined boundaries or isolated wolf populations. We considered a range of area sizes (radius 50-km to 300-km from study area centroids) and selected a 200-km radius because *human density* and *habitat quality* were less variable in comparison with small or large radii, and it is more congruent with wolf dispersal distance[49,50]. *Human density* was considered to be the number of people per 1000-km$^2$[51], and was used as a proxy for the presence of unvaccinated dogs and synanthropic animals[52]. *Habitat quality* was a proxy for the presence of carnivore hosts, and was a continuous variable calculated as the product of: percent forest cover[53], percent area with slope ≤ 20° [54], and density of hard edges (e.g., cutblocks, pipeline cuts, forest edges; R package *landscapemetrics*[55]). These habitat characteristics were selected because they were considered positive predictors of carnivore presence, such as grizzly bears, lynx, bobcat, coyotes, with a focus on wolves[56–68]. While this proxy for carnivore presence is imperfect as carnivore distributions varied over our sampling distribution, and carnivores may select for different landscape features at different scales, it captures important features where wolves and other carnivores may interact, and therefore where cross-species pathogen transmission may occur. Finally, *sex* (male or female) was recorded during captures.

Before building the *complete model*, all variables were screened for collinearity using Spearman's correlation coefficient (ρ). Human density and wolf density were highly correlated (ρ = 0.62; Supplementary Fig. S3, S4) and thus were not included in the same model; however, as we were interested in the effects of both wolf and human density on pathogen dynamics, we ran the complete model both ways (i.e., with either wolf density or human density). All variables other than latitude and longitude were retained (i.e., correlation < 0.4). Latitude was highly correlated with human density (ρ = – 0.79) and moderately correlated with wolf density (ρ = – 0.36) and habitat quality (ρ = – 0.33, Fig. 2, S3). Longitude was moderately correlated with human density (ρ = 0.37), habitat quality (ρ = 0.30), and proportion of black wolves (ρ = – 0.33, Fig. 2, Supplementary Fig. S3). Our models were as follows (note that the divider between *year* and *study area* denotes the nested structure *study area* + *study area*year*):

*Complete model.*

$$\begin{aligned}\text{Probability}\left(\text{exposure}\right) \sim & \; \textit{wolf density} \text{ or } \textit{human density} + \textit{habitat type} \\ & + \textit{pack size} + \textit{age class} + \textit{sex} + \textit{color} + \left(\textit{study area} \,|\, \textit{year}\right)\end{aligned}$$

*Geographic model.*

$$\text{Probability}(\text{exposure}) \sim \textit{latitude} + \textit{longitude} + \textit{age class} + \textit{study area}$$

Continuous variables were standardized prior to model implementation (subtract the variable mean and divide by the standard deviation, Gelman and Hill[69], Menard[70]). This centers all variables (mean = 0), and deviations from the mean are represented in standard deviations. Standardizing puts all continuous variables on the same scale, allowing for direct comparisons and simplifying interpretation. All models converged using the bobyqa optimizer.

**Model evaluation.** Models were evaluated by root mean square error (RMSE) and area under the receiver-operator curve (AUC). RMSE and AUC provide different, important model evaluation. RMSE is a measure of model fit as it calculates the error between the observed data and the fitted model, whereas AUC provides a measure of the classification accuracy of the model; both criteria use model fixed effects. To calculate AUC, the false positive rate (1—specificity) is plotted against the true-positive rate (sensitivity); AUC = 0.5 indicates no discrimination, AUC > 0.5 indicates that the true positive rate is higher than the false-positive rate, and AUC > 0.8 indicates excellent discrimination[71]. We compared the testing set and training set RMSE and AUC using four-fold cross validation[72] (see Supplementary Information for training and testing group information). Supplementary Figure S5 and Table S6 display the mean RMSE and AUC across the four datasets (training and testing) per pathogen and model.

Model fit assessments included: training and testing set RMSE and AUC estimates, pseudo-R2 values (calculated with fixed effects only), Maximum Likelihood estimator convergence, and p-values (i.e., hypothesis testing, Table 2). Predictor variables were considered statistically significant at an alpha value of ≤ 0.05. The geographic and complete models, parameter estimation, and their evaluations used all (non-missing) data.

## Results

**Dataset.** We sampled 1839 wolves from 17 study areas to comprise the final dataset, with 134 wolves resampled, totaling 1973 rows of data. The mean number of samples per study area was 116 (95% confidence interval [CI] 90–142), ranging from 10 (SE AK) to 383 (YNP), but most study areas had between 50 and 150 samples. Most study areas were sampled for 10 years (95% CI 8.5–11.8, range = 2–25) and, on average, 12 wolves were sampled per year (95% CI 11–13). Collectively, study areas had a mean wolf density of 13 wolves/1000-km$^2$ (95% CI 11–16, range = 3.4–34.0) and a mean of 6.3 wolves per pack (95% CI 5.8–6.8, range = 3.7–9.6). Habitat quality with respect to the presence of carnivore hosts was similar in most study areas, although a few populations stood out as low quality (AK PEN, Ellesmere Island), and others as high quality (ONT, SNF). Human density was more variable: some study areas had < 11 people/1000-km$^2$ (AK PEN, Ellesmere, N NWT) and others had > 3000 people/1000-km$^2$ (MI, SNF, YNP, GTNP, MT), with a mean of ~ 1600 people/1000-km$^2$.

Most wolves sampled were adults (44%), and pups and subadults were equally sampled (28% each). Males and females were nearly equally sampled (52% and 48%, respectively), and there were more than twice as many gray wolves sampled (70%) as black wolves (30%). Some metadata were missing, in particular coat color, and missing information tended to be grouped by population (Supplementary Table S4).

Adenovirus was the most widespread and prevalent pathogen (mean seroprevalence 86.2%, sd = 8.0%, range = 73.5–100%), followed by herpesvirus (mean seroprevalence 79.5%, sd = 11.3%, range = 57.1–94.3%, Fig. 3). *N. caninum* was the least common pathogen (mean seroprevalence 24.8%, sd = 24.4%, range = 0–74.7%, Fig. 3), and *T. gondii* was moderately prevalent across study areas (mean seroprevalence 51.5%, sd = 20.5%, range = 26.9–87.6%, Fig. 3). Distemper virus was relatively uncommon (mean seroprevalence 22.7%, sd = 18.0%, range = 0–55.6%, Fig. 3), but as an epizootic virus, overall seroprevalence is a poor representation of viral pressure or dynamics. We identified clear peaks in distemper seroprevalence in most populations that were sampled for at least five consecutive years (Supplementary Fig. S2). Evidence of exposure to parvovirus was the most variable (mean seroprevalence 73.8%, sd = 25.0%, range = 10.0–100%). Interestingly, parvovirus tended to be enzootic (e.g., BAN JAS, GTNP, MT, SNF, YNP) or common but variable (e.g., BC, DENALI, MEXICAN, MI, SS NWT, YUCH), but was uncommon in some study areas (e.g., AK PEN, SE AK), or patterns were unclear (e.g., INT AK, ELLES, N NWT, ONT, Fig. 3, Supplementary Fig. S2).

**Model results.** The coefficient estimates (β) for latitude were negative for all pathogens except adenovirus and distemper where β ~ 0. However, latitude was only a statistically significant predictor of *N. caninum* exposure such that the probability of exposure to *N. caninum* decreased appreciably as latitude increased across North America (i.e., northward, Fig. 4A, Supplementary Fig. S14, Table S7). The effect of longitude was variable: the coefficient estimates for longitude were negative for adenovirus and herpesvirus, positive for parvovirus and *N. caninum*, and approximately zero for distemper and *T. gondii* (Fig. 4B). Longitude was only a statistically significant predictor of herpesvirus exposure such that the probability of exposure to herpesvirus decreased as longitude increased across North America (i.e., eastward)—although statistically significant, the effect size of longitude on herpesvirus was relatively small as herpesvirus was common in our sampled study areas (mean seroprevalence ~ 80% Fig. 3, S10). Pseudo-R$^2$ values (Cragg-Uhler approximation, see SI) were lower for *geographic models* compared with *complete models* for the adenovirus, distemper, and herpesvirus; *geographic model* pseudo-R$^2$ was higher for the *N. caninum complete model*; pseudo-R$^2$ values were equal for both models for parvovirus and *T. gondii*. In general, the selected predictor variables accounted for a larger proportion of the variation in exposure than latitude and longitude.

The effect of *habitat quality* on pathogen exposure varied and tended to be small ($\beta < 0$ adenovirus, distemper, herpesvirus, *T. gondii*; $\beta > 0$ parvovirus, *N. caninum*); *habitat quality* was only considered a statistically significant predictor of canine distemper (Fig. 4C). Increasing *human density* was significantly and positively related to the

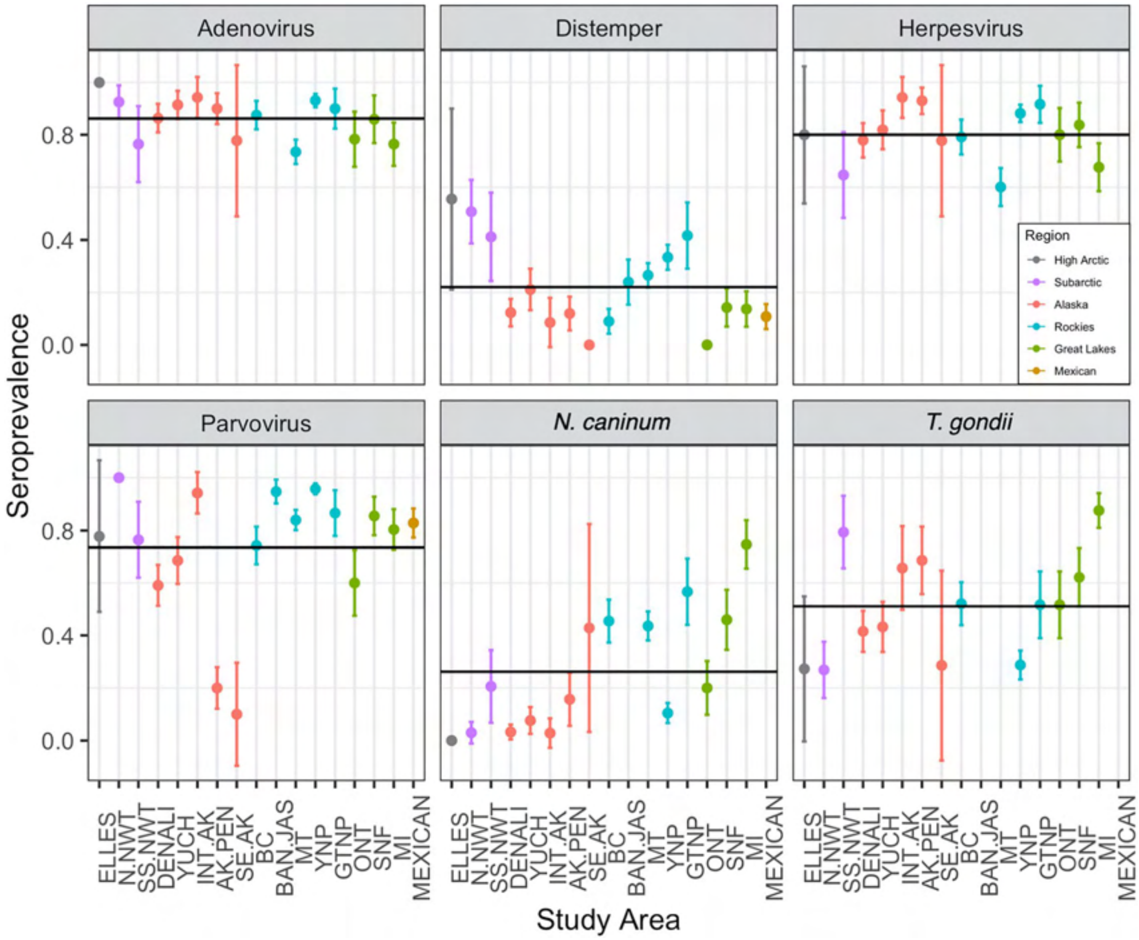


**Figure 3.** A panel plot displaying seroprevalence estimates, and 95% confidence intervals, for each pathogen tested from each study area[39]. Study areas are listed approximately north (left) to south (right) on the x-axis and grouped by region: High Arctic (gray), Subarctic (purple), Alaska (red), central Rocky Mountains (turquoise), Great Lakes (green), and Mexican (gold) (see Fig. 1 caption for study area abbreviations). The horizontal lines show the grand mean seroprevalence for each pathogen. Note that not all study areas were tested for each pathogen.

probability of pathogen exposure for all pathogens ($\beta > 0$, $p \le 0.05$ except *T. gondii*; Fig. 4D). *Human density* had large effects on distemper, parvovirus, and *N. caninum*—for example, the probability a wolf was seropositive for distemper increased 68% over the *human density* range assessed (Supplementary Fig. S9). Similarly, *wolf density* was positively related to the probability of pathogen exposure for all pathogens ($\beta > 0$), except *T. gondii* ($\beta \sim 0$), and was a statistically significant predictor of pathogen exposure for pathogens except parvovirus and *T. gondii* (Fig. 4E). The effect of *pack size* on probability of exposure was variable ($\beta < 0$ *N. caninum, T. gondii;* $\beta > 0$ adenovirus, herpesvirus, parvovirus; $\beta \sim 0$ distemper), but these effects were small and statistically insignificant (Fig. 4F). Contrary to our predictions, probability of pathogen exposure was invariant to *coat color* and *sex* such that effect sizes were small and statistically insignificant (Fig. 4H,G); the exception was that gray wolves had a slightly higher probability of exposure to *N. caninum* than black wolves. As expected, seroprevalence increased with age for all pathogens (Fig. 4I,J). See SI *Model Results* (Supplementary Table S7) for additional modeling outputs.

We performed a four-fold cross validation whereby 13 study areas were used as the training set and the remaining four study areas were used as the testing set (Supplementary Fig. S5, Table S6). Testing set RMSE values were higher than RMSE values from models built using the training set, indicating that predictive power was weaker than explanatory power, as expected[72]. This also suggests that model fit was not highly dependent on which study areas were used in the training or testing sets. *Geographic models* had marginally higher RMSE and lower AUC than *complete models*, indicating slightly poorer fit and classification power. Regardless of model, exposure to some pathogens was better explained than others (e.g., poorest fit for *T. gondii*, best fit for adenovirus and herpesvirus). RMSE values were fairly high across all models, meaning that there was a significant amount of variation in pathogen exposure that was unaccounted for—especially *T. gondii*. This was also evident in that random effects accounted for a notable portion of the variation in pathogen exposure (Fig. 5), and pseudo-$R^2$ values were fairly low (<0.4).

Models had moderate power to correctly classify an individual as positive or negative for pathogen exposure (mean training set AUC = 0.69, mean testing set AUC = 0.67). For pathogens other than *T. gondii*, AUC dropped, on average, 2–4% from training to testing sets when evaluating the same pathogen; the training set AUC was,

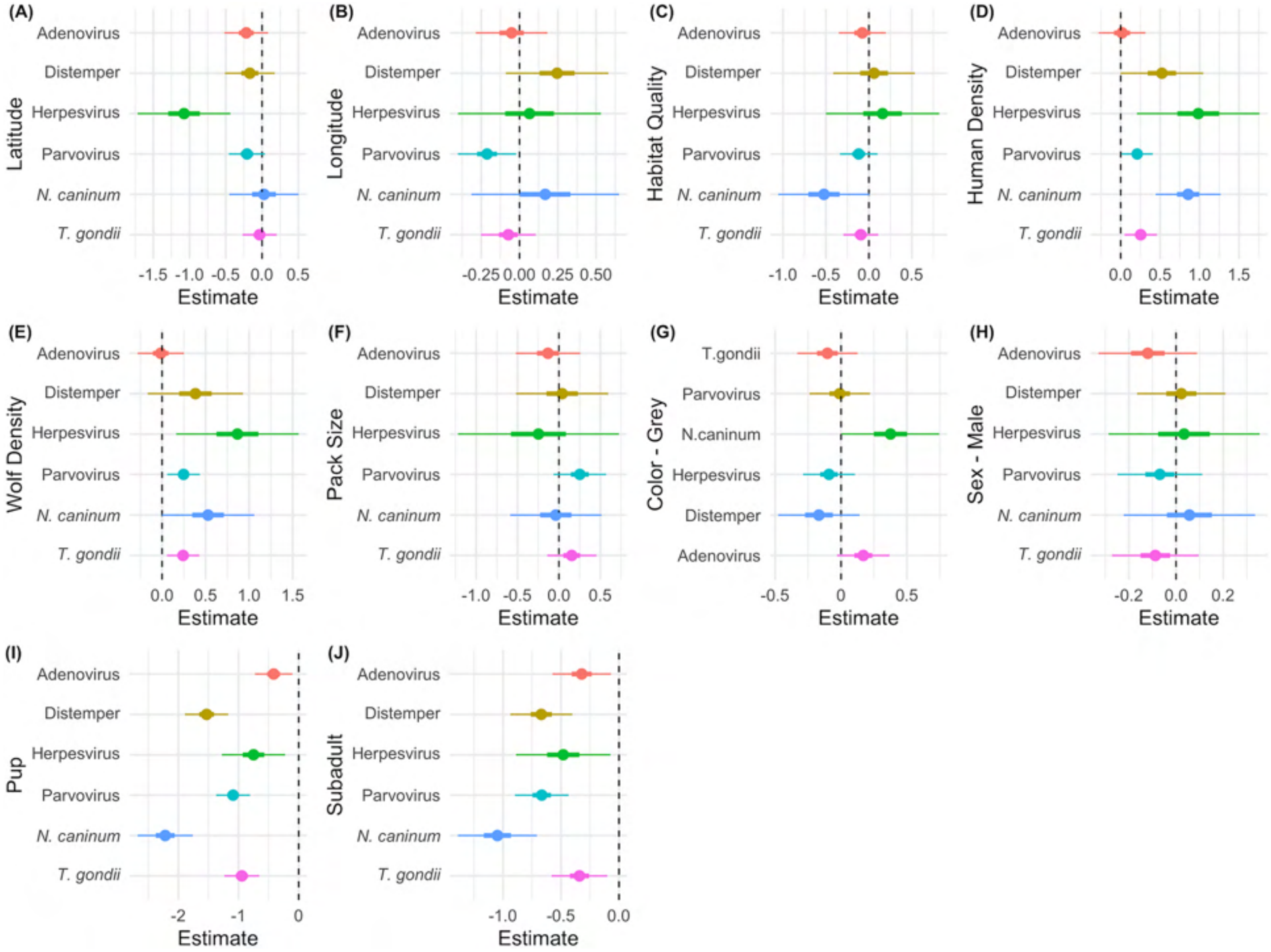


**Figure 4.** Coefficient estimates (log-odds) of the fixed effects in the (**A**,**B**) *geographic* and (**C**–**J**) *complete models* by pathogen (colors)[39]. Thick and thin lines are 50% and 95% confidence intervals, respectively. Categorical variables are interpreted as the effect of: (**G**) gray wolves with respect to black, (**H**) males with respect to females, and (**I**) pups and (**J**) subadults with respect to adults.

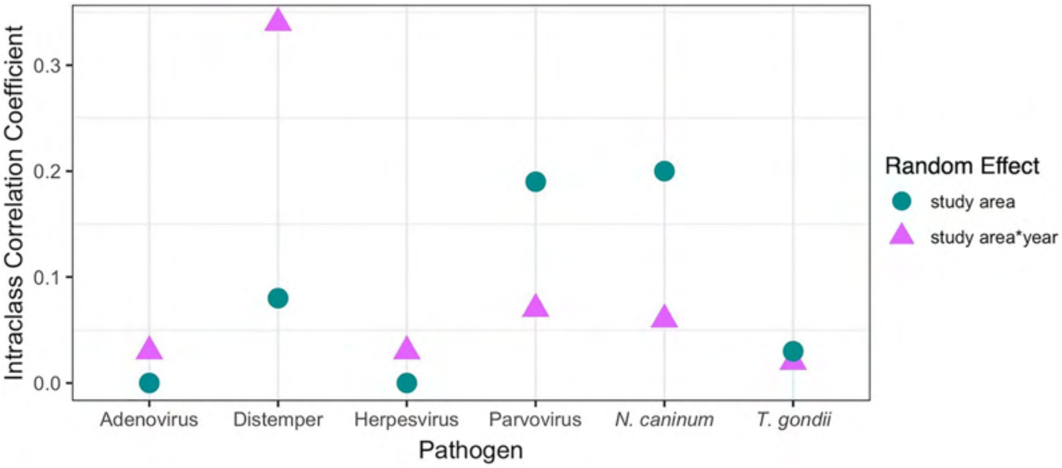


**Figure 5.** Intraclass correlation coefficient values for the random effects from the *complete models*: *study area* (teal circles) and *study area*year* (purple triangles)[39].

on average, about 4% higher using *complete models* compared with *geographic models*, and the testing set AUC improved 2.3%. *Complete models*, therefore, provided modest improvements to the *geographic models*.

**Random effects.** Random effects (*study area* and *study area*year*) accounted for a notable portion of the deviance in exposure status (range = 0–33%, mean = 9%). We explicitly compared the random effects by calculating intraclass correlation coefficient, which describes the proportion of variance in exposure probability that the grouping accounts for (i.e., *study area* or *study area*year*, Fig. 5). The effect of *study area* and *study area*year* differed by pathogen, but we can generalize that: (1) *study area* was most important for *N. caninum* and parvovirus, (2) *study area*year* was most important for distemper virus due to its epizootic nature, (3) random effects were not very important for pathogens that were universally prevalent (e.g., adenovirus and herpesvirus), and (4) both *study area* and *study area*year* did not account for very much variation in *T. gondii* exposure, potentially because it was fairly common in all sampled study areas (Fig. 3). For example, in Yellowstone, the odds of distemper exposure differed up to tenfold among years (Supplementary Fig. S18A), whereas *T. gondii* exposure was stable (Supplementary Fig. S18B). Parvovirus exposure was most variable across study areas, but there was still some fluctuation within study areas. In other words, parasite exposure was more dependent on spatial dynamics whereas epizootic viruses were more dependent on temporal dynamics.

We can also draw conclusions from assessing the *study area* random intercepts, which provides a comparison to the baseline, or grand mean, probability of pathogen exposure across North American wolf populations (Supplementary Fig. S19, S20). The probability of contracting *N. caninum* increased significantly from north to south; *T. gondii* was more variable, and wolves from Michigan and South Slave Northwest Territories had particularly high odds of exposure. Epizootic viruses (i.e., parvovirus and distemper) had less predictable latitudinal trends, but Great Lakes and Alaska wolves generally had lower odds of exposure. Wolves in the central Rocky Mountains (except British Columbia) were more likely to be seropositive for both parvovirus and distemper, and South Slave Northwest Territories and Mexican wolves also had higher probability of distemper exposure. Adenovirus and herpesvirus antibodies were highly prevalent across all study areas sampled (often > 75% seroprevalence, Fig. 3), thus all intercept estimates hovered around the grand mean.

## Discussion

Spatial variation in pathogen infections in wide-ranging hosts have been described by latitudinal gradients[5–7,11,12,73,74]. While latitude may predict pathogen dynamics, it does not elucidate the underlying mechanisms. This is largely because necessary datasets to assess mechanisms of exposure are difficult to acquire across a species' geographic range. Our objectives were to describe the spatial variation in seroprevalence of gray wolves spanning the North American continent, identify which variables best predict pathogen exposure, and expand our understanding about the mechanisms driving pathogen dynamics. Specifically, we focused on the effect of latitude as a primary driver of spatial variation in seroprevalence. To this end, we compiled an expansive serological dataset that captures the natural variation in pathogen seroprevalence as well as variables at the ecosystem, population, and individual scales (Figs. 1, 2). The effect size of latitude was greatest for *N. caninum* exposure, and compared with the other study areas, *N. caninum* seroprevalence trends most closely tracked latitude (Figs. 3, 4). Study areas in close proximity were more likely to be similar; for example, Great Lakes wolves had a lower probability of exposure for distemper and parvovirus, whereas wolves in the Arctic and central Rocky Mountains had higher probabilities. Our results highlight that individual host characteristics, as well as inherent features of ecosystems, determine pathogen exposure risk.

Human density was correlated with an increased probability of exposure of the four viruses of interest and *N. caninum*. Human density may be a proxy for density of unvaccinated dogs or synanthropic animals that act as reservoirs for infectious diseases that spill over into wolves[52]. Domestic dogs in Africa are the primary reservoir for canine distemper and rabies, and are responsible for major epizootics from these diseases in other wildlife species following spillover[23,25,75,76]. Across North America, we expect that dogs and synanthropic wildlife (e.g., raccoons, skunks, rodents) are important reservoirs for transmitting canine distemper, parvovirus, and *T. gondii*. Once spillover has occurred, wolf contact rate (i.e., density) must be high enough for wolf-wolf transmission. This might explain why we observed higher distemper and parvovirus seroprevalence in populations with both high human and wolf densities: Yellowstone, Grand Teton, and Banff and Jasper National Parks. However, dog and human densities may not always covary—for instance, dog density is high in areas where dog sledding is popular (Alaska, Northwest Territories), but human density is low. Additionally, some populations did not follow this rule and warrant further investigation, such as the South Slave region of the Northwest Territories that had low wolf, human, and carnivore density, yet high distemper seroprevalence, and Mexican wolves that displayed relatively high seroprevalence and risk of exposure despite low wolf density.

We predicted that study areas with larger pack sizes would have higher pathogen seroprevalence, which has been demonstrated in primates[77,78]. On the other hand, larger packs may aid in individual recovery from non-immunizing, chronic infections such as *N. caninum*, similar to wolves with sarcoptic mange[15]. However, mean pack size was not an important predictor of exposure to any pathogen in our models. We suggest that any effect of pack size on exposure risk may have been obscured by averaging across groups.

We predicted that better quality habitats would be more speciose and thus multi-host pathogens would occur at higher prevalences[79–81]. Interestingly, our results demonstrate a weak negative correlation between habitat quality and exposure probability. Our habitat quality index may have been a poor proxy for habitat quality, or not representative of quality habitat for other competent hosts. In reality, understanding the dynamics of multi-host pathogens requires knowledge about host contact rates, transmission, and pathogen reservoirs.

We expected black wolves to have a higher probability of pathogen exposure, in particular, canine distemper virus. Mechanistically this could occur because black wolves have improved immune responses to respiratory pathogens, and heterozygote black wolves have higher survival rates than their gray counterparts, especially in years of canine distemper virus[29–31,81,82]. Thus if black wolves survived pathogen infections at a higher rate, there would be more seropositive black wolves than gray wolves. We found that wolves in western study areas experienced more frequent distemper outbreaks and had a high proportion of black wolves (> 30%, Fig. 2, Supplementary Fig. S2). Wolves in the Great Lakes region experienced reduced pressure from distemper, and accordingly, had a much lower proportion of black wolves (< 5%, Fig. 2, Supplementary Fig. S2). However, wolf phenotype in the Great Lakes may also be influenced by historical hybridization with eastern wolves (*C. l. lycaon*)[83]. Still, coat color was not a significant predictor of exposure to any pathogen except *N. caninum*. This does not preclude a relationship between coat color and pathogen infections, and potentially suggests that pathogen pressure may predict coat color, which would reverse the response and predictor variable compared to our GLMMs.

*Neospora caninum* was the only pathogen we investigated that showed a strong latitudinal gradient in risk of pathogen exposure (Fig. 4) and mean seroprevalence (Fig. 3). We postulate that this corresponds to the proportion of white-tailed deer (*Odocoileus virginianus*) in the local wolf diets. The *N. caninum* cervid-canid lifecycle is well established[84], and white-tailed deer are considered to be the *N. caninum* reservoir[27,85]. *N. caninum* has been detected at low levels in North American caribou (*Rangifer tarandus*), elk (*Cervus canadensis*), bison (*Bison bison*), mule deer (*Odocoileus hemionus*), and moose (*Alces alces*)[27,86–89], but robust and widespread sampling is generally lacking. Based on our findings, it appears that the probability of *N. caninum* exposure varies with white-tailed deer consumption: higher in the Great Lakes region (mean seroprevalence 47%) where wolves primarily consume white-tailed deer and moose, moderate in the central Rocky Mountains (mean seroprevalence 39%) where wolves opportunistically consume deer, and uncommon in Alaska, the Northwest Territories, and Nunavut (mean seroprevalence 12%) where white-tailed deer do not occur[49,90–97]. This supports the notion that white-tailed deer are the natural hosts for *N. caninum*, although livestock consumption may also play a role, and both should also be evaluated such as adding diet data or deer/livestock density into future models.

The *complete models* provided modest improvements to the *geographic models* in terms of model fit and predictive power, indicating that mechanistic variables described a greater proportion of the observed variation in pathogen exposure than geography alone. More importantly, this provides a stronger interpretation of the drivers of pathogen exposure. However, serological data and corresponding host metadata are logistically challenging to collect and compile, thus our results also suggest that, for some host–pathogen systems, information from adjacent wolf populations may provide decent insight into pathogen dynamics.

## Conclusion

Elucidating the biogeographic patterns of pathogen exposure in a single host species across its distribution provides us with a deeper understanding of the mechanisms driving exposure, how these drivers predictably vary through space and time, and potential effects on host population dynamics or individual vital rates. We identified human density as a major driver of pathogen exposure at a continental scale. Anthropogenic environments create opportunities for aggregations of reservoir hosts and pathogen persistence, which in turn can affect wildlife—even wildlife that purposefully avoid human activity centers, like gray wolves[63,98]. Large-scale pathogen patterns have not been previously identified for the gray wolf, and here we show that regional rather than latitudinal patterns of seroprevalence were supported, with antibodies to viral pathogens more commonly identified among wolves in the Rocky Mountains whereas antibodies to parasites were more commonly identified among wolves in the Great Lakes region. This work builds upon previous studies and will hopefully serve as a catalyst for additional investigations into carnivore disease ecology, multi-host transmission dynamics, and biogeographic wildlife surveillance.

## Data accessibility

All data and code are publicly available on the Dryad Repository https://doi.org/10.5061/dryad.5hqbzkh51.

Received: 27 October 2020; Accepted: 29 December 2020

## References

1. Ferrari, M. J. *et al.* The dynamics of measles in sub-Saharan Africa. *Nature* **451**, 679–684 (2008).
2. Hudson, P. J. *et al.* Trophic interactions and population growth rates: Describing patterns and identifying mechanisms. *Philos. Trans. R. Soc. B Biol. Sci.* **357**, 1259–1271 (2002).
3. Thieltges, D. W., Ferguson, M. A. D., Jones, C. S., Leslie, R. & Poulin, R. Biogeographical patterns of marine larval trematode parasites in two intermediate snail hosts in Europe. *J. Biogeogr.* **36**, 1493–1501 (2009).
4. Bryan, H. M. *et al.* Seasonal and biogeographical patterns of gastrointestinal parasites in large carnivores: Wolves in a coastal archipelago. *Parasitology* **139**, 781–790 (2012).
5. Hosseini, P. R., Dhondt, A. A. & Dobson, A. Seasonality and wildlife disease: how seasonal birth, aggregation and variation in immunity affect the dynamics of Mycoplasma gallisepticum in house finches. *Proc. R Soc. London Ser. B Biol. Sci.* **271**, 2569–2577 (2004).
6. Guernier, V., Hochberg, M. E. & Guégan, J. F. Ecology drives the worldwide distribution of human diseases. *PLoS Biol.* **2**, 740–746 (2004).
7. Nunn, C. L., Altizer, S. M., Sechrest, W. & Cunningham, A. A. Latitudinal gradients of parasite species richness in primates. *Divers. Distrib.* **11**, 249–256 (2005).
8. Merino, S. *et al.* Haematozoa in forest birds from southern Chile: Latitudinal gradients in prevalence and parasite lineage richness. *Austral. Ecol.* **33**, 329–340 (2008).
9. Benejam, L., Alcaraz, C., Sasal, P., Simon-Levert, G. & García-Berthou, E. Life history and parasites of the invasive mosquitofish (*Gambusia holbrooki*) along a latitudinal gradient. *Biol. Invasions* **11**, 2265–2277 (2009).
10. Seabloom, E. W., Borer, E. T., Mitchell, C. E. & Power, A. G. Viral diversity and prevalence gradients in North American Pacific Coast grasslands. *Ecology* **91**, 721–732 (2010).

11. Bonds, M. H., Dobson, A. P. & Keenan, D. C. Disease ecology, biodiversity, and the latitudinal gradient in income. *PLoS Biol.* **10**, e1001456 (2012).
12. Kriger, K. M., Pereoglou, F. & Hero, J. M. Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. *Conserv. Biol.* **21**, 1280–1290 (2007).
13. Peterson, R. O., Thomas, N. J., Thurber, J. M., Vucetich, J. A. & Waite, T. A. Population limitations and the wolves of Isle Royale. *J. Mammal.* **97**, 828–841 (1998).
14. Almberg, E. S., Mech, L. D., Smith, D. W., Sheldon, J. W. & Crabtree, R. L. A serological survey of infectious disease in Yellowstone National Park's canid community. *PLoS ONE* **4**, e7042 (2009).
15. Almberg, E. S. *et al.* Social living mitigates the costs of a chronic illness in a cooperative carnivore. *Ecol. Lett.* **18**, 660–667 (2015).
16. Brandell, E. E. *et al.* Infectious diseases in Yellowstone's Wolves. In *Yellowstone Wolves: Science and Discovery in the World's First National Park* (eds. Smith, D. W., Stahler, D. R. & MacNulty, D. R.) 121–133 (The University of Chicago Press, 2020).
17. Watts, D. E. & Benson, A. M. Prevalence of antibodies for selected canine pathogens among wolves (*Canis lupus*) from the Alaska Peninsula, USA. *J. Wildl. Dis.* **52**, 506–515 (2016).
18. Carstensen, M. *et al.* A serosurvey of diseases of free-ranging gray wolves (*Canis lupus*) in Minnesota, USA. *J. Wildl. Dis.* **53**, 459–471 (2017).
19. Anderson, R. M. & May, R. M. Regulation and stability of host-parasite population interactions: I. Regulatory processes. *J. Anim. Ecol.* **47**, 219–247 (1978).
20. Silbernagel, E. R., Skelton, N. K., Waldner, C. L. & Bollinger, T. K. Interaction among deer in a chronic wasting disease endemic zone. *J. Wildl. Manag.* **75**, 1453–1461 (2011).
21. Gehrt, S. D. Raccoons and allies. In *Wild Mammals of North America: Biology, Management, and Conservation* (eds. Feldhamer, G., Thompson, B. & Chapman, J.) 611–633 (2003).
22. McFarlane, R., Sleigh, A. & McMichael, T. Synanthropy of wild mammals as a determinant of emerging infectious diseases in the Asian-Australasian region. *EcoHealth* **9**, 24–35 (2012).
23. Woodroffe, R. *et al.* Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (*Lycaon pictus*). *PLoS ONE* **7**, e30099 (2012).
24. Knobel, D. L., Butler, J. R., Lembo, T., Critchlow, R. & Gompper, M. E. Dogs, disease, and wildlife. In *Free-Ranging Dogs and Wildlife Conservation* (ed. Gompper, M. E.) (Oxford University Press, Oxford, 2014).
25. Viana, M. *et al.* Dynamics of a morbillivirus at the domestic–wildlife interface: Canine distemper virus in domestic dogs and lions. *Proc. Natl. Acad. Sci.* **112**, 1464–1469 (2015).
26. Bianco, A. *et al.* Two waves of canine distemper virus showing different spatio-temporal dynamics in Alpine wildlife (2006–2018). *Infect. Genet. Evol.* **84**, 104359 (2020).
27. Dubey, J. P., Schares, G. & Ortega-Mora, L. M. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.* **20**, 323–367 (2007).
28. Anderson, T. M. *et al.* Molecular and evolutionary history of melanism in North American gray wolves. *Science* **323**, 1339–1343 (2009).
29. Candille, S. I. *et al.* A β-defensin mutation causes black coat color in domestic dogs. *Science* **318**, 1418–1423 (2007).
30. Coulson, T., Macnulty, D. R., Stahler, D. R., Wayne, R. K. & Smith, D. W. Modeling effects of environmental change on wolf population dynamics, trait evolution, and life history. *Science* **334**, 1275–1278 (2011).
31. Hedrick, P. W., Stahler, D. R. & Dekker, D. Heterozygote advantage in a finite population: Black color in wolves. *J. Hered.* **105**, 457–465 (2014).
32. Altizer, S., Davis, A. K., Cook, K. C. & Cherry, J. J. Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. *Can. J. Zool.* **82**, 755–763 (2004).
33. Biek, R. *et al.* Factors associated with pathogen seroprevalence and infection in Rocky Mountains cougars. *J. Wildl. Dis.* **42**, 606–615 (2006).
34. Härkönen, T., Harding, K., Rasmussen, T. D., Teilmann, J. & Dietz, R. Age- and sex-specific mortality patterns in an emerging wildlife epidemic: The phocine distemper in European harbour seals. *PLoS ONE* **2**, e887 (2007).
35. Guerra-Silveira, F. & Abad-Franch, F. Sex bias in infectious disease epidemiology: Patterns and processes. *PLoS ONE* **8**, e62390 (2013).
36. McDonald, J. L., Smith, G. C., McDonald, R. A., Delahay, R. J. & Hodgson, D. Mortality trajectory analysis reveals the drivers of sex-specific epidemiology in natural wildlife–disease interactions. *Proc. R. Soc. B Biol. Sci.* **281**, 20140526 (2014).
37. Williams, E. S. & Barker, I. K. (eds) *Infectious Diseases of Wild Mammals* (Wiley, New York, 2001).
38. USGS. North America Political Boundaries. (2006). Available at: https://www.sciencebase.gov/catalog/item/4fb555ebe4b04cb937751db9.
39. Justice-Allen, A. & Clement, M. J. Effect of canine parvovirus and canine distemper virus on the Mexican wolf (*Canis lupus baileyi*) population in the USA. *J. Wildl. Dis.* **55**, 682–688 (2019).
40. Nelson, B. *et al.* Prevalence of antibodies to canine parvovirus and distemper virus in wolves in the Canadian Rocky Mountains. *J. Wildl. Dis.* **48**, 68–76 (2012).
41. R Core Team. R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing). (2019 v3.6.3). Available at: https://www.R-project.org.
42. Bates, D., Maechler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
43. Gelman, A. & Loken, E. The garden of forking paths: Why multiple comparisons can be a problem, even when there is no "fishing expedition" or "p-hacking" and the research hypothesis was posited ahead of time. *Psychol. Bull.* **140**, 1272–1280 (2013).
44. Fuller, T. K. & Murray, D. L. Biological and logistical explanations of variation in wolf population density. *Anim. Conserv.* **1**, 153–157 (1998).
45. Fuller, T. K. & Sievert, P. R. Carnivore demography and the consequences of changes in prey availability. In *Conservation biology series - Cambridge* 163–178 (2001).
46. MacNulty, D. R., Tallian, A., Stahler, D. R. & Smith, D. W. Influence of group size on the success of wolves hunting bison. *PLoS ONE* **9**, 1–8 (2014).
47. Barber-Meyer, S. M., Mech, L. D., Newton, W. E. & Borg, B. L. Differential wolf-pack-size persistence and the role of risk when hunting dangerous prey. *Behaviour* **153**, 1473–1487 (2016).
48. Gipson, P. S., Ballard, W. B., Nowak, R. M. & Mech, L. D. Accuracy and precision of estimating age of gray wolves by tooth wear. *J. Wildl. Manag.* **64**, 752 (2000).
49. Fuller, T. K., Mech, L. D. & Cochrane, J. F. Wolf population dynamics. In *Wolves: Behavior, Ecology, and Conservation* (eds Mech, L. D. & Boitani, L.) 161–191 (University of Chicago Press, Chicago, 2003).
50. Jimenez, M. D. *et al.* Wolf dispersal in the Rocky Mountains, Western United States: 1993–2008. *J. Wildl. Manag.* **81**, 581–592 (2017).
51. NASA Socioeconomic Data and Applications Center. Gridded Population of the World (GPW), v4. *EarthData* (2015). Available at: https://sedac.ciesin.columbia.edu/data/set/gpw-v4-population-count-rev11/data-download.
52. Millán, J. *et al.* Patterns of exposure of Iberian wolves (Canis lupus) to canine viruses in human-dominated landscapes. *EcoHealth* **13**, 123–134 (2016).

53. North American Land Change Monitoring System 30m, 2010–2015 (Landsat). *Commission of Environmental Cooperation* (2015). Available at: http://www.cec.org/north-american-land-change-monitoring-system/.
54. USGS EROS Archive—Digital Elevation—Global 30 Arc-Second Elevation (GTOPO30). *Earth Resources Observation and Science (EROS) Center* (1996).
55. Hesselbarth, M. H. K., Sciaini, M., With, K. A., Wiegand, K. & Nowosad, J. landscapemetrics: an open-source R tool to calculate landscape metrics. *Ecography* **42**, 1648–1657 (2019).
56. Poole, K. G., Wakelyn, L. A. & Nicklen, P. N. Habitat selection by lynx in the Northwest Territories. *Can. J. Zool.* **74**, 845–850 (1996).
57. Nielsen, S. E., Boyce, M. S., Stenhouse, G. B. & Munro, R. H. M. Modeling grizzly bear habitats in the yellowhead ecosystem of Alberta: Taking autocorrelation seriously. *Ursus* **13**, 45–56 (2001).
58. Arjo, W. M. & Pletscher, D. H. Coyote and wolf habitat use in northwestern Montana. *Northwest Sci.* **78**, 24–32 (2004).
59. Oakleaf, J. K. *et al.* Habitat selection by recolonizing wolves in the northern Rocky Mountains of the United States. *J. Wildl. Manag.* **70**, 554–563 (2006).
60. Hebblewhite, M. & Merrill, E. Modelling wildlife-human relationships for social species with mixed-effects resource selection models. *J. Appl. Ecol.* **45**, 834–844 (2008).
61. Roever, C. L., Boyce, M. S. & Stenhouse, G. B. Grizzly bears and forestry II: Grizzly bear habitat selection and conflicts with road placement. *For. Ecol. Manag.* **256**, 1262–1269 (2008).
62. Houle, M., Fortin, D., Dussault, C., Courtois, R. & Ouellet, J. P. Cumulative effects of forestry on habitat use by gray wolf (*Canis lupus*) in the boreal forest. *Landsc. Ecol.* **25**, 419–433 (2010).
63. Mayor, S. J., Schneider, D. C., Schaefer, J. A. & Mahoney, S. P. Habitat selection at multiple scales. *Ecoscience* **16**, 238–247 (2009).
64. Milakovic, B. *et al.* Habitat selection by a focal predator (*Canis lupus*) in a multiprey ecosystem of the northern Rockies. *J. Mammal.* **92**, 568–582 (2011).
65. Kittle, A. M. *et al.* Wolves adapt territory size, not pack size to local habitat quality. *J. Anim. Ecol.* **84**, 1177–1186 (2015).
66. Kittle, A. M. *et al.* Landscape-level wolf space use is correlated with prey abundance, ease of mobility, and the distribution of prey habitat. *Ecosphere* **8**, e01783 (2017).
67. Morin, S. J., Bowman, J., Marrotte, R. R. & Fortin, M. J. Fine-scale habitat selection by sympatric Canada lynx and bobcat. *Ecol. Evol.* **10**, 9396–9409 (2020).
68. O'Neil, S. T., Vucetich, J. A., Beyer, D. E., Hoy, S. R. & Bump, J. K. Territoriality drives preemptive habitat selection in recovering wolves: Implications for carnivore conservation. *J. Anim. Ecol.* **89**, 1433–1447 (2020).
69. Gelman, A. & Hill, J. *Data analysis using regression and multilevel/hierarchical models* (Cambridge University Press, 2007).
70. Menard, S. Standards for standardized logistic regression coefficients. *Soc. Forces* **89**, 1409–1428 (2011).
71. Hosmer, D. W. & Lemeshow, S. *Applied Logistic Regression* (Wiley, New York, 2000).
72. Hastie, T., Tibshirani, R. & Friedman, J. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction* (Springer, Berlin, 2009).
73. Finkelman, B. S. *et al.* Global patterns in seasonal activity of influenza A/H3N2, A/H1N1, and B from 1997 to 2005: Viral coexistence and latitudinal gradients. *PLoS ONE* **2**, e1296 (2007).
74. Nguyen, D. *et al.* Fungal disease incidence along tree diversity gradients depends on latitude in European forests. *Ecol. Evol.* **6**, 2426–2438 (2016).
75. Rhodes, C. J., Atkinson, R. P. D., Anderson, R. M. & Macdonald, D. W. Rabies in Zimbabwe: reservoir dogs and the implications for disease control. *Philos. Trans. R Soc. Lond. Ser. B Biol. Sci.* **353**, 999–1010 (1998).
76. Lembo, T. *et al.* Exploring reservoir dynamics: A case study of rabies in the Serengeti ecosystem. *J. Appl. Ecol.* **45**, 1246–1257 (2008).
77. Nunn, C. L., Altizer, S., Jones, K. E. & Sechrest, W. Comparative tests of parasite species richness in primates. *Am. Nat.* **162**, 597–614 (2003).
78. Nunn, C. L. & Heymann, E. W. Malaria infection and host behavior: A comparative study of Neotropical primates Malaria infection and host behavior. *Behav. Ecol. Sociobiol.* **59**, 30–37 (2005).
79. Begon, M., Bowers, R. G., Kadianakis, N. & Hodgkinson, D. E. Disease and community structure: the importance of host self-regulation in a host-host-pathogen model. *Am. Nat.* **139**, 1131–1150 (1992).
80. Power, A. G. & Mitchell, C. E. Pathogen spillover in disease epidemics. *Am. Nat.* **164**, S79–S89 (2004).
81. Keesing, F., Holt, R. D. & Ostfeld, R. S. Effects of species diversity on disease risk. *Ecol. Lett.* **9**, 485–498 (2006).
82. Schweizer, R. M. *et al.* Natural selection and origin of a melanistic allele in North American gray wolves. *Mol. Biol. Evol.* **35**, 1190–1209 (2018).
83. Wilson, P. J., Grewal, S. K., Mallory, F. F. & White, B. N. Genetic characterization of hybrid wolves across Ontario. *J. Hered.* **100**, S80–S89 (2009).
84. Gondim, L. F. P. *et al.* Transmission of *Neospora caninum* between wild and domestic animals. *J. Parasitol.* **90**, 1361–1365 (2004).
85. Dubey, J. P. *et al.* Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in white-tailed deer (*Odocoileus virginianus*) from Iowa and Minnesota using four serologic tests. *Vet. Parasitol.* **161**, 330–334 (2009).
86. Stieve, E., Beckmen, K., Kania, S. A., Widner, A. & Patton, S. *Neospora caninum* and *Toxoplasma gondii* antibody prevalence in Alaska wildlife. *J. Wildl. Dis.* **46**, 348–355 (2010).
87. Pruvot, M., Hutchins, W. & Orsel, K. Statistical evaluation of a commercial *Neospora caninum* competitive ELISA in the absence of a gold standard: Application to wild elk (*Cervus elaphus*) in Alberta. *Parasitol. Res.* **113**, 2899–2905 (2014).
88. Bondo, K. J. *et al.* Health survey of boreal caribou (*Rangifer tarandus caribou*) in northeastern British Columbia, Canada. *J. Wildl. Dis.* **55**, 544–562 (2019).
89. Donahoe, S. L., Lindsay, S. A., Krockenberger, M., Phalen, D. & Šlapeta, J. A review of neosporosis and pathologic findings of *Neospora caninum* infection in wildlife. *Int. J. Parasitol. Parasites Wildl.* **4**, 216–238 (2015).
90. Huggard, D. J. Prey selectivity of wolves in Banff National Park I. Prey species. *Can. J. Zool.* **71**, 130–139 (1993).
91. Hebblewhite, M., Paquet, P. C., Pletscher, D. H., Lessard, R. B. & Callaghan, C. J. Development and application of a ratio estimator to estimate wolf kill rates and variance in a multiple-prey system. *Wildl. Soc. Bull.* **31**, 933–946 (2003).
92. Adams, L. G. *et al.* Are inland wolf-ungulate systems influenced by marine subsidies of Pacific salmon?. *Ecol. Appl.* **20**, 251–262 (2010).
93. Latham, A. D. M., Latham, M. C., McCutchen, N. A. & Boutin, S. Invading white-tailed deer change wolf-caribou dynamics in northeastern Alberta. *J. Wildl. Manag.* **75**, 204–212 (2011).
94. Metz, M. C., Smith, D. W., Vucetich, J. A., Stahler, D. R. & Peterson, R. O. Seasonal patterns of predation for gray wolves in the multi-prey system of Yellowstone National Park. *J. Anim. Ecol.* **81**, 553–563 (2012).
95. Merkle, J. A., Polfus, J. L., Derbridge, J. J. & Heinemeyer, K. S. Dietary niche partitioning among black bears, grizzly bears and wolves in a multi-prey ecosystem. *Can. J. Zool.* **95**, 663–671 (2017).
96. Gable, T. D., Windels, S. K., Bruggink, J. G. & Barber-Meyer, S. M. Weekly summer diet of gray wolves (*Canis lupus*) in northeastern Minnesota. *Am. Midl. Nat.* **179**, 15–27 (2018).
97. O'Donovan, S. A., Budge, S. M., Hobson, K. A., Kelly, A. P. & Derocher, A. E. Intrapopulation variability in wolf diet revealed using a combined stable isotope and fatty acid approach. *Ecosphere* **9**, e02420 (2018).
98. Whittington, J., St. Clair, C. C. & Mercer, G. Spatial responses of wolves to roads and trails in mountain valleys. *Ecol. Appl.* **15**, 543–553 (2005).

## Acknowledgements

We thank the wildlife professionals that contributed wolf sera, serology data, and metadata to this project: Erin Stahler, L. David Mech, Dean Beyer, Erin Largent, Susan Dicks, John Oakleaf, the Mexican Wolf Project, Lindsey Dreese, Brent Patterson, Emily Almberg, Montana Fish Wildlife and Parks, Shari Willmott, and the countless unlisted biologists that collected these samples and data through the decades. Financial support includes: E.E.B. and P.J.H. endowment from Verne Willaman; E.E.B. and P.C.C. U.S. Geological Survey (Grant G17AC00427); D.W.S., D.R.S., and D.R.M. NSF LTREB grant DEB–1245373 and many donors to Yellowstone Forever, especially Annie and Bob Graham and Valerie Gates; M.H. Parks Canada and NSF LTREB award 1556248; G.R. Pittman-Robertson Federal Aid in Wildlife Restoration Program and the State of Alaska general funds; K.B. Federal Aid in Support of Wildlife Restoration and Alaska Dept. of Fish and Game; M.A. and H.S. British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Habitat Conservation Trust Foundation, Forest Enhancement Society of British Columbia; D.R.M and M.A. Polar Continental Shelf Project and National Geographic Society; M.L.J.G. the Office of the Director, National Institutes of Health under award number NIH T32OD010993; T.W. Ontario Ministry of Natural Resources and Forestry; B.L.B. National Park Service; A.K. GNWT Environmental Stewardship Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

## Author contributions

E.E.B., P.C.C., D.W.S., and P.J.H. formulated the hypotheses and designed the project. E.E.B. conducted the data collection, performed all analyses, and wrote the first draft of the manuscript. All authors provided samples, metadata, and contributed substantially to manuscript development and revisions.

## Additional information

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-81192-w.

**Correspondence** and requests for materials should be addressed to E.E.B.

# Impacts of Landscape Change on Wolf Restoration Success: Planning a Reintroduction Program Based on Static and Dynamic Spatial Models

CARLOS CARROLL,* MICHAEL K. PHILLIPS,† NATHAN H. SCHUMAKER,‡
AND DOUGLAS W. SMITH§

*Klamath Center for Conservation Research, P.O. Box 104, Orleans, CA 95556, U.S.A., email carlos@sisqtel.net
†Turner Endangered Species Fund, 1123 Research Drive, Bozeman, MT 59718, U.S.A.
‡U.S. Environmental Protection Agency, Western Ecology Division, Corvallis, OR 97333, U.S.A.
§Yellowstone Center For Resources, Yellowstone National Park, P.O. Box 168, Mammoth, WY 82190, U.S.A.

**Abstract:** *Mammalian carnivores are increasingly the focus of reintroduction attempts in areas from which they have been extirpated by historic persecution. We used static and dynamic spatial models to evaluate whether a proposed wolf reintroduction to the southern Rocky Mountain region (U.S.A) would advance recovery by increasing species distribution beyond what might be expected through natural range expansion. We used multiple logistic regression to develop a resource-selection function relating wolf distribution in the Greater Yellowstone region with regional-scale habitat variables. We also used a spatially explicit population model to predict wolf distribution and viability at several potential reintroduction sites within the region under current conditions and under two contrasting predictions of future landscape change. Areas of the southern Rocky Mountains with resource-selection-function values similar to those of currently inhabited areas in Yellowstone could potentially support >1000 wolves, 40% within protected areas and 47% on unprotected public lands. The dynamic model predicted similar distribution under current conditions but suggested that development trends over 25 years may result in the loss of one of four potential regional subpopulations and increased isolation of the remaining areas. The reduction in carrying capacity due to landscape change ranged from 49% to 66%, depending on assumptions about road development on public lands. Although much of the wolf population occurs outside core protected areas, these areas remain the key to the persistence of subpopulations. Although the dynamic model's sensitivity to dispersal parameters made it difficult to predict the probability of natural recolonization from distant sources, it suggested that an active reintroduction to two sites within the region may be necessary to ensure low extinction probability. Social carnivores such as the wolf, which often require larger territories than solitary species of similar size, may be more vulnerable to environmental stochasticity and landscape fragmentation than their vagility and fecundity would suggest.*

## Impacto del Cambio de Paisaje sobre el Exito de Restauración de Lobos: Planeación de Programas de Reintroducción Utilizando Modelos Espaciales Estáticos y Dinámicos

**Resumen:** *Con mayor frecuencia, los mamíferos carnívoros son el foco de intentos de reintroducción en áreas de las que han sido extirpados por persecución histórica. Utilizamos modelos espaciales estáticos y dinámicos para evaluar si la propuesta de reintroducción de lobos a la región sur de las Montañas Rocallosas (E.U.A.) haría progresar la recuperación al incrementar la distribución de la especie más allá de lo que pudiera esperarse por su expansión natural. Usamos regresión logística múltiple para desarrollar una función recurso-selección que relacionó la distribución de lobos en la región de Greater Yellowstone con variables de hábitat a escala regional. También utilizamos un modelo poblacional espacialmente explícito para predecir la distribución y viabilidad de lobos en varios sitios potenciales de reintroducción dentro de la región bajo condiciones actuales y bajo dos predicciones contrastantes de cambios futuros en el paisaje. Las*

---

*Paper submitted November 12, 2001; revised manuscript accepted June 25, 2002.*

Conservation Biology, Pages 536–548
Volume 17, No. 2, April 2003

*áreas del sur de las Montañas Rocallosas con valores recurso-selección similares a los de áreas actualmente habitadas en Yellowstone potencialmente podrían albergar >1000 lobos, con 40% en áreas protegidas y 47% en tierras públicas no protegidas. El modelo dinámico predijo una distribución similar bajo condiciones actuales pero sugirió que las tendencias de desarrollo a 25 años pudieran resultar en la pérdida de una de cuatro potenciales subpoblaciones regionales y en el incremento en el aislamiento de las áreas remanentes. La reducción en la capacidad de carga debido a cambios en el paisaje varió de 49 a 66% dependiendo de la construcción proyectada de caminos en tierras públicas. Aunque buena parte de la población de lobos ocurre fuera de las áreas protegidas núcleo, estas áreas siguen siendo la clave para la persistencia de las subpoblaciones. Aunque la sensibilidad a los parámetros de dispersión del modelo dinámico dificultó la predicción de la probabilidad de recolonización a partir de fuentes lejanas, sugirió que puede ser necesaria una reintroducción activa en dos sitios dentro de la región para asegurar una baja probabilidad de extinción. Carnívoros sociales, tales como los lobos, que a menudo requieren territorios mayores que especies solitarias de similar tamaño, pueden ser más vulnerables a la estocacidad ambiental y a la fragmentación del paisaje de lo que pudieran sugerir su vagilidad y fecundidad.*

## Introduction

Mammalian carnivores such as the gray wolf (*Canis lupus*) have increasingly been the focus of restoration efforts in North America and several other continents (Breitenmoser et al. 2001). Large carnivores merit conservation attention in their own right (Gittleman et al. 2001). Because they may also be particularly sensitive to fragmentation effects that will eventually influence a larger suite of species (Woodroffe & Ginsberg 1998), examining the effects of human-induced landscape change on carnivores may also provide more general guidelines for regional-scale conservation. The wolf was extirpated from the Rocky Mountains of the United States during the early to middle 1900s (Young & Goldman 1944). In the 1980s, wolves reoccupied portions of the northern Rocky Mountain region (U.S.A) through natural recolonization from Canada (Ream et al. 1991). In the 1990s, wolves were re-introduced to the Greater Yellowstone Ecosystem (GYE), central Idaho, and Arizona (Bangs et al. 1998; Brown & Parsons 2001).

Wolves locate their home ranges in areas with adequate prey and low levels of human interference (Mladenoff et al.1995). Human-caused mortality often comprises 80–95% of total mortality (Fuller 1989). Roads, by increasing human access, negatively affect wolf populations at local, landscape, and regional scales (Fuller 1989; Mladenoff et al. 1995). Ungulates such as elk (*Cervus elaphus*), deer *(Odocoileus virginianus* and *O. hemionus*), moose (*Alces alces*), and bighorn sheep (*Ovis canadensis*) make up the bulk of the wolf's diet (Fuller 1989). Prey density explains up to 72% of the variation in wolf density in areas where anthropogenic mortality is low (Fuller 1989). Because wolves in mountainous regions such as the western United States often concentrate activities in forested valley bottoms where snow condition and prey availability are optimal (Singleton 1995), topographic and snowfall data may be correlated with habitat productivity.

Wolves reach sexual maturity at an early age and have large litters. The species' flexible social structure allows pack structure, fecundity, and dispersal to respond to shifts in population density and prey abundance (Fuller 1989; Weaver et al. 1996). However, wolves, like many other large carnivores, require large areas to support viable populations, and the social structure of the wolf may make limits to habitat area even more important because social animals require larger territories than solitary animals of similar size (Woodroffe & Ginsberg 1998). Social structure may also increase the effects of demographic stochasticity by limiting reproduction to the dominant pair within a breeding group (Vucetich et al. 1997).

Regional habitat suitability can be predicted in a geographic information system (GIS) by combining data on different components of wolf habitat quality, such as prey availability and human-associated mortality risk (Mladenoff et al. 1995; Harrison & Chapin 1998). These static habitat models provide a snapshot of habitat quality and potential population distribution. Alternately, nonspatial dynamic viability models (Lacy 1993) use summary information on habitat characteristics to predict carrying capacity and other habitat-related parameters over time. Combining both spatial habitat information and demographic data in a dynamic model results in a spatially explicit population model (SEPM) (Karieva & Wennergren 1995; Schumaker 1998). Such models can be used to evaluate area and connectivity factors and predict source-sink behavior, but they can be sensitive to errors in poorly known parameters such as dispersal rate (Karieva et al. 1996). Nevertheless, they provide qualitative insights into factors, such as variance in population size, that are difficult to explore using static spatial models. In contrast, static habitat models and nonspatial demographic viability models can provide robust results even when data on a species' demography and habitat associations are limited.

We used two types of spatial models to help evaluate whether a wolf reintroduction to the southern Rocky Mountain region (U.S.A.) would advance recovery goals by increasing species distribution and viability beyond what might be expected through natural range expansion. We also compared the viability of reintroduced populations at several potential reintroduction sites within the region under two contrasting predictions of future landscape change.

Species-reintroduction efforts generally require large investments of time and resources. If spatial models such as those evaluated here can provide robust predictions of reintroduction success, they may become important tools in planning the recovery of endangered species. In addition, model results may provide more-general insights into the vulnerability of wide-ranging species to human-associated landscape change.

## Methods

### Study Area

The study area covered 280,000 km$^2$ in the Rocky Mountains of the western United States and included the Southern Rocky Mountain (SRM) ecoregion (Shinneman et al. 2000) and adjacent areas (Fig. 1). Areas to the northwest that link the study area with current wolf range in the GYE were also analyzed but were not included in the summary statistics. Mean elevation is 2300 m, ranging from approximately 1200 m in the Colorado River canyon to approximately 4200 m in the mountains of central Colorado. The climate ranges from semiarid in the southwestern portion of the region to continental on the eastern margin. Mean annual precipitation is 1500 mm, and mean annual snowfall is 2700 mm (Daly et al. 1994). Major vegetation types include evergreen needleleaf forests, aspen (*Populus* spp.) parklands, sagebrush (*Artemisia* spp.) shrublands, and grasslands (Shinneman et al. 2000).

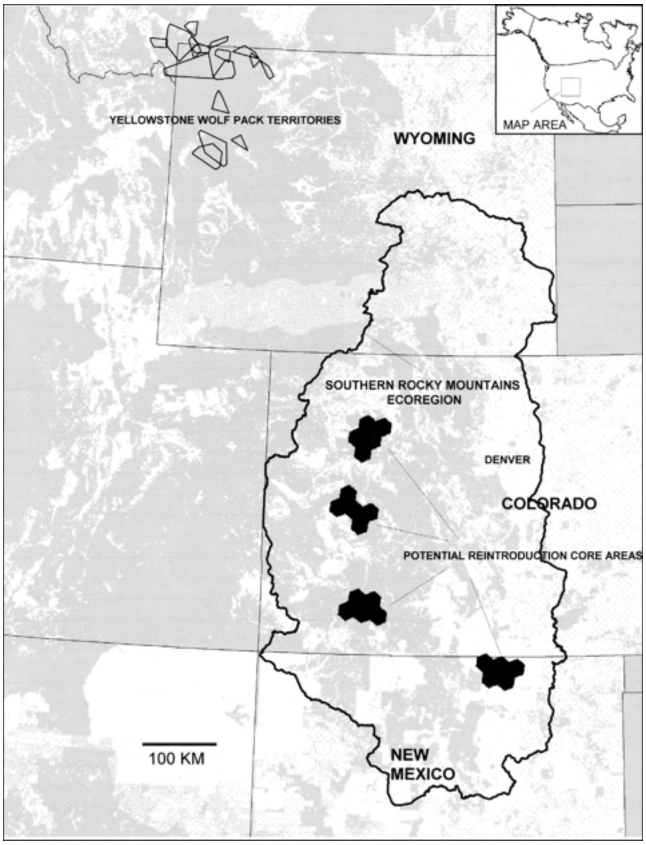


*Figure 1. Study area in the U.S. southern Rocky Mountains, with public lands shown in gray and candidate wolf reintroduction sites in black.*

Public lands make up 53% of the region (Fig. 1). Designated protected areas, which comprise 20% of public lands, are primarily located in higher elevations along the continental divide (Gap Analysis Program, unpublished data), which also hold most other unprotected areas with few roads. Prey such as elk and deer are most abundant on lower-elevation public lands to the west of these areas.

Historically important economic activities such as mining, livestock production, and agriculture remain significant but are increasingly eclipsed by other employment categories such as the service industry and the retail trade (Shinneman et al. 2000; Theobald 2000). Livestock production, a land use which potentially conflicts with large-carnivore restoration, is concentrated in west-central and especially northwest Colorado. Grazing is permitted on the majority of public lands, including those designated as wilderness. Public lands and adjacent private lands also may experience high levels of recreational use.

Mean population density in the SRM region is approximately 9 persons/km$^2$ (Shinneman et al. 2000). Portions of the study area, such as western Colorado, have among the highest rates of human population growth in the United States, resulting in conversion of forest and large agricultural holdings to low-density residential development (Theobald 2000). Although private lands form less than half of the landscape, their rapid development may disproportionately affect key areas, such as productive riparian corridors, and geographically fragment public lands (Theobald 2000).

### Static Model

We compared spatial data on the boundaries of wolf pack territories (Fig. 1) in the GYE (annual minimum convex polygon, 2000 data) with habitat characteristics to predict wolf distribution in the SRM region. Habitat variables, which are reviewed in detail in Carroll et al. (2001*a*), included vegetation, satellite imagery metrics derived from the MODIS sensor (Huete et al. 1997), topography, climate, and human-impact variables. The MODIS data were used to derive the "tasseled-cap" indices of brightness, greenness, and wetness (Crist & Cicone 1984), which are correlated to varying degrees with ecological factors such as net primary productivity and which have proved useful in modeling wildlife distributions (Mace et al. 1999; Carroll et al. 2001*a*). We used MODIS data from both midsummer and early winter to incorporate seasonal changes in resource availability and phenology. All GIS layers were generalized to a 1-km$^2$ resolution for the analysis. Multiple logistic regression was used to compare habitat variables at points within territories with those at points outside territories within the GYE. We selected an optimal multivariable model using the Bayesian information criterion (BIC), a diagnostic statistic that penalizes for overfitting (Schwarz 1978). We used the coefficients from the final model to calculate a resource-selection function (RSF) $w(x)$ for used (occurrences) and unused resources (Manly et al. 1993; Boyce & McDonald 1999), which is referred to subsequently as the static model. Although the resulting RSF predictions were produced at the resolution of the input data (1 km$^2$), we used these predictions to compare the relative capacity of larger landscapes of over 500 km$^2$ to support wolves. This larger scale, which approximates the average size of wolf pack territories in the region (D. Smith, unpublished data), was also the scale of the predictions produced by the dynamic model described below.

We used a two-step process for estimating potential wolf population size that first delineated potential wolf distribution and then estimated wolf abundance within those areas based on auxiliary data on prey density (Mladenoff & Sickley 1999). We predicted the potential size of wolf populations that might inhabit areas with high habitat suitability as predicted by the static model, based on an equation relating wolf density to prey density (Fuller 1989; Mladenoff & Sickley 1999): wolf density/1000 km$^2$ = 4.19*DEPU/km$^2$, where DEPU, or deer-equivalent prey units, were derived from elk and deer abundance data (Colorado Division of Wildlife Resources 1997; New Mexico Game and Fish, unpublished data; Wyoming Game and Fish, unpublished data).

### Dynamic Model

We performed population viability analyses by using a modified version of the program PATCH (Schumaker 1998), which links the survival and fecundity of female territorial animals to GIS data on mortality risk and habitat productivity measured at the scale of the individual territory. The model tracks the population through time as individuals are born, disperse, reproduce, and die, predicting population size, time to extinction, and migration and colonization rates. Territories are allocated by intersecting the GIS data with an array of hexagonal cells. We modified the model to associate each hexagon with a single wolf pack rather than with an individual territory holder. The GIS maps were assigned weights based on the relative fecundity and survival rates expected in the various habitat classes, as described below. Survival and reproductive rates are then supplied to the model as a population projection matrix. The model scales the matrix values based on the hexagon scores, with poorer habitat translating into lower scores and thus higher mortality rates and lower reproductive output. Each hexagon can then be assigned a value for lambda, the finite rate of increase, indicating its expected source-sink properties.

Conceptual models were used to estimate relative fecundity and survival. The fecundity model was based on tasseled-cap greenness (Mace et al. 1999), which has been shown to be correlated with ungulate density (Carroll et al. 2001*b*). The fecundity metric incorporated the negative effect of terrain (slope) on prey availability (Carroll et al. 2001*b*). We used a habitat model rather than directly using prey data because we could obtain relatively consistent prey data for the SRM region but not for the larger region analyzed in the PATCH model simulations. A metric combining road density, local human population density, and interpolated human population density (Merrill et al. 1999) predicted mortality risk. Survival was also proportionately increased in parks as a result of lack of hunting and a consequent lower lethality of humans.

We were able to build a strong link between the GIS habitat data and demographic parameters because a large number of published field studies with estimates of fecundity and survival are available (e.g., Ballard et al. 1987; Fuller 1989; Pletscher et al. 1997). We calibrated the demographic rates assigned in PATCH so that, for example, wolves in an area showing levels of habitat productivity and human impacts similar to those of Yellowstone National Park would be assigned fecundity and mortality values near those actually recorded there. Even for a well-studied species such as the wolf, however, generalizing local demographic data to a regional scale based on habitat models requires assumptions that strongly affect resulting model predictions. Because the dynamic model, unlike the static model, was developed without the use of the GYE pack-territory data, we validated the wolf distribution predicted by PATCH with that pack-territory data.

Mean and maximum demographic rates are shown in Table 1. Fecundity is given as the number of female offspring per pack. Expected rates (Table 1) are given as averaged over the entire region, including areas with low suitability for wolves. Because most areas assigned rates at the lower end of the scale remain unoccupied in the simulations, packs actually show higher mean rates and a smaller range of rates than are shown in Table 1. The expected lambda values were predicted by scaling the Leslie matrix based on the hexagon scores. These values (Fig. 2a) show that most of the region is expected to be at least weak source habitat. Mean expected lambda was 1.062 and maximum expected lambda was 1.274.

We modified PATCH to better reflect wolf demography by allowing territory holders to be social rather than solitary. This social structure added demographic resilience because individuals from the same pack could rapidly replace territory holders (alpha females) that die, and it strongly influenced movement rates and patterns. We assumed fecundity to be independent of pack size because no general relationship between the two factors has been

**Table 1. Demographic values used in the PATCH simulations of wolf population dynamics in the southern Rocky Mountains.***

| | *Age* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *0* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* |
| Maximum fecundity | 0.00 | 0.00 | 2.29 | 3.21 | 3.21 | 3.21 | 3.21 | 3.21 | 3.21 | 1.15 |
| Mean fecundity | 0.00 | 0.00 | 1.20 | 1.68 | 1.68 | 1.68 | 1.68 | 1.68 | 1.68 | 0.60 |
| Maximum survival | 0.46 | 0.86 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.69 | 0.46 |
| Mean survival | 0.37 | 0.69 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.55 | 0.37 |

** Fecundity is given as number of female offspring per pack. Mean values are averaged over the entire region, including areas that did not support wolves in the subsequent simulations. Maximum values are before adjustment for environmental stochasticity.*

documented (Ballard et al. 1987). As pack size increases, individual wolves in PATCH have a greater tendency to disperse and search for new available breeding sites. The probability of leaving a pack is a quadratically increasing function, with high dispersal probabilities as pack size approaches the theoretical maximum. Setting the maximum at 24 adults resulted in observed maximum pack sizes of 8–11 adults. C. C., unpublished data). Packs in the GYE currently average 5.9 adults (D. W. S., unpublished data). The size of hexagons or pack territories used in the PATCH model was 500 $\mathrm{km}^2$. The mean territory size of GYE packs in 2000 was 545.6 $\mathrm{km}^2$ ($n$ = 16, range = 154-1675 $\mathrm{km}^2$, SD = 504.0) (D. Smith, unpublished data).

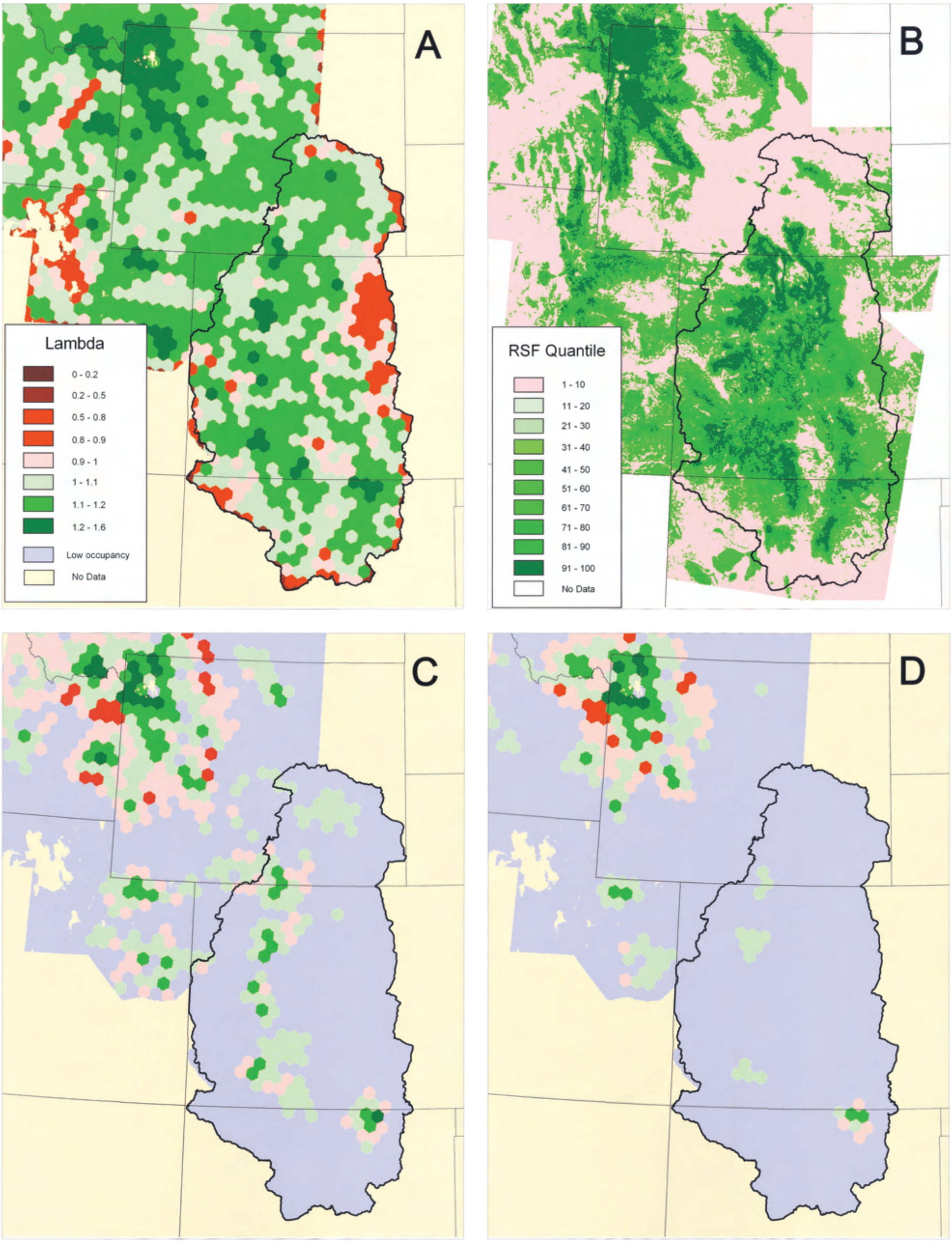
We simulated dispersal by using a directed random walk with a maximum dispersal distance of 250 km. Movement decisions in a directed random walk combine varying proportions of randomness, correlation (tendency to continue in the direction of the last step), and attraction to higher quality habitat, but without knowledge of habitat quality beyond the immediately adjacent territories.

We modeled environmental stochasticity by drawing the maximum Leslie matrix values (Table 1) from a truncated normal distribution with coefficients of variation of 30% for fecundity, 40% for pup mortality, and 30% for adult mortality (Ballard et al. 1987; Fuller 1989). We compared those outcomes to results from simulations conducted without environmental stochasticity. One thousand replicate simulations of 200 years were conducted per scenario. In addition to assessing model sensitivity to environmental stochasticity, we explored the plausibility of the model's predicted wolf distributions by comparing them with known distribution in other areas of the Rocky Mountains (C.C., unpublished data).

The PATCH model allows the landscape to change through time. Hence, the user can quantify the consequences of landscape change for population viability and examine changes in vital rates and occupancy patterns that result from habitat loss or fragmentation. We used this feature to explore the consequences for wolves of road development and human population growth during the period 2000–2025. Census data were available for the period 1990–2000. We predicted human population growth from 2000 to 2025 based on growth rates from 1990 to 2000, but we adjusted the predicted 2025 population to match state-level Census Bureau predictions (U.S. Census Bureau, unpublished data). Road density was predicted to increase at 1% per year (Theobald et al. 1996). The landscape scenarios we evaluated included: (A) current conditions, (B) human population as of 2025, with increased road development on private lands only, and (C) human population as of 2025, with increased road development on private and unprotected public lands. A new map including all landscape change over the period 2000–2025 was introduced into the simulation at year 25, rather than incrementally over 25 yearly time steps.

In addition to using PATCH to assess the overall potential of the region to support wolf populations (carrying capacity), we modeled specific reintroduction options to assess transient dynamics such as the probability of extinction and the probability of an area being colonized by wolves from a specific reintroduction site. Based on a review of the literature (Bennett 1994; Martin et al. 1999), field knowledge, and preliminary modeling results, we chose to compare reintroduction areas in southwestern, west-central, and northwestern Colorado and northern New Mexico (Fig. 1). Within each of these general regions, the 2500-$\mathrm{km}^2$ area exhibiting the highest long-term potential occupancy rates in PATCH was selected as the candidate reintroduction core sites (Fig. 1). We also combined the individual core sites to construct four composite

*Figure 2. Predicted distribution and demography of wolves in the southern Rocky Mountain region under the differing models used in this study: (a) expected demographic potential for wolves as predicted by the scaled Leslie matrix used as input to the PATCH simulations; (b) relative probability of occupancy by wolves as predicted by a resource-selection function (RSF) developed from wolf distribution data in the Greater Yellowstone Ecosystem; (c) potential distribution and demography of wolves as observed in the PATCH simulations under current landscape conditions; and (d) potential distribution and demography of wolves as observed in the PATCH simulations under future scenario C (development on both public and private lands through 2025). Only those areas with a predicted probability of occupancy of >50% are shown in (c) and (d).*


A
Lambda
0 - 0.2
0.2 - 0.5
0.5 - 0.8
0.8 - 0.9
0.9 - 1
1 - 1.1
1.1 - 1.2
1.2 - 1.6
Low occupancy
No Data
B
RSF Quantile
1 - 10
11 - 20
21 - 30
31 - 40
41 - 50
51 - 60
61 - 70
71 - 80
81 - 90
91 - 100
No Data
C
D

reintroduction options: (1) natural recovery from northern Rocky Mountain populations, (2) reintroduction to northern New Mexico, (3) option 2 plus reintroduction to southwestern Colorado, and (4) option 3 plus reintroduction to northwestern Colorado. We approximated the standard reintroduction protocol (Bangs & Fritts 1996) by introducing five breeding-age females in the first year and setting survival for the first five years at close to 100%, under the assumption that new animals would be released to compensate for mortality among the initial releases.

## Results

### Static Model

We selected an optimal RSF model ($-2\text{LL} = 2034$, $\chi^2 = 3897$, $\text{df} = 11$, $p < 0.001$) of the form

$$w(x) = \exp(0.1369949\text{GREEN} - 0.07082755\text{WET} + 1.286847\text{ELK} + 0.004094865\text{SNOW} - 5.523428{*}10^{-7}\text{SNOW}^2 + 0.2073694\text{SLOPE} - 0.0117322\text{SLOPE}^2 + 0.3847016\text{PUBLIC} + 0.4901291\text{WILD} + 2.877422\text{PARK}),$$

where GREEN is MODIS July greenness, WET is MODIS November wetness, ELK is elk winter range, SNOW is annual snowfall, SLOPE is slope in degrees, and PUBLIC, WILD, and PARK are the general public, wilderness, and park management classes.

Extrapolating the model southward from the GYE into Colorado produces a predicted distribution map (Fig. 2b) showing that wolf habitat similar to that occupied within the GYE is found in a band running across northwestern Colorado and also within southwestern Colorado. Based on this model, 46.7% of the region's wolves would be found within general public lands, followed by 40.0% within parks and wilderness areas, and 13.3% on private, unprotected land. The overall number of wolves potentially supported within habitat in the SRM region with RSF values similar to currently inhabited habitat in the GYE (the upper 10% areal quantile of RSF values) is 1305, according to the estimation method based on the model of Fuller (1989), which makes use of the auxiliary data on prey abundance for the SRM region. For comparison, the number of wolves potentially supported within the three Colorado reintroduction core areas of 2500 km$^2$ in size, according to the Fuller (1989) model, ranges from 97 individuals for northern New Mexico to 75 wolves for southwestern Colorado, 102 for west-central Colorado, and 155 for northwestern Colorado.

### Dynamic Model

#### CURRENT CARRYING CAPACITY: LANDSCAPE SCENARIO A

Under current conditions, strong source areas are present in all of the potential reintroduction sites and on the Wyoming-Colorado border (Fig. 2c). Weaker source habitat between the three western Colorado sites facilitates their connectivity, whereas the northern New Mexico site is more isolated. Based on this model, 41.8% of the region's wolf packs would be found within general public lands, followed by 38.8% on private unprotected land, and 18.7% within parks and wilderness areas. The mean number of packs predicted by the PATCH simulations for the larger subregions surrounding the core reintroduction sites ranged from 21.6 for northern New Mexico to 23.1 for west-central Colorado, 32.2 for southwestern Colorado, and 42.6 for northwestern Colorado. Adjusting the PATCH estimate to account for both sexes of adults and for the percentage of packs composed of pups (range 35–67%, $\bar{x} = 46\%$; Fuller 1989) resulted in a total population estimate of 1486. This figure may be high because mean percentage of pups in packs is based on both autumn and winter data, whereas the wolf density model is based on late winter data (Fuller 1989). The mean lambda observed in the simulations is 0.999, which is lower than the mean expected lambda of 1.062 as a result of the influence of infrequently occupied territories, which tend to show a lambda of near 1.000. Weighting the estimate by the probability that a pack territory is occupied in a particular year gives a mean lambda of 1.017. The maximum lambda observed in the simulations is 1.215, which is also lower than the maximum expected lambda of 1.274. Wolf distribution predicted by the dynamic model is significantly correlated with the observed location of the wolf pack territories in the GYE ($R_s = 0.35$, $p < 0.001$).

#### FUTURE CARRYING CAPACITY: LANDSCAPE SCENARIO B

Human population growth, coupled with road development on private lands only, reduced the carrying capacity of the region by 49.2% to a total wolf population estimate of 755. Mean number of packs predicted by the PATCH simulations was 9.7 for northern New Mexico, 9.2 for west-central Colorado, 18.9 for southwestern Colorado, and 14.3 for northwestern Colorado. Although we report these as point estimates, we emphasize that model predictions are best used in a relative sense to rank potential sites and management options. Based on this model, 43.8% of the region's wolf packs would be found within general public lands, followed by 32.3% on private, unprotected land, and 23.9% within parks and wilderness areas.

#### FUTURE CARRYING CAPACITY: LANDSCAPE SCENARIO C

Human population growth, coupled with road development on both private and unprotected public lands, reduced the wolf carrying capacity of the region by 66.3% to a total wolf population estimate of 501. Areas with a >50% likelihood of occupancy remained in all potential reintroduction areas except in west-central Colorado (Fig. 2d). Connectivity, in the form of permanently occupied "stepping-stone" areas, no longer existed between reintroduction areas, although long-distance dispersal by floaters could still occur. Due to the high proportion of private lands in northern New Mexico, the results from both future landscape scenarios were similar in that region. Mean number of packs predicted by the PATCH simulations was 10.7 for northern New Mexico, 4.3 for west-central Colorado, 9.8 for southwestern Colorado, and 9.7 for northwestern Colorado. Based on this model, 38.8% of the region's wolf packs would be found within general public lands, followed by 34.5% on private, unprotected land, and 26.7% within parks and wilderness areas.

A comparison of predicted wolf distribution between simulations using identical mean demographic rates with or without incorporating environmental stochasticity (Fig. 3) showed that year-to-year variation in demographic rates had a strong effect on the likelihood that wolves would occupy habitat areas with higher edge-to-area ratios (i.e., smaller core areas and linear linkages connecting major core areas).

### Reintroduction Options

Under current habitat conditions (landscape scenario A), most options show a low probability of extinction. Excluding the natural recolonization option, option 2 has the highest extinction probability at 6.4% (Table 2). Under future landscape scenario B, extinction probability is 16.6% for option 2, 4.5% for option 3, and 1.7% for option 4. Under future landscape scenario C, extinction probability is 13.7% for option 2, 5.3% for option 3, and 2.2% for option 4.

Mean number of packs within the southern Rocky Mountains at year 200 is estimated at 1.7 for the natural recolonization option, 21.6 for option 2, 51.4 for option 3, and 93.1 for option 4 (Fig. 4), given current landscape scenario A. Increasing the maximum dispersal distance parameter from 250 to 500 or 1500 km resulted in estimates of 3.3 or 6.1 packs, respectively, for the natural recolonization option. Under future landscape scenario B, mean number of packs within the southern Rocky Mountains at year 200 is estimated at <1 for the natural recolonization option, 9.7 for option 2, 28.0 for option 3, and 41.1 for option 4 (Fig. 4). Under future landscape scenario C, mean number of packs within the southern Rocky Mountains at year 200 is estimated at <1 for the natural recolonization option, 10.7 for option 2, 20.5 for option 3, and 29.4 for option 4 (Fig. 4).

When the separate reintroduction areas are consid-

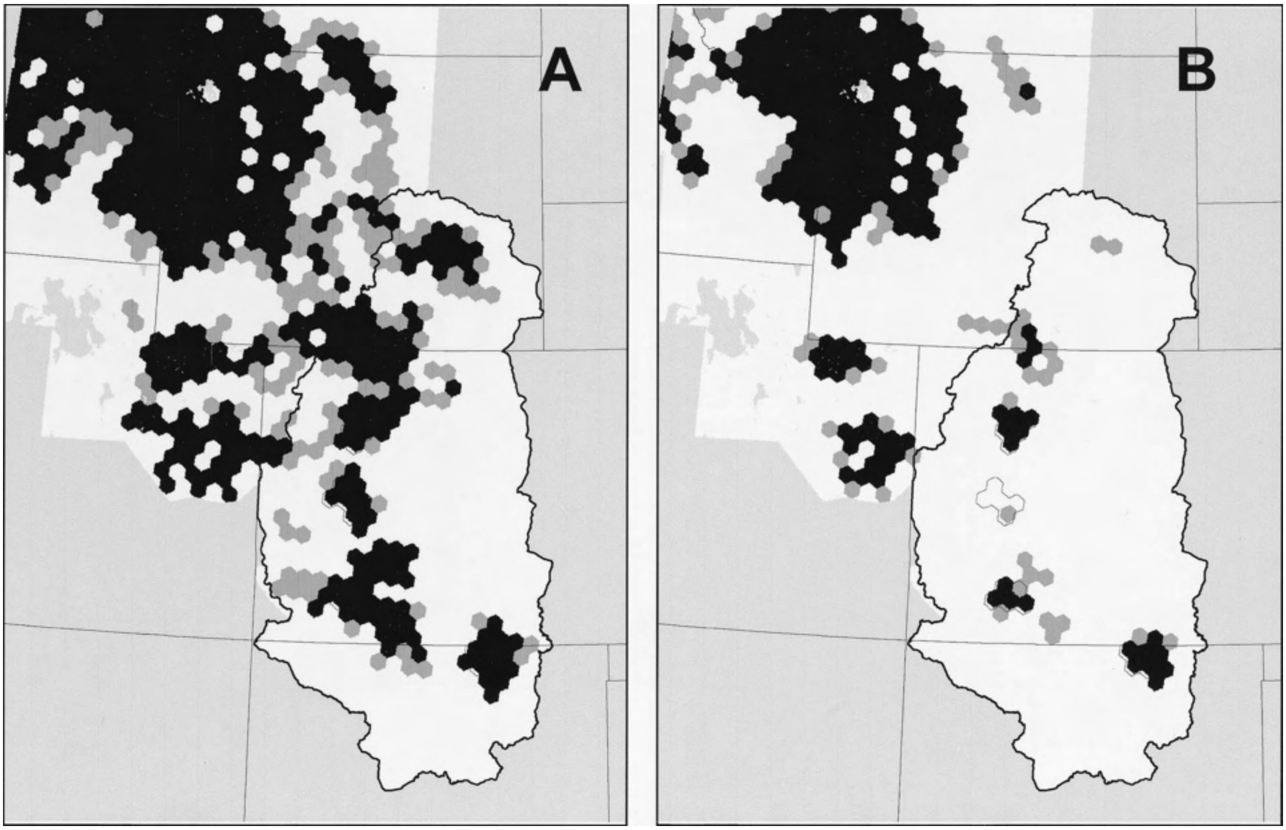


*Figure 3. Contrasts between predicted wolf distribution in simulations with and without environmental stochasticity under (a) current conditions (scenario A) and (b) future conditions (scenario C, development on both public and private lands through 2025). Areas in black are occupied in simulations with environmental stochasticity, whereas areas in gray are occupied only in the absence of environmental stochasticity.*

**Table 2. Relative ranking of potential wolf reintroduction areas in terms of model predictions, with Yellowstone National Park added for comparison.**

| | *Model* | | | | | |
|---|---|---|---|---|---|---|
| *Area* | *mean RSF*[a] *value* | *occupancy probability*[b] *(PATCH), 2000* | *occupancy probability, 2025 (scenario B)* | *occupancy probability, 2025 (scenario C)* | *extinction probability (PATCH), 2000* | *extinction probability, 2025 (scenario C)* |
| Northern New Mexico | 15.7 | 80.0 | 67.6 | 67.1 | 6.7 | 14.5 |
| Southwest Colorado | 12.0 | 75.9 | 68.4 | 53.1 | 13.3 | 31.6 |
| West-central Colorado | 17.3 | 64.4 | 37.3 | 23.8 | 26.7 | 58.8 |
| Northwestern Colorado | 21.9 | 78.7 | 58.1 | 54.8 | 11.4 | 31.6 |
| Yellowstone National Park | 221.6 | 99.1 | 98.7 | 98.6 | <1 | <1 |

[a] *Resource selection function.*

[b] *Occupancy probability is given as averaged over all pack territories in an area.*

ered alone (Fig. 5), rather than as part of the multisite options, extinction probability is 26.7% for a reintroduction to only west-central Colorado, 13.3% for southwestern Colorado, 11.4% for northwestern Colorado, and 6.7% for northern New Mexico (Table 2).

Under future landscape scenario B, extinction probability is 26.3% for a reintroduction to only west-central Colorado, 12.5% for southwestern Colorado, 11.8% for northwestern Colorado, and 16.6% for northern New Mexico. Under future landscape scenario C, extinction probability increases, reaching 58.8% for a reintroduction to only west-central Colorado, 31.6% for southwestern Colorado, 31.6% for northwestern Colorado, and 14.5% for northern New Mexico (Table 2).

## Discussion

Spatial models such as ours contribute a new perspective to population viability analysis and endangered species recovery planning. The resource-selection-function (RSF) model extracts new information from the successful reintroduction of wolves into a neighboring region, revealing regionally specific habitat associations not evident in more-general models or those adapted from the central United States (e.g., Mladenoff et al.1995). The RSF model may be too specific, however, because wolves in the GYE, which were reintroduced in 1995, have not yet dispersed to inhabit a full range of potential habitats, and some characteristics of currently occupied habitat (e.g., association with boreal forest types) may be coincidental rather than actual limiting factors. The probability of such extrapolation error grows as distance increases from the source of the wolf-distribution data, the GYE.

The spatially explicit population model (SEPM) allows a greater level of biological realism because it integrates data on demography and habitat and can explore the response of wolf populations to new habitat scenarios and examine long-term viability requirements, which may differ from short-term requirements for occupation of habitat. How-

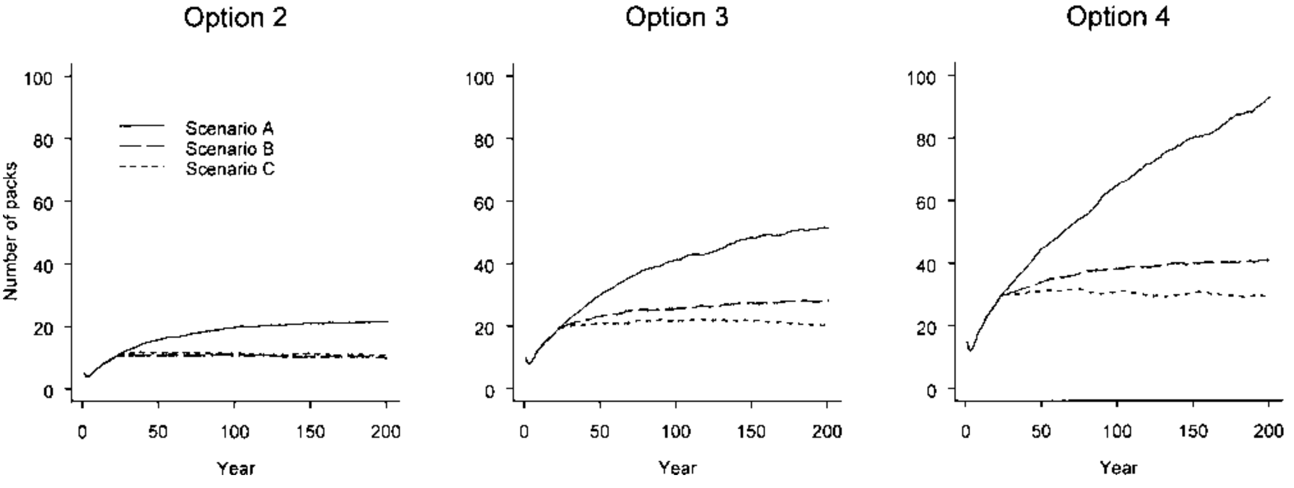


*Figure 4. Mean wolf population trajectory as predicted by the PATCH model for three reintroduction options under current and future conditions. Option 2 would involve reintroduction of wolves to northern New Mexico, option 3 would add a second reintroduction site in southwestern Colorado, and option 4 would add a third reintroduction site in northwestern Colorado.*

ever, this model's added complexity may make its results more sensitive to variation in poorly known parameters such as dispersal behavior (Karieva et al. 1996). This increased variability makes SEPMs more appropriate for ranking management options than predicting actual population levels. Both the static and dynamic models provide a structure for considering restoration potential and making qualitative comparisons between regions. Both approaches are also useful for generating testable hypotheses that can be refined in an adaptive management context based on new field research, improved modeling techniques, and data from successful and unsuccessful restoration efforts and natural recolonization events (Murphy & Noon 1992).

Contrasts between the static and dynamic model predictions derive in part from how they quantify human impacts. The static model includes management status as a surrogate for human impacts, rather than the road or human population variables used as input to the PATCH model. Road density is one of the most significant univariate predictors of wolf distribution in the north-central United States (Mladenoff et al. 1995). Low road density, along with public land ownership, forested land cover, and high elk density are also significantly correlated with the locations of wolf-pack territories in the U.S. northern Rockies (Houts 2000). The absence of road density from the multivariate RSF model we developed is the result of collinearity between habitat variables, specifically the negative correlation between road density and other significant variables such as snowfall and slope. Candidate multivariate models that included road density along with the latter variables often resulted in positive coefficients for road density, which would have caused poor model generality when extrapolated to areas of high road density outside the GYE.

The remaining variables included in the static model were consistent with field knowledge of wolf habitat associations and with previous static carnivore habitat models (Carroll et al. 2001*a*). Because wolves are coursing predators, they generally avoid areas with steep slopes where prey vulnerability is low (Paquet et al. 1996). Areas of high snowfall also limit winter movement because of the wolf's high foot loading compared with snow-adapted predators such as the lynx (Paquet et al. 1996). Fall wetness is correlated with early snow cover, accounting for its negative coefficient here. Both elk winter range and summer greenness are indicators of prey productivity (Carroll et al. 2001*a*).

The static and dynamic models give similar estimates of the potential size of the wolf population in the SRM region, but the spatial distribution of predicted wolf abundance differs between the models. The static model predicts that a larger proportion of the region's wolves occurs within parks and wilderness areas than does the PATCH model. Because it ignores the influence of social structure (e.g., interpack aggression) on limiting wolf density, the static model likely overpredicts density in highly suitable areas.

The results of the PATCH model emphasize the importance of mortality risk in limiting wolf distribution. Although wolves may be more demographically resilient than some large carnivores (Weaver et al. 1996), adult survival is still of overriding importance. Contrasts between the predictions of the static and dynamic models derive primarily from area effects and the effects of landscape change. Small, isolated areas of predicted habitat in the static model are rarely occupied in the dynamic model. In contrast, predicted occupancy in areas adjacent to other populations is higher in the PATCH model than in the static model because wolves there benefit from a demographic rescue effect (Brown & Kodric-Brown 1977). For example, the re-introduction area in northwestern Colorado benefits from its location within a larger constellation of habitat patches. Incorporating landscape change into the dynamic model caused areas such as southwestern Colorado, with relatively low human population growth and a high proportion of protected areas, to increase their ranking under future conditions as other more threatened areas become degraded (Table 2). The reintroduction location in northern New Mexico is unique in that it is a highly protected core area that has little risk of future degradation but is surrounded by a relatively unprotected and at-risk regional landscape. Although the core protected area there is larger than areas that support isolated wolf populations, its regional value for wolf conservation may be affected by loss of connectivity.

Model predictions may be inaccurate if extensive land uses, such as public-lands grazing, are more important than intensive land uses, such as development trends, in limiting wolf restoration. Most mortalities in the early stages of restoration of wolves to the GYE have been associated with control of livestock depredation (Bangs et al. 1998). Our model effectively assumes that the risk of encounters between wolves and livestock is correlated with other human-impact factors such as roads, or that intensive human impacts form more-important long-term limiting factors. It can be argued that it is relatively easy to reduce public-lands grazing through changes in management policy but more difficult to reverse development once it occurs. Wolves in the north-central United States, which are in a later stage of range expansion, are more limited by intensive than extensive land use (Mladenoff et al. 1995). However, patterns of low-density public-lands grazing in the western United States may create greater potential for livestock depredation there. Livestock grazing on U.S. Forest Service lands increases from south to north in the SRM region (Bennett 1994). This may place wolves in northwestern Colorado at greater short-term risk than shown in our model and hinder dispersal between the GYE and Colorado.

Turning from the individual sites to the composite reintroduction options, our results suggest that adding a second release site results in a large reduction in extinction probability. Of the options we considered, option 3 of reintroduction to two sites in northern New Mexico and

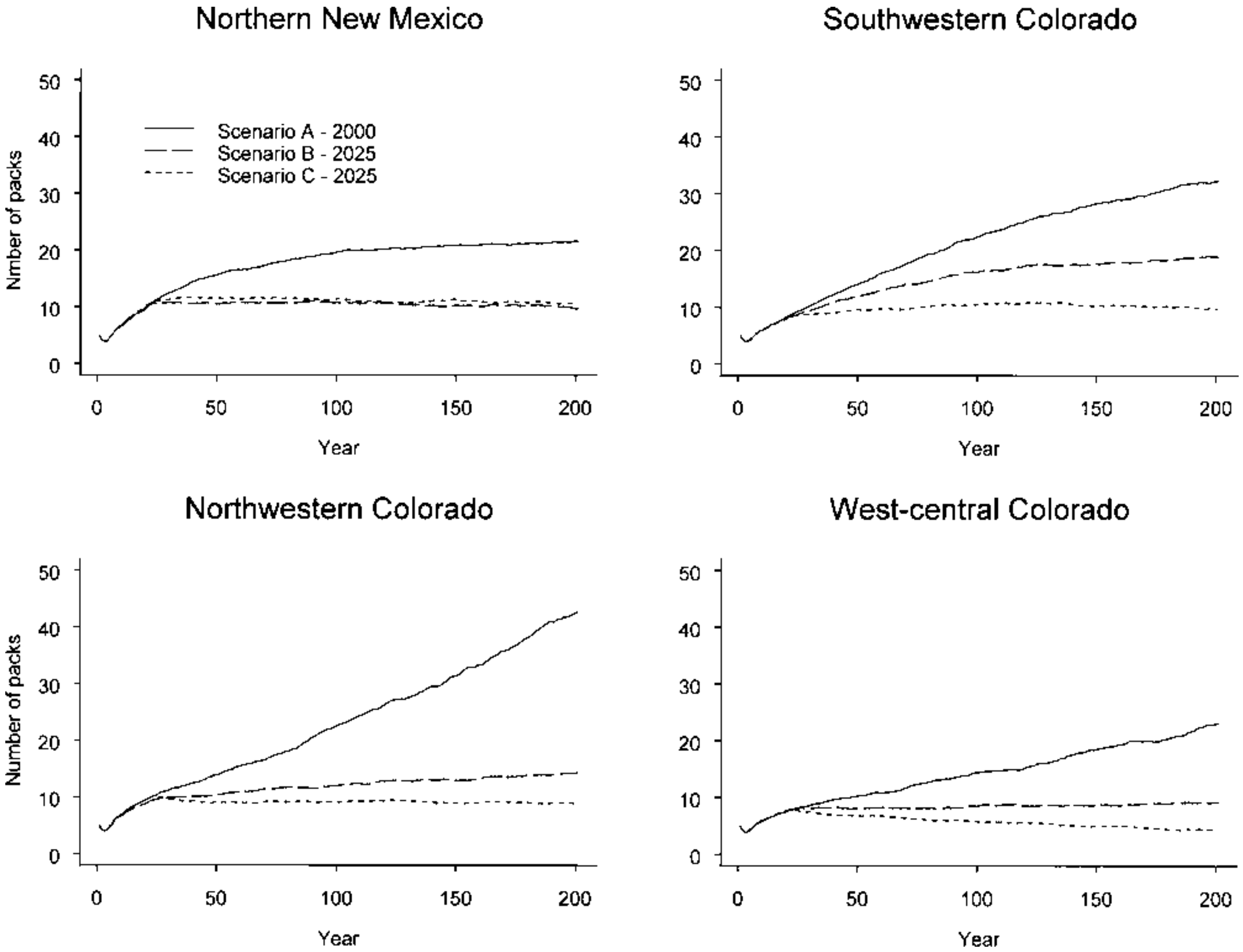


*Figure 5. Mean wolf population trajectory as predicted by the PATCH model for the individual candidate reintroduction areas under current and future conditions.*

southwestern Colorado may best balance the goals of maximizing the probability of success and minimizing the cost of restoration. The prospects for long-term viability with this option under future scenario C (94.7%) compare favorably with those for wolves in the GYE (>99%). Therefore, despite the effects of landscape change, our results suggest a high potential for successful wolf restoration to the SRM region.

### Lessons for Reintroduction Planning

Overall, a consistent message emerges from our two models regarding what portions of the SRM have the highest potential to support wolves and how that potential compares with previous reintroduction areas. Comparison of the RSF and PATCH results from Colorado with those from the GYE and Idaho suggest that the latter two areas are unique in the western United States in the size of their core areas. Resource-selection-function values for the GYE are an order of magnitude greater than those for the Colorado reintroduction sites (Table 2). Although wolves often occur outside core protected areas, they may depend on them for long-term population persistence (Fritts & Carbyn 1995; Haight et al. 1998). Semi-developed, mixed-ownership landscapes such as those found in western Colorado may both support high prey densities and create a high risk of human-caused wolf mortality (Mladenoff et al. 1997). More effort and time may be necessary for wolves in Colorado to reach the population levels seen in the GYE and Idaho after relatively short reintroduction efforts. However, wolf-recovery efforts in the north-central United States suggest that, given favorable human attitudes, wolves can coexist with development at surprisingly high levels. In Wisconsin, wolves currently inhabit more area than was predicted in empirical habitat models based on road density (Mladenoff et al. 1999). Both the RSF and PATCH results suggest that a large proportion of the packs of the SRM region will be found on general public lands rather than in parks or wilderness, but the PATCH results suggest that core refugia remain the key to whether a particular reintroduction area can maintain wolves under future conditions.

The smaller size and greater isolation of core refugia in Colorado compared with those of the GYE and Idaho will likely make maintenance of connectivity between subpopulations of greater importance (Haight et al. 1998). The reduction in carrying capacity caused by landscape change in the SRM is two and four times that seen in the GYE and southern Canadian Rockies, respectively (C.C., unpublished), pointing to the higher pace of development in the SRM, its isolation from more northerly populations, and its current status closer to the threshold for large-carnivore persistence.

Because wolf dispersal behavior is too complex to model realistically, we must consider the variation in results due to dispersal behavior when evaluating model output. Wolves may exhibit a pattern of dispersal termed "stratified diffusion," a mixture of short-distance dispersal that expands existing colonies and long-distance dispersal that creates new colonies (Shigesada & Kawasaki 1997). Although the PATCH model cannot currently predict this type of mixed-dispersal dynamics, it may be instructive regarding the influence of short-distance dispersal on source-sink dynamics within a region. For example, the contrasts in predicted population trajectories between the subregions (Fig. 5) relate to the dispersion of core habitat and the level of connectivity in each area. Occasional long-distance dispersal events may cause the long-term distribution predicted in the PATCH reintroduction options to be achieved more rapidly. The relative levels of connectivity shown by the different scenarios are significant, however, in that they show that even if dispersers from the GYE may potentially colonize Colorado over the long term, the level of connectivity between the GYE and Colorado is low enough that a separate Colorado reintroduction would significantly hasten establishment of that population. This may be important given the pace of landscape change in the region. Our results suggest that although their sensitivity to dispersal parameters make spatially explicit population models unsuitable for some aspects of reintroduction planning, they nevertheless can provide other insights not available from less complex models.

Comparison of the model results also suggests more-general guidelines for large-carnivore conservation. Lambda values observed in the dynamic model were lower than expected from the model input. These more pessimistic predictions are the result of area and connectivity factors that reduce the potential of small and isolated habitat patches to support species with large area requirements. A comparison of the spatial distribution of expected (Fig. 2a) and observed (Fig. 2c) sources and sinks suggests that weak source habitat isolated from strong sources has a low probability of occupancy. The key territories occupying strong source habitat tend to consist of large packs in our model. As the size and demographic value of these packs is reduced with future landscape change, they are less able to support peripheral packs. The high lambda of the largest packs is the inverse of what might be predicted by a density-dependent PVA model, but it is consistent with patterns observed in the GYE population.

An additional reduction in potential occurs when environmental stochasticity is incorporated in the model because territories located in the periphery are most affected by stochastic factors (Fig. 3). This "extinction vortex" (Gilpin & Soulé 1986) is an example of the novel results provided by combining spatial and demographic data. As predicted (Woodroffe & Ginsberg 1998), this effect is more noticeable in the wolf than in solitary large carnivores such as the grizzly bear (C.C., unpublished data) and may be generally relevant to other threatened social carnivores such as the African wild dog (*Lycaon pictus*) (Creel & Creel 1998). This reduction in the viability of peripheral populations may partially negate the added resilience (sensu Weaver et al. 1996) conferred by the wolf's high fecundity and vagility. Our results suggest that dynamic models and less complex models such as resource-selection functions can be complementary tools for the design of reintroduction strategies for carnivores and other area-sensitive species in increasingly human-dominated landscapes.

## Acknowledgments

We thank K. Kunkel, W. Martin, B. Miller, B. Noon, P. Paquet, and one anonymous reviewer for helpful reviews of the manuscript and M. Thornton of the University of Montana for assistance with conversion of the MODIS data. R. Noss and P. Paquet provided helpful comments on model development in the course of a collaboration on a companion study of carnivore viability in the northern Rocky Mountains. This study was funded by the Turner Endangered Species Fund.

### Literature Cited

Ballard, W. B., J. S. Whitman, and C. L. Gardner. 1987. Ecology of an exploited wolf population in south-central Alaska. Wildlife Monographs **98:**1–54.

Bangs, E. E., and S. H. Fritts. 1996. Reintroducing the gray wolf to central Idaho and Yellowstone National Park. Wildlife Society Bulletin **24:**402–413.

Bangs, E. E., S. H. Fritts, J. A. Fontaine, D. W. Smith, K. M. Murphy, C. M. Mack, and C. C. Niemeyer. 1998. Status of gray wolf restoration in Montana, Idaho, and Wyoming. Wildlife Society Bulletin **26:**785–798.

Bennett, L. E. 1994. Colorado gray wolf recovery: a biological feasibility study. University of Wyoming Fish and Wildlife Cooperative Research Unit, Laramie.

Boyce, M. S., and L. L. McDonald. 1999. Relating populations to habitats using resource selection functions. Trends in Ecology & Evolution **14:**268–272.

Breitenmoser, U., C. Breitenmoser-Würsten, L. N. Carbyn, and S. M. Funk. 2001. Assessment of carnivore reintroductions. Pages 241–281 in J. L. Gittleman, S. M. Funk, D. W. MacDonald, and R. K. Wayne, editors. Carnivore conservation. Cambridge University Press, Cambridge, United Kingdom.

Brown, J. H., and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration on extinction. Ecology **58:**445–449.

Brown, W. M., and D. R. Parsons. 2001. Restoring the Mexican gray wolf to the desert southwest. Pages 169–186 in D. Maehr, R. Noss, and J. Larkin, editors. Large mammal restoration: ecological and social implications. Island Press, Washington, D.C.

Carroll, C., R. F. Noss, and P. C. Paquet. 2001*a*. Carnivores as focal species for conservation planning in the Rocky Mountain region. Ecological Applications **11:**961–980.

Carroll, C., R. F. Noss, N. H. Schumaker, and P. C. Paquet. 2001*b*. Is the restoration of wolf, wolverine, and grizzly bear to Oregon and California biologically feasible? Pages 25–46 in D. Maehr, R. Noss, and J. Larkin, editors. Large mammal restoration: ecological and social implications. Island Press, Washington, D.C.

Colorado Division of Wildlife Resources (CDOW). 1997. Big game hunting statistics: a Colorado outdoors special edition. CDOW, Denver.

Creel, S., and N. M. Creel. 1998. Six ecological factors that may limit African wild dogs, *Lycaon pictus.* Animal Conservation **1:**1–9.

Crist, E. P., and R. C. Cicone. 1984. Application of the tasseled cap concept to simulated thematic mapper data. Photogrammetric Engineering and Remote Sensing **50:**343–352.

Daly, C., R. P. Neilson, and D. L. Phillips. 1994. A statistical-topographic model for mapping climatological precipitation over mountainous terrain. Journal of Applied Meteorology **33:**140–158.

Fritts, S. H., and L. N. Carbyn. 1995. Population viability, nature reserves, and the outlook for gray wolf conservation in North America. Restoration Ecology **3:**26–38.

Fuller, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs **105:**1–41.

Gilpin, M. E., and M. E. Soulé. 1986. Minimum viable populations: the processes of species extinction. Pages 19–34 in M. E. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts.

Gittleman, J. L., S. M. Funk, D. W. MacDonald, and R. K. Wayne, editors. 2001. Carnivore conservation. Cambridge University Press, Cambridge, United Kingdom.

Haight, R. G., D. J. Mladenoff, and A. P. Wydeven. 1998. Modeling disjunct gray wolf populations in semi-wild landscapes. Conservation Biology **12:**879–888.

Harrison, D. J., and T. G. Chapin. 1998. Extent and connectivity of habitat for wolves in eastern North America. Wildlife Society Bulletin **26(4):**767–775.

Houts, M. E. 2000. Modeling gray wolf habitat in the Northern Rocky Mountains using GIS and logistic regression. M.S. thesis. University of Kansas, Lawrence.

Huete, A. R., H. Q. Liu, K. Batchily, and W. van Leeuwen. 1997. A comparison of vegetation indices over a global set of TM images for EOS-MODIS. Remote Sensing of the Environment **59:**440–451.

Karieva, P., and U. Wennergren. 1995. Connecting landscape patterns to ecosystem and population processes. Nature **373:**299–302.

Karieva, P., D. Skelly, and M. Ruckelshaus. 1996. Reevaluating the use of models to predict the consequences of habitat loss and fragmentation. Pages 156–166 in S. T. A. Pickett, R. S. Ostfeld, M. Schachak, and G. E. Likens, editors. The ecological basis of conservation: heterogeneity, ecosystems, and biodiversity. Chapman and Hall, New York.

Lacy, R. C. 1993. VORTEX: a computer simulation model for population viability analysis. Wildlife Research **20:**45–65.

Mace, R. D., J. S. Waller, T. L. Manley, K. Ake, and W. T. Wittinger. 1999. Landscape evaluation of grizzly bear habitat in western Montana. Conservation Biology **13:**367–377.

Manly, B. F. J., L. L. McDonald, and D. L. Thomas. 1993. Resource selection by animals. Chapman and Hall, New York.

Martin, B., R. Edward, and A. Jones. 1999. Mapping a future for wolves in the southern Rockies. Southern Rockies Wolf Tracks **7(2):**1–12.

Merrill, T., D. J. Mattson, R. G. Wright, and H. B. Quigley. 1999. Defining landscapes suitable for restoration of grizzly bears (*Ursus arctos*) in Idaho. Biological Conservation **87:**231–248.

Mladenoff, D. J., and T. A. Sickley. 1999. Assessing potential gray wolf restoration in the northeastern United States: a spatial prediction of favorable habitat and potential population levels. Journal of Wildlife Management **62:**1–10.

Mladenoff, D. J., T. A. Sickley, R. G. Haight, and A. P. Wydeven. 1995. A regional landscape analysis and prediction of favorable gray wolf habitat in the northern Great Lakes region. Conservation Biology **9:**279–294.

Mladenoff, D. J., R. G. Haight, T. A. Sickley, and A. P. Wydeven. 1997. Causes and implications of species restoration in altered ecosystems: a spatial landscape projection of wolf population recovery. BioScience **47:**21–31.

Mladenoff, D. J., T. A. Sickley, and A. P. Wydeven. 1999. Predicting gray wolf landscape recolonization: logistic regression models vs. new field data. Ecological Applications **9:**37–44.

Murphy, D. D., and B. R. Noon. 1992. Integrating scientific methods with habitat planning: reserve design for Northern Spotted Owls. Ecological Applications **2:**3–17.

Paquet, P. C., J. Wierzchowski, and C. Callaghan. 1996. Effects of human activity on gray wolves in the Bow River Valley, Banff National Park, Alberta. Chapter 7 in J. Green, C. Pacas, S. Bayley, and L. Cornwell, editors. A cumulative effects assessment and futures outlook for the Banff Bow Valley. Prepared for the Banff Bow Valley Study, Department of Canadian Heritage, Ottawa, Ontario.

Pletscher, D. H., R. R. Ream, D. K. Boyd, M. W. Fairchild, and K. E. Kunkel. 1997. Population dynamics of a recolonizing wolf population. Journal of Wildlife Management **61:**459–465.

Ream, R. R., M. W. Fairchild, D. K. Boyd, and D. H. Pletscher. 1991. Population dynamics and home range changes in a colonizing wolf population. Pages 349–366 in R. B. Keiter and M. S. Boyce, editors. The greater Yellowstone ecosystem: redefining America's wilderness heritage. Yale University Press, New Haven, Connecticut.

Schumaker, N. H. 1998. A user's guide to the PATCH model. EPA/600/R-98/135. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oregon.

Schwarz, G. 1978. Estimating the dimension of a model. Annals of Statistics **6:**461–464.

Shigesada, N., and K. Kawasaki. 1997. Biological invasions: theory and practice. Oxford series in ecology and evolution. Oxford University Press, Oxford, United Kingdom.

Shinneman, D., R. McClellan, and R. Smith. 2000. The state of the southern Rockies ecoregion. The Southern Rockies Ecosystem Project, Nederland, Colorado.

Singleton, P.H. 1995. Winter habitat selection by wolves in the North Fork of the Flathead River Basin, Montana and British Columbia. M.S. thesis. University of Montana, Missoula.

Theobald, D. M. 2000. Fragmentation by inholdings and exurban development. Pages 155–174 in R. L. Knight, F. W. Smith, S. W. Buskirk, W. H. Romme, and W. L. Baker, editors. Forest fragmentation in the southern Rocky Mountains. University Press of Colorado, Boulder, Colorado.

Theobald, D. M., H. Gosnell, and W. E. Riebsame. 1996. Land use and landscape change in the Colorado mountains II: a case study of the East River Valley, Colorado. Mountain Research and Development **16(4):**407–418.

Vucetich, J. A., R. O. Peterson, and T. A. Waite. 1997. Effects of social structure and prey dynamics on extinction risk in gray wolves. Conservation Biology **11:**957–965.

Weaver, J. L., P. C. Paquet, and L. F. Ruggiero. 1996. Resilience and conservation of large carnivores in the Rocky Mountains. Conservation Biology **10:**964–976.

Woodroffe, R., and J. R. Ginsberg. 1998. Edge effects and the extinction of populations inside protected areas. Science **280:**2126–2128.

Young, S. P., and E. A. Goldman. 1944. Wolves of North America. American Wildlife Institute, Washington, D.C.

# Defining Recovery Goals and Strategies for Endangered Species: The Wolf as a Case Study

CARLOS CARROLL, MICHAEL K. PHILLIPS, CARLOS A. LOPEZ-GONZALEZ, AND NATHAN H. SCHUMAKER

*We used a spatially explicit population model of wolves (*Canis lupus*) to propose a framework for defining rangewide recovery priorities and finer-scale strategies for regional reintroductions. The model predicts that Yellowstone and central Idaho, where wolves have recently been successfully reintroduced, hold the most secure core areas for wolves in the western United States, implying that future reintroductions will face greater challenges. However, these currently occupied sites, along with dispersal or reintroduction to several unoccupied but suitable core areas, could facilitate recovery of wolves to 49% of the area in the western United States that holds sufficient prey to support wolves. That percentage of the range with recovery potential could drop to 23% over the next few decades owing to landscape change, or increase to 66% owing to habitat restoration efforts such as the removal of some roads on public lands. Comprehensive habitat and viability assessments such as those presented here, by more rigorously defining the Endangered Species Act's concept of "significant portion of range," can clarify debate over goals for recovery of large carnivores that may conflict with human land uses.*

*Keywords:* Canis lupus, *conservation planning, Endangered Species Act, reintroduction, spatially explicit population model*

**As human impacts on the biosphere increase, con**servation biology must increasingly focus not only on preserving the current distribution of biodiversity but also on restoring species to areas from which they have been extirpated (figure 1). The success of restoration efforts depends in part on clarification of both the normative and the technical components of recovery goals (Breitenmoser et al. 2001). For example, the level of extinction risk tolerated or the extent of historic range to which recovery is desired are normative decisions guided by laws such as the US Endangered Species Act (ESA; 16 USC 1531–1540 [1988]). Once these normative aspects are resolved, conservation science can help identify which restoration strategy is most likely to ensure the desired level of recovery. Many of the species listed under the ESA are narrowly distributed endemics that can be protected by preserving a limited number of sites (Dobson et al. 1997). It is more difficult to define recovery goals for species such as the gray wolf (*Canis lupus*), which have large area requirements for viable populations, and whose protection may conflict with existing land uses such as livestock production. The scientific methodology used to define recovery goals and strategies for endangered species has not fully integrated recent technical advances in conservation biology, such as spatially explicit population models (SEPMs; Dunning et al. 1995). We present an example of such an analysis applied to the wolf, a high-profile endangered species whose proposed recovery goals (68 Federal Register 15804–15875) have recently been the subject of litigation (*Defenders of Wildlife v. Norton,* Civ. 03-1348-JO [2005]; *National Wildlife Federation v. Norton,* 03-CV-340 [2005]), to demonstrate how these methods can introduce key scientific knowledge into the debate over recovery goals and facilitate the decisionmaking process by illustrating the efficacy of alternate management scenarios.

Although the ESA of 1973 was the third in a series of laws aimed at protecting imperiled species, it was the first to offer protection to any species in danger of extinction throughout all or a significant portion of its range. By including the phrase "significant portion of its range," Congress signaled its intent that listed species should not simply be saved from extinction, but rather recovered so that populations inhabit relatively large areas (i.e., significant portions) of suitable habitat within historic ranges. Case law (*Defenders of Wildlife v. Norton,* 258 F.3d 1136 [2001], 239 F. Supp. 2d 9 [2002], Civ. 03-1348-JO [2005]; *National Wildlife Federation v. Norton,* 03-CV-340 [2005]) and previous delisting actions by the US Fish and Wildlife Service (USFWS) are consistent with this intent, as the 15 taxa that have been declared recovered since passage of the ESA were generally widely distributed at the time of delisting. This expectation was buttressed when Con-

---

*Carlos Carroll (e-mail: carlos@klamathconservation.org) is with the Klamath Center for Conservation Research, Orleans, CA 95556, and is conservation science advisor for the Wilburforce Foundation in Seattle. Michael K. Phillips is with the Turner Endangered Species Fund, 1123 Research Drive, Bozeman, MT 59718. Carlos A. Lopez-Gonzalez is with the Universidad Autonoma de Querétaro, Querétaro, Mexico 76010. Nathan H. Schumaker is with the US Environmental Protection Agency, Corvallis, OR 97333.*

*Figure 1. In 1995, M. K. P. (the second author) and others released the first wolves to inhabit Yellowstone National Park in 70 years. Endangered species recovery efforts often involve reintroduction of animals to unoccupied but potentially suitable habitat. However, the extent of historic range to which reintroduction is needed is a controversial issue for formerly widespread species such as the wolf whose restoration may conflict with human land uses, such as livestock grazing on public lands. Photograph: National Park Service/Jim Peaco.*

gress defined the term "species" to include "any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature" (ESA section 3[15]). The policy of recognizing distinct population segments (DPSs) allows for protective measures before the occurrence of large-scale declines that would necessitate listing a species or subspecies throughout its entire range (61 Federal Register 4722).

In the late 1950s, the number of gray wolves inhabiting the conterminous United States reached an all-time low, with fewer than 1000 wolves occupying less than 1% of the species' historic range in northeastern Minnesota and the adjacent Isle Royale National Park (Phillips et al. 2004). Three decades after passage of the ESA, owing to the expansion of populations in Minnesota and Canada and to reintroduction efforts in the northern Rocky Mountains (USFWS 1994) and the southwestern United States (USFWS 1996), about 4500 wolves occupy about 5% of the species' historic range in the conterminous United States (figure 2). In response to this improved conservation status, in April 2003 the USFWS published a reclassification rule that divided the lower 48 states into three DPSs (figure 2), retaining the experimental–nonessential population areas in the northern Rocky Mountains (USFWS 1994), but elsewhere downlisting the eastern and western gray wolf DPSs from endangered to threatened and indicating that recovery objectives for both had been met (68 Federal Register 15804–15875). However, in 2005, two federal court rulings vacated and enjoined the rule on the basis, in part, that it lacked comprehensive consideration of the phrase "significant portion of range" and misapplied the DPS policy (*Defenders of Wildlife v. Norton,* Civ. 03-1348-JO [2005]; *National Wildlife Federation v. Norton,* 03-CV-340 [2005]). When considered with the two earlier rulings cited above, this indicates that future recovery plans for wolves and other listed species should be guided by a rangewide determination of habitat suitability and relevant principles of conservation planning. The three principles of representation (establishing populations across the full array of potential habitats), resiliency (protecting populations large enough to remain viable), and redundancy (saving enough different populations that some can be lost without a loss of the species) are widely invoked guidelines for ensuring conservation of threatened species, even in the face of geographically widespread threats such as climate change (Shaffer and Stein 2000). By broadening recovery criteria to encompass representation, these principles recognize that a single population may not represent species recovery, even if it is large enough to be significantly resilient to extinction. For wide-ranging species such as the wolf, the importance of connectivity (protecting linkage areas, especially those that enhance viability by connecting larger with smaller populations) may justify its addition as a fourth principle for defining recovery goals (Soulé and Terborgh 1999).

In the 2003 proposed rule, the USFWS conflated the concepts of population viability and recovery. The claim that the ESA mandates only maintaining a species' viability (preventing extinction) rather than effecting recovery was first made in a 1986 revision to the regulations governing ESA enforcement (50 CFR 402), but has been repeatedly rejected by the courts (Suckling and Taylor 2005). This distinction is especially important for species such as the wolf or grizzly bear (*Ursus arctos*) that currently occupy a small portion of their historic range, because ESA mechanisms for maintaining viability restrict only "take" of individuals or occupied habitat, whereas ESA mechanisms for effecting recovery may restrict the destruction of unoccupied but suitable habitat and call for proactive measures to promote population reestablishment (Suckling and Taylor 2005). Although the bulk of the ESA's language addresses recovering individual species, Congress also included language that mandates the conservation of ecosystems on which listed species depend. Because of this, some researchers have proposed an additional guideline for recovery planning, the principle of ecological effectiveness (Soulé et al. 2005). An ecologically effective population contains enough individuals with a wide enough geographic distribution to reestablish the species' role in ecosystems. The argument for reestablishing ecologically effective populations is most persuasive in the case of the wolf and other "keystone" species that strongly influence ecosystem function through interspecific interactions such as predation (figure 3). For example, the return of wolves to Yellowstone has triggered a cascade of top-down effects on that ecosystem (Smith et al. 2003). Wolf predation has reduced the ability of elk to concentrate browsing on preferred species such as aspen (*Populus tremuloides*), leading to the recovery of riparian vegetation and associated species (Ripple and Beschta 2004). Because the wolf is a keystone species that was historically widespread throughout the western United States, yet whose recovery may conflict with current land-use practices such as livestock grazing on public lands, it provides an ideal case study of the role of conservation science in clarifying species recovery goals. We first present an example of a rangewide analysis for the wolf in the western contiguous United States, and then describe the use of an SEPM to help define recovery goals and strategies at a finer scale for the southwestern DPS (SWDPS) for the gray wolf (figure 2).

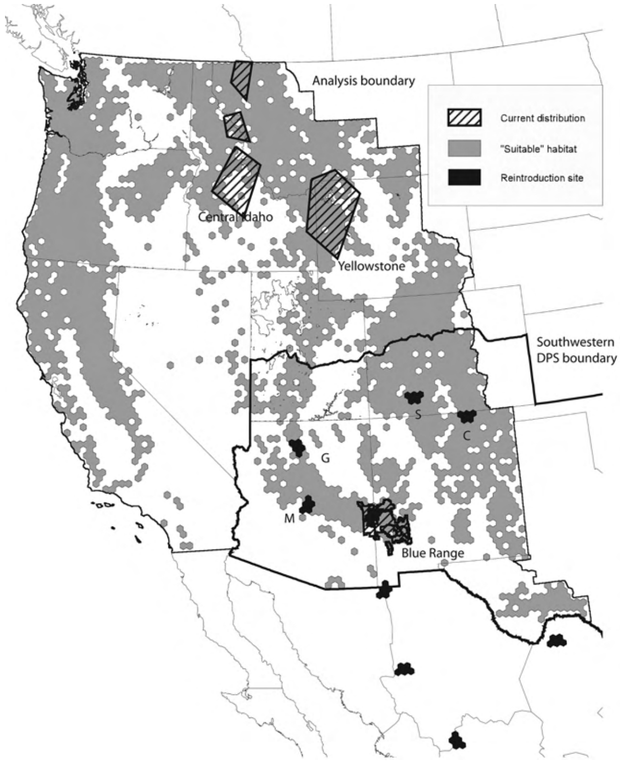


*Figure 2. Map of the analysis area and the approximate location of wolf (*Canis lupus*) populations in the western United States, including the existing Yellowstone, central Idaho, and Blue Range reintroduction sites and the population in northwestern Montana established by dispersal from Canada. Nearly all of the area within the analysis boundary is within the historic geographic range boundary of the gray wolf, although more arid areas typically held few wolves. The extent of habitat defined as "suitable" in this analysis (meeting the productivity threshold that allows breeding in the PATCH model) is shown in gray. The boundaries of the southwestern distinct population segment, or DPS (as proposed by the US Fish and Wildlife Service [68 Federal Register 15804–15875]), are shown. Locations within the southwestern DPS evaluated as potential reintroduction sites in this study are shown in black. Abbreviations: C, Carson; G, Grand Canyon; M, Mogollon; S, San Juan Mountains.*

## Rangewide analysis for the western United States

We analyzed potential wolf habitat and population viability across the western contiguous United States, from the western edge of the Great Plains to the Pacific Ocean, an area of about 2,800,000 square kilometers (km$^2$) (figure 2). The structure of the SEPM (PATCH, or program to assist in tracking critical habitat) and input habitat models used in this study are described in detail elsewhere (Schumaker 1998, Carroll et al. 2001a, 2001b, 2003a, 2003b) and summarized here (box 1). We calibrated habitat rankings to specific demographic values based on field studies from areas that showed similar habitat quality to the habitat classes in the SEPM input layers (Ballard et al. 1987, Fuller 1989, Hayes and Harestad 2000, Fuller et al. 2003, Smith et al. 2004). Because the analysis covers a large and ecologically diverse region, the geographic information system, or GIS, models for fecundity and survival must use general habitat data that are available in every state. This is a lesser problem for the survival input layer, because roads and human population have a similar negative effect on large carnivore survival in diverse habitats (Thiel 1985, Fuller et al. 2003). A metric combining road density, local human population density, and interpolated human population density (Merrill et al. 1999) predicted survival in the spatially explicit population modeling (figure 4b).

Estimating wolf fecundity (reproductive rates) across the western United States is more difficult. Abundance estimates of ungulate prey are not collected in some areas of the western United States, and where they do exist, they show strong

*Figure 3. Wolves in Yellowstone have reduced the ability of elk to concentrate foraging on aspen, cottonwood, and other favored species, thus allowing the recovery of key riparian vegetation and its associated biota. Restoring such top-down ecosystem processes involving wolves and other keystone species may require ecologically effective populations (i.e., populations that are larger and more widespread than would be necessary to ensure viability of the species itself). Photograph: Bob Landis.*

### Box 1. Spatially explicit population models.

Conservation planners assess the distribution of wildlife habitat (including potentially suitable but currently unoccupied areas) with the aid of computer models of varying complexity. Broadly speaking, large carnivores such as the wolf can persist in areas where there is sufficient food and where persecution by humans is low (Fuller et al. 2003). A simple model of recovery potential could therefore highlight large roadless areas with sufficient productivity or extensive forest habitat. More complex spatially explicit population models (SEPMs) might also begin with data on road density and productivity, but would then integrate additional information on species characteristics such as demographic rates and dispersal behavior. For example, social carnivores, such as the wolf, often require larger territories than solitary species of similar size, and may thus be more vulnerable to landscape fragmentation (Carroll et al. 2003a). Unlike the simpler model, an SEPM can provide insights on the effects of population size and connectivity on viability and can help identify the locations of population sources and the degree of threat to those areas from landscape change (figure 4a; Carroll et al. 2003b).

PATCH (program to assist in tracking critical habitat), the SEPM used here, is designed for studying territorial vertebrates. It links the survival and fecundity of individual animals to geographic information system (GIS) data on mortality risk and habitat productivity at the scale of an individual or pack territory (Schumaker 1998). Territories are allocated by intersecting the GIS data with an array of hexagonal cells (figure 4c). The different habitat types in the GIS maps are assigned weights based on the relative levels of fecundity and survival expected in those habitat classes. Base survival and reproductive rates, derived from published field studies, are then supplied to the model as a population projection matrix (box 2; Caswell 2001). The model scales these base matrix values using the mean of the habitat weights within each hexagon, with lower means translating into lower survival rates or reproductive output (figure 4c). Each individual in the population is tracked through a yearly cycle of survival, fecundity, and dispersal events (figure 4a). Environmental stochasticity is incorporated by drawing each year's base population matrix from a randomized set of matrices whose elements were drawn from a beta (survival) or normal (fecundity) distribution (coefficients of variation given in box 2). Adult organisms are classified as either territorial or floaters. The movement of territorial individuals is governed by a parameter for site fidelity, but floaters must always search for available breeding sites. As pack size increases, pack members in the model have a greater tendency to disperse and search for new available breeding sites (Carroll et al. 2003a). Movement decisions use a directed random walk that combines varying proportions of randomness, correlation, and attraction to higher-quality habitat (Schumaker 1998).

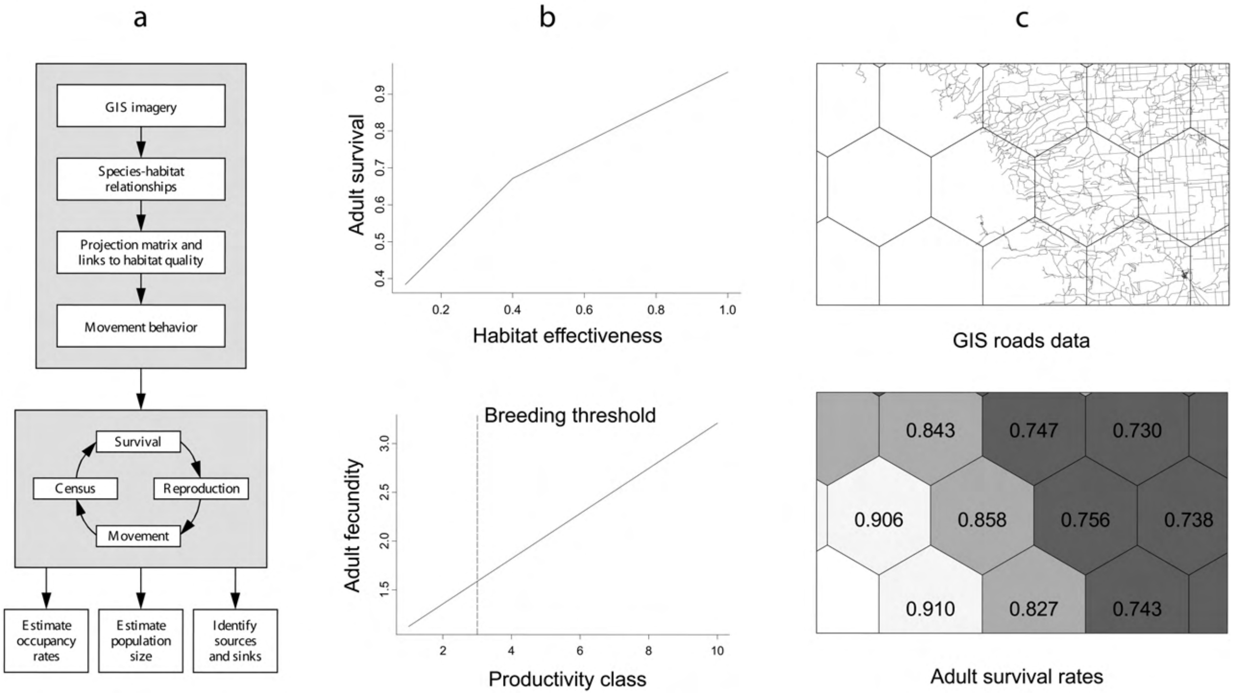


*Figure 4. Spatially explicit population models (SEPMs) represent population processes by tracking the spatial location of individuals and landscape features. (a) A flowchart of the simulation process in PATCH, the SEPM used in this study. (b) Graphs of the relationship between GIS-based habitat values and demographic values for fecundity (given as females produced per pack) and survival for wolves. (c) Territories are allocated by overlaying an array of hexagonal cells on GIS habitat data. For the wolf, data on roads are used in combination with human population data to calculate the metric of habitat effectiveness used to scale wolf survival rates. Abbreviation: GIS, geographic information system.*

inconsistencies across state boundaries. Therefore, as a surrogate for fecundity, we used tasseled-cap greenness (Crist and Cicone 1984), a metric derived from MODIS (Moderate Resolution Imaging Spectroradiometer) satellite imagery from mid-July 2003 and 2004 (Wharton and Myers 1997). "Pseudohabitat" variables such as greenness that are derived directly from unclassified satellite imagery are correlated to varying degrees with ecological factors such as net primary productivity and green phytomass (Cihlar et al. 1991, Merrill et al. 1993, White et al. 1997), and thus with abundance of ungulate prey species, although this relationship is weakened by phenological variation between years and by spatial variation in the percentages of bare ground and of dry biomass (Merrill et al. 1993). Summer greenness values are strongly correlated with ungulate density in the northern Rocky Mountains and Pacific Northwest (Carroll et al. 2001b, 2003a), and with carnivore habitat in other regions (Mace et al. 1999, Carroll et al. 2001a). However, the link between greenness and prey abundance may be less general across the larger and more ecologically varied region addressed in this study than is the well-established link between prey abundance and wolf density (Fuller et al. 2003). Therefore, to avoid overestimation of prey abundance in nonforest habitats, we used data on vegetation type to rate forest habitat higher than shrubland habitat with similar greenness values. Nonnatural (agricul-

**Box 2. Parameters used in the PATCH model of wolf population dynamics in the western United States.**

Territory size: 504 square kilometers (km$^2$)
Maximum dispersal distance: 750–1500 km
Survival rates (maximum):

- Young, year 0: 0.46
- Subadult, year 1: 0.86
- Adult, > 2 years: 0.96
- At senescence (year 8): 0.69

Fecundity rates (maximum number of female offspring per adult female or pack):

- Subadult, year 1: 0
- Adult, year 2: 2.29
- Adult, > 3 years: 3.21

Coefficient of variation in demographic rates:

- Fecundity: 30%
- Pup (year 0) mortality: 40%
- Adult mortality: 30%

tural and urban) habitat was given zero habitat value. Because wolves are coursing predators that avoid steep terrain, the wolf fecundity model also incorporated the negative effect of slope on prey vulnerability (Paquet et al. 1996, Carroll et al. 2001b).

The results of the PATCH model are generally more sensitive to the demographic parameters used, and to how these parameters were assigned to habitat classes, than to variation in other parameters, such as dispersal distance (Carroll et al. 2003b). The large body of published research on relationships between wolf demographics and habitat (e.g., as reviewed in Fuller et al. 2003) strengthens the power of conceptual models such as those used here. In previous studies, SEPM predictions of wolf distribution were strongly correlated with wolf distributions as recorded in regional-scale field surveys (Carroll et al. 2003a). This is most likely because large carnivore distribution is strongly limited by human influences, for which easily mapped attributes such as road density are good surrogates (Carroll et al. 2001a). Such "pattern-oriented" calibration of complex spatial models may in some cases reduce uncertainty due to poorly known demographic parameters (Wiegand et al. 2004).

The landscape-change scenarios we used estimated potential change in human-associated impact factors (e.g., roads and human population) by proportionately increasing road density and by increasing human population on the basis of current trends derived from a time series of human census data. Census data were available for the period 1990–2000 (USCB 1991). We predicted human population growth from 2000 to 2025 based on growth rates from 1990 to 2000, but adjusted the predicted 2025 population to match state-level predictions based on more complex socioeconomic models. Human population in the area of our analysis is predicted to grow 42%, from 62 million to 88 million, in the period 2000–2025. Because available road data are of varying dates, it is not possible to assemble a regional chronosequence of road distribution and determine county-level rates of increase in roads. Therefore, the road density parameters incorporate an increase of 1% per year (proportional to the current road density at the 1-km$^2$ scale) across the study area. We chose to use a rate (1% per year) that is half of that seen in the most rapidly growing portions of our study region (e.g., western Colorado; Theobald et al. 1996). Similarly, we used a simplified habitat restoration scenario that assessed the effects of removing 1% of the roads on public lands per year.

We treated human impacts within strictly protected areas (parks with no hunting or trapping) as less lethal than in other areas, because of the lack of incidental mortality from hunters in those areas. In the landscape-change analysis, we also treated all protected areas (including those with hunting) differently from unprotected habitat in that we assumed no increase in road density over time. The simulations began with animals inhabiting all suitable habitat. We define "suitable habitat" as the areas with sufficient food resources to support reproduction (i.e., fecundity values above the threshold value for breeding; figure 2). The threshold determining the extent of suitable habitat was based on the historic distribution and abundance of wolves and their prey, which was low in semiarid, nonforested regions of the Great Basin and Sonoran Desert (Young and Goldman 1944). By the end of the 200-year simulations, animals persisted only in "occupiable" habitat, which we define as the areas with greater than 50% potential for long-term occupation despite the presence of human impacts (figure 5). Thus "current" predictions depict, not the number of animals now inhabiting an area, but the capacity of current habitat conditions to support a resident wolf population over the long term (200 years).

The five landscape scenarios examined (table 1) were as follows:

1. Scenario A: Current conditions (i.e, potential long-term viability given current habitat conditions).
2. Scenario B: Future conditions (with human population as of 2025), with increased road development on private lands only.
3. Scenario C: Future conditions (with human population as of 2025), with increased road development on both private and unprotected public lands.
4. Scenario D: Current conditions (with human population as of 2000), with decreased road development on public lands.
5. Scenario E: Future conditions (with human population as of 2025), with decreased road development on public lands and increased road development on private lands.

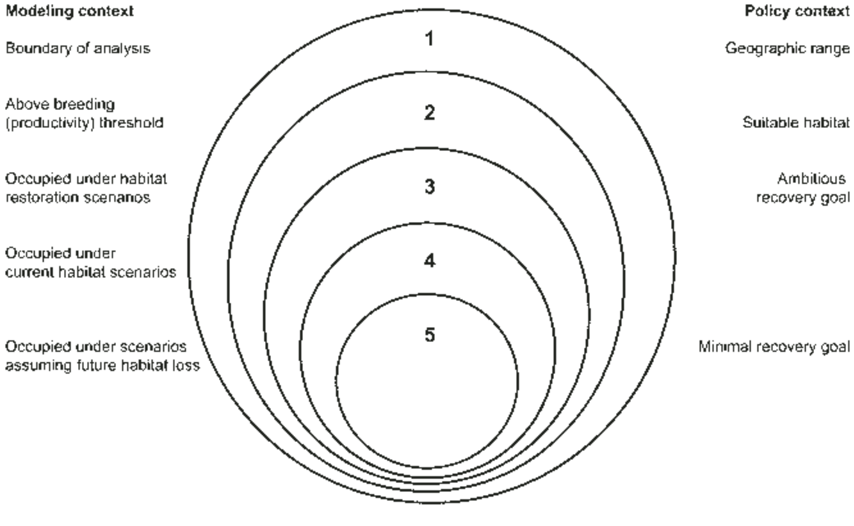


***Figure 5. Conceptual diagram of the relationship between the various geographic levels of range occupancy as defined by the application of spatially explicit population models to evaluate recovery thresholds.***

*Table 1. Levels of human impacts used to parameterize wolf survival in alternate scenarios using the PATCH (program to assist in tracking critical habitat) model.*

| | Parameter | | |
|---|---|---|---|
| Scenario | Human population | Roads on public land | Roads on private land |
| A | Current level (2000) | Current level (2000) | Current level (2000) |
| B | Predicted level (2025) | Current level (2000) | Predicted level (2025) |
| C | Predicted level (2025) | Predicted level (2025) | Predicted level (2025) |
| D | Current level (2000) | Potential level given road closure/removal on public lands[a] | Current level (2000) |
| E | Predicted level (2025) | Potential level given road closure/removal on public lands[a] | Predicted level (2025) |

*Note:* Wolf survival was parameterized to vary inversely to levels of human population and road density.

a. Assumes closure or removal on 1% of public lands per year for 25 years.

Although any restoration of public lands would take place over time, we included scenario D to help separate the contrasting effects of this restoration of public lands and the continued degradation of private lands. Scenario E depicts a high-contrast landscape with restored core areas of public lands embedded in a generally unfavorable environment of heavily roaded private lands.

## Analysis at the scale of a distinct population segment

We next evaluated restoration strategies at the scale of a DPS. The SWDPS encompasses the states of Arizona, New Mexico, southern Utah, southern Colorado, and western Texas and Oklahoma, as well as adjacent areas in northern Mexico that were part of the historic range of the Mexican wolf (*C. lupus baileyi;* figure 2). The Mexican wolf has been the focus of conservation concern due to its high level of genetic distinctiveness and the fact that it is extinct in the wild, with the exception of a small population reintroduced to the Blue Range of Arizona and New Mexico in 1998 (Brown and Parsons 2001). We used the SEPM to evaluate the adequacy of a recovery goal similar to that established for the gray wolf in the northern Rocky Mountains: the creation of three wolf populations of at least 100 individuals each (USFWS 1987). We compared the wolf distribution achieved by this goal with the extent of suitable habitat and ecoregions in the DPS. Ecoregions are commonly used as surrogates for biogeographic gradients (Groves 2003). These analyses, as in the earlier rangewide assessment, were based on the long-term potential of an area to support wolf populations, as predicted by the PATCH simulations. Because management actions to remove wolves often arise from livestock depredation, we added a scenario that incorporated data on levels of cattle grazing into the mortality risk metric for wolves. We also modeled specific reintroduction options to assess transient dynamics such as the probability of extinction and the probability of an area being colonized by dispersers from a specific reintroduction site (Carroll et al. 2003a). We evaluated the sensitivity of results to varying assumptions as to maximum dispersal distance. We performed 1000 simulations of 200 years each for each reintroduction scenario.

We identified eight potential reintroduction sites, four in the United States and four in Mexico, based on the results of initial SEPM simulations. Here we discuss only the results for the US sites: Carson (northern New Mexico), the Grand Canyon (northern Arizona), the Mogollon Rim (central Arizona), and the San Juan Mountains (southwestern Colorado; figure 2). A fifth site in the Blue Range Wolf Recovery Area (BRWRA; Arizona and New Mexico) was also included to provide comparability with current recovery program results. Each of these sites was evaluated in detail by simulating the effects of releasing wolves at that site alone. Each reintroduction site comprised five adjacent potential wolf territories, totaling 2500 km$^2$. We approximated the standard reintroduction protocol (Bangs and Fritts 1996) by introducing five breeding-age females in the first year and setting survival for the first 5 years at close to 100% under the assumption that new animals would be released to replace mortality among the initial releases.

## Results of rangewide analysis

The habitat quality threshold used in the SEPM simulations resulted in 44% of the western United States being judged suitable for breeding (i.e., having sufficient prey to support territorial wolves). The proportion of that "suitable" habitat likely (> 50% probability) to be occupied by wolves was 49% under current conditions (scenario A; figure 6a), 32% under future conditions without new roads on public lands (scenario B; a decrease of 35%), 23% under future conditions with development on public lands (scenario C; figure 6b; a decrease of 53%), 61% under current conditions with road closure or removal on some public lands (scenario D; figure 6c; an increase of 25%), and 45% under future conditions with road removal on public lands (scenario E; a decrease of 8%). The potential size of the wolf population in the western United States was predicted to be close to 7000 under current conditions, with a decrease of 29% under scenario B, a decrease of 44% under scenario C, an increase of 24% under scenario D, and a decrease of 6% under scenario E.

Under current conditions, the states of Montana, Colorado, Wyoming, and Idaho have the largest potential wolf populations, followed by Arizona, Utah, and New Mexico (figure 7). Rather than artificially dividing habitat by state lines, one can also identify distinct population centers from the SEPM results (figure 6a). The largest wolf populations could inhabit the Greater Yellowstone ecosystem (GYE) and central Idaho (figure 6), both areas in which wolf reintroduction has already achieved notable success (Phillips et al. 2004). Population centers of the second rank (smaller size) are found in north-

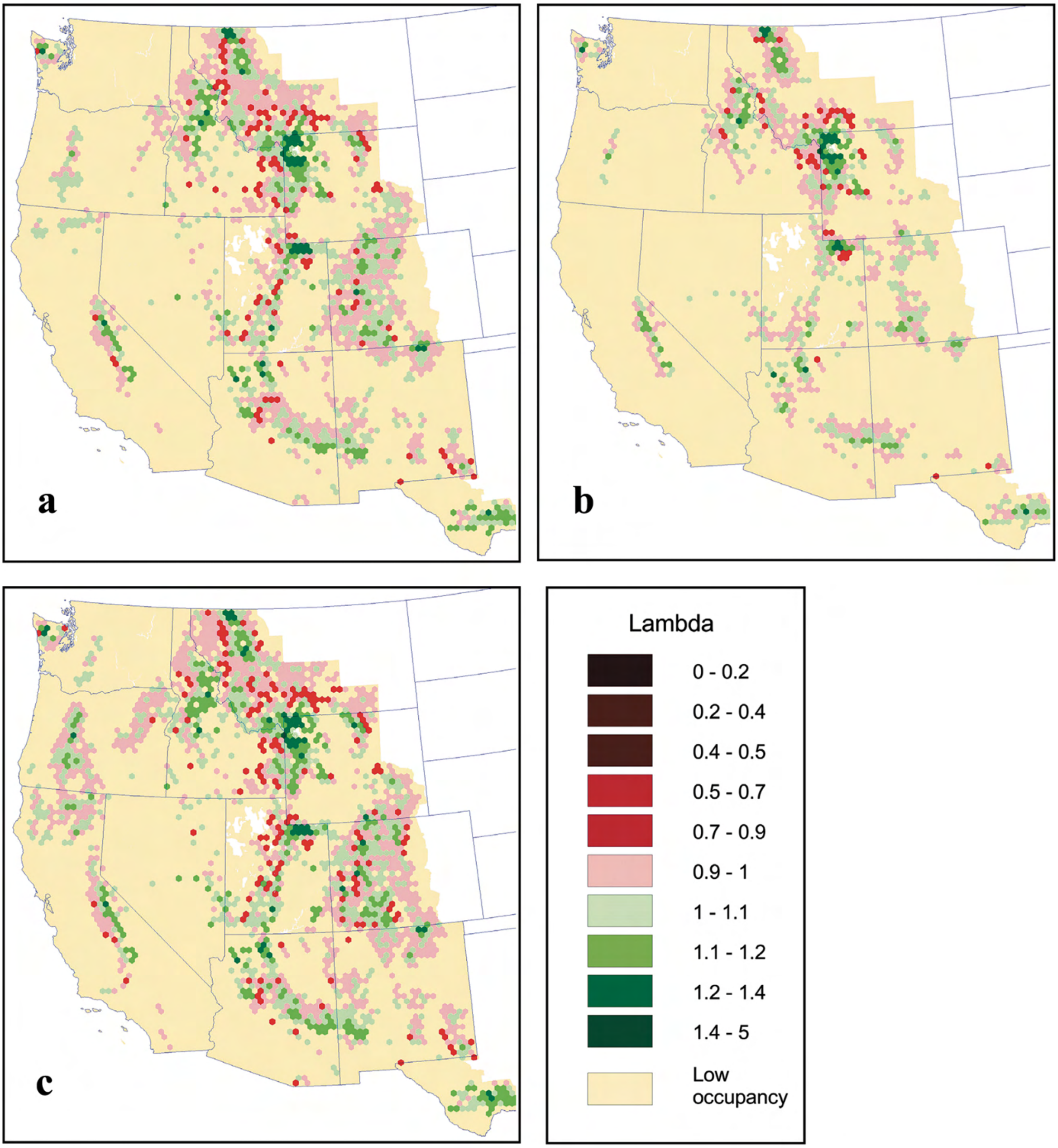


*Figure 6. Potential distribution and demography of wolves as predicted by the PATCH model in the western United States under three landscape scenarios: (a) scenario A, current conditions (i.e., potential long-term viability given current habitat conditions); (b) scenario C, future conditions, with human population as of 2025, with increased road development on both private and unprotected public lands; and (c) scenario D, current conditions, with human population as of 2000, with restoration (reduction in roads) on public lands. Those areas with a predicted probability of occupancy of less than 25% are shown as "low occupancy." Some of these areas are infrequently occupied (i.e., between 25% and 50% of the simulations) but are shown to illustrate potential landscape linkages.*

western Montana and western Colorado, of the third rank in the Blue Range and Utah's high plateaus region, and of the fourth rank in Oregon's Cascades. The populations most vulnerable to landscape change (as reflected by percentage decline from scenario A to scenario C) are those in Colorado and Oregon (figure 6). The New Mexico wolf population also declines dramatically under landscape change (figure 6b) but is supported by its connections to Colorado and Arizona populations. The populations that most benefit from road removal on public lands (scenarios D and E) are those in (a) western Oregon and northern California, (b) Colorado and New Mexico, and (c) western Montana (figures 6c, 7).

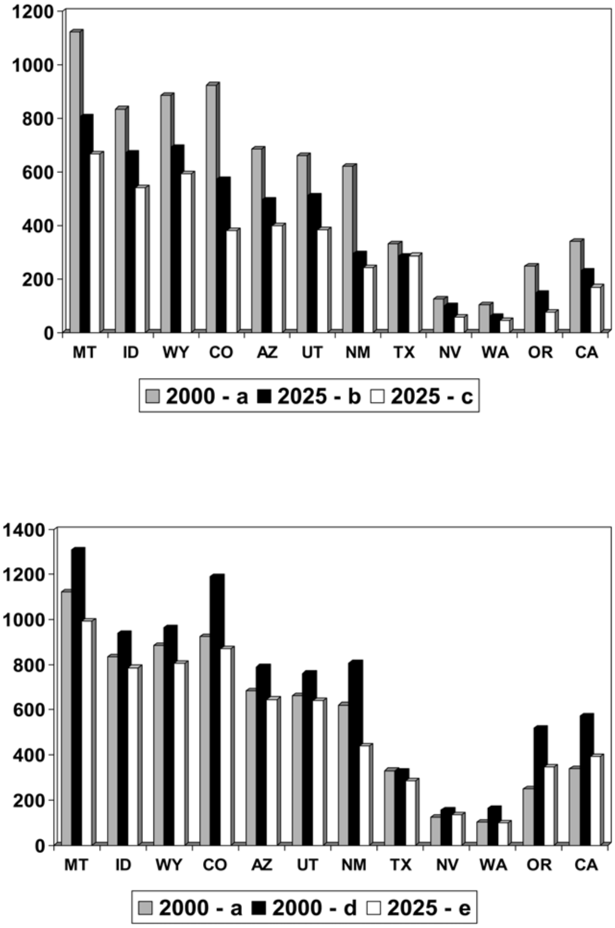


*Figure 7. Potential wolf population size, by state, under one scenario for current conditions (2000a), two habitat degradation scenarios (above; 2025b, 2025c), and two habitat restoration scenarios (below; 2000d, 2025e) of the PATCH model, as shown in table 1.*

## Results of analysis at the scale of a distinct population segment

In addition to the current reintroduced population in the Blue Range, the Grand Canyon reintroduction site showed a high probability of success (low extinction rates) and rapid geographic expansion (table 2). Several other reintroduction sites showed higher, but still relatively low, extinction rates. If we assumed that two additional reintroduction projects, in addition to the current Blue Range program, were conducted in the Grand Canyon and Carson sites, then three populations of 100 wolves each would occupy 5.24% of the SWDPS's suitable habitat, and 7.86% of its occupiable habitat (as defined above and in figure 5). Moreover, 5, or 38.5%, of the SWDPS's 13 ecoregions (Bailey 1995) would contain wolves (as a result of two reintroduction sites lying in more than one ecoregion). The probability that a reintroduction at a single site will fail (extinction probability) under scenario A ranges from near zero (0 of 1000 simulations) for the Blue Range and Grand Canyon sites to near 10% for the Mogollon Rim and San Juan Mountains sites (table 2). Under scenario C, the extinction probability for the Mogollon and San Juan Mountains sites increases to 16%–20%. The probability of extinction for the Blue Range, Grand Canyon, and Carson sites increases slightly but remains low (< 3%; table 2). Occupancy of the larger (10,000-km$^2$) restoration zone surrounding each 2500-km$^2$ reintroduction site gives a sense of the extent of suitable habitat that might be important in the early stages of population establishment. The Blue Range restoration zone has the highest occupancy, at 72.5%, followed closely by the Carson and Grand Canyon zones (table 2). The Grand Canyon zone is more resilient to landscape change than the Blue Range or Carson; thus, it shows the highest wolf population density among US restoration zones under scenario C (table 2). A scenario that incorporated cattle density as an additional mortality risk factor resulted in a similar ranking of restoration zones, except that the San Juan Mountains zone appeared less vulnerable, and thus only the Mogollon zone showed high relative extinction risk.

*Table 2. Comparative summary of results of analysis of potential wolf restoration zones (areas of 10,000 square kilometers in size surrounding initial reintroduction sites) in the southwestern distinct population segment for the gray wolf.*

| | Population | | Occupancy (%), | Lambda, | Extinction risk (%) | | Vulnerability, |
|---|---|---|---|---|---|---|---|
| Reintroduction site | Scenario A | Scenario C | scenario A | scenario A | Scenario A | Scenario C | scenarios A–C/scenario A |
| Blue Range | 92 | 67 | 72.5 | 1.04 | 0 | 1.4 | 27.2 |
| Carson | 84 | 66 | 68.2 | 1.04 | 0.8 | 2.7 | 21.4 |
| Grand Canyon | 91 | 79 | 68.5 | 1.06 | 0 | 0.4 | 13.2 |
| Mogollon Rim | 71 | 45 | 60.3 | 1.00 | 8.6 | 15.8 | 36.6 |
| San Juan Mountains | 79 | 51 | 63.6 | 1.04 | 10.5 | 19.6 | 35.4 |

*Note:* See the text for a definition of PATCH (program to assist in tracking critical habitat) scenarios A through C.

The regional population size achieved at the end of the SEPM reintroduction simulations (year 200) gives an indication of the ability of a particular reintroduction site to enhance the broader regional population, an ability that is due to factors such as ease of dispersal to other suitable habitat. The Grand Canyon site achieves the highest regional population within the US SWDPS. As a result of sink habitat and other barriers to population spread, the largest regional US population achieved from a single reintroduction is only 59.9% of the maximum population size achieved in the equilibrium scenario (scenario A) that began with all habitat occupied. However, a regional population of 89.3% of the maximum population size is eventually achieved by using three reintroduction sites (Blue Range, Grand Canyon, and Carson). At the end of the 200-year simulations, this reintroduced population occupied 54.3% to 57.5% (depending on assumptions about dispersal distance) of the US SWDPS's suitable habitat under scenario A, 26.3% to 26.6% under scenario C, and 100% of the region's ecoregions under both scenarios. Population predictions in peripheral areas with fragmented habitat were most sensitive to alternate assumptions about maximum dispersal distance (e.g., New Mexico, with 13% relative change), with most other areas showing less than 5% relative change. Extinction probability at individual reintroduction sites was not sensitive to dispersal parameterization, with a doubling of maximum dispersal distance from 750 to 1500 km generally producing changes in extinction risk of less than 0.5% (absolute percentage), with a maximum of 1.6% change.

## Using model results to inform policy

Advances in conservation science since the passage of the ESA have provided scientists and managers with a better understanding of the factors, such as interpopulation connectivity, necessary for successful reintroductions and for the long-term viability of reintroduced populations (Breitenmoser et al. 2001). For example, a key element of the Northwest Forest Plan, designed to facilitate recovery of the northern spotted owl (*Strix occidentalis caurina*), was the recognition that the viability of any particular owl subpopulation was dependent on the successful establishment of territories by dispersing individuals, and hence on the size and connectivity of habitat patches across the landscape (Noon and McKelvey 1996). Such a regional-scale perspective on processes such as loss of connectivity has been difficult to achieve with simpler models of habitat suitability, but is now possible with SEPMs that combine spatial data such as satellite imagery with information from the field on how well animals survive and reproduce in different habitats. Because SEPMs such as the PATCH model (Schumaker 1998) can incorporate changes in landscapes over time, they are also more useful than simpler models in forecasting how species' populations might respond to alternative futures in which current trends either continue or instead are slowed or reversed through habitat protection and restoration.

Complex spatial viability models such as SEPMs may be more biologically realistic than simpler tools, but their realism has a cost: SEPM results may suffer from increased sensitivity to a lack of detailed demographic, habitat, and movement data (Kareiva et al. 1996). We found that population predictions in peripheral areas were most sensitive to alternate assumptions about maximum dispersal distance, and that extinction probability at individual reintroduction sites was not sensitive to dispersal parameterization. Nonetheless, it is important to assess which conservation questions can or cannot be answered with relative confidence in the face of model uncertainty. For example, the minimum threshold of food (prey) availability at which wolves can persist is poorly known (Fuller at al. 2003). Therefore, especially in semiarid areas of the West, the exact population estimates from PATCH, which are strongly affected by where this threshold is set, should be viewed with caution (Carroll et al. 2005). However, because we know more about habitat security thresholds for large carnivores, the proportion of this "suitable" habitat that the model predicts as occupied is more informative (figure 6). In general, population viability analysis tools such as SEPMs are more suitable for comparing alternative management options and suggesting qualitative insights about population structure and threat processes than for providing exact population estimates (McCarthy et al. 2003). As knowledge of wolf–habitat relationships is gathered through long-term field studies in areas such as Yellowstone (Smith et al. 2004), SEPM results can be updated to predict future population distribution more accurately.

For species for which demographic data are too sparse to parameterize SEPMs, simpler, static models of habitat suitability may still be useful for guiding recovery planning. Even for these species, SEPMs may be valuable as heuristic tools to generate hypotheses concerning limiting factors and regional population structure. Emergent characteristics of the regional landscape, such as interpopulation connectivity, are likely to be significant for wide-ranging species and poorly addressed by static models. Connectivity in SEPMs depends on both the strength of the source habitat and the permeability of the intervening landscape (Carroll forthcoming), and thus SEPMs more realistically portray factors fragmenting carnivore populations in the western United States. Wolves in threatened habitat patches, unlike those in the boreal "mainland" of their distribution, cannot expect a large rescue effect (Brown and Kodric-Brown 1977) from surrounding regions. Landscape change in the western United States thus can quickly result in a loss of connectivity. In our SEPM results, semi-isolated (e.g., Oregon) and fragmented (e.g., Colorado) wolf populations show greater threats than they would in a static model of habitat suitability (figure 6). Counterintuitively, landscape change has a greater negative impact on wolves (a 35% to 53% decrease in occupied habitat) than on grizzly bears (a 24% to 40% decrease) in the SEPM simulations. Although currently wolves can occupy a broader spectrum of the landscape than grizzly bears, more of this matrix is threatened by landscape change than are the core areas used by grizzlies. The loss of such high proportions of potential wolf habitat as a result of landscape change in the western United States over the next quarter-century suggests that absent the protection of important habitat, many western landscapes will become unsuitable for the species, and possibly for other large carnivores as well.

The SEPM results can help planners evaluate the extent of currently "occupiable" and potentially restorable habitat across a species' range. They reveal a potential wolf population structure that combines two highly resilient core areas (the GYE and central Idaho) and several smaller cores, with many peripheral areas that may be dependent on dispersal from core areas for their initial colonization, their continued demographic rescue, or both (Brown and Kodric-Brown 1977). An optimal strategy for establishing representative wolf populations might therefore be based on initial reintroductions to a geographically well-distributed set of core areas (e.g., the current reintroduction areas in the GYE, Idaho, and the Blue Range [figure 2], plus the Grand Canyon and western Colorado). This would seek to maximize the area of peripheral habitat affected by dispersal from the core reintroductions. Secondary targets for reintroductions, to achieve representation and buttress redundancy, would be regions that lack large core areas, but might be unlikely to be rapidly recolonized because of their distance from initial reintroduction sites (e.g., the Oregon Cascades). The high relative vulnerability to future threats and high potential benefit from restoration actions would justify more aggressive habitat protection in Colorado and Oregon, where protected public lands are fragmented and embedded in a rapidly developing matrix of private lands.

Because wolf habitat, as depicted in the SEPM results, is not distributed uniformly across the western United States, it makes sense to break the region into several subareas, each of which might support tightly interacting populations and be linked loosely with the other subareas by infrequent dispersal. Such areas include (a) the northern Rockies, (b) Colorado, (c) the Southwest (Arizona, New Mexico, and portions of Utah), and (d) the Pacific states (figures 2, 6a). These regions could serve as the basis for DPSs or multistate management coordination areas. Ecological barriers, such as expanses of unsuitable habitat, are more appropriate for delineating DPSs than geographic divisions, such as state boundaries (*National Wildlife Federation v. Norton,* 03-CV-340 [2005]). However, management decisions such as delisting proposals that affect a particular DPS should also take into account the broader rangewide context for recovery. For example, even infrequent dispersal between DPSs may be important for initial recolonization and subsequent genetic interchange. The SEPM results suggest that important areas for maintaining population connectivity, both within and among DPSs, include (a) linkages between the three northern Rockies populations (central Idaho, the GYE, and northwestern Montana), (b) linkages along an arc of mountainous habitat extending southward from the GYE to the Blue Range (Arizona and New Mexico) and southward into Mexico, and (c) a linkage between Colorado and the Uintas of northern Utah (figure 6a). Connectivity between central Idaho and the Oregon Cascades is more tenuous but is strongly enhanced by road removal on public lands (figure 6c). Our results suggest that the potential still exists to recreate a metapopulation of wolves stretching from Canada to Mexico. Similar habitat analyses for adjacent regions of Mexico will allow binational coordination of recovery efforts (Carroll et al. 2005). Expanding analyses beyond the United States is difficult because of inconsistencies in habitat data. However, planners should be aware that truncating analysis at the US border may affect results for areas dependent on dispersal from source habitat outside the United States. For example, inclusion of Mexico and western Canada in the wolf analysis increases predicted occupancy in southern Arizona and northeastern Washington.

SEPM results such as those reported here are also relevant to planners at the DPS scale, in that they make it possible to consider recovery throughout the DPS, rather than constrained within artificially defined recovery areas. For example, current regulations require that wolves dispersing outside of the 17,546 km$^2$ BRWRA (figure 2) be recaptured, a policy that has severely impeded the success of the recovery program (Oakleaf et al. 2004). The inadequacy of the BRWRA alone to support a self-sustaining population, and the likelihood of high dispersal rates, could have been anticipated on the basis of SEPM results showing fragmented source habitat within the BRWRA but sufficient additional habitat northwest of the area (figure 6a). Our results suggest that at least two more reintroduction sites will be necessary to achieve recovery within the SWDPS, because of the more fragmented nature of regional wolf habitat there when compared with the northern Rockies. This fragmentation is due to the natural isolation of forest habitat on mountain ranges in this semiarid region, as well as other anthropogenic barriers to dispersal. Although all four candidate reintroduction sites have low enough extinction risk that they can be included in further planning for wolf recovery, the vulnerability to landscape change of the Mogollon Rim and San Juan Mountains sites, and the relative isolation of the Carson site from the bulk of wolf habitat in the region, may make it advisable to pair any of these three sites with a second site to ensure the establishment of a well-distributed, viable population.

Although it achieves viability (resiliency and redundancy) goals, the potential recovery goal of three populations of 100 wolves each achieves a relatively low level of representation in the short term. However, the eventual wolf distribution achieved from a three-site reintroduction approach appears adequate, at least under the assumption that current habitat conditions do not deteriorate. The central issue then becomes the role of federal versus state management of wildlife during the recovery process, and the appropriate stage for transfer of regulatory authority from the federal to the state level, given the ESA mandate to ensure that a recovered species occupies a significant portion of range. A state plan sufficient to ensure this mandate would most likely be more precautionary than those approved by the USFWS to date.

In their efforts to restore imperiled species and ecosystems, planners must be both ambitious and realistic. Inadequacy and lack of rigor in current ESA recovery plan goals (Gerber and Hatch 2002) are due in part to a shifting-baseline effect (Jackson et al. 2001) that limits the "realistic" range of goals from considering the historic extent of suitable habitat. As Leonard and colleagues (2005) concluded on the basis of genetic analysis, "restoration goals for grey wolves in the western contiguous US include far less area and target vastly lower population sizes than existed historically." The population estimates from the SEPM scenarios reported here are far more ambitious than current recovery goals but at least an order of magnitude lower than historic population estimates (Leonard et al. 2005), and should thus fall within the range of options considered in recovery planning.

To clarify the debate over wolf recovery goals, suitable habitat might be divided into three categories: (1) areas that can be occupied by wolves despite current human impacts and anticipated habitat loss (figure 5, zone 5), (2) areas that are unlikely to support wolves even with substantial habitat restoration or policy change (figure 5, zone 2), and (3) intermediate areas where long-term wolf recovery might require proactive conservation measures (e.g., road removal and restriction of lethal control in response to livestock depredation) (figure 5, zones 3 and 4). While recovery goals must incorporate the ESA mandate concerning significant portion of range, beyond this threshold a normative decision must be made as to what level of biologically suitable habitat should be made occupiable by mitigating human impacts. Our results suggest that more ambitious recovery goals (up to about two-thirds of suitable habitat occupied) may be feasible. Closure or removal of roads on public lands greatly enhances wolf recovery in regions such as Colorado and Oregon that have high ecosystem productivity but currently lack large core areas. Although wolves could inhabit portions of these states without habitat restoration, their distribution might be too restricted to fulfill ESA mandates.

Ecological effectiveness is the most ambitious of the five guiding principles for recovery, as it speaks to abundance as well as distribution (Soulé et al. 2005). Unlike the concept of "significant portion of range," ecological effectiveness is only implicitly mandated by the ESA's charge to conserve the ecosystems on which endangered species depend. Although the role of wolves as keystone species presents a particularly strong argument for restoration of ecologically effective populations, conservation science has increasingly highlighted the high proportion of threatened species that may strongly influence ecosystem function (Soulé et al. 2005), and the high value to humankind of the services arising from functioning ecosystems (Daily 1997). The normative debate over recovery goals for wolves, although tied to the specific legal context of the ESA, thus illuminates a larger debate over the necessity for "rewilding," a reversal of the trend toward increasing human domination of Earth's natural ecosystems (Vitousek et al. 1997, Soulé and Noss 1998).

## Acknowledgments

This study was funded by the Wilburforce Foundation and the Turner Endangered Species Fund. The authors' understanding of the implications of Endangered Species Act language regarding "significant portion of range" benefited greatly from discussions with John Vucetich. Doug Smith, Tracy Scheffler, and three anonymous reviewers also provided helpful comments. The information in this document has been funded in part by the US Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory's Western Ecology Division and approved for publication. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

## References cited

Bailey R. 1995. Descriptions of the Ecoregions of the United States. 2nd ed. Washington (DC): USDA Forest Service. Miscellaneous Publication 1391.

Ballard WB, Whitman JS, Gardner CL. 1987. Ecology of an exploited wolf population in south-central Alaska. Wildlife Monographs 98: 1–54.

Bangs EE, Fritts SH. 1996. Reintroducing the gray wolf to central Idaho and Yellowstone National Park. Wildlife Society Bulletin 24: 402–413.

Breitenmoser U, Breitenmoser-Würsten C, Carbyn LN, Funk SM. 2001. Assessment of carnivore reintroductions. Pages 241–281 in Gittleman JL, Funk SM, MacDonald DW, Wayne RK, eds. Carnivore Conservation. Cambridge (United Kingdom): Cambridge University Press.

Brown JH, Kodric-Brown A. 1977. Turnover rates in insular biogeography: Effect of immigration on extinction. Ecology 58: 445–449.

Brown WM, Parsons DR. 2001. Restoring the Mexican gray wolf to the desert Southwest. Pages 169–186 in Maehr D, Noss RF, Larkin J, eds. Large Mammal Restoration: Ecological and Sociological Challenges in the 21st Century. Washington (DC): Island Press.

Carroll C. Linking connectivity to viability: Insights from spatially-explicit population models of large carnivores. In Crooks K, Sanjayan MA, eds. Connectivity Conservation. Cambridge (United Kingdom): Cambridge University Press. Forthcoming.

Carroll C, Noss RF, Paquet PC. 2001a. Carnivores as focal species for conservation planning in the Rocky Mountain region. Ecological Applications 11: 961–980.

Carroll C, Noss RF, Schumaker NH, Paquet PC. 2001b. Is the return of the wolf, wolverine, and grizzly bear to Oregon and California biologically feasible? Pages 25–46 in Maehr D, Noss RF, Larkin J, eds. Large Mammal Restoration: Ecological and Sociological Challenges in the 21st Century. Washington (DC): Island Press.

Carroll C, Phillips MK, Schumaker NH, Smith DW. 2003a. Impacts of landscape change on wolf restoration success: Planning a reintroduction program using dynamic spatial models. Conservation Biology 17: 536–548.

Carroll C, Noss RF, Paquet PC, Schumaker NH. 2003b. Use of population viability analysis and reserve selection algorithms in regional conservation plans. Ecological Applications 13: 1773–1789.

Carroll C, Phillips MK, Lopez Gonzalez CA. 2005. Spatial Analysis of Restoration Potential and Population Viability of the Wolf (*Canis lupus*) in the Southwestern United States and Northern Mexico. Orleans (CA): Klamath Center for Conservation Research. (2 December 2005; *www.klamathconservation.org*)

Caswell H. 2001. Matrix Population Models: Construction, Analysis, and Interpretation. Boston: Sinauer.

Cihlar J, St.-Laurent L, Dyer J. 1991. Relation between the normalized difference vegetation index and ecological variables. Remote Sensing of the Environment 35: 279–298.

Crist EP, Cicone RC. 1984. Application of the tasseled cap concept to simulated thematic mapper data. Photogrammetric Engineering and Remote Sensing 50: 343–352.

Daily G, ed. 1997. Nature's Services: Societal Dependence on Natural Ecosystems. Washington (DC): Island Press.

Dobson AP, Rodriguez JP, Roberts WM, Wilcove DS. 1997. Geographic distribution of endangered species in the United States. Science 275: 550–553.

Dunning JB, Stewart DJ, Danielson BJ, Noon BR, Root TL, Lamberson RH, Stevens EE. 1995. Spatially explicit population models: Current forms and future uses. Ecological Applications 5: 3–11.

Fuller TK. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs 105: 1–41.

Fuller TK, Mech LD, Cochrane JF. 2003. Wolf population dynamics. Pages 161–191 in Mech LD, Boitani L, eds. Wolves: Behavior, Ecology, and Conservation. Chicago: University of Chicago Press.

Gerber LR, Hatch LT. 2002. Are we recovering? An evaluation of recovery criteria under the US Endangered Species Act. Ecological Applications 12: 668–673.

Groves C. 2003. Drafting a Conservation Blueprint: A Practitioner's Guide to Planning for Biodiversity. Washington (DC): Island Press.

Hayes RD, Harestad AS. 2000. Demography of a recovering wolf population in the Yukon. Canadian Journal of Zoology 78: 36–48.

Jackson JBC, et al. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293: 629–63.

Kareiva P, Skelly D, Ruckelshaus M. 1996. Reevaluating the use of models to predict the consequences of habitat loss and fragmentation. Pages 156–166 in Pickett STA, Ostfeld RS, Schachak M, Likens GE, eds. The Ecological Basis of Conservation: Heterogeneity, Ecosystems, and Biodiversity. New York: Chapman and Hall.

Leonard JA, Vilà C, Wayne RK. 2005. Legacy lost: Genetic variability and population size of extirpated US grey wolves (*Canis lupus*). Molecular Ecology 14: 9–17.

Mace RD, Waller JS, Manley TL, Ake K, Wittinger WT. 1999. Landscape evaluation of grizzly bear habitat in western Montana. Conservation Biology 13: 367–377.

McCarthy MA, Andelman SJ, Possingham HP. 2003. Reliability of relative predictions in population viability analysis. Conservation Biology 17: 982–989.

Merrill EH, Bramble-Brodahl MK, Marrs RW, Boyce MS. 1993. Estimation of green herbaceous phytomass from Landsat MSS data in Yellowstone National Park. Journal of Range Management 46: 151–157.

Merrill T, Mattson DJ, Wright RG, Quigley HB. 1999. Defining landscapes suitable for restoration of grizzly bears (*Ursus arctos*) in Idaho. Biological Conservation 87: 231–248.

Noon BR, McKelvey KS. 1996. Management of the spotted owl: A case history in conservation biology. Annual Review of Ecology and Systematics 27: 135–162.

Oakleaf JK, Stark D, Overy P, Smith N. 2004. Mexican wolf recovery: Technical component of the five-year program review and assessment. Albuquerque (NM): US Fish and Wildlife Service.

Paquet PC, Wierzchowski J, Callaghan C. 1996. Effects of human activity on gray wolves in the Bow River Valley, Banff National Park, Alberta. Chapter 7 in Green J, Pacas C, Bayley S, Cornwell L, eds. A Cumulative Effects Assessment and Futures Outlook for the Banff Bow Valley. Ottawa (Canada): Department of Canadian Heritage.

Phillips MK, Bangs EE, Mech LD, Kelly BT, Fazio BB. 2004. Extermination and recovery of the red wolf and grey wolf in the contiguous United States. Pages 297–309 in MacDonald DW, Sillero-Zubiri C, eds. Biology and Conservation of Wild Canids. New York: Oxford University Press.

Ripple WJ, Beschta RL. 2004. Wolves and the ecology of fear: Can predation risk structure ecosystems? BioScience 54: 755–766.

Schumaker NH. 1998. A User's Guide to the PATCH Model. Corvallis (OR): US Environmental Protection Agency. EPA/600/R-98/135.

Shaffer ML, Stein B. 2000. Safeguarding our precious heritage. Pages 301–322 in Stein BA, Kutner LS, Adams JS, eds. Precious Heritage: The Status of Biodiversity in the United States. Oxford (United Kingdom): Oxford University Press.

Smith DW, Peterson RO, Houston DB. 2003. Yellowstone after wolves. BioScience 53: 330–340.

Smith DW, Stahler DR, Guernsey DS. 2004. Yellowstone Wolf Project: Annual Report, 2003. Yellowstone National Park (WY): National Park Service. YCR-NR-2004-04.

Soulé M, Noss R. 1998. Rewilding and biodiversity: Complementary goals for continental conservation. Wild Earth 8: 18–28.

Soulé ME, Terborgh J. 1999. Continental Conservation: Scientific Foundations of Regional Reserve Networks. Washington (DC): Island Press.

Soulé ME, Estes JA, Miller B, Honnold DL. 2005. Strongly interacting species: Conservation policy, management, and ethics. BioScience 55: 168–176.

Suckling KF, Taylor M. 2005. Critical habitat and recovery. In Goble DD, Scott JM, Davis FW, eds. The Endangered Species Act at Thirty: Renewing the Conservation Commitment. Washington (DC): Island Press.

Theobald DM, Gosnell H, Riebsame WE. 1996. Land use and landscape change in the Colorado mountains, II: A case study of the East River Valley, Colorado. Mountain Research and Development 16: 407–418.

Thiel RP. 1985. Relationship between road densities and wolf habitat suitability in Wisconsin. American Midland Naturalist 113: 404.

[USCB] US Census Bureau. 1991. Census of the United States: 1990. Washington (DC): USCB.

[USFWS] US Fish and Wildlife Service. 1987. Northern Rocky Mountain Wolf Recovery Plan. Denver (CO): USFWS.

———. 1994. The Reintroduction of Gray Wolves to Yellowstone National Park and Central Idaho: Final Environmental Impact Statement. Denver (CO): USFWS.

———. 1996. Reintroduction of the Mexican Wolf within Its Historic Range in the Southwestern United States: Final Environmental Impact Statement. Albuquerque (NM): USFWS.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM. 1997. Human domination of Earth's ecosystems. Science 277: 494–499.

Wharton SW, Myers MF. 1997. MTPE EOS Data Products Handbook, vol. 1. Greenbelt (MD): NASA Goddard Space Flight Center. Publication 902.

White JD, Running SW, Nemani R, Keane RE, Ryan KC. 1997. Measurement and remote sensing of LAI in Rocky Mountain montane ecosystems. Canadian Journal of Forest Research 27: 1714–1727.

Wiegand T, Revilla E, Knauer F. 2004. Dealing with uncertainty in spatially explicit population models. Biodiversity and Conservation 13: 53–78.

Young SP, Goldman EA. 1944. Wolves of North America, pt. 2: Classification of Wolves. Washington (DC): American Wildlife Institute.

# Wolf Delisting Challenges Demonstrate Need for an Improved Framework for Conserving Intraspecific Variation under the Endangered Species Act.

CARLOS CARROLL, DANIEL J. ROHLF, BRIDGETT M. VONHOLDT, ADRIAN TREVES, AND SARAH A. HENDRICKS

*Recent advances in genomics have increased our understanding of geographic patterns of intraspecific variation and the importance of this variation in enhancing species' potential to adapt to novel threats. However, as part of an effort to limit the scope of the Endangered Species Act (ESA), the US government has proposed the removal of the gray wolf from the list of protected species on the basis of a claim that the statute permits a species to be declared recovered given the existence of a single presently secure population. We rebut this interpretation and propose a framework for the conservation of adaptive potential that builds on current agency practice in delineating subspecific recovery units and reconciles the definition of significance in the statute's "distinct population segment" and "significant portion of range" clauses. Such a coordinated policy would enhance the ESA's effectiveness in stemming loss of biodiversity in the face of climate change and other factors altering Earth's ecosystems.*

*Keywords: adaptive potential, Canis lupus, conservation genomics, distinct population segment, recovery planning*

**Although the US Endangered Species Act (ESA; 16** U.S.C. §§ 1531–44) is among the world's most influential biodiversity protection statutes, key aspects of how the law should be implemented remain contested. A central issue involves the appropriate level of ambition for recovery of formerly widely distributed species such as the North American gray wolf (*Canis lupus*; figure 1; Enzler and Bruskotter 2009, Carroll et al. 2010). If the ESA aims only to prevent the complete extinction of a species, is the existence of a single secure population sufficient to declare a species recovered? Alternately, does a species need to achieve recovery in all or a majority of its historical range before it can be removed (delisted) from the list of protected species? If the purpose of the statute lies somewhere between these bounds, how can appropriate recovery goals be established? These questions resonate beyond the US context because they address how best to conserve variation below the level of the taxonomic groupings (species and subspecies) typically acknowledged in conservation statutes of other nations (Laikre et al. 2016, vonHoldt et al. 2018, Hendricks et al. 2019a).

Although Congress and federal agencies have long recognized the importance of conserving intraspecific variation, recent agency actions, exemplified by a 2019 proposal to delist the gray wolf (84 FR 9648), suggest a shift away from biologically informed policy (Lambert 2019). In this Forum, we use the 2019 delisting proposal to demonstrate that recent inconsistent implementation of the ESA's mandate for the conservation of intraspecific variation undermines the conservation outcomes intended by Congress. We propose a more consistent and transparent framework that coordinates the two elements of the ESA that authorize the conservation of intraspecific variation: the distinct population segment (DPS; see supplemental table S1 for a definition of terms) and significant portion of range (SPR) clauses, while building on current agency guidance for delineating subspecific recovery units. Rather than representing a detailed policy proposal or a comprehensive review of case law in the present article, we synthesize

*BioScience* 71: 73–84.

doi:10.1093/biosci/biaa125
Advance Access publication 29 October 2020

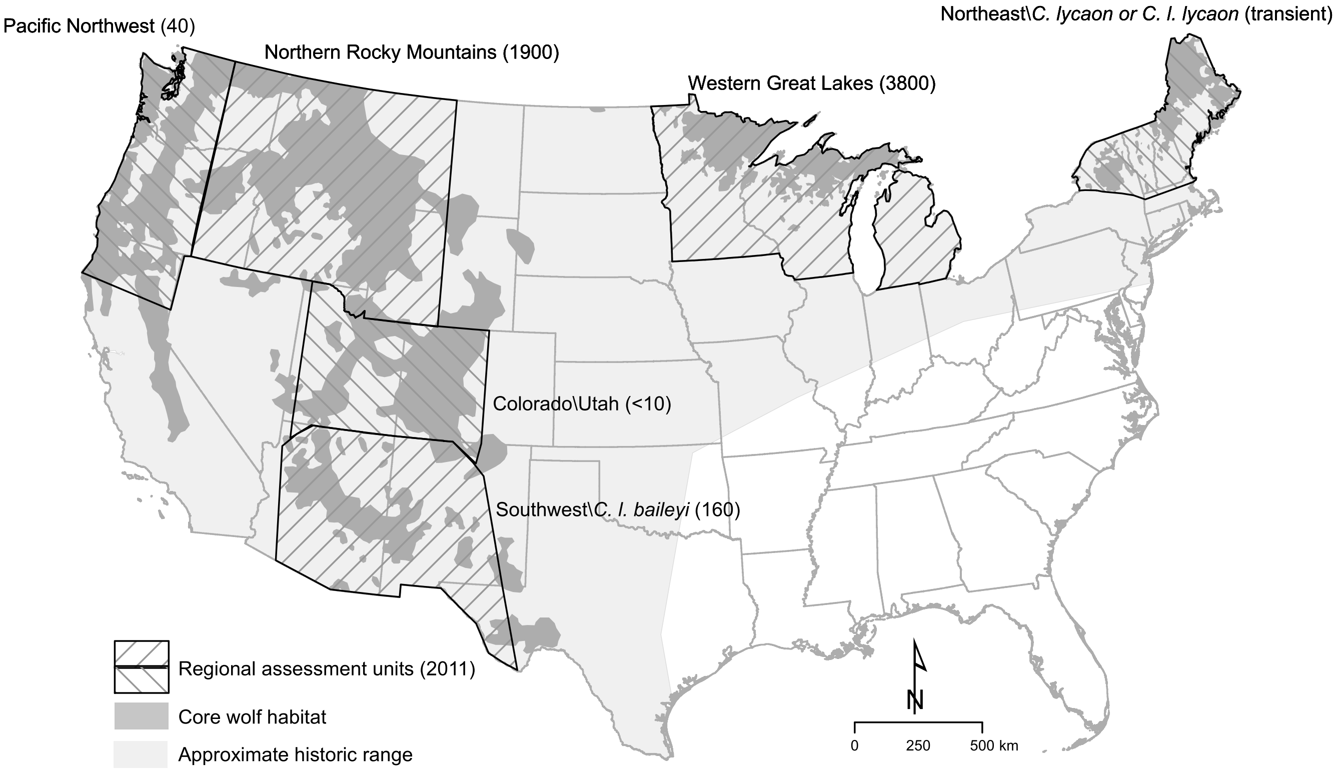


***Figure 1. Map of regional assessment units used in the 2008–2011 national wolf strategy process (Runge 2011). Current estimates of population numbers in each assessment unit (USFWS 2019) are given in parentheses and are approximate particularly in two units (Western Great Lakes and Northern Rocky Mountains) with recent changes in census methods. Wolf packs in Washington and Oregon are divided between the Pacific Northwest and Northern Rocky Mountain assessment units, with most falling within the latter unit. Distribution of potential core habitat is as delineated by CBD and HSUS (2018) based on published regional habitat models. Many areas of potential core habitat currently lack wolves, and many areas of historical range outside of core habitat could be inhabited by wolves given sufficiently low anthropogenic mortality.***

information from the fields of conservation genetics, wildlife ecology, and endangered species law to advance the discussion and resolution of conceptual issues regarding the conservation of intraspecific variation under the ESA.

## Why is conservation of intraspecific variation important?

Why would a statute designed to protect the nation's biodiversity, such as the ESA, mandate the conservation of multiple populations of widely distributed species rather than a museum piece approach (Vucetich and Nelson 2014) based on preserving a single narrowly distributed population? The ESA's preamble mentions an array of "esthetic, ecological, educational, historical, recreational, and scientific" benefits provided by maintaining a species presence throughout substantial proportions of its range (Carroll et al. 2010, Nelson et al. 2016). Science also increasingly supports the conclusion that preserving multiple populations furthers conservation efforts by enhancing adaptive potential, the genetic variability that allows species to adapt in the face of climate change and other factors altering Earth's ecosystems (Funk et al. 2019). The conservation of multiple genetically distinct ecotypes (i.e., populations adapted to a particular habitat) in a metapopulation structure across a species's range enhances metapopulation connectivity and allows gene flow and the exchange of adaptive variants among populations, enhancing the adaptive potential of the metapopulation as a whole (Crandall et al. 2000, Hoffmann and Sgro 2011, Hamilton and Miller 2015, vonHoldt et al. 2018, Hendricks et al. 2019a).

Quantitative models have been developed to predict how gene flow among populations enhances adaptive potential and reduces extinction risk in species experiencing environmental shifts because of climate change or other factors (Funk et al. 2019, Razgour et al. 2019). In addition, the conservation of adaptive potential has long been recognized as forming part of "an ethical imperative to provide for the continuation of evolutionary processes" (Soulé 1985), with value extending beyond its immediate role in lowering extinction risk over the relatively short time horizons typically considered in population viability analyses (Wolf et al. 2015).

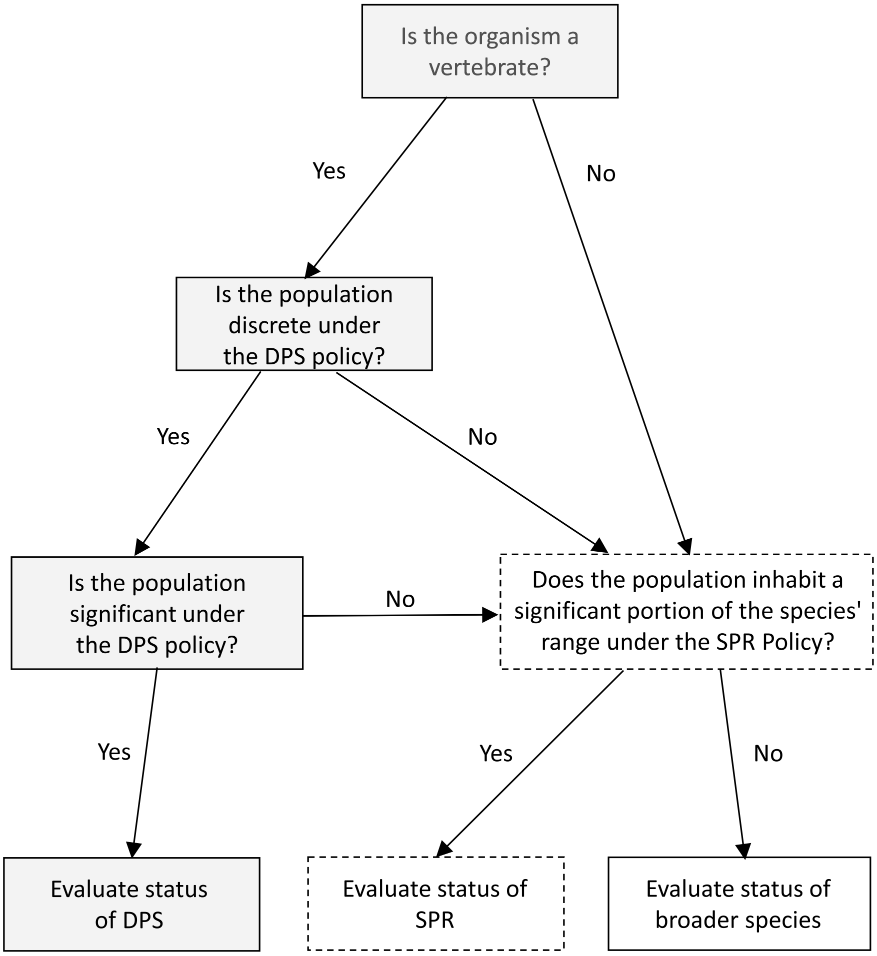


*Figure 2. Flow diagram illustrating the proposed framework for designation of intraspecific conservation units under the US Endangered Species Act (ESA). Gray filled boxes represent decision steps currently taken when the Services evaluate whether a population constitutes a distinct population segment (DPS), a designation that the ESA limits to vertebrate species. Dashed boxes represented decision steps taken under the proposed "significant portion of range" (SPR) policy. Definitions of significance under the DPS and proposed SPR policy would be substantially similar but may diverge in emphasis as was described in the text.*

## Conservation of intraspecific variation via the ESA's distinct population segment clause

Although the ESA predates the modern genetics research described above, lawmakers indicated their support for conserving intraspecific variation via the act's DPS and SPR clauses. Initially, almost all ESA listings were of entire species and subspecies, although the act did include language allowing listings of "any other group… in common spatial arrangement that interbreed when mature." In 1978, Congress clarified the law to allow listing of "distinct population segments" (DPS) of vertebrate species (16 U.S.C. §1532(3.16)), although lawmakers directed that DPS designation be used "sparingly."

In 1996, the Services (the US Fish and Wildlife Service [FWS] and its counterpart, the National Marine Fisheries Service [NMFS]) finalized a policy that evaluates a population's "discreteness" and "significance" to its taxon in order to decide whether the population qualifies for protection as a DPS (61 FR 4722; figure 2). Similar frameworks based on discreteness and significance were subsequently adopted outside of the United States, such as in Canada's policy for identifying designatable units within species and subspecies (COSEWIC 2018).

The DPS policy's factors for determining what constitutes a significant population include evidence that the population persists in a unique ecological setting, that the loss of the population would result in a significant gap in the range of the taxon, that the population represents the only surviving natural occurrence of a taxon that may exist as an introduced population outside its historical range, and that the population's genetic characteristics differ markedly from those of other populations (Waples et al. 2018). Discreteness requires either marked separation from other populations of the same taxon as a consequence of physical, physiological, ecological, or behavioral factors or delimitation by international governmental boundaries with important differences in management or conservation status (61 FR 4722). Recognizing that population connectivity rates fall along a continuum, the DPS policy's standard for a discrete population requires "marked" rather than complete separation (61 FR 4722). For example, NMFS identifies distinct populations for salmonid species even though a small proportion of returning fish will reproduce within adjacent regions rather than the natal population (Waples 2006).

## Recolonizing species and hybridizing lineages pose challenges for delineating intraspecific conservation units

A large proportion of litigation concerning conservation of intraspecific variation under the ESA (table 1) relates to gray wolf delisting. This is due not only to the fraught politics surrounding this species but also to aspects of its distribution and systematics. Defining intraspecific conservation units for species such as the wolf that have been extirpated from the majority of their historical range is more complex than for species that are declining but remain extant across

Table 1. A timeline of gray wolf listing and delisting related actions.

| Year | Action | Conservation unit | Reference |
|---|---|---|---|
| 1967 | *C. l. lycaon* listed. | Subspecies | 32 FR 4001, 11 March 1967 |
| 1973 | *C. l. irremotus* listed. | Subspecies | 38 FR 14678, 4 June 1973 |
| 1974 | *C. l. lycaon* listed. | Subspecies | 39 FR 1171, 4 January 1974 |
| 1976 | *C. l. baileyi* listed as Endangered. | Subspecies | 41 FR 17736, 28 April 1976 |
| 1976 | *C. l. monstrabilis* listed as Endangered. | Subspecies | 41 FR 24064, 14 June 1976 |
| 1978 | *C. lupus* in lower 48 United States (except Minnesota) and Mexico reclassified as Endangered. | Species | 43 FR 9607, 9 March 1978 |
| 1978 | *C. lupus* in Minnesota reclassified as Threatened. | State population | 43 FR 9607, 9 March 1978 |
| 2003 | *C. lupus* Eastern, Western, and Southwestern DPS designated and reclassified. | DPS | 68 FR 15804, 1 April 2003 |
| 2005 | *C. lupus* DPS Rule vacated. | DPS | Defenders of Wildlife v. Norton, 354 F. Supp. 2d 1156 (D. Or. 2005); National Wildlife Federation v. Norton, 386 F. Supp. 2d 553 (D. Vt. 2005) |
| 2007 | *C. lupus* WGL DPS designated and delisted. | DPS | 72 FR 6052, 8 February 2007 |
| 2008 | *C. lupus* WGL delisting rule vacated. | DPS | Humane Society of the United States v. Kempthorne, 579 F. Supp. 2d 7 (D.D.C. 2008) |
| 2008 | *C. lupus* NRM DPS designated and delisted. | DPS | 73 FR 10514, 27 February 2008 |
| 2008 | *C. lupus* NRM Rule vacated. | DPS | Defenders of Wildlife v. Hall, 565 F. Supp. 2d 1160 (D. Mont. 2008) |
| 2008 | Protections for *C. lupus* WGL and NRM DPS reinstated. | DPS | 73 FR 75356, 11 December 2008 |
| 2009 | *C. lupus* WGL DPS designated and delisted. | DPS | 74 FR 15070, 2 April 2009 |
| 2009 | *C. lupus* WGL DPS delisting rule vacated. | DPS | Humane Society of the United States v. Salazar, 1:09–CV–1092–PLF (D.D.C. 2009) |
| 2009 | *C. lupus* NRM DPS (except Wyoming) designated and delisted. | DPS | 74 FR 15123, 2 April 2009. |
| 2009 | Protections for *C. lupus* WGL DPS reinstated. | DPS | 74 FR 47483, 16 September 2009 |
| 2010 | *C. lupus* NRM DPS delisting rule vacated. | DPS | Defenders of Wildlife v. Salazar, 729 F. Supp. 2d 1207 (D. Mont. 2010) |
| 2010 | Protections for *C. lupus* NRM DPS reinstated. | DPS | 75 FR 65574, 26 October 2010 |
| 2011 | *C. lupus* NRM DPS delisted by Congress. | DPS | Public Law 112–10 and 76 FR 25590, May 5, 2011 |
| 2011 | *C. lupus* WGL DPS designated and delisted. | DPS | 76 FR 81666, 28 December 2011 |
| 2012 | *C. lupus* in Wyoming delisted. | State population | 77 FR 55530, 10 September 2012 |
| 2014 | *C. lupus* WGL DPS delisting rule vacated. | DPS | Humane Society of the US v. Jewell, 76 F. Supp. 3d 69, 110 (D.D.C. 2014) |
| 2014–2017 | *C. lupus* Wyoming delisting rule vacated but reinstated on appeal. | State population | Defenders of Wildlife v. Jewell, 68 F. Supp. 3d 193 (D.D.C. 2014), Defenders of Wildlife v. Zinke, 849 F.3d 1077 (D.C. Cir. 2017) |
| 2013 | Delisting of *C. lupus* in lower 48 United States (except NRM and WGL DPS) and Mexico proposed. | Species | 78 FR 35664, 13 June 2013 |
| 2015 | *C. l. baileyi* listed as endangered. | Subspecies | 80 FR 2488 and 80 FR 2512, 16 January 2015 |
| 2015 | Protections for *C. lupus* WGL DPS and C. lupus in Wyoming reinstated. | State population | 80 FR 9218, 20 February 2015 |
| 2017 | Delisting of *C. lupus* in Wyoming reinstated. | State population | 82 FR 20284, 1 May 2017 |
| 2019 | *C. lupu*s delisting in lower 48 United States (except NRM DPS and C. l. baileyi) and Mexico proposed. | Species | 84 FR 9648, 15 March 2019 |

*Source:* Adapted from 2019 proposed delisting rule (84 FR 9648). *Abbreviations:* DPS, Distinct Population Segment; NRM, Northern Rocky Mountains; WGL, Western Great Lakes.

their historical range. The range of a species is inherently difficult to define, being contingent on timeframe as well as spatial scale (Gaston and Fuller 2009). For example, when the FWS initially proposed to delist the gray wolf in 2013, no breeding pairs of wolves existed in California, and therefore, under the Services' definition, the state was not within the species's range (78 FR 35664). However, by the time of the 2019 proposal, at least one breeding pair was known to inhabit California, and the FWS considered the state as within the species's range (84 FR 9653).

The conservation of such small recolonizing populations is important in part because their genetic composition can diverge rapidly from that of the source population, given the small number of founders. This divergence provides a rapid mechanism for novel and potentially adaptive genetic variants to originate and be acted on by natural selection. An example in North American wolves is provided by the historic spread of the allele controlling black coat color, which correlates with enhanced fitness during canine disease outbreaks (Schweizer et al. 2018).

In addition, canids such as the gray wolf can hybridize and form extensive zones of intergradation, which poses challenges for policies that involve assigning subspecies and genetic groupings to disjunct geographic areas (Leonard et al. 2005, vonHoldt et al. 2011). For example, the Great Lakes wolf population—on which the 2019 delisting rule depends for its claim that the gray wolf is recovered—is an admixture with contributions from up to three canid species (*C. lupus*, *Canis latrans*, and putative *Canis lycaon*; Heppenheimer et al. 2018).

Although the conservation of intergradation zones is important for maintaining adaptive potential (Leonard et al. 2005), populations in these areas may not meet the DPS policy's standard for discreteness (i.e., marked geographic or genetic separation). Recent genetic research has concluded that evolutionary relationships in canids and some other taxa resemble a web of life because of historical and possibly ongoing genetic exchange, rather than a tree of life defined by reproductive isolation (vonHoldt et al. 2018), implying that the discreteness standards in the DPS policy may not be well suited for protecting admixed populations important to the overall taxon. Such genomic admixture can be a rich source of beneficial alleles, which quickly boost genetic variation in recently bottlenecked populations (vonHoldt et al. 2018).

## Conservation of intraspecific variation via the ESA's significant portion of range clause

Lawmakers also included within the ESA a second clause supporting the conservation of intraspecific variation, which has proved more challenging for the Services to implement than was the DPS clause. The ESA of 1973 differed from two previous versions of the law (P.L. 89–669 [1966], P. L. 91–135 [1969]) in recognizing that endangerment has a geographic component and in extending legal protections to species "at risk of extinction throughout all or a significant portion of its range" (16 U.S.C. §1532(3.6)). The SPR clause suggests that Congress intended that managers interpret the concept of endangerment more broadly than an entire species facing the risk of extinction (Wolf et al. 2015). From this perspective, recovery requires not only that a species exist but also that it be present across all "significant" portions of its range (Carroll et al. 2010).

The ambiguity of the ESA's SPR clause, coupled with ongoing controversy concerning the geographic component of recovery under the statute, have led to numerous legal challenges to delisting proposals (table 1). Two related themes have emerged from the series of SPR-related court decisions, many of which involved the gray wolf. The first revolves around the meaning of the term *range* in the SPR clause. The courts, although deferring to the Services' desire to interpret the term *range* as indicating current rather than historical range, have nonetheless required the agency to consider loss of historical range when assessing a species's viability (Enzler and Bruskotter 2009, *Humane Society v. Zinke,* 865 F. 3d 585 [2017]).

Second, in several decisions stretching over two decades (from *Defenders of Wildlife v. Norton,* 258 F. 3d 1136 [2001] to *Center for Biological Diversity v. Everson*, 1:15-cv-00477 [2020]), the courts have concluded that the Services must interpret the term *significant* in the SPR clause in such a way that it is not rendered duplicative; that is, a species in peril throughout all of its range must somehow differ from a species in danger of extinction throughout just a significant portion of its range (Enzler and Bruskotter 2009). Although the Services have made multiple attempts to establish policy defining SPR, several court decisions have concluded that the most recent (2014) SPR policy (79 FR 37577), like previous efforts, runs counter to congressional intent (*Humane Society of the United States v. Jewell,* Case No. 13–186 [2014]), and the policy has been vacated nationwide (*Desert Survivors v. US Dept. of the Interior,* 231 F. Supp. 3d 368 [2017]). The courts concluded that the 2014 policy did not distinguish between a species at risk in a SPR and one at risk throughout its range, because it made SPR status contingent on a conclusion that extirpation of a regional population would place the entire species at risk of endangerment in the relatively short timeframe represented by the Services' definition of the "foreseeable future."

## Recovery units as a tool for conserving intraspecific variation

The Services have also developed guidance for delineating "recovery units" as an additional tool for conserving intraspecific variation. A recovery unit is "a special unit of the listed entity that is geographically or otherwise identifiable and is essential to the recovery of the entire listed entity, i.e., recovery units are individually necessary to conserve genetic robustness, demographic robustness, important life history stages, or some other feature necessary for long-term sustainability of the entire listed entity" (NMFS 2018). The Services often evaluate whether a regional population merits recovery unit status on the basis of whether it contributes to a species's resiliency, redundancy, and representation (Evans et al. 2020 [preprint] doi:10.1101/2020.03.15.991174). These 3R criteria suggest that a species, to be considered recovered, should be present in many large populations arrayed across a range of ecological settings (Shaffer and Stein 2000). Recovery units are especially appropriate "for species occurring across wide ranges with multiple populations or varying ecological pressures in different parts of their range," for "ensuring conservation of the breadth of a species's genetic variability… necessary to provide adaptive flexibility," "reestablishing historical or maintaining current genetic flow," and “encompassing current and historical population and habitat distributions” (NMFS 2018). The clause “necessary for long-term sustainability” is not strictly defined in the context of recovery unit designation. Nonetheless, unlike the invalid definition of SPR used in the Services’ 2014 policy, it is clearly distinct from the threshold used to judge whether a species is at risk of extinction throughout its range.

Although the existing recovery unit guidance provides a tool for conserving intraspecific variation, several shortcomings in its current implementation limit its effectiveness. The delineation of recovery units is discretionary, representing only about 2% of ESA-listed species, and is biased toward specific taxonomic groups (Evans et al. 2020 [preprint] doi:10.1101/2020.03.15.991174). Although the recovery unit guidance for defining intraspecific variants is relevant to defining SPR, the Services have not linked recovery units to the courts’ requirement that the agencies consider SPR in listing and delisting decisions. Although the recovery guidance states that “some recovery units may qualify as a DPS,” there is no clear decision tree to help planners decide which option to select (NMFS 2018). In theory, recovery units should inform consultations under the ESA’s section 7 regarding whether an action by another federal agency places a species in jeopardy, but this frequently does not occur (Evans et al. 2020 [preprint] doi:10.1101/2020.03.15.991174).

The wolf example detailed below, in which the FWS proposed to delist a widely distributed species on the basis of the recovery of a single population (a proposal at odds with the practice for other species), reinforces the conclusion of Evans and colleagues ([preprint] doi:10.1101/2020.03.15.991174) as to the “need for standardized practice regarding the use of recovery units” (see box 1). We propose that explicitly linking the delineation of intraspecific conservation units to the ESA’s SPR mandate would increase consistency, limit the broad discretion (and consequent opportunity for inappropriate political influence) that characterizes the Services’ current approach, and provide the foundation of an SPR policy that could withstand judicial review.

## Toward a consistent and effective framework for conserving intraspecific variation under the ESA

The current implementation of the ESA falls short in protecting intraspecific variation when faced with ecological and genetic complexities such as those described above. A more integrated approach to evaluating potential DPS and SPR can help overcome these challenges and prevent species such as the gray wolf from falling through the cracks. At first glance, the context of how *significant* is used in the SPR clause differs from how the term is used in the DPS policy. In the case of SPR, *significance* refers to a geographic area inhabited by a population (i.e., its range), whereas in the case of DPS, it refers to characteristics of the population itself. However, insights from landscape genetics, which maps population characteristics to environmental features, could allow the Services to interpret the term *significant* in a more consistent manner in relation to both the DPS policy and the SPR clause.

We propose a framework under which the relevant Service would consider both geography and genetics in assessing whether a population is in danger of extinction or likely to become endangered in the foreseeable future in a “significant” portion of its range. Figure 2 shows the decision tree that the framework envisions. If the Services were assessing a species that appeared to be under threat in only a portion of its range, but the species either was not a vertebrate or did not show marked isolation (i.e., discreteness), they would consider both of the following factors in assessing whether that portion of the species’s range is significant: a) the geographic extent of the area in which the population is imperiled, compared with both the species’s current and historical distributions and b) the current or potential future genetic distinctiveness and adaptive potential of the imperiled population.

This means of incorporating genetics into the assessment of SPR is also consistent with the DPS policy’s consideration of a population segment’s genetic characteristics compared with the species as a whole in assessing whether the population is “significant.” Such an approach in the context of assessing SPR would resemble current guidance on identifying recovery units (NMFS 2018) but would establish a consistent science-based policy linked to delisting rather than an ad hoc application of recovery guidance. By encompassing geography as well as genetics, this analytical approach allows the Services to also consider the range of “esthetic, ecological, educational, historical, recreational, and scientific” benefits cited in the ESA as provided by a species’s presence across its range (Carroll et al. 2010). We consider in supplement S1 the related question of what regulatory actions follow if the Services find a species endangered or threatened within only a significant portion of its range.

Even if recolonizing populations of formerly widely distributed species have not yet diverged genetically, their significance can be evaluated in a forward-looking manner as contingent on a degree of differentiation great enough for evolutionarily important contrasts to accumulate in the future (Waples 2006). Bowen (1998) coined the term *geminate evolutionary unit* to describe a regional population that shows morphological, behavioral, or biogeographical differentiation but does not yet show genetic divergence at neutral loci. Such a population can be considered significant on the basis of its ability to contribute to future evolutionary potential—for example, because of colonization of a new habitat (e.g., as defined by ecoregions or climatic zones) with novel selective pressures. For example, the North Cascades region of Washington State, which may currently contain only transient grizzly bears, has nonetheless been the object of substantial recovery planning efforts in part because it represents a unique ecological and evolutionary context for the species within the contiguous United States (USFWS and NPS 2017).

## Box 1. Distinguishing significant units within a species' distribution.

Federal agencies have employed a variety of approaches to identify intraspecific conservation units on the basis of how they contribute to a species's intraspecific variation and adaptive potential (Funk et al. 2019). Recovery units are often delineated on the basis of general ecosystem or habitat boundaries that are hypothesized to be relevant to adaptive variation in the species. The recovery plan for the northern spotted owl (*Strix occidentalis caurina*) designated 11 recovery units on the basis of the physiographic provinces found within the species's distribution (figure 3a; USFWS 2011). For species whose distribution has contracted, planners may consider the breadth of ecoregions encompassed by their historic distribution. The status assessment for the rusty-patched bumblebee (*Bombus affinis*) evaluated current and historical representation of the species in all ecoregions within its historical range, and projected the number of "representation units" (a surrogate for adaptive potential) that the species would inhabit under contrasting management scenarios (figure 3b; Szymanski et al. 2016a). Units can alternately be delineated on the basis of genetic data when such information is sufficient. In its status assessment of the eastern massasauga rattlesnake (*Sistrurus catenatus*), the FWS identified three genetically distinct regional units needed to maintain the adaptive potential of the species (figure 3c; Szymanski et al. 2016b).

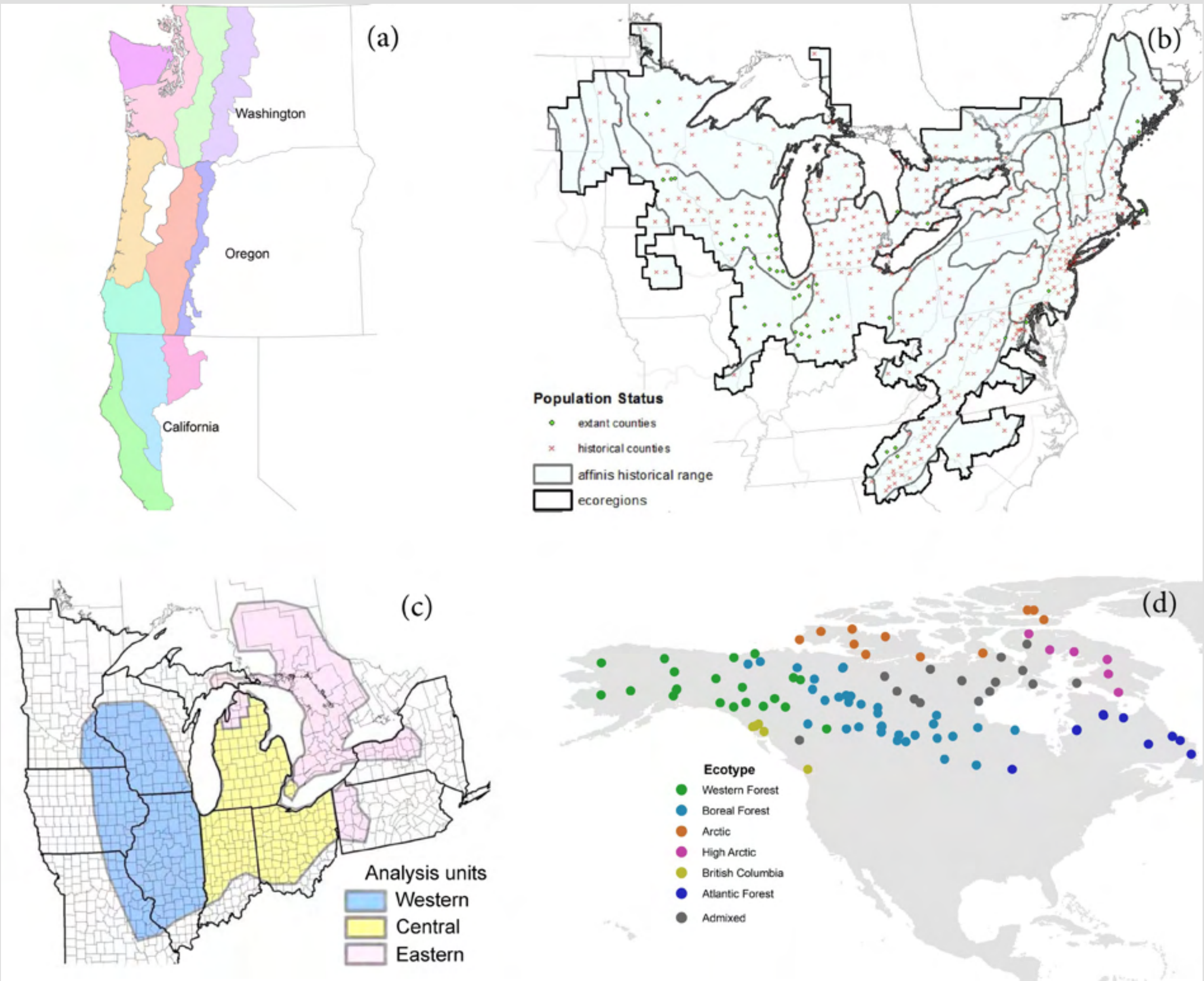


*Figure 3. Examples of consideration of the significance of portions of a species's range in terms of their contributions to the species's adaptive potential: (a) the recovery plan for the Northern Spotted Owl (USFWS 2011) designated 11 recovery units on the basis of the physiographic provinces found within the species's distribution; (b) the status assessment for the rusty-patched bumblebee (Szymanski et al. 2016a) projected the number of representation units the species would inhabit under contrasting management scenarios; (c) the status assessment for the eastern massasauga rattlesnake (Szymanski et al. 2016b) identified three genetically distinct regional units needed to maintain the adaptive potential of the species; and (d) Schweizer and colleagues (2016) delineated six significant ecotypes for wolves inhabiting Canada and Alaska on the basis of associations between genetic clusters and 12 environmental variables. Source: (a–c) USFWS, (d) Rena Schweizer.*

**Box 1. Continued.**

Although many examples of delineating subspecific units are based solely on either genetic analysis or habitat discontinuities, stronger inferences can be drawn by using environmental data in combination with genetic information (Funk et al. 2012, Hendricks et al. 2019a). Landscape genomics techniques, such as selection tests and genotype-by-environment associations, provide powerful methods for distinguishing significant adaptive variants and ecotypes on the basis of the degree of adaptive differentiation between them (Carmichael et al. 2007, vonHoldt et al. 2011, Funk et al. 2012, Schweizer et al. 2016, Hendricks et al. 2019a).

Schweizer and colleagues (2016) were able to accurately infer the genetic cluster to which a gray wolf belonged on the basis of the habitat type (as defined by 12 environmental variables) where it was collected, allowing the delineation of six significant ecotypes for wolves inhabiting Canada and Alaska (figure 3d). Hendricks and colleagues (2019b) similarly used a maximum entropy approach to model distinct coastal and interior environmental niches for wolves in the US Pacific Northwest. The most relevant type of genetic information may differ when delineating DPS versus SPR. Intraspecific conservation units such as DPS, whose genetics have been shaped by both historical isolation (i.e., discreteness) and adaptive processes, can be delineated using both neutral loci and loci under selection (Funk et al. 2012). Other less-isolated but significant subunits of species (SPR) can be delineated primarily using loci that exhibit signatures of divergent selection (Funk et al. 2012).

The question remains as to how finely to divide a species's range—that is, how to discern "significant" intraspecific adaptive variants. DPS designation has been criticized as being partially subjective because there is no universally accepted threshold for the level of differentiation that confers evolutionary significance (Waples 1995). Although this criticism necessarily extends to identification of potential SPR, model selection metrics such as the Deviance Information Criterion (Gao et al. 2011) are frequently used to determine the best-supported number of clusters or subunits within a sample on the basis of genetic and environmental data. As Winker (2010) states, "the process of diagnosing states that exist along a continuum of differentiation can be difficult and contentious and necessarily has some arbitrariness; professional standards can be developed so that such diagnoses are objective."

The elements we propose the Services consider in identifying a "significant" portion of a species's range are also identified in the DPS policy as relevant to assessing a discrete population's significance. Therefore, it is feasible to coordinate the definitions of significance in the DPS and SPR policies. For example, recognition of evolutionary potential and the importance of geography are inherent in the DPS policy's consideration of the significance of unique ecological settings and potential gaps in a species's range created by the loss of a population, respectively. However, our framework (figure 2) allows for differences in emphasis and implementation to remain between the DPS and SPR policies' definitions of significance. In addition, although existing guidance regarding recovery units informs our proposed SPR definition, the Services could retain the flexibility to identify recovery units for the purposes of recovery planning and implementation, even if such units were not identified as SPR.

## Gray wolf listing and delisting demonstrates the need for a consistent approach to conserving intraspecific variation

The several subspecies of North American gray wolf were among the earliest taxa listed as endangered under the ESA. The FWS shifted in 1978 to listing the wolf at the species level, except the Mexican wolf subspecies (*Canis lupus baileyi*), which remains listed separately (43 FR 9610). As wolf population numbers increased under ESA protection, the FWS repeatedly sought to remove some or all of the US population from the list of endangered and threatened species, only to be blocked by the courts in at least nine separate decisions since 2005 (table 1). The successive wolf delisting proposals have been characterized by scientific as well as legal controversy. The FWS withdrew a 2013 delisting proposal after a panel of scientific peer reviewers found flaws in the agency's taxonomic analysis (NCEAS 2014). A panel of invited scientific peer reviewers (including two of the present authors, CC and AT) also found significant shortcomings in the 2019 delisting proposal (Atkins 2019).

A notable feature of the successive delisting proposals is that they have varied widely in how they defined appropriate gray wolf conservation units, ranging from a focus on *C. lupus* as a whole to a focus on one or more DPS or populations inhabiting individual states (table 1). The most recent (2019) delisting proposal asserted that gray wolves in the contiguous United States (except for the separately listed *C. l. baileyi*) no longer merit ESA protection, on the basis of the premise that the agency can delist a species when a single regional population (in this case wolves inhabiting the Great Lakes states; figure 1) has recovered to a status the agency deems presently secure (84 FR 9683).

The roughly 4000 wolves estimated to inhabit the Great Lakes region constitute approximately two-thirds of the total population currently inhabiting the contiguous United States (figure 1). But is total population the only relevant metric for assessing the conservation status of a species? The Great Lakes population occupies only 3 of the at least 17 states within the species's historical range that hold substantial areas of habitat (figure 1). The approximately 2000 wolves inhabiting the Northern Rocky Mountain (NRM) region form the only other large regional population within the contiguous Untied States (figure 1). Because the US Congress passed legislation (Pub. L. No. 112-10, § 1713, 125 Stat. 38) removing ESA protections from the NRM population (the only instance of such legislative delisting since the ESA's passage), that population is counterintuitively not part of the listed entity considered in the 2019 proposal.

By arguing in the 2019 proposal that "wolves that occur outside the Great Lakes area… are not necessary for the recovered status of the gray wolf entity" (84 FR 9683), the FWS took a dramatic step away from its policy at the time it consolidated wolf subspecies into a single listing in 1978, when the agency offered "the firmest assurance that it will continue to recognize valid biological subspecies for purposes of its research and conservation programs" (43 FR 9610). The FWS's evolving position on wolf delisting exemplifies how the agency has moved away from Congress's vision of an ESA that protects intraspecific variation toward a more politically expedient approach predicated on a misrepresentation of the extent of intraspecific variation found in most geographically widespread species. For example, the FWS justified the central premise of the 2019 wolf delisting proposal—that wolf populations outside the Great Lakes region do not contribute to recovery—to a large degree on an assertion that the North American wolf population is genetically unstructured because the wolf's ability to disperse long distances would prevent genetic variation among subpopulations (84 FR 9685).

The development of high-throughput genotyping methods over the last decade has enabled an increasingly detailed analysis of historical and current population structure of North American wolves (Hendricks et al. 2019a). Wolf populations are now known to be characterized by complex genetic clines at several spatial scales, driven by historical biogeographic factors, isolation by distance, and association with particular ecosystems (Geffen et al. 2004, Carmichael et al. 2007, vonHoldt et al. 2011, Schweizer et al. 2016). Environmental factors related to climate zones significantly contribute toward genetic isolation by distance in North American gray wolves, likely through habitat matching decisions made by dispersers (Geffen et al. 2004). Environment factors, along with intraspecific competition for prime territories, resources, and access to reproduction, result in a nested structuring of genetic variation at both the continental and regional scales (Carmichael et al. 2007, vonHoldt et al. 2011, Schweizer et al. 2016).

## Distinct population segment policy as applied to the wolf

The highly structured North American wolf population revealed by genetic analyses has implications for determining whether conservation units below the species level are appropriate under the ESA (vonHoldt et al. 2011, Hendricks et al. 2019a). Wolf habitat in the contiguous United States is discontinuous enough to allow identification of DPS for some regional populations, despite occasional dispersal between regions (Carroll et al. 2006, CBD and HSUS 2018). For example, the FWS itself concluded in 2007 that despite "occasional individual wolves or packs [that] disperse among populations," Northern Rocky Mountain wolves were markedly separated from other regional wolf populations (73 FR 10519).

In 2008, the FWS embarked on an effort to develop a comprehensive national strategy for gray wolf conservation by identifying appropriate wolf listing units within the broader continental distribution of the species (76 FR 26086). This national strategy was necessary because earlier proposals to remove protections for individual regional wolf populations by piecemeal designation and delisting of a single DPS within the larger range had been rejected by the courts (table 1; Alexander 2010). Five assessment units, including several potential DPS, were identified throughout the contiguous United States (figure 1). Although this closed-door process involving federal and state agencies lacked the inclusivity and scientific guidelines typical of recovery teams (PEER 2013), it nonetheless attempted (but never finalized) a comprehensive analysis of what recovery efforts might be appropriate in the different regions that include habitat for the species (Runge 2011).

In contrast, the 2019 proposed delisting rule did not attempt a comprehensive analysis of potential DPS status for regional populations but instead asserted that no regional wolf populations meet the DPS policy's standard for discreteness because the entire range of the gray wolf in the contiguous United States constitutes a single metapopulation (a term used in the rule in the broad sense of subpopulations linked by immigration and emigration). However, the Great Lakes and Pacific wolf populations, situated at the periphery of currently occupied wolf range, are separated by 1800 kilometers (km), much of which is transformed by agriculture. Although wolves inhabiting the Northern Rocky Mountains could provide an intermediate stepping stone population, any genetic interchange between these distant groups would necessarily be indirect and attenuated, allowing substantial genetic divergence (Schweizer et al. 2016).

The FWS has identified DPS for other large mammalian carnivores such as the grizzly bear (*Ursus arctos horribilis*) and does not claim that grizzly bear recovery in one region renders recovery efforts elsewhere unnecessary. Connectivity between regional grizzly bear populations, far from precluding DPS designation, has been judged by the FWS to be essential to long-term genetic health and recovery of those populations (82 FR 30502). The degree of genetic differentiation between regional wolf populations (e.g., between the NRM and Great Lakes populations) resembles that between grizzly bears inhabiting separate DPS in the Northern Rocky Mountains (vonHoldt et al. 2011, Cronin and MacNeil 2012).

Because average natal dispersal of male and female wolves (114 and 78 km; Boyd and Pletscher 1999) is several times that of male and female grizzly bears (42 and 14 km; Proctor et al. 2004), several grizzly bear DPS might occur within a single wolf DPS, as has been the case in the Northern Rocky Mountains. However, the entire gray wolf distribution in the contiguous United States cannot be considered a single genetically undifferentiated population, as was proposed in the 2019 delisting rule. The divergence in application of the DPS policy to grizzly bears and gray wolves demonstrates the need for a more consistent application of the DPS policy. Our proposed framework coordinating the DPS and SPR policies would not require modification of the existing DPS policy's criteria but, rather, their consistent application even to controversial species such as the wolf, enabled by strengthened support for scientific integrity from agency leadership (Carroll et al. 2017).

### Significant portion of range as applied to the wolf

While acknowledging the absence to date of a legally sufficient definition of SPR, the FWS in the 2019 proposed wolf rule attempted to satisfy future judicial review by evaluating whether regional populations outside the Great Lakes are "significant." To support its claim that recovery of the Great Lakes population allows the agency to delist wolves throughout the contiguous United States, the FWS concluded that any currently listed wolf population found outside the Great Lakes region is not significant "because it is not biologically important" because of the small size of peripheral populations and the purported lack of genetic differentiation within the North American wolf population (84 FR 9648). This conclusion requires both a particularly narrow reading of the 3R criteria and a misrepresentation of research regarding wolf genetic population structure.

Under our proposed framework (figure 2), DPS could be identified for regional wolf populations that showed marked separation from other populations, whereas wolf populations inhabiting intergradation zones might instead qualify for delineation as SPR. The coastal Pacific Northwest (western Washington and Oregon and northern California; figure 1), one of the five regions assessed in the 2008 process, provides an example of a regional wolf population that meets the DPS discreteness criterion (figure 2). Marked separation can be established for this regional population as a consequence of several factors: physical (separation from larger inland populations by areas of nonhabitat), ecological (occupation of coastal rainforest ecosystems), genetic (unique genetic contributions from wolves from coastal British Columbia; Hendricks et al. 2019b), and an international governmental boundary separating US populations from coastal wolves in Canada that have different management status. Once discreteness has been established, wolves in the Pacific Northwest could merit significance because of their persistence in a unique ecological setting, which is used as a proxy for adaptive genetic differences, as well as the fact that loss of the population would result in a significant gap in the range of the taxon (Carroll et al. 2001, Waples et al. 2018).

The Colorado and Utah assessment unit considered in the 2008 process (figure 1), which historically formed a zone of intergradation between northern and southwestern wolf subspecies (Leonard et al. 2005), provides an example of an area that should be evaluated as a SPR, even if it is found to not show marked separation from adjacent populations (figure 2). This region, although currently supporting only a handful of wolves, represents a valid SPR because it holds abundant suitable habitat in a unique ecological setting (based on ecoregions or climate zones) subject to novel selective pressures (Carroll et al. 2006). Although we recognize that policy alone cannot ensure against inappropriate political influence in agency rulemaking, a coherent approach to DPS and SPR evaluation would be more likely to withstand litigation than the current ad hoc approach to wolf delisting, and more likely to result in the robust conservation outcomes envisioned by the lawmakers who drafted the ESA.

### Conclusions

In 2019, the US federal administration enacted sweeping changes to regulations interpreting the ESA that limit the statute's reach (83 FR 35174, Lambert 2019). The 2019 wolf delisting proposal forms part of this effort to advance a minimalist interpretation of the ESA's mandate, in that its central premise goes beyond what is necessary to support wolf delisting and seeks to establish a precedent that the ESA allows for a narrow view of what constitutes recovery of widely distributed species. By extending the assumptions of previous agency policy regarding the significant portion of range clause to their extreme, the proposed wolf delisting rule highlights the degree to which the conservation of intraspecific variation is central to ESA implementation and underlines the need to develop more effective policy concerning this issue. If applied generally to other species, the 2019 rule's approach to ESA implementation would represent a significant scaling back of recovery efforts for widely distributed species that would increase both short-term vulnerability and long-term loss of adaptive potential.

The recovery of formerly widely distributed species such as the wolf poses practical challenges for delisting and recovery planning (Treves and Bruskotter 2011). In some instances, an approach that requires continued federal management of the species throughout its range until the weakest regional population is secure may consume scarce conservation resources. An efficient strategy for recovery of such species could allow reduction of regulatory protections in regions that already hold abundant populations while maintaining protections in other regions that hold small recolonizing populations. The strategies we propose, based respectively on DPS and SPR designation, represent complementary approaches to achieving this flexibility that build on the Services' existing standards for evaluating the significance of regional populations under the DPS policy and recovery unit guidance. Our proposed approach has relevance beyond the United States in the context of international regulations such as the European Union's Habitats Directive, which requires member states to achieve "favorable conservation status" for protected species without clarifying at what scale this status is to be achieved (Laikre et al. 2016).

When initially defining their resiliency, redundancy and representation criteria, Shaffer and Stein (2000) noted that successful conservation “will require identifying conservation targets not simply as species and communities but as the complexes of populations, communities, and environmental settings that are the true weave of biodiversity.” As advances in genomics increase our understanding of patterns of intraspecific variation, the conservation of adaptive potential merits increased emphasis as a key element in achieving the ESA’s goal of “saving all the pieces” (Leopold 1968).

## Acknowledgments

We thank Brett Hartl, Phillip Hedrick, Ya-Wei Li, Timothy Preso, and Robert Wayne for comments that improved the manuscript, and the Wilburforce Foundation for support to CC.

## Supplemental material

Supplemental data are available at *BIOSCI* online.

## References cited

Alexander K. 2010. Gray Wolves under the Endangered Species Act (ESA): Distinct Population Segments and Experimental Populations. Congressional Research Service. Report no. 49.

Atkins North America. 2019. Summary Report of Independent Peer Delisting Reviews for the US Fish and Wildlife Service Gray Wolf Delisting Review. Atkins North America. www.fws.gov/endangered/improving_esa/peer_review_process.html.

Bowen BW. 1998. What is wrong with ESUs? The gap between evolutionary theory and conservation principles. Journal of Shellfish Research 17: 1355–1358.

Boyd DK, Pletscher DH. 1999. Characteristics of dispersal in a colonizing wolf population in the central Rocky Mountains. Journal of Wildlife Management 63: 1094–1108.

Carmichael LE, Krizan J, Nagy JA, Fuglei E, Dumond M, Johnson D, Veitch A, Berteaux D, Strobeck C. 2007. Historical and ecological determinants of genetic structure in arctic canids. Molecular Ecology 16: 3466–3483.

Carroll C, Noss RF, Schumaker NH, Paquet PC. 2001. Is the return of the wolf, wolverine, and grizzly bear to Oregon and California biologically feasible? Pages 25–46 in Maehr D, Noss RF, Larkin J, eds. Large Mammal Restoration: Ecological and Sociological Challenges in the 21st Century. Island Press.

Carroll C, Phillips MK, Lopez-Gonzalez CA, Schumaker NH. 2006. Defining recovery goals and strategies for endangered species: The wolf as a case study. BioScience 56: 25–37.

Carroll C, Vucetich JA, Nelson MP, Rohlf DJ, Phillips MK. 2010. Geography and recovery under the US Endangered Species Act. Conservation Biology 24: 395–403.

Carroll C, et al. 2017. Defending the scientific integrity of conservation-policy processes. Conservation Biology 31: 967–975

[CBD and HSUS] Center for Biological Diversity and Humane Society of the United States. 2018. Petition to Maintain Protections for Gray Wolves (*Canis lupus*) in the Lower 48 States as Endangered or Threatened “Distinct Population Segments” under the Endangered Species Act. CBD. www.biologicaldiversity.org/campaigns/gray_wolves/pdfs/Wolf-Petition-12-17-2018.pdf.

Crandall K, Bininda-Emonds O, Mace G, Wayne RK. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15: 290–295.

Cronin MA, MacNeil MD. 2012. Genetic relationships of extant brown bears (Ursus arctos) and polar bears (Ursus maritimus). Journal of Heredity 103: 873–881.

[COSEWIC] Committee on the Status of Endangered Wildlife in Canada. 2018. Guidelines for Recognizing Designatable Units. COSEWIC. cosewic.ca/index.php/en-ca/reports/preparing-status-reports/guidelines-recognizing-designatable-units.

Enzler SA, Bruskotter JT. 2009. Contested definitions of endangered species: The controversy regarding how to interpret the phrase a significant portion of a species range. Virginia Environmental Law Journal 27: 1–67.

Funk W, Forester BR, Converse SJ, Darst C, Morey S. 2019. Improving conservation policy with genomics: A guide to integrating adaptive potential into US Endangered Species Act decisions for conservation practitioners and geneticists. Conservation Genetics 20: 115–134.

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW. 2012. Harnessing genomics for delineating conservation units. Trends in Ecology and Evolution 27: 489–496.

Gao H, Bryc K, Bustamante CD. 2011. On identifying the optimal number of population clusters via the deviance information criterion. PLOS ONE 6: e21014.

Gaston KJ, Fuller RA. 2009. The sizes of species’ geographic ranges. Journal of Applied Ecology 46: 1–9.

Geffen E, Anderson MJ, Wayne RK. 2004. Climate and habitat barriers to dispersal in the highly mobile grey wolf. Molecular Ecology 13: 2481–2490.

Hamilton JA, Miller JM. 2015. Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology 30: 33–41.

Hendricks SA, Schweizer RM, Wayne RK. 2019a. Conservation genomics illuminates the adaptive uniqueness of North American gray wolves. Conservation Genetics 20: 29–43.

Hendricks SA, et al. 2019b. Natural re-colonization and admixture of wolves (*Canis lupus*) in the US Pacific Northwest: Challenges for the protection and management of rare and endangered taxa. Heredity 122: 133–149.

Heppenheimer E, et al. 2018. Population genomic analysis of North American eastern wolves (*Canis lycaon*) supports their conservation priority status. Genes 9: 606.

Hoffmann AA, Sgrò CM. 2011. Climate change and evolutionary adaptation. Nature 470: 479–485.

Laikre L, Olsson F, Jansson E, Hössjer O, Ryman N. 2016. Metapopulation effective size and conservation genetic goals for the Fennoscandian wolf (*Canis lupus*) population. Heredity 117: 279.

Lambert J. 2019. Trump administration weakens Endangered Species Act. Nature (12 August 2019). www.nature.com/articles/d41586-019-02439-1.

Leonard JA, Vila C, Wayne RK. 2005. Legacy lost: Genetic variability and population size of extirpated US grey wolves (*Canis lupus*). Molecular Ecology 14: 9–17.

Leopold A. 1968. From A Sand County Almanac (1949). Oxford University Press.

[NCEAS] National Center for Ecological Analysis and Synthesis 2014. Review of proposed rule regarding status of the wolf under the Endangered Species Act. NCEAS. www.fws.gov/science/pdf/Peer-Review-Report-of-Proposed-rule-regarding-wolves.pdf.

Nelson MP, Vucetich JA, Bruskotter JT. 2016. Ecological value and the US Endangered Species Act: Comment on Waples et al. (2015). Endangered Species Research 30: 187–190.

[NMFS] National Marine Fisheries Service. 2018. Interim Endangered and Threatened Species Recovery Planning Guidance. US Department of the Interior.

[PEER] Public Employees for Environmental Responsibility. 2013. Politics Dominated Wolf De-Listing Meetings: Science Was Afterthought in Developing Preferred Alternatives for Wolf Recovery. PEER. www.peer.org/news/news-releases/2013/06/26/politicsdominated-wolf-de-listing-meetings.

Proctor MF, McLellan BN, Strobeck C, Barclay RMR. 2004. Gender-specific dispersal distances of grizzly bears estimated by genetic analysis. Canadian Journal of Zoology 82: 1108–1118.

Razgour O, Forester B, Taggart JB, Bekaert M, Juste J, Ibáñez C, Puechmaille SJ, Novella-Fernandez R, Alberdi A, Manel S. 2019. Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. Proceedings of the National Academy of Sciences 116: 10418–10423.

Runge M. 2011. SW Wolf SDM Workshop: Background and Purpose. US Geological Survey Patuxent Wildlife Research Center. www.peer.org/assets/docs/6_27_13_Wolf_Webinar.pdf.

Schweizer RM, Vonholdt BM, Harrigan R, Knowles JC, Musiani M, Coltman D, Novembre J, Wayne RK. 2016. Genetic subdivision and candidate genes under selection in North American grey wolves. Molecular Ecology 25: 380–402.

Schweizer RM, et al. 2018. Natural selection and origin of a melanistic allele in North American gray wolves. Molecular Biology and Evolution 35: 1190–1209.

Shaffer ML, Stein BA. 2000. Safeguarding our precious heritage. Pages 301–321 in Shaffer ML, Stein BA, eds. Precious Heritage: The Status of Biodiversity in the United States. Oxford University Press.

Soulé ME. 1985. What is conservation biology? BioScience 35: 727–734.

Szymanski J, Smith T, Horton A, Parkin M, Ragan L, Masson G, Olson E, Gifford K, Hill L. 2016a. Rusty Patched Bumble Bee (*Bombus Affinis*) Species Status Assessment. US Fish and Wildlife Service.

Szymanski J, Pollack C, Ragan L, Redmer M, Clemency L, Voorhies K, JaKa J. 2016b. Species Status Assessment for the Eastern Massasauga Rattlesnake (*Sistrurus Catenatus*). US Fish and Wildlife Service.

Treves A, Bruskotter J. 2011. Gray wolf conservation at a crossroads. BioScience 61: 584–585.

[USFWS and NPS] US Forest and Wildlife Service and National Parks Service. 2017. North Cascades Ecosystem Draft Grizzly Bear Restoration Plan and Environmental Impact Statement. USFWS and NPS. parkplanning.nps.gov/projectHome.cfm?projectId=44144.

[USFWS] US Fish and Wildlife Service. 2011. Revised Recovery Plan for the Northern Spotted Owl (*Strix occidentalis caurina*). USFWS.

[USFWS] US Fish and Wildlife Service. 2019. Gray wolf Biological Report: Information on the Species in the Lower 48 United States. USFWS.

vonHoldt BM, Brzeski KE, Wilcove DS, Rutledge LY. 2018. Redefining the role of admixture and genomics in species conservation. Conservation Letters 11: e12371.

vonHoldt BM, et al. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. Genome Research 21: 1294–1305.

Vucetich JA, Nelson MP. 2014. Conservation, or Curation? New York Times (20 August 2014) www.nytimes.com/2014/08/21/opinion/conservation-or-curation.html.

Waples RS. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. American Fisheries Society Symposium 17: 8–27.

Waples RS. 2006. Distinct population segments. Pages 127–149 in Scott JM, Goble DD, Davis FW, eds. The Endangered Species Act at Thirty: Conserving Biodiversity in Human-Dominated Landscapes. Island Press.

Waples RS, Pacifici K, Kays R, Fredrickson RJ, Mills LS. 2018. Is the red wolf a listable unit under the US Endangered Species Act? Journal of Heredity 109: 585–597.

Winker K. 2010. Subspecies represent geographically partitioned variation, a gold mine of evolutionary biology, and a challenge for conservation. Ornithological Monographs 67: 6–23.

Wolf S, Hartl B, Carroll C, Neel MC, Greenwald DN. 2015. Beyond PVA: Why recovery under the Endangered Species Act is more than population viability. BioScience 65: 200–207.

---

*Carlos Carroll (carlos@klamathconservation.org) is affiliated with the Klamath Center for Conservation Research, in Orleans, California. Daniel J. Rohlf is affiliated with the Earthrise Law Center, in the Lewis and Clark Law School, in Portland, Oregon. Bridgett M. vonHoldt is affiliated with the Department of Ecology and Evolutionary Biology, at Princeton University, in Princeton, New Jersey. Adrian Treves is affiliated with the Nelson Institute for Environmental Studies, at the University of Wisconsin, in Madison, Wisconsin. Sarah A. Hendricks is affiliated with the Institute for Bioinformatics and Evolutionary Studies, at the University of Idaho, in Moscow, Idaho.*

OPEN ACCESS Freely available online

# Meta-Analysis of Relationships between Human Offtake, Total Mortality and Population Dynamics of Gray Wolves (*Canis lupus*)

**Scott Creel*, Jay J. Rotella**

Department of Ecology, Montana State University, Bozeman, Montana, United States of America

## Abstract

Following the growth and geographic expansion of wolf (*Canis lupus*) populations reintroduced to Yellowstone National Park and central Idaho in 1995–1996, Rocky Mountain wolves were removed from the endangered species list in May 2009. Idaho and Montana immediately established hunting seasons with quotas equaling 20% of the regional wolf population. Combining hunting with predator control, 37.1% of Montana and Idaho wolves were killed in the year of delisting. Hunting and predator control are well-established methods to broaden societal acceptance of large carnivores, but it is unprecedented for a species to move so rapidly from protection under the Endangered Species Act to heavy direct harvest, and it is important to use all available data to assess the likely consequences of these changes in policy. For wolves, it is widely argued that human offtake has little effect on total mortality rates, so that a harvest of 28–50% per year can be sustained. Using previously published data from 21 North American wolf populations, we related total annual mortality and population growth to annual human offtake. Contrary to current conventional wisdom, there was a strong association between human offtake and total mortality rates across North American wolf populations. Human offtake was associated with a strongly additive or super-additive increase in total mortality. Population growth declined as human offtake increased, even at low rates of offtake. Finally, wolf populations declined with harvests substantially lower than the thresholds identified in current state and federal policies. These results should help to inform management of Rocky Mountain wolves.

**Citation:** Creel S, Rotella JJ (2010) Meta-Analysis of Relationships between Human Offtake, Total Mortality and Population Dynamics of Gray Wolves (*Canis lupus*). PLoS ONE 5(9): e12918. doi:10.1371/journal.pone.0012918

**Editor:** Geoffrey Clayton Trussell, Northeastern University, United States of America

**Received** April 30, 2010; **Accepted** August 22, 2010; **Published** September 29, 2010

**Funding:** This work was funded by the National Science Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

* E-mail: screel@montana.edu

## Introduction

### Status of US Wolf Populations

Following their extirpation by direct harvesting across most of the United States, gray wolves (*Canis lupus*) were among the 14 mammals originally listed by the U.S. Fish and Wildlife Service under the Endangered Species Preservation Act of 1966. This legal protection was renewed under the Endangered Species Act of 1973, and wolves are now considered endangered in 16 states. Following steady growth of the wolf population of the Western Great Lakes region, this population segment was down-listed to threatened status in 1978. A proposal for delisting in Minnesota and Michigan was initiated in 2000 and remains under legal appeal. Following reintroduction into Yellowstone National Park and central Idaho in 1995–1996, wolves in the Northern Rocky Mountains Recovery Area grew to a minimum of 1,645 wolves at the end of 2008 [1]. This population segment (including all or parts of Idaho, Montana, Oregon, Utah, Washington, Wyoming) was delisted in 2009 [2], a decision that also remains under appeal. Legal authority for wolf management passed from the US Fish and Wildlife Service to state agencies in this region, and public hunting seasons were initiated in Idaho and Montana, with quotas of 255 (220+35 within the Nez Perce Tribal Treaty Area) and 75 wolves, respectively [3–6]. These quotas represent an annual harvest of 20% of the regional population. Quotas were filled in 7 of 12 Idaho regions with a total harvest of 188 wolves. Montana's wolf season closed after 23 days with the quota 96% filled. Together with wolves killed in predator control operations (145 in Montana and 93 in Idaho), humans killed 44% of Montana's wolves and 37.1% of the two-state population in 2009. In March 2010, Montana liberalized its policy for control of wolves that prey on livestock, no longer requiring confirmation by state wildlife officials before wolves near livestock carcasses are trapped or shot. In July 2010, Montana increased the public hunting quota by a factor of 2.5, from 75 to 186 wolves. Idaho is now considering similar changes to wolf management policy.

Predator control and sport hunting are well-established tools to manage large carnivores and broaden societal acceptance of wolves, but to our knowledge it is unprecedented for a species to move this rapidly from highly protected to heavily-hunted, and it remains important to quantitatively assess the probable consequences of these policies as carefully as possible (regardless of the intended outcome). In general, stakeholders calling for reductions in wolf numbers are concerned about three issues: livestock losses, effects on ungulates (particularly elk) and human safety. In 2008 and 2009, Northern Rocky Mountain wolves were responsible for an average of 203 confirmed kills of cattle (from a population of approximately 5.9 million cattle) and 538 confirmed kills of sheep, or 0.8 cows/wolf pack/year and 2.2 sheep/wolf pack/year [1]. Elk numbers in some areas have declined in parallel with wolf recolonization, particularly in locations with locally high wolf density such as portions of the Greater Yellowstone Ecosystem [7,8], though elk numbers have remained stable or increased in many other areas during the period of wolf recovery [9]. For example, 60% of Montana elk management units were above target population density in 2002, despite liberalized hunting regulations [9]. Wolves have not killed or physically injured people in the Northern Rocky Mountains (NRM) since reintroduction. Current state policies for NRM wolf management focus mainly on providing hunting opportunity, reducing population sizes, and maintaining populations large enough to avoid reclassification as endangered [3–6]. Analysis of the relationship between harvest, survival rates and population growth is useful if these objectives (or broader objectives related to predator conservation and ecosystem function) are to be met.

Here, we use previously published data [1,10] from 21 North American wolf populations (including the recently delisted wolves of the Northern Rocky Mountains) to evaluate relationships between human offtake, mortality and population growth of wolves, and consider the implications for policy.

### Human Offtake and Total Mortality in Wolves

Mortality due to hunting can increase a population's total death rate (additive mortality) or be compensated by density-dependent reductions in non-harvest mortality factors, thus having little effect on overall mortality (compensatory mortality). Williams et al. [11] and Lebreton [12] provide excellent reviews of compensatory and additive mortality. Formally, harvest mortality is fully additive when the regression of total mortality on harvest rate [with slope $=\hat{\beta}$ and intercept $=\hat{m}(0)$] yields $\frac{\hat{\beta}}{1-\hat{m}(0)}=1$. When $\frac{\hat{\beta}}{1-\hat{m}(0)}=0$, a harvest is fully compensatory [up to a threshold harvest $=\hat{m}(0)$, the rate of mortality with no harvest]. A harvest is partially additive when $0<\frac{\hat{\beta}}{1-\hat{m}(0)}<1$, and super-additive when $\frac{\hat{\beta}}{1-\hat{m}(0)}>1$. A super-additive harvest increases total mortality beyond the effect of direct killing itself, through social disruption or the loss of dependent offspring.

It is widely argued that human-caused wolf mortality is mainly compensatory, with little effect on wolf dynamics until a large proportion of the population is harvested. Haight et al. [13] summarized that "natural mortality decreases when a wolf population is harvested" and "sustainable harvest rates of 30%–50% have been estimated for free ranging populations" (p. 850). Mech [14] stated that "most human-caused mortality is compensatory" (p.74). In the most comprehensive prior analysis of this question, Fuller et al. [10] concurred that "the principle of compensation operates in wolf populations" (p. 185). Using data from 18 wolf populations, Fuller et al. regressed total mortality on human-caused mortality, and concluded that human-caused mortality was largely compensatory. However, the slope ($\hat{\beta}=0.73$) and intercept ($\hat{m}(0)=0.20$) they reported yield $\frac{\hat{\beta}}{1-\hat{m}(0)}=0.91$, indicating that human harvest was almost fully additive. Thus, there is reason to reconsider the inference that human-caused wolf mortality is primarily compensatory.

## Methods

We tested relationships between the rates of population growth, total mortality and human-caused mortality. To assemble data we began with the 18 populations examined by Fuller et al. [10] in their comprehensive 2003 review. For consistency in the data examined across studies, we used the values that Fuller et al. tabulated (see their Table 6.8) from prior single-population studies, and we retained their decision to divide the data from one population (Isle Royale) into two subsets, based on changes in long-term population trajectory. We tabulated data from United States Fish and Wildlife Service annual reports [1] for wolves in the three segments of the Northern Rocky Mountains (NRM) Recovery Area (Greater Yellowstone, $N=11$ years, 1998–2008; Central Idaho, $N=8$ years, 2001–2008, Northwest Montana, $N=10$ years, 1999–2008). Changes across years in the method of tabulating data in USFWS annual reports yielded different sample sizes for the three segments of the NRM metapopulation. Finally, we used Google Scholar and Scopus to search on the keywords 'wolf' and '*Canis lupus*', and for the names of all of the authors of studies tabulated by Fuller et al [10] (their Table 6.8). This search yielded no additional studies with the requisite data. Collectively, these procedures yielded 48 estimates of population growth, harvest rate and total mortality rate from 21 populations (19 estimates as in Fuller et al.'s [10] Table 6.8, and 29 estimates for NRM wolves from USFWS annual reports through 2009 [1]).

Our analyses test two basic hypotheses. First, was total mortality affected by human offtake, and if so, what was the form of the relationship? Second, was the population growth rate ($\lambda$) affected by human offtake, and if so, what was the form of the relationship? To test the relationship of harvest to population growth, we evaluated a set of *a priori* models using Akaike's Information Criterion corrected for sample size (AICc). To test the relationship of harvest to mortality (which was approximately binomially distributed), we used quasi-AICc (QAICc) values, with variances adjusted for over-dispersion using the estimated value of c-hat from a quasi-binomial model with a linear link function, and taking the number of population means ($N=48$) as the sample size to avoid pseudo-replication. Annual reports from the USFWS [1] allowed us to tabulate data from NRM populations as annual means. Data from other populations were multi-year means (following Fuller et al. [10]). We weighted each estimate by sample size to account for variation in the amount of information and the precision of each estimate, and we show the standard error (whiskers) of each population estimate (point) in Figures 1 & 2. Below, we discuss the possible effects of sampling error on the inferences from these models.

Tables 1–3 identify and describe the set of *a priori* models for each analysis. Briefly, each analysis included a set of plausible generalized linear and nonlinear (e.g., breakpoint and general additive models) relationships and tested for regional differences in slopes and intercepts. In each model set, the linear models formalized the hypothesis that human offtake causes additive changes in the rate of survival or population growth, and the breakpoint and general additive models formalized the hypothesis that the effects of offtake are partially or completely compensated. Both model sets included an intercept-only model, to evaluate the explanatory power of the best-supported models in comparison to a null hypothesis of no relationship between harvest and the dependent variable.

From the perspective of collating data for meta-analysis, we did not suspect that reporting bias against 'negative' results would be an important issue for the publication of data on rates of harvest, total mortality or population growth, because most of the original studies were descriptive in nature, and for the Northern Rockies, raw data were reported in a standardized fashion in annual reports. For most of the original studies, it is likely that some wolves were killed illegally and not reported. Because illegal killing cannot be quantified, our analyses are based on reported offtake (which is a rational basis for management decisions about wolf harvest quotas). For data from NRM populations [1], we included 'missing' radiocollared animals (but not known dispersers) in the number of total deaths. With this method, any undetected illegal killing of a radiocollared wolf would contribute to the estimated total mortality rate but not to the estimated human killing rate. However, the number of missing wolves was a small proportion (typically 5–10%) of known mortality, and large carnivores go missing for reasons other than illegal killing (e.g., failure of VHF transmitters, long distance dispersal, natural mortality with transmitter damage). For non-NRM populations, methods of monitoring varied, so the extent and direction of biases due to unreported illegal killing is unknown. Issues related to unreported harvesting and the dynamics of wolves merit further study.

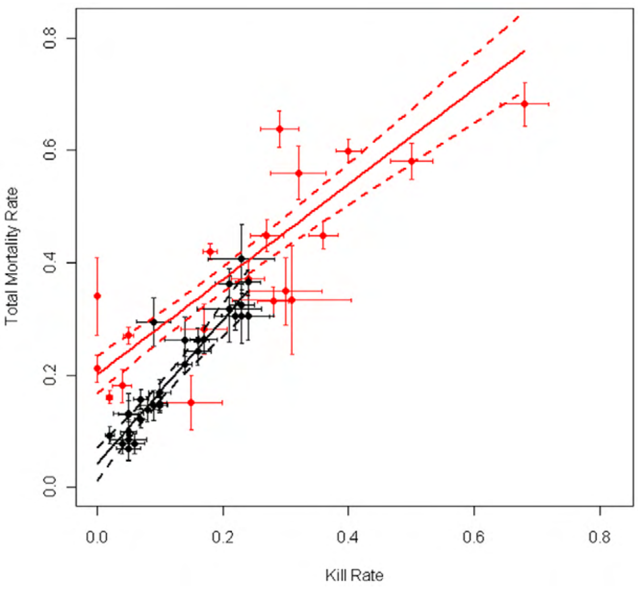


**Figure 1. The relationship between total annual mortality and human offtake for wolves in the Northern Rocky Mountains Recovery Area (black) and other populations (red).** Points are annual means for the Northern Rocky Mountains data, and multi-year means for other populations. The bars on each point show one standard error. The relationships shown are from the best-supported model in Table 1, a linear relationship with separate slopes and intercepts for the two subsets of data. Dashed lines show 95% confidence bands, accounting for overdispersion by multiplying the variance by the inflation factor (c-hat) from the best-supported model.
doi:10.1371/journal.pone.0012918.g001

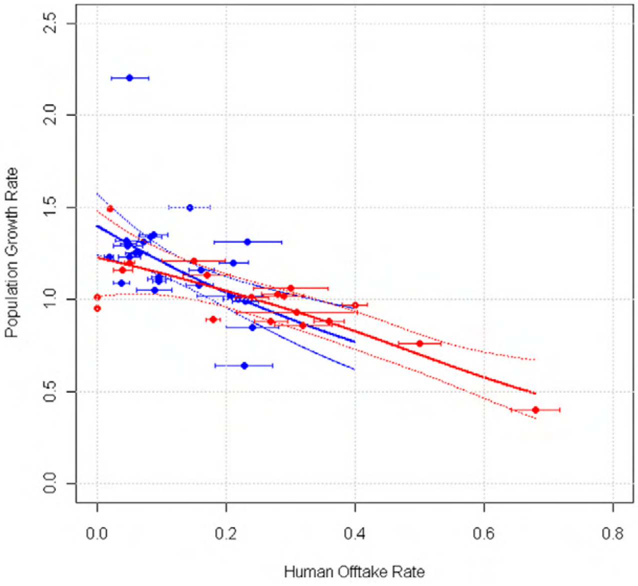


**Figure 2. The relationship between population growth (λ) and annual human offtake for wolves in the Northern Rocky Mountains Recovery Area and other populations.** Points show annual means for the Northern Rocky Mountains (blue), and multi-year means for other populations (red). Bars show one standard error. Because three models were similarly supported by the data (Table 3), solid lines show the model-averaged function based on all models with Akaike weights ≥0.01. Dashed lines show 95% confidence bands for the model-averaged functions. Blue: Northern Rocky Mountains. Red: Other populations.
doi:10.1371/journal.pone.0012918.g002

## Results and Discussion

### Human Offtake and the Annual Mortality Rate

There is a strong association between human offtake and total mortality rate across North American wolf populations. The best-supported model of the relationship between total mortality and human caused mortality was linear, with slopes that differed for wolves in the NRM and elsewhere (Table 1). Human-caused mortality has been lower for NRM wolves than in most other populations (Fig. 1) but has exceeded 20% killed in some years through predator control, while under Endangered Species Act regulations. From the best model (Fig. 1), $\frac{\hat{\beta}}{1-\hat{m}(0)}$ was 1.34 for NRM wolves (96% CI: 1.11 to 1.56, after inflating variances to account for estimated overdispersion) and 1.06 (95% CI: 0.92 to 1.20, again adjusted for overdispersion) for other populations (Table 2). These results suggest that mortality due to humans was not compensatory but highly additive or even super-additive. Super-additivity might be expected from the consequences of breeder mortality in wolves [15]. In a study of 10 populations, pup survival declined with decreasing pack size, 38% of packs disbanded following loss of a breeder, and only 47% of packs that lost a breeder reproduced in the subsequent year (9% reproduced after loss of both breeders) [15]. These consequences of social disruption are sufficiently large to compound the direct effect of mortality due to hunting, particularly when packs are small, so that a high proportion of adults are breeders. In 2008, 120 (69%) of 173 packs in the NRM held 4 or fewer adults [1], so that randomly killed adults would have ≥50% probability of being breeders. If these mechanisms do underlie super-additivity, the full effects of harvesting might not be manifest until the following year (or longer).

Models of compensatory mortality predict that the total mortality rate is initially constant as harvest increases, and then begins to rise above a threshold harvest rate equal to $\hat{m}(0)$. Contrary to this prediction, models with a change in slope (breakpoint and general additive models) did not fit the data well as linear models (Table 1). A general additive model fit only slightly worse than the linear model (Table 1), but its curvature was slight, and in the direction opposite that predicted by a model of compensatory mortality. These results provide further evidence that human-caused mortality was additive rather than compensatory. Finally, harvest can only be compensatory (in the sense of 'competing risks') when the rate of offtake is less than or equal to the rate of mortality in the absence of harvest, $\hat{m}(0)$, but mortality rates in the absence of harvest are low for wolves (as for most long-lived large mammals). Using estimates from the best model (Table 2), $\hat{m}(0)$ was 0.04±0.015 (SE) for the NRM and

**Table 1.** (A) Comparison of models of the relationship between total annual mortality and human-caused mortality for wolves in North America.

| Model description[1] | Log Likelihood | K[2] | QAICc[3] | ΔQAICc | ω[4] |
|---|---|---|---|---|---|
| i. Regional intercept & slopes | −225.13 | 5 | 122.31 | 0.00 | 0.69 |
| ii. Gen additive model by region | −212.17 | 9.02 | 123.88 | 1.57 | 0.31 |
| iii. Breakpoint model by region | −310.69 | 5 | 164.99 | 42.68 | 0.00 |
| iv. Common intercept & slope | −354.55 | 3 | 182.87 | 60.56 | 0.00 |
| v. Common breakpoint model | −378.79 | 3 | 194.96 | 72.65 | 0.00 |
| vi. Single intercept only | −965.62 | 2 | 485.70 | 363.40 | 0.00 |

[1] Expanded model descriptions:
(i) Generalized linear model (binomial errors with identity link) that allowed different slopes and intercepts for the relationship between total mortality and human offtake for two regions (wolves in the Northern Rocky Mountains (NRM) recovery area and wolves in previously-studied populations),
(ii) General additive model that allowed regional differences, fit in the 'mgcv' package of R with cross-validation used to determine the optimum amount of smoothing. GAM models allow curvilinear functions if the data support curvature.
(iii) Generalized linear model (binomial errors with identity link) that allowed the slope to change at a breakpoint and allowed regional differences,
(iv) Generalized linear model (binomial errors with identity link) with no regional effect.
(v) Generalized linear model (binomial errors with identity link) that allowed the slope to change at a breakpoint with no regional effect,
(vi) Constant total mortality (no effect of human offtake on total mortality).

[2] Number of parameters in the model (non-integer values are expected for general additive models).

[3] *QAICc calculated using c-hat = 4, the estimated overdispersion value obtained from a quasi-binomial model and using the number of mortality rates ($N = 48$) as the sample size.

[4] Akaike model weight.

doi:10.1371/journal.pone.0012918.t001

0.20±0.017 for other populations, so there was little scope for harvest mortality to be compensatory, especially for NRM wolves.

A recent re-analysis of the data for non-NRM populations [16] also concluded that 'human take does not share a compensatory interaction with natural mortality', because natural mortality did not decline with increasing human offtake. A recent analysis of the correlates of mortality in a large sample of radiocollared NRM wolves [17] reported that human killing accounted for a minimum of 54% of wolf mortality between 1982 and 2004, but did not directly test the relationship between human offtake and total mortality.

In studies that examine responses to harvest at a relatively small spatial scale, immigration can compensate for mortality due to harvest [10,16]. However, this mechanism is fundamentally different than compensatory reductions in non-harvest mortality, because compensatory immigration simply involves movement of individuals onto a study site from locations off of the study site. When we consider the dynamics of the entire population, this movement does not truly compensate for harvest mortality, because gains in one pack are offset by losses in another. Indeed, if dispersing wolves have lower rates of survival than pack-living wolves (as in other social carnivores [18]), then an increase in dispersal would further reduce mean survival for the population as a whole, rather than compensating.

**Table 2.** Intercepts and regression coefficients from the best model of total mortality as a function of human-caused mortality in North American wolf populations (see Table 1 for model selection using QAICc scores).

| Parameter | Estimate | Std. Error | Lower 95% C.L. | Upper 95% C.L. |
|---|---|---|---|---|
| ***Intercept*** $\hat{m}(0)$ | | | | |
| Northern Rocky Mountains | 0.041 | 0.015 | 0.011 | 0.071 |
| Other Populations | 0.200 | 0.017 | 0.167 | 0.234 |
| ***Slope*** $\hat{\beta}$ | | | | |
| Northern Rocky Mountains | 1.285 | 0.127 | 1.036 | 1.534 |
| Other Populations | 0.849 | 0.069 | 0.714 | 0.983 |

This is a generalized linear model (binomial errors, identity link) with a linear relationship between total mortality and human-caused mortality, and regional differences in the parameters of this relationship.

doi:10.1371/journal.pone.0012918.t002

## Human Offtake and Wolf Population Growth Rates

Given that mortality due to hunting was strongly additive or super-additive, we tested the effect of harvest on population growth rates, an analysis that incorporates the possibility that reproduction might increase to offset human-caused mortality. The literature on wolf harvesting includes many estimates of the proportion of a wolf population that must be killed to reduce wolf numbers. These studies often conclude that a harvest of 28%–50% of a wolf population is required to make a population decline. For example, Mech (2001) stated that "wolf populations can sustain annual winter harvest rates of 28%–47%" (p. 74), and "it is important for all to recognize that a moderate to large kill of wolves from the general population will have little limiting or reducing effect on the population" (p. 75) [14]. Adams et al. [16] concluded that "population trends were not correlated with annual human take ≤29%" (p. 1). With respect to policy, the 2003 delisting decision by the USFWS [2] stated that "the levels of documented human-caused mortality in the Northern Rocky Mountains have not, at this time, been significant enough to cause declines in the wolf population *or to slow overall wolf population growth*" ( p. 15851, emphasis added). Mirroring these conclusions, state management plans for NRM wolf populations [3,5] state that "wolf populations can apparently withstand human-caused mortality of 28%–50% without declining" (Idaho) and "wolf populations can apparently withstand human-caused mortality rates of 28%–35% without declining" (Montana). Why the state policies identify different upper limits is not clear, but the policies concur that harvests up to 35% are sustainable. The federal policy goes further, stating that human offtake has not slowed population growth in NRM wolves.

**Table 3.** Comparison of models of the relationship between annual population growth and human-caused mortality for wolves in North America.

| Model description[1] | Log Likelihood | K[2] | $R^2$adj[3] | ΔAICc | ω[4] |
|---|---|---|---|---|---|
| i. General additive model by region | 20.63 | 6.15 | 0.59 | 0.00 | 0.63 |
| ii. Common intercept & slope | 15.92 | 3 | 0.53 | 1.40 | 0.31 |
| iii. Regional intercept & slopes | 16.64 | 5 | 0.52 | 4.91 | 0.05 |
| iv. Regional intercepts, no slopes | 2.29 | 3 | 0.14 | 28.66 | 0.00 |
| v. Single intercept only | −1.51 | 2 | 0.00 | 33.96 | 0.00 |

[1] Expanded model descriptions:
(i) General additive model (GAM) that allowed regional differences, fit in the 'mgcv' package of R with cross-validation used to determine the optimum amount of smoothing. GAM models allow curvilinear functions if the data support curvature.
(ii) General linear model (normal errors with log link) with no regional effect on slope and intercept.
(iii) General linear model (normal errors with log link) that allowed regional differences in the slope and intercept.
(iv) Constant total mortality (no effect of human offtake on total mortality), with regional differences.
(v) Constant total mortality (no effect of human offtake on total mortality).
[2] Number of parameters in the model (non-integer values are expected for general additive models).
[3] The coefficient of determination ($R^2$) adjusted for degrees of freedom.
[4] Akaike model weight.
doi:10.1371/journal.pone.0012918.t003

We evaluated these statements using information theory to compare models of population growth ($\lambda$) as a function of human harvest, for NRM wolves and other populations (Table 3). All models supported by the data (Table 3) showed that population growth declined across all observed levels of human-caused mortality, which included low levels (Fig. 2). Because three models had reasonable support from the data (Table 3), we used model averaging (Figs. 2 & 3) to estimate the maximum offtake expected to yield $\lambda \geq 1$. For NRM wolves, the maximum stable offtake was 0.224 (model-averaged 95% CI: 0.177–0.335). For other populations, the maximum stable offtake was 0.245 (model-averaged 95% CI: 0.149–0.343). These estimates coincide well with the simple observation that NRM wolf populations have declined three times in the past decade, in each case with human harvests of 23%–24% (Fig 2). Better understanding of harvest effects can help managers achieve population goals. In July 2010, the Montana Fish Wildlife and Parks Commission approved an increase in the wolf harvest from 75 to 186 wolves. On the basis of internal analysis, the Montana Department of Fish Wildlife & Parks predicted that this harvest would, in combination with predator control killing continuing at past levels, cause a 13% decrease in wolf numbers. A harvest of 186 wolves together with 145 killed through predator control would yield a total offtake of 331 wolves, or 63% of the Montana population (which was estimated to number 524 at the end of 2009). The data in Fig 2 suggest that a direct killing rate of 0.63 would typically produce a decline substantially greater than 13%.

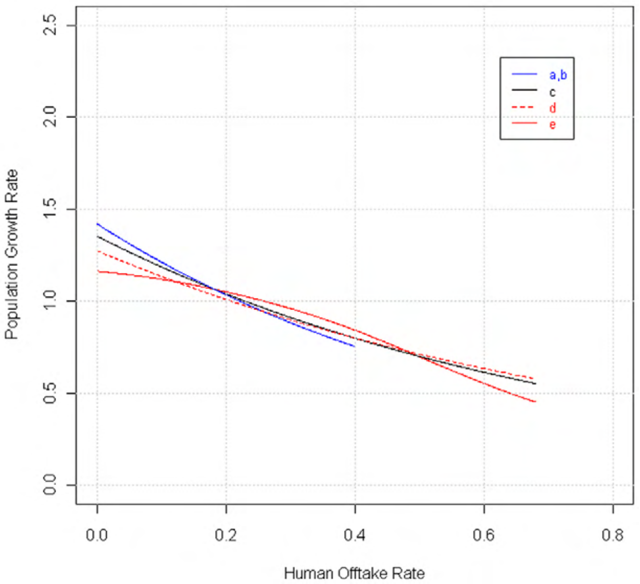


**Figure 3. The individual models that were averaged to produce the functions in Figure 2 were highly congruent in their estimates of the offtake that yields $\lambda = 1$.** a,b: GLM and GAM for Northern Rockies (these models were identical), c: GLM for all data combined, d,e: GLM and GAM for other populations.
doi:10.1371/journal.pone.0012918.g003

Because wolf populations in the Northern Rocky Mountains have grown since reintroduction, we tested whether growth slowed as population sizes increased. Overall, the NRM population has increased 15-fold over the past 15 years, providing unusually broad scope to test for density-dependent changes in the growth rate. Despite this, population growth was not detectably related to population size in the Northern Rocky Mountains ($\hat{\beta} = -0.06 \pm 0.15$ S.E., Wald statistic = 0.19, P = 0.66), and a model of linear density dependence was 5.5 AICc units worse than a model of linear harvesting effects on population growth. Density-dependence underlies compensation, so these observations reinforce the expectation that harvesting is not likely to increase reproduction or decrease natural mortality by reducing competition for resources, within the range of wolf densities seen to date. Although the data to date do not reveal clear density dependence, simply inspecting the growth curve gives some indication that NRM population growth may have slowed since 2007 [1]. If so, a reduced growth rate might indicate the incipient emergence of density dependent growth driven by resource competition. Contrary to this hypothesis, the survival of radiocollared NRM wolves increased with population density [17], rather than decreasing as would be expected with density dependent growth. Slower growth since 2007 could also be due to increased offtake by humans, if the rate of offtake is positively related to population density ($\hat{\beta} = 0.08 \pm 0.05$ S.E., Wald statistic = 2.69, P = 0.10). Between 1982 and 2004, human killing accounted for a minimum of 54% of total mortality for radiocollared NRM wolves [17], revealing that human offtake was more strongly limiting than all other factors combined, at least with respect to survival. (Anthropogenic effects are a dominant limiting factor for many large carnivores, world-wide.) If human offtake holds wolves at densities below the region's ecological carrying capacity, then it is plausible that density dependence will remain weak or equivocal.

Our analysis is based on comparison of multiple populations, rather than changes through time in a single population. Prior studies of human harvesting and its effect on wolf dynamics [10,16] were also based on comparison across populations, so the differences in our inferences are not due to this distinction. Comparisons across populations have a broader scope of inference than single-population studies, but correlations across populations can be affected by uncontrolled heterogeneity among sites. By including models that allowed different slopes and intercepts for NRM wolves and other populations, we incorporated heterogeneity to the degree possible with the data in hand. We encourage further research to test whether human offtake still appears to be largely additive or super-additive with hierarchical models that more completely account for differences among populations.

Our results confirm that wolf populations can grow while being harvested. However, point estimates for the maximum offtake rate associated with stable wolf populations are below the thresholds identified by recent state wolf management plans. Moreover, sustainable harvest is probably lower than our estimates, for two reasons. First, our models are based on deterministic estimates of population growth, which typically over-estimate true stochastic growth rates [19]. Second, estimated human offtake has an associated variance in these data (Fig. 2), and the effect of variance in an independent variable is to bias a regression's slope toward zero. For these reasons, we encourage further work on this topic, especially analysis with direct data on the survival of known individuals.

The management of wolves is controversial, and recent experience in the Rocky Mountains shows that any policy will face opposition from at least one constituency. Different stakeholders desire different numbers of wolves on the landscape. In structured decision-making it is important to isolate ecological analysis that considers the likely outcome of a policy from the discussion that considers whether or not that outcome is desirable [20]. Here, we have attempted to correct several broad misconceptions about the quantitative relationships between harvest intensity, mortality and population growth rates of wolves. The meta-analysis suggests that the effect of human-caused mortality on wolf dynamics is greater than suggested by current management plans (see references [21,22] for similar recent inferences about the role of human offtake in the dynamics of large felids including African lions, *Panthera leo*, and North American cougars, *Panthera concolor*). These results should help to inform wolf management, in conjunction with other important considerations about the interactions of wolves with ungulate prey, livestock, people, and ecosystems.

## Conclusions

In summary, it appears that: (1) Wolves can be harvested sustainably within limits. (2) Examined across populations, human killing of wolves is generally not compensatory, as has been widely argued. Management policies should not assume that an increase in human-caused mortality will be offset by a decline in natural mortality. (3) Rather, the effect of harvesting on wolf mortality appears highly additive to super-additive. Evidence for super-additive mortality is stronger for wolves in the recently-delisted Northern Rocky Mountains Recover Area, which often live in small packs. (4) Estimated sustainable harvest levels from this meta-analysis are lower than current Northern Rocky Mountain management plans suggest, and lower than the 2009 rate of offtake for the Northern Rockies. While some wolf populations might maintain constant population size at the harvest intensities considered sustainable by current state management plans, our results suggest that such harvests will generally cause wolf populations to decline. (5) The relationship of population growth rates to killing rates suggest that a proposed 2.5-fold increase in wolf harvest for 2010 is likely to reduce population size by a greater amount than management policy statements for Montana have stated. (6) The effects of harvesting on population growth may not be fully manifest in one year. These results should help with the development of policies for the management of wolves, particularly newly-delisted wolf populations in the Northern Rocky Mountains. The basic point that harvest mortality cannot be highly compensatory via substitution of mortality under conditions of low natural mortality (as in most long-lived species [12]) should be clearly expressed in policies for the management of large carnivores. Finally, these results highlight the ongoing need to fully incorporate quantitative analysis of available data in the development of conservation and management policies.

## Acknowledgments

We respectfully acknowledge the extensive field work conducted by all of the original studies of wolf mortality and population dynamics whose published data contributed to this meta-analysis.

## Author Contributions

Conceived and designed the experiments: SC. Performed the experiments: SC. Analyzed the data: SC JJR. Wrote the paper: SC JJR.

## References

1. US Fish and Wildlife Service (2009) Rocky Mountain Wolf Recovery 2008 Interagency Annual Report. 1-362. USFWS Rocky Mountain Gray Wolf Website (accessed 2010) http://www.fws.gov/mountain-prairie/species/mammals/wolf/annualrpt09/index.html.
2. US Fish and Wildlife Service (2009) Final Rule To Identify the Northern Rocky Mountain Population of Gray Wolf as a Distinct Population Segment and To Revise the List of Endangered and Threatened Wildlife. Federal Register 74: 15123–15188.
3. Montana Department of Fish Wildlife and Parks (2003) Montana Gray Wolf Conservation and Management Plan. Helena: Montana Fish Wildlife and Parks. 420 p.
4. Montana Department of Fish, Wildlife and Parks (2009) 2009 Montana Hunting Regulations: Wolf. Helena: Montana Fish Wildlife and Parks. 4 p.
5. Idaho Legislative Wolf Oversight Committee (2002) Idaho Wolf Conservation and Management Plan. Boise: Idaho Fish and Game. 85 p.
6. Idaho Department of Fish and Game (2009) 2009 Wolf Hunting Seasons and Rules. Boise: Idaho Fish and Game. 4 p.
7. Creel S, Christianson D, Liley S, Winnie J (2007) Effects of predation risk on reproductive physiology and demography in elk. Science 315: 960.
8. Hebblewhite M, Pletscher D, Paquet P (2002) Elk population dynamics in areas with and without predation by recolonizing wolves in Banff National Park, Alberta. Can J Zool 80: 789–799.
9. Hamlin K (2005) Montana Final Elk Management Plan. Helena: Montana Fish Wildlife & Parks. 397 p.
10. Fuller TK, Mech LD, Cochrane JF (2003) Wolf population dynamics. In: Mech LD, Boitani L, eds. Wolves: Behavior, Ecology and Conservation. Chicago: University of Chicago Press.
11. Williams BK, Nichols JD, Conroy MJ (2002) Analysis and Management of Animal Populations. London: Academic Press. 817 p.
12. Lebreton J-D (2005) Dynamical and statistical models for exploited populations. Aust and New Zealand J Stat 47: 49–63.
13. Haight RG, Travis LE, Nimerfro K, Mech LD (2002) Computer simulations of wolf removal strategies for animal damage control. Wildl Soc Bull 30: 844–852.

14. Mech LD (2001) Managing Minnesota's recovered wolves. Wildl Soc Bull 29: 70–77.
15. Brainerd SM, Andren H, Bangs EE, Bradley EE, Fontaine JA, et al. (2008) The effects of breeder loss on wolves. J Wildl Manage 72: 89–98.
16. Adams LG, Stephenson RO, Dale BW, Ahgook RT, Demma DJ (2008) Population dynamics and harvest characteristics of wolves in the central Brooks Range, Alaska. Wildl Monogr 170: 1–25.
17. Smith DW, Bangs EE, Oakleaf JK, Mack C, Fontaine J, et al. (2010) Survival of colonizing wolves in the northern Rocky Mountains of the United States, 1982–2004. J Wildl Manage 74: 620–634.
18. Creel S, Creel NM (2002) The African Wild Dog: Behavior, Ecology and Conservation. Princeton: Princeton University Press. 341 p.
19. Morris WF, Doak DF (2002) Quantitative Conservation Biology. Sunderland: Sinauer. 480 p.
20. Martin J, Runge MC, Nichols JD, Lubow BC, Kendall WL (2009) Structured decision making as a conceptual framework to identify thresholds for conservation and management. Ecol Appl 19: 1079–1090.
21. Packer C, Brink H, Kissul BM, Maliti H, Kushnir H, et al. (2010) Effects of trophy hunting on lion and leopard populations in Tanzania. Cons Biol (advanced online). DOI 10.1111/j.1525-1739.2010.01576.x.
22. Packer C, Kosmala M, Cooley HS, Brink H, Pintea L, et al. (2009) Sport hunting, predator control and conservation of large carnivores. PLoS ONE 4: e5941. DOI: 10.1371/journal.pone.0005941.

11-2004

# Predators in Natural Fragments: Foraging Ecology of Wolves in British Columbia’s Central and North Coast Archipelago

C. T. Darimont
*University of Victoria*

M. H. H. Price
*University of Victoria*

N. N. Winchester
*University of Victoria*

J. Gordon-Walker
*Raincoast Conservation Society*

P. C. Paquet
*University of Calgary*

---

## Recommended Citation

Darimont, C. T., Price, M. H. H., Winchester, N. N., Gordon-Walker, J., & Paquet, P. C. (2004). Predators in natural fragments: foraging ecology of wolves in British Columbia's central and north coast archipelago. Journal of Biogeography, 31(11), 1867-1877.

*Journal of Biogeography* (*J. Biogeogr.*) (2004) **31**, 1867–1877

ORIGINAL ARTICLE

# Predators in natural fragments: foraging ecology of wolves in British Columbia's central and north coast archipelago

C. T. Darimont[1,2]*, M. H. H. Price[1], N. N. Winchester[1], J. Gordon-Walker[2] and P. C. Paquet[2,3]

[1]*Department of Biology, University of Victoria, Victoria,* [2]*Raincoast Conservation Society, Bella Bella, B.C. and* [3]*Faculty of Environmental Design, University of Calgary, Calgary, Alberta, Canada*

*Correspondence: C. T. Darimont, Department of Biology, University of Victoria, PO Box 3020, Victoria, B.C. Canada, V8W 3N5.
E-mail: cdarimon@uvic.ca

## ABSTRACT

**Aim** Predator–prey dynamics in fragmented areas may be influenced by spatial features of the landscape. Although little is known about these processes, an increasingly fragmented planet underscores the urgency to predict its consequences. Accordingly, our aim was to examine foraging behaviour of an apex mammalian predator, the wolf (*Canis lupus*), in an archipelago environment.

**Location** Mainland and adjacent archipelago of British Columbia, Canada; a largely pristine and naturally fragmented landscape with islands of variable size and isolation.

**Methods** We sampled 30 mainland watersheds and 29 islands for wolf faeces in summers 2000 and 2001 and identified prey remains. We examined broad geographical patterns and detailed biogeographical variables (area and isolation metrics) as they relate to prey consumed. For island data, we used Akaike Information Criteria to guide generalized linear regression model selection to predict probability of black-tailed deer (main prey; *Odocoileus hemionus*) in faeces.

**Results** Black-tailed deer was the most common item in occurrence per faeces (63%) and occurrence per item (53%) indices, representing about 63% of mammalian biomass. Wolves consumed more deer on islands near the mainland (65% occurrence per item) than on the mainland (39%) and outer islands (45%), where other ungulates (mainland only) and small mammals replaced deer. On islands, the probability of detecting deer was influenced primarily by island distance to mainland (not by area or inter-landmass distance), suggesting limited recolonization by deer from source populations as a causal mechanism.

**Main conclusions** Although sampling was limited in time, consistent patterns among islands suggest that population dynamics in isolated fragments are less stable and can result in depletion of prey. This may have important implications in understanding predator–prey communities in isolation, debate regarding wolf–deer systems and logging in temperate rain forests, and reserve design.

**Keywords**
**Archipelago, British Columbia, black-tailed deer, *Canis lupus*, conservation, foraging, fragmentation, islands, *Odocoileus hemionus*, wolf.**

## INTRODUCTION

Islands have been considered natural laboratories to study evolutionary and ecological process (Gorman, 1979; Williamson, 1981). Investigations of oceanic archipelagos have revealed how island communities are related to area, isolation and other island characteristics (e.g. MacArthur & Wilson, 1967; Abbott, 1974; Kadmon & Pulliam, 1993; Conroy *et al.*, 1999). Biogeographical features, however, may also exert influence at the population level, including the mediation of predator–prey dynamics on islands or in other fragmented systems (Kareiva, 1990; Kareiva & Wennergren, 1995; Dolman & Sutherland, 1997). A predator's niche breadth can be predicted by the diversity and abundance of potential prey species (MacArthur & Pianka, 1966), which may differ among islands. The limitations imposed by island geography, for example, may restrict prey species available to predators. For some consumers, however, foraging constraints on small or isolated oceanic islands may be mitigated by nutrient subsidies from the ocean, as recent investigations have revealed the coupled nature of marine-terrestrial ecosystems (Polis & Hurd, 1995; Rose & Polis, 1998; Reimchen, 2000).

The amplitude of predator and prey fluctuations reflect ecological conditions (Ricklefs, 1990), which may be unique in archipelagos or in other systems fragmented naturally or by humans. Predator–prey dynamics in isolation may be volatile, resulting in large amplitudes of predator and prey, or the extirpation of predator, prey, or both (Taylor, 1984). For example, the wolf-moose (*Canis lupus – Alces alces)* system on 540 km$^2$ Isle Royale, Michigan, is separated by 36 km to the mainland. There, wolves and moose have experienced extreme fluctuations in abundance that is at least partially associated with their interaction (Peterson *et al.*, 1984; Peterson & Page, 1988; Vucetich & Peterson, in press). Similar process has also been examined with smaller taxa in experimental designs. Populations of an herbivorous spider mite (*Tetranychus urticae*) and a predatory mite (*Phytoseiulus persimilis*) are highly unstable on isolated bean plants (*Phaseolus lunatus*) and can ultimately result in extinction of both (McCauley *et al.*, 2000).

Knowledge about predator–prey dynamics in patchy landscapes is valuable because the planet is becoming increasingly fragmented by human activities (Saunders *et al.*, 1991; Fahrig, 1997, 2003). Moreover, predators are more likely to decline or become extinct in fragments (Woodroffe & Ginsberg, 1998), possibly resulting in mesopredator release and other ecosystem-wide consequences (Crooks & Soulé, 1999; Terborgh *et al.*, 2001). Conversely, even the effects of native predators on endangered prey can be severe in fragmented environments (Schneider, 2001). Consequently, archipelagos may provide model systems in which to predict the effects of size and isolation on predator–prey dynamics.

The temperate rain forest archipelago of British Columbia (BC) is an ideal system in which to address the influence of area and isolation of fragments on predator–prey systems. This remote and nearly pristine region is naturally fragmented, comprised of dozens of islands < 0.1 to > 13 km apart (Fig. 1). Here, the wolf-black-tailed deer (*Odocoileus hemionus*) association forms the dominant mammalian predator–prey system, in which both animals can occupy all islands, at least ephemerally (Darimont & Paquet, 2000, 2002). Herein, we examine spatial variability of resource use during spring and summer by examining wolf faeces from BC's central and north coast mainland and 29 islands of the adjacent archipelago. Theory of predator–prey systems in fragments suggests that area and isolation effects can strongly influence population dynamics, including processes associated with the depletion of prey (Kareiva, 1990; Kareiva & Wennergren, 1995; Dolman & Sutherland, 1997; McCauley *et al.*, 2000). Moreover, the marine-terrestrial interface and the heterogeneous landscape of our study area offer a broad potential niche to wolves. Accordingly, on smaller and/or more isolated islands, we predicted a departure from a diet dominated by their main prey (deer) to one that includes considerable use of alternative resources. Our objectives herein are to identify prey species consumed by wolves of British Columbia's archipelago during spring and summer and to examine variability in wolf foraging behaviour as it relates to area and isolation of islands.

## METHODS

### Study area

We collected wolf faeces on BC's coast between the Kshwan Valley (55 °37′ N, 129 °48′ W) in the north and the Koeye River (51 °46′ N, 127 °53′ W) in the south (Fig. 1). This large, nearly roadless, and mostly unsettled region is bounded by the Coast Mountain range and Pacific Ocean to the east and west, respectively. Most of the low elevation forest is within the Coastal Western Hemlock biogeoclimatic zone (Pojar & Mackinnon, 1994). Habitat heterogeneity in these temperate rain forests corresponds to landscape variability, which includes the following general regions: mountainous mainland, topographically complex inner islands, and flatter outer islands. Island sizes range from 5.0 km$^2$ (Moore) to 2295 km$^2$ (Princess Royal), distances to mainland 250 m to 13.05 km, and distances among landmasses 0.05–7.25 km (Fig. 1).

Potential prey base is diverse, including black-tailed deer, moose, mountain goat (*Oreamnos americanus*), beaver (*Castor canadensis*), black bear (*Ursus americanus*), river otter (*Lontra canadensis*), plus smaller mustelids, rodents and birds. Five species of spawning salmonids (*Onchorynchus* spp.), crustaceans, molluscs and marine mammals are also available to wolves (Darimont & Paquet, 2000, 2002), although salmon were not yet spawning widely when sampling occurred.

### Faecal collection

During June and July 2000, and June and August 2001, we collected faeces in 30 mainland watersheds and on 29 islands (typically one to two sites per island). Sampling sites were selected non-randomly but were well distributed throughout the study area (Fig. 1). At each location, we surveyed beaches, estuaries and forests of the beach fringe, often on wildlife trails. We also surveyed logging roads when encountered, circumnavigated beaver ponds, and walked forest ridgelines. Surveys rarely extended > 5 km inland.

We stored faeces in plastic bags and froze them until analysis at the University of Victoria. Faeces can decompose rapidly in this wet environment (Wallmo *et al.*, 1962; C.T. Darimont *unpublished data*). Therefore, we assumed the samples represented late spring and summer diets of wolves.

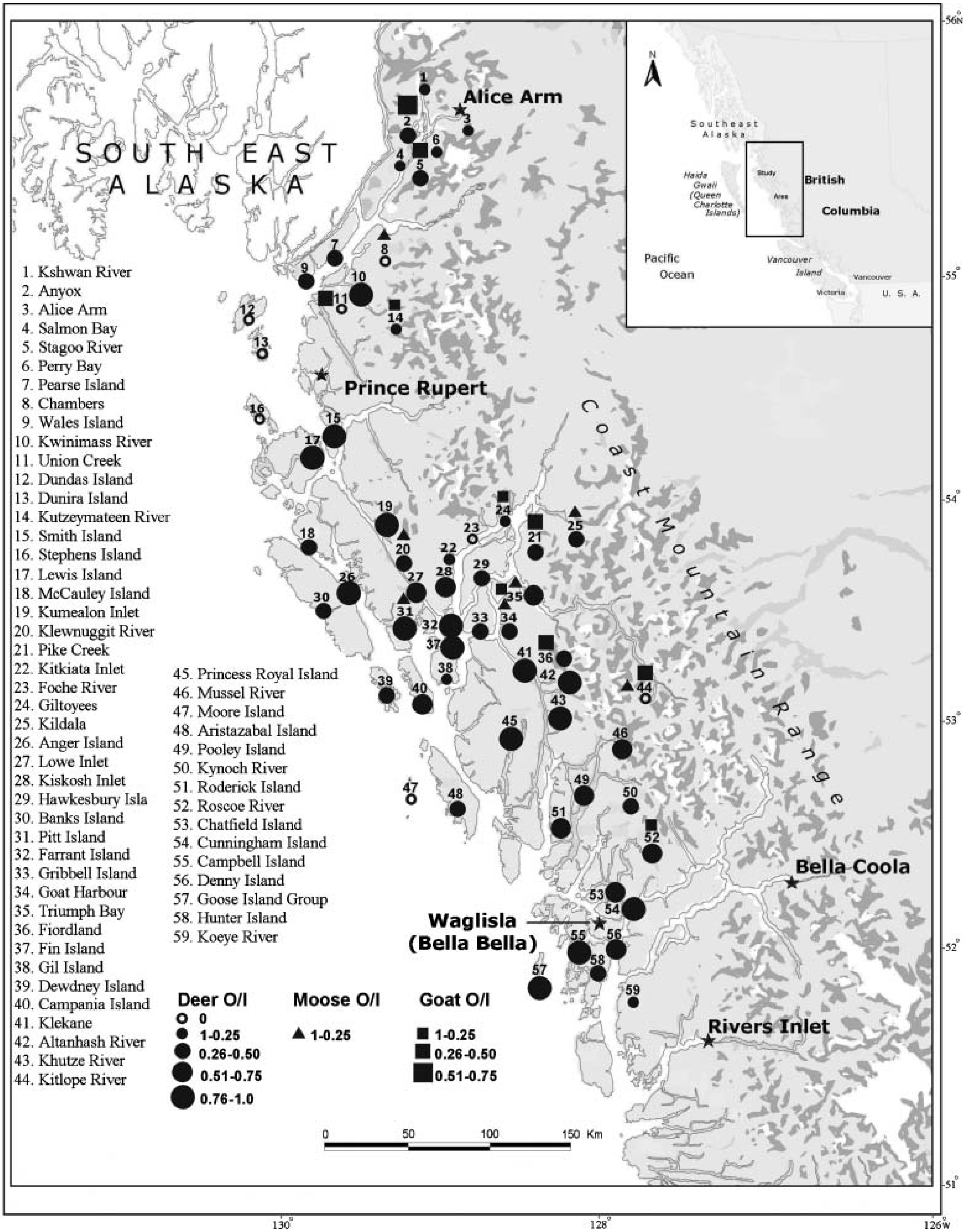


**Figure 1** Study area where wolf (*Canis lupus*) faeces were collected in coastal British Columbia, Canada, during summers 2000 and 2001. Fifty-nine sampled islands and mainland watersheds numbered. Sampling extended from the Kshwan River (1) in the north to the Koeye River (59) in the south. Also shown are symbols denoting occurrence per item data for ungulates consumed (deer, *Odocoileus hemionus*; moose, *Alces alces* and goat, *Oreamnos americanus*).

### Prey item identification and reporting

Identification of prey followed Ciucci *et al.* (1996) and Kohira & Rexstad (1997). Samples were autoclaved, then soaked and rinsed in a 1 mm mesh sieve until only hair, bone fragments and other macroscopic components remained. Mammalian prey was identified by comparing hair in faeces with voucher samples and use of dichotomous keys and dissecting microscope (magnification 20–40×; Mathiak, 1938; Mayer, 1952; Stains, 1958). When identification was uncertain ($n = 60$ scats), scale imprints from a few guard hairs melted in acetate were examined using a compound microscope (magnification 40–400×). Non-mammal prey, such as fish, bird, and marine invertebrates, were identified by bones, teeth, feathers and shell fragments. Birds and small rodents (i.e. rodents smaller than beaver) were not identified further than class and order respectively. To eliminate inter-observer bias, only one person identified prey remains (MP) and only after a lengthy training period (*c.* 40 h). We estimated precision by re-sampling approximately 10% of samples ($n = 59$), in which prey remains were consistently identified in 58 cases (98%).

We report occurrence per faeces (O/F) index for comparison with published literature but use the occurrence per item (O/I) index in statistical tests as the former can be problematic because it exceeds unity when summed (Kohira & Rexstad, 1997). These two metrics are important when faeces often contain more than one item (see Results). O/F is the frequency by which an item occurs in faecal samples, whereas O/I is the item's frequency among all items identified in all faeces combined. We also estimated mammalian biomass consumed using a regression equation estimated by Weaver (1993): $Y = 0.439 + 0.008X$, where $Y$ is the estimated biomass of prey consumed per faecal sample and $X$ is the mass of prey. Although masses may differ among age and sex classes, and wolves may kill unequal ratios of these classes, we used mean masses of adults reported in Cowen & Guiguet (1975) and assumed a 1 : 1 sex ratio. For deer, however, we distinguished between adults and fawns for biomass calculations using diagnostic hair diameter and colour characters, which are useful until the autumn (Scott, 1979). By necessity, biomass estimates excluded non-mammalian prey ($n = 124$ of 705 items identified).

### Statistical analyses

#### *General geographical patterns in foraging ecology*

We tested for general geographical patterns in foraging ecology among three areas that are associated with general habitat differences: mainland, inner islands, and outer islands. We defined inner islands as those directly adjacent to the mainland and outer islands as those that are not, irrespective of distance to mainland. This classification is consistent with mainland, southern inner island, and southern outer island biogeographical sub-regions defined in southeast Alaska, which are based on presence of endemic species and unique combinations of native taxa (MacDonald & Cook, 1996). We compared O/I indices for deer, other ungulates (moose, goat), and small mammals among these areas using ANOVA or Kruskal–Wallis tests. We repeated these tests with indices relating to the proportion of total biomass represented by these taxa.

#### *Examining area and isolation effects on islands*

General geographical patterns, although informative, cannot adequately address the influence of area and isolation on predator–prey dynamics. For example, an inner island, next to the mainland, may be more isolated from other landmasses by water barriers compared with a collection of nearby outer islands. Thus we examined how biogeographical parameters, area (AREA), distance to mainland (MDIST), and inter-landmass distance (LDIST) affected the probability of deer occurring in faecal remains on islands. We used these two isolation metrics to disentangle the possible influences of distance from the mainland, which may be the ultimate source for prey colonization, and distance to other landmasses, which likely provide the most available sources for prey colonization.

We measured MDIST as the shortest island-to-mainland distance or sum of island-to-island distances to mainland excluding distances across islands, whichever was shorter (Conroy *et al.*, 1999). LDIST was the minimum distance to landmasses > 75 km$^2$ (either mainland or island), roughly the size of Coronation Island, in nearby southeast Alaska, on which a small population of wolves existed for 8 years (Klein, 1996). All geographical parameters were estimated using marine charts (Canadian Hydrographic Service, Ottawa, Ontario) and Geographic Information Systems (Darimont & Paquet, 2002).

We formed exploratory *a priori* hypotheses to explain how these biogeographical features would affect the probability of deer occurring in wolf faeces on islands, which were based on our knowledge of the area and ecological theory described above. From these hypotheses, we developed a set of candidate generalized linear regression models (binary logistic form). These were restricted to combinations of one to three of the identified (and untransformed) parameters and two-way interaction terms. We considered islands as the experimental unit, with the number of faeces containing deer as events and the total number of faeces as trials. A Hosmer–Lemeshow goodness-of-fit statistic based on the global model showed the data did not depart from a logistic-regression model ($P = 0.475$). Multicollinearity diagnostics suggested only weak interdependencies among predictor variables (Variance Inflation Factors range: 1.076–1.709). For each model, we calculated Akaike Information Criteria, adjusted for small sample sizes (AIC$_c$), following the formula: $\mathrm{AIC}_c = -2(\log \text{likelihood}) + 2K + 2K(K + 1)/(n - K - 1)$, where $K$ is the number of parameters and $n$ the number of sampled islands. We then evaluated $\Delta\ \mathrm{AIC}_c$ to select best approximating model(s) and make appropriate inference, using $\Delta\ \mathrm{AIC}_c < 2$ to describe the top model set (offering substantial level of empirical support). Finally, we summed Akaike weights ($\omega_i$) across the top model set for each variable to rank them by importance (Burnham & Anderson, 1998; Anderson *et al.*, 2001). Tests were performed using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) and SAS 8 (SAS Inc., Cary, NC, USA).

## RESULTS

Coastal wolves showed a wide dietary niche across the heterogeneous landscape (Table 1). We collected a mean of 8.77 samples at mainland sites (range 1–36; SD = 1.65; $n = 263$) and 11.45 at island sites (range 1–64; SD = 3.24; $n = 332$). Of 705 food items identified, black-tailed deer was the most common item in both occurrence/faeces and occurrence/item indices, followed by salmon, mountain goat, bird, mustelids, intertidal organisms, black bear, beaver, mink, moose and small mammals (Table 1). Biomass estimates demonstrated a different order of occurrence, with ungulates (deer, goat, moose) representing a combined 82.3% of mammal biomass consumed (Table 1). Grizzly bear (*Ursus arctos*), fisher (*Martes pennanti*), harbour seal (*Phoca vitulina*) and wolf each occurred once. We could not identify six items.

Foraging patterns of wolves differed among areas. Fifteen species occurred in mainland samples and 13 in island samples. Mountain goat, grizzly bear and fisher were found exclusively at mainland locations, whereas the sample containing seal was collected on an island. Goat remains were restricted to areas in or near rocky inlets, whereas moose remains, although near inlets, had a greater distribution, including on one island (Fig. 1).

Differences we observed in detection of deer and non-deer prey provide evidence of major changes in predation regime among geographical areas. Generally, deer dominated the diet on inner islands whereas other ungulates (goat, moose) and small mammals collectively occurred approximately as often as deer at mainland and outer island sites. Differences in occurrence/item among mainland, island, and outer island sites for deer approached significance (ANOVA; $F_{2,56} = 2.967$, $P = 0.060$) and was highest on inner islands, but differed little between mainland and outer islands (Fig. 2a). Similarly, proportion of total mammalian biomass represented by deer also was highest on inner islands and varied little between mainland and outer islands sites (ANOVA; $F_{2,56} = 6.972$, $P = 0.002$; Fig. 2b). Other ungulates (moose, goat) represented significantly higher occurrence/item (Kruskal–Wallis *H*-test; $\chi^2 = 14.961$; $P = 0.001$; Fig. 2a) and proportion of mammalian biomass (Kruskal–Wallis *H*-test; $\chi^2 = 14.885$; $P = 0.001$; Fig. 2b) on mainland sites compared with extremely low or nil values on inner and outer islands respectively. In place of deer, wolves also foraged on small mammals, which generally occured more frequently at mainland and outer island sites compared with inner islands (Fig. 2a,b), but this difference was not significant in occurrence/item (Kruskal–Wallis *H*-test; $\chi^2 = 1.775$; $P = 0.412$) or proportion of total mammalian biomass (Kruskal–Wallis *H*-test; $\chi^2 = 1.120$; $P = 0.571$).

Model selection and multimodel inference suggest that among island sites, isolation was more important than area in predicting departure from a diet dominated by deer.

**Table 1** Prey items identified in 595 wolf (*Canis lupus*) faeces collected summers 2000 and 2001 on the mainland and archipelago of coastal British Columbia

| | Mainland sites | | | Island sites | | | All sites combined | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Prey taxa | *n* | O/F (%) | O/I (%) | *n* | O/F (%) | O/I (%) | *n* | O/F (%) | O/I (%) | Biomass (%) |
| *Odocoileus hemionus* (deer) | 124 | 47.1 | 39.5 | 250 | 75.3 | 63.9 | 374 | 62.7 | 53.0 | 64.6 |
| *Onchorynchus* spp. (salmon) | 21 | 8.0 | 6.7 | 25 | 7.5 | 6.4 | 46 | 7.7 | 6.5 | N/A |
| *Oreamnos americanus* (goat) | 37 | 14.1 | 11.8 | 0 | 0.0 | 0.0 | 37 | 6.2 | 5.3 | 9.1 |
| *Aves* (birds) | 15 | 5.7 | 4.8 | 22 | 6.6 | 5.6 | 37 | 6.2 | 5.3 | N/A |
| *Martes americana* (marten) | 20 | 7.6 | 6.4 | 14 | 4.2 | 3.6 | 34 | 5.7 | 4.8 | 2.9 |
| *Mustela erminea* (ermine) | 25 | 9.5 | 8.0 | 8 | 2.4 | 2.1 | 33 | 5.5 | 4.7 | 2.7 |
| *Lontra canadensis* (otter) | 10 | 3.8 | 3.2 | 15 | 4.5 | 3.8 | 25 | 4.2 | 3.6 | 2.1 |
| Intertidal organisms | 13 | 4.9 | 4.1 | 12 | 3.6 | 3.1 | 25 | 4.2 | 3.6 | N/A |
| *Ursus americanus* (bear) | 9 | 3.4 | 2.9 | 9 | 2.7 | 2.3 | 18 | 3.0 | 2.6 | 5.8 |
| *Castor canadensis* (beaver) | 6 | 2.3 | 1.9 | 10 | 3.0 | 3.0 | 16 | 2.7 | 2.3 | 2.1 |
| *Mustela vison* (mink) | 4 | 1.5 | 1.3 | 12 | 3.6 | 3.1 | 16 | 2.7 | 2.3 | 1.3 |
| *Alces alces* (moose) | 11 | 4.2 | 3.5 | 1 | 0.0 | 0.0 | 12 | 2.0 | 1.7 | 8.6 |
| Small rodents | 5 | 1.9 | 1.6 | 3 | 0.9 | 0.8 | 8 | 1.3 | 1.1 | 0.7 |
| Vegetation | 9 | 3.4 | 2.9 | 5 | 1.5 | 1.3 | 14 | 2.4 | 2.2 | N/A |
| Other* | 5 | 1.9 | 1.6 | 5 | 1.5 | 1.3 | 10 | 1.7 | 1.4 | N/A |
| Total | 314 | 119 | 100 | 391 | 117 | 100 | 705 | 118 | 100 | 100 |

*Other represents single occurrence of brown bear (*Ursus arctos*), wolf, seal (*Phoca vitulina*) and fisher (*Martes pennanti*), plus six unidentified remains.

*n*, Number of items; O/F, occurrence/faeces; O/I, occurrence/item.

Biomass estimates are proportion of total mammalian biomass. Taxa organised by decreasing O/F and O/I for all sites combined.

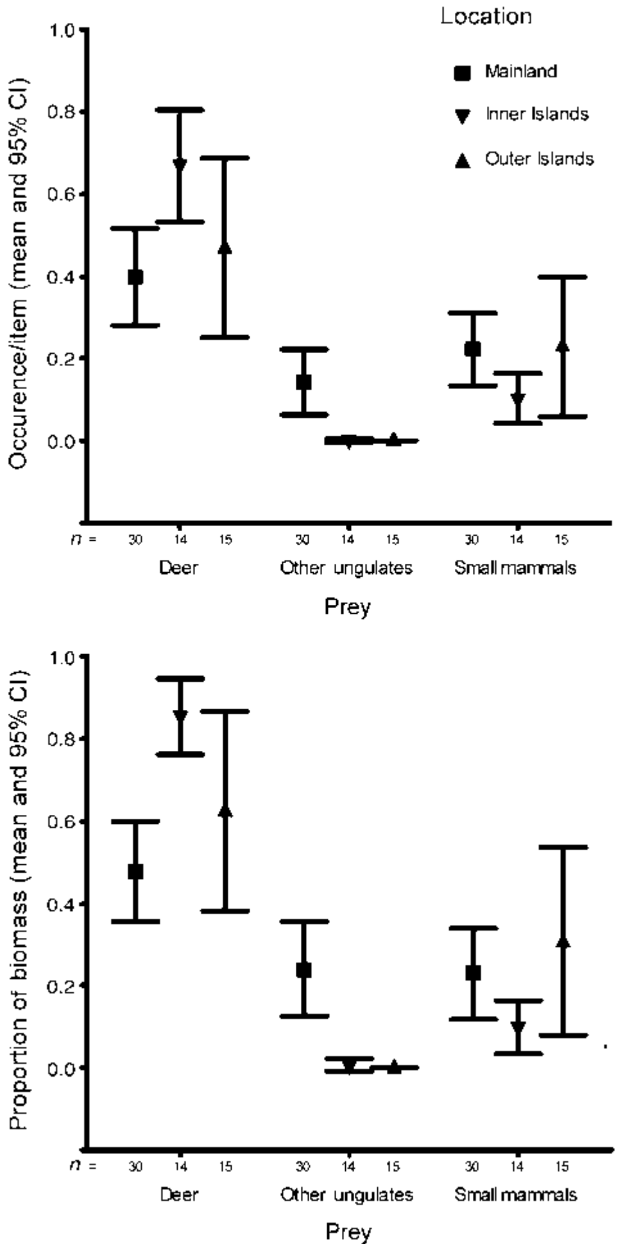


**Figure 2** (a) Mean occurrence per item and (b) mean proportion of total mammalian biomass of prey detected in 595 wolf (*Canis lupus*) faeces from 59 sampling locations on the islands and mainland coast of British Columbia, Canada, summers 2000 and 2001. 'Small mammals' included river otter (*Lontra canadensis*), marten (*Martes americanus*), (*ermine Mustela ermina*), mink (*Mustela vison*), and rodent (Rodentia spp.). 'Other ungulates' were goat (*Oreamnos americanus*) and moose (*Alces alces*). Deer are *Odocoileus hemionus.*

Specifically, the probability of wolves foraging on deer declined primarily with increased island isolation from the mainland, but less so with isolation from other landmasses and smaller island area (Table 2; Fig. 3). All five models in the top model set (0–2 $\Delta$ AIC$_c$) contained MDIST. Considerable ambiguity, however, existed among top models, which had similar Akaike weights ($\omega_{i=1 \text{ to } 5} = 0.21$ to 0.08; Table 2). Moreover, these top models explained a similar proportion of the variance (Nagelkerke $R^2$ range = 0.218–0.242; note that pseudo $R^2$ values for logistic regression are lower than would be expected in a linear model; Table 2). In cases when the data do not strongly support a single best model, however, the one with fewest parameters is often worth most consideration, following the rule of parsimony (Burnham & Anderson, 1998). Accordingly, we consider model 4, containing only the intercept and MDIST, as a preferred model (Table 2).

The top model set can still make robust multimodel inference (Burnham & Anderson, 1998); summing the Akaike weights across top models ranked the variable MDIST ($\Sigma\omega_i = 0.74$) higher than LDIST and AREA ($\Sigma\omega_i =$ 0.35 and 0.27) by factors of 2.11 and 2.74 respectively. Moreover, the strength of coefficients associated with isolation metrics was much higher than those for area, which approached zero (Table 2). Interaction terms MDIST × LDIST ($\Sigma\omega_i = 0.20$) and AREA × MDIST ($\Sigma\omega_i = 0.20$) were less important.

## DISCUSSION

Islands provide ideal model systems for studying predator–prey interactions (e.g. Peterson *et al.*, 1984). Often, however, isolated islands lack predators and even on less isolated islands mammalian carnivores are relatively rare (Williamson, 1981; Alcover & McMinn, 1994). Consequently, our knowledge of predator–prey dynamics in isolated systems is limited. Moreover, our ecological knowledge of coastal temperate rain forests of North America is in its infancy (MacDonald & Cook, 1996). Herein we examine the foraging ecology of BC's coastal wolves and provide additional insight into predator–prey dynamics in fragmented landscapes. Specifically, we partitioned the variability we observed to spatial features of the landscape, both on a gross geographical scale (mainland, inner and outer islands) and with finer resolution by disentangling the effects of area and isolation.

Across their holarctic distribution, wolves hunt a diverse suite of animals (Paquet & Carbyn, 2003; Peterson & Ciucci, 2003). Here we show high trophic diversity and variability among wolves within a single biome. On BC's coast, we observed a minimum of 14 terrestrial mammals, a marine mammal, salmon, birds and marine invertebrates in diet. We did not detect any 'species richness' effects (narrower dietary niche for wolves on isolated islands), perhaps because these prey taxa are not as sensitive as others to biogeographical effects of isolation. Prey detected across this landscape greatly exceeds the number of items identified in earlier studies in the same biome [Scott & Shackleton, 1980 (Vancouver Island; 3 items); Milne *et al.*, 1989 (Vancouver Island; 4 items); Kohira & Rexstad, 1997 (Southeast Alaska; 11 items)]. This difference may reflect our greater geographical span of sampling and a more rigorous laboratory protocol.

**Table 2** Top generalized linear regression model set to predict the probability of deer remains occurring in wolf faeces collected on islands of British Columbia ($n = 29$ islands), with model structure, deviance, and corresponding $\Delta$ AIC$_c$ and Akaike weight ($\omega_i$). MDIST is island distance to mainland, LDIST is island distance to another landmass > 75 km$^2$, and AREA is area of island. Shown also are parameter coefficients, their standard errors, and model Nagelkerke $R^2$

| Model form | Deviance | $\Delta$ AIC$_C$ | $\omega_i$ | $\beta_0$ | SE | $\beta_1$ | SE | $\beta_2$ | SE | $\beta_3$ | SE | $\beta_4$ | SE | Nagelkerke $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\beta_0 + \beta_1$(MDIST) + $\beta_2$(LDIST) + $\beta_3$(MDIST × LDIST) | 312.107 | 0.000 | 0.21 | 2.156** | 0.244 | −0.444** | 0.109 | −0.616* | 0.242 | 0.075* | 0.032 | – | – | 0.242 |
| $\beta_0 + \beta_1$ (MDIST) + $\beta_2$ (AREA) + $\beta_3$ (MDIST × AREA) | 312.184 | 0.077 | 0.20 | 1.593** | 0.243 | −0.317** | 0.104 | 0.001* | 0.000 | −0.001* | 0.000 | – | – | 0.242 |
| $\beta_0 + \beta_1$ (MDIST) + $\beta_2$ (LDIST) | 315.514 | 0.700 | 0.14 | 1.988** | 0.202 | −0.363** | 0.106 | −0.351 | 0.204 | – | – | – | – | 0.229 |
| $\beta_0 + \beta_1$ (MDIST) | 318.384 | 1.072 | 0.12 | 1.915** | 0.197 | −0.494** | 0.093 | – | – | – | – | – | – | 0.218 |
| $\beta_0 + \beta_1$ (MDIST) + $\beta_2$ (AREA) | 311.862 | 1.960 | 0.08 | 1.716** | 0.246 | −0.469** | 0.094 | −0.001 | 0.000 | – | – | – | – | 0.225 |

*$P < 0.05$, **$P < 0.01$.

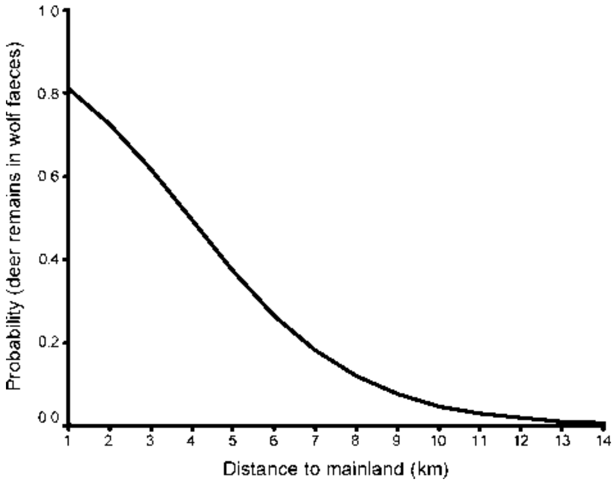


**Figure 3** Probability of deer (*Odocoileus hemionus*) remains occurring in wolf (*Canis lupus*) faeces on islands as a function of their distance to the mainland (MDIST). Samples collected in coastal British Columbia, summers 2000 and 2001. Equation: $Y = 1.915 - 0.494 \times \text{MDIST}$, which forms the simplest model in our top model set based on Akaike Information Criteria ranking.

Additional spatial and temporal aspects of our sampling may explain differences with previous studies. Sampling within 5 km of shoreline may explain the abundance of smaller prey consumed. For example, marine invertebrates accounted for approximately 4% of prey items we detected. In addition, river otter and mink, which accounted for a combined 5.9%, are primarily occupants of the beach fringe. Moreover, we detected beaver in < 3% of scats, which differs greatly from other North American studies in which they occurred much more commonly (Voigt *et al.*, 1976; Fuller, 1989; Thurber & Peterson, 1993). This too may reflect our sampling bias of omitting much of the inland area, and/or a naturally low abundance of beaver in this conifer-dominated landscape (McCabe, 1948). Also, because faecal samples represented late spring and summer diet, more than half a year of dietary information was excluded. Early runs of spawning salmon accounted for the second highest proportion of prey consumed by wolves (6.5%) and recent stable isotope and behavioural evidence suggest that salmon runs during late summer and autumn support a major seasonal shift in the foraging of coastal wolves (Szepanski *et al.*, 1999; Darimont & Reimchen, 2002; Darimont *et al.*, 2003).

Major changes in the predation regime occurred across broad geographical categories, possibly because of associated habitat differences. Wolves consume less deer in rocky mainland areas compared with nearby but less mountainous inner islands (Figs 1 & 2). This may relate to low deer biomass per area on the mainland where elevations > 1100 m are common, altitudes at which deer in nearby southeast Alaska are known not to occur (Schoen & Kirchhoff, 1985). Although no similar data exist for BC's coast, deer densities in southeast Alaska are lower on the mainland coast compared with adjacent inner islands (Kirchhoff, 1996). Alternatively or concomitantly, the lower observed frequency of deer in wolf diet on the mainland can be attributed to a more species-rich prey assemblage in mainland watersheds. For example, mountain goat and moose are two large terrestrial mammals that predominantly occupy mainland habitats and provided an additional food resource.

Predator–prey dynamics in fragmented landscapes may be determined by area of fragments, their isolation, and system-specific factors, which in coastal BC may include carrying capacity for deer. Similar to mainland areas, habitat on outer islands may support fewer deer. Thus, the lower frequency of this item in wolf diet may reflect lower availability. We consider this unlikely to serve as the full explanation. Similar outer islands, but those lacking wolves, in southeast Alaska and Haida Gwaii, BC, support (or supported before wolf introduction) high populations of deer (Reimchen *et al.*, in press; Kirchhoff, 1994, 1996; Klein, 1996). Likewise, on a recent survey of the Goose Group of islands (*c.* 25 km$^2$), an outer archipelago at least 7 km from the nearest habitable island, we noted an absence of wolves and severe over browsing of vegetation by deer, suggesting high deer densities (Darimont & Paquet, 2000, 2002). Deer density data for islands in this archipelago would aid in evaluating this hypothesis.

Among biogeographical parameters to predict the occurrence of deer in wolf faeces, we found isolation, specifically distance to mainland, to be more important than area, likely because it influences dispersal. Regardless of carrying capacity, predators may deplete resources in isolated fragments if colonization by prey is limited. We consider this a plausible hypothesis for wolf-deer systems on isolated islands of coastal BC. We postulate that greater distances to mainland reduce immigration rates by deer, predisposing island populations to sustained predation by wolves. Neither area nor the interaction between isolation and area was very important, perhaps because the ecological conditions we deduce from our one-time sampling represent a steady state: over time wolves regulate deer on isolated islands to low abundance, regardless of island size.

Deer are excellent dispersers, however, capable of swimming across water bodies with intense and frequent wave and tidal action (Wallmo, 1981). Reimchen *et al.* (in press) examined deer colonization rates to offshore islands of Haida Gwaii, BC, using microsatellite markers. These authors made a conservative estimate of persistent dispersal of about one deer per year to Skaang Gwaii and Reef Islands, isolated by 2 and 6 km respectively. If colonization rates are similar in BC, our data suggest this is too infrequent to prevent depletion of deer prey on isolated islands. Mainland distance may have been more important than our inter-landmass metric because it estimates distance from the probable ultimate source populations for deer prey.

Our single sampling of this archipelago revealed a pattern of decreased occurrence of deer as prey with isolation, to which we attribute top–down effects coupled with limited recolonization by deer. To be certain, one must sample islands before and after wolf colonization and estimate deer abundance and wolf foraging ecology over time. In the 1960s, a small experiment addressed these parameters by introducing four wolves to the 73 km$^2$ Coronation Island, southeast Alaska, 900-m from another landmass (Klein, 1996). After reaching a peak of 13 wolves in 4 years, the population fell to one, having apparently reduced deer numbers significantly. During this time, wolves foraged extensively on smaller mammals, seals and intertidal organisms (Klein, 1996). The last wolf was shot in the late 1960s, ending the experiment, and the deer population has since rebounded (Person *et al.*, 1996).

Others have demonstrated the consequences of insularity on mammalian predator–prey communities on islands but the dynamics of these systems vary. On Isle Royale, fluctuations of wolves and moose have been extreme but this system has persisted for over 50 years (Peterson *et al.*, 1984; Peterson & Page, 1988; Vucetich & Peterson *in press*). In contrast, Kauhala & Auniola (2001) suggested that raccoon dogs (*Nyctereutes procyonoides*) can extirpate frog populations on some islands in the Finnish Archipelago, as they are easy prey to capture and occur frequently in mainland diet. Migration by predators among landmasses may also be important; by switching between islands and mainland areas from winter to summer, foxes can stabilize fluctuations in hare numbers on Swedish islands, but the effect depends on how often ice permits foxes to recolonize islands and numerical response by predators (Angerbjörn, 1989).

Wolves may persist on isolated islands because alternate prey are available. When deer are scarce (and other large mammals like goat and moose absent), smaller prey such as mink, river otter and bird, appear to be important dietary items (Table 1; Fig. 2). Many of these taxa are either aquatic or volant and not likely as affected by isolation that may limit migration by larger, terrestrial prey.

Future studies in BC's archipelago, combining stable isotope and faecal analyses and occurring over several seasons, may provide better insight into predator–prey dynamics in this fragmented marine landscape. Notably, if combined with microsatellite genetic markers, we may learn how food resources influence presence, movements and demographical fates of individuals over time and assess how water barriers among islands may affect metapopulation dynamics (Hanski & Gilpin, 1991; Hanski, 1991); such frameworks for other large mammals in habitat patches have recently been developed (e.g. Elmhagen & Angerbjörn, 2001). Notably, although water barriers may constrain dispersal of predator and prey, this study suggests the ocean also provides food. In this respect, for wolves and likely other animals, BC's islands are not fragments within a totally inhospitable matrix, to which other islands have been likened (Brotons *et al.*, 2003; see also Dunning *et al.*, 1992; Fahrig, 1997).

This study has implications for conservation of predator–prey systems regionally and beyond. Deer constitute the majority of diet for BC's coastal wolves and salmon is an important seasonal resource (this study; Kohira & Rexstad, 1997; Szepanski *et al.*, 1999; Darimont & Reimchen, 2002;

Darimont *et al.*, 2003). Mounting evidence, however, suggests that carrying capacity for deer is reduced by clearcut logging in west coast temperate rain forests (Wallmo & Schoen, 1980; Alaback, 1982; Rose, 1982; Schoen *et al.*, 1984, 1988; Van Horne *et al.*, 1988). Likewise, many Pacific Northwest salmon stocks have declined dramatically because of the modification of spawning habitat by logging and over-exploitation by the fishing industry (National Resources Council, 1996). If current planning processes aim to preserve this remnant population of wolves in its current form (Darimont & Paquet, 2002), we suggest that plans include significant protection of critical habitat for deer and salmon, especially on islands. Moreover, in any ecosystem, a system of reserves must have appropriate connectivity to permit gene flow (Soulé & Simberloff, 1986). Our data suggest that connectivity should also be considered to accommodate fluctuations in population structure to prevent predator–prey disequilibria, to which fragments may already be predisposed.

## ACKNOWLEDGMENTS

This study took place in the Traditional Territories of several First Nation groups, from whom we sought permission before research begun. We are extremely grateful to the Raincoast Conservation Society for financial and logistical support as well as the Heiltsuk Nation for their participation. We are obliged to Chris Genovali, Ian and Karen McAllister, Misty MacDuffee, Gudrun Pfleuger, Anita Rocamora, Chester Starr, and Erin Urton for fieldwork, and skippers Jean-Marc Leguerrier and Dave Lutz. We also thank Richard Ring, Becky Wiggen and Eric Lofroth. The Defenders of Wildlife, McCaw Foundation, National Geographic Society, University of Montana Paquet Wildlife Fund, Wilburforce, Vancouver Foundation, World Wildlife Fund Canada and private donors kindly provided funding. While preparing the manuscript, CTD was supported by a Natural Sciences and Engineering Research Council (NSERC) – Industrial Post-graduate Scholarship and NNW by a NSERC operating grant. We thank B. Anholt, R. Page, D. Person, T. Reimchen and two reviewers for comments that greatly improved the manuscript.

## REFERENCES

Abbott, I. (1974) Numbers of plant, insect and land bird species on nineteen remote islands in the Southern Hemisphere. *Biological Journal of the Linnaean Society*, **6,** 143–152.

Alaback, P.B. (1982) Dynamics of understory biomass in Sitka spruce-western hemlock forests of southeast Alaska. *Ecology*, **63,** 1932–1948.

Alcover, J.A. & McMinn, M. (1994) Predators of vertebrates on islands. *Bioscience*, **44,** 12–18.

Anderson, D.R., Link, W.A., Johnson, D.H. & Burham, K.P. (2001) Suggestions for presenting the results of data analyses. *Journal of Wildlife Management*, **65,** 373–378.

Angerbjörn, A. (1989) Mountain hare populations on islands: effects of predation by red fox. *Oecologia*, **81,** 335–340.

Brotons, L., Mönkkönen, M. & Martin, J.L. (2003) Are fragments islands? Landscape context and density–area relationships in Boreal forest birds. *American Naturalist*, **162,** 343–357.

Burnham, K.P. & Anderson, D.R. (1998) *Model selection and inference: a practical information-theoretic approach*. Springer-Verlag, New York, NY, USA.

Ciucci, P., Boitani, L., Pelliccioni, E.R., Rocco, M. & Guy, I. (1996) A comparison of scat-analysis methods to assess the diet of the wolf *Canis lupus*. *Wildlife Biology*, **2,** 37–48.

Conroy, C.J., Demboski, J.R. & Cook, J.A. (1999) Mammalian biogeography of the Alexander Archipelago of Alaska: a north temperate nested fauna. *Journal of Biogeography*, **26,** 343–352.

Cowen, I.M. & Guiguet, C.J. (1975) *The mammals of British Columbia*. British Columbia Provincial Museum, Victoria, BC.

Crooks, K.R. & Soulé, M.E. (1999) Mesopredator release and avifaunal extinctions in a fragmented system. *Nature*, **400,** 563–565.

Darimont, C.T. & Paquet, P.C. (2000) *The gray wolves (Canis lupus) of British Columbia's coastal rainforests: findings from year 2000 pilot study and conservation assessment.* Prepared for the, Raincoast Conservation Society, Victoria, BC. Available on http://www.raincoast.org.

Darimont, C.T. & Paquet, P.C. (2002) The gray wolves, *Canis lupus*, of British Columbia's Central and North Coast: distribution and conservation assessment. *Canadian Field-Naturalist*, **116,** 416–422.

Darimont, C.T. & Reimchen, T.E. (2002) Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. *Canadian Journal of Zoology*, **80,** 1638–1642.

Darimont, C.T., Reimchen, T.E. & Paquet, P.C. (2003) Foraging behaviour by gray wolves on salmon streams in coastal British Columbia. *Canadian Journal of Zoology*, **81,** 349–353.

Dolman, P.M. & Sutherland, W.J. (1997) Spatial patterns of depletion imposed by foraging vertebrates: theory, review and meta-analysis. *Journal of Animal Ecology*, **66,** 481–494.

Dunning, J.B., Danielson, J.B. & Pulliam, H.R. (1992) Ecological processes that affect populations in complex landscapes. *Oikos*, **65,** 169–175.

Elmhagen, B. & Angerbjörn, A. (2001) The applicability of metapopulation theory to large mammals. *Oikos*, **94,** 89–100.

Fahrig, L. (1997) Relative effects of habitat loss and fragmentation on species extinction. *Journal of Wildlife Management*, **61,** 603–610.

Fahrig, L. (2003) Effects of habitat fragmentation on diversity. *Annual Review of Ecological Systems*, **34,** 487–515.

Fuller, T.K. (1989) Population dynamics of wolves in north-central Minnesota. *Wildlife Monographs*, **105,** 1–41.

Gorman, M.L. (1979) *Island ecology*. Chapman and Hall, New York, NY, USA.

Hanski, I. (1991) Single-species metapopulation dynamics: concepts, models and observations. *Biological Journal of the Linnaean Society*, **42,** 17–38.

Hanski, I. & Gilpin, M. (1991) Metapopulation dynamics: brief history and conceptual domain. *Biological Journal of the Linnaean Society*, **42,** 3–16.

Kadmon, R. & Pulliam, H.R. (1993) Island biogeography: effects of geographical isolation on species composition. *Ecology*, **74,** 977–981.

Kareiva, P. (1990) Population dynamics in spatially complex environments: theory and data. *Philosophical Transactions of the Royal Society of London, Series B. Biological Sciences*, **330,** 175–190.

Kareiva, P. & Wennergren, U. 1995. Connecting landscape patterns to ecosystem and population processes. *Nature*, **373,** 299–302.

Kauhala, K. & Auniola, M. (2001) Diet of raccoon dogs in summer in the Finnish archipelago. *Ecography*, **24,** 151–156.

Kirchhoff, M.D. (1994) *Effects of forest fragmentation on deer in southeast Alaska*. Unpublished Management Report. Alaska Department of Fish and Game, Juneau, AK, USA.

Kirchhoff, M.D. (1996) *Deer pellet-group surveys in southeast Alaska*. Unpublished Management Report. Alaska Department of Fish and Game, Douglas, AK, USA.

Klein, D.R. (1996) The introduction, increase and demise of wolves on Coronation Island, Alaska. *Ecology and conservation of wolves in a changing world* (ed. by L.N. Carbyn, S.H. Fritts and D.R. Seip), pp. 275–280. Canadian Circumpolar Institute, University of Alberta, Edmonton, AB.

Kohira, M. & Rexstad, E.U. (1997) Diets of wolves, *Canis lupus*, in logged and unlogged forests of southeastern Alaska. *Canadian Field-Naturalist*, **111,** 429–435.

MacArthur, R.H. & Pianka, E.R. (1966) On optimal use of a patchy environment. *American Naturalist*, **100,** 603–609.

MacArthur, R.H. & Wilson, E.O. (1967) *The theory of island biogeography: monographs in population biology*. Princeton University Press, Princeton, NJ, USA.

MacDonald, S.O. & Cook, J.A. (1996) The land mammal fauna of Southeast Alaska. *Canadian Field-Naturalist*, **110,** 571–598.

Mathiak, H.A. (1938) A key to hairs of the mammals of southern Michigan. *Journal of Wildlife Management*, **2,** 251–268.

Mayer, W.V. (1952) The hair of California mammals with the keys to the dorsal guard hairs of California mammals. *American Midland Naturalist*, **38,** 480–512.

McCabe, T.T. (1948) Beaver on the northern British Columbian islands. *Canadian Field-Naturalist*, **62,** 72–74.

McCauley, E., Kendall, B.E., Janssen, A., Wood, S., Murdoch, W.W., Hosseini, P., Briggs, C.J., Ellner, S.P., Nisbet, R.M., Sabelis, M.W. & Turchin, P. (2000) Inferring colonization processes from population dynamics in spatially structured predator–prey systems. *Ecology*, **81,** 3350–3361.

Milne, D.G., Harestad, A.S. & Atkinson, K. (1989) Diets of wolves on northern Vancouver Island. *Northwest Science*, **63,** 83–86.

National Resources Council (1996) *Upstream: salmon and society in the Pacific Northwest*. National Academy Press, Washington, DC, USA.

Paquet, P.C. & Carbyn, L.N. (2003) Wolf, *Canis lupus. Wild mammals of North America: biology, management, and conservation* (ed. by G.A. Feldhamer, B.C. Thompson and J.A. Chapman), Johns Hopkins University Press, Baltimore, MD, USA.

Person, D.K., Kirchhoff, M.D., Van Ballenberghe, V., Iverson, G.C. & Grossman, E. 1996. *The Alexander Archipelago wolf: a conservation assessment*. General Technical Report PNW-GTR-384. USDA Forest Service, Portland, OR, USA.

Peterson, R.O. & Ciucci, P. (2003) The wolf as a carnivore. *Wolves. Behaviour, ecology and conservation* (ed. by L.D. Mech and L. Boitani), pp. 104–130. University of Chicago Press, Chicago, IL, USA.

Peterson, R.O. & Page, R.E. (1988) The rise and fall of Isle Royale wolves. *Journal of Mammalogy*, **69,** 89–99.

Peterson, R.O., Page, R.E. & Dodge, K.M. (1984) Wolves, moose and allometry of population cycles. *Science*, **224,** 1350–1352.

Pojar, J. & Mackinnon, A. (1994) *Plants of coastal British Columbia, including Washington, Oregon, and Alaska*. Lone Pine Publishing, Vancouver, BC.

Polis, G.A. & Hurd, S.D. (1995) Extraordinarily high spider densities on islands: flow of energy from the marine to terrestrial food webs and the absence of predation. *Proceedings of the National Academy of Sciences of the United States or America*, **92,** 4382–4386.

Reimchen, T.E. (2000) Some ecological and evolutionary aspects of bear–salmon interactions in coastal British Columbia. *Canadian Journal of Zoology*, **78,** 448–457.

Reimchen, T.E., Nelson, R.J. & Smith, C.J. (in press) Estimating deer colonization rates to offshore islands of Haida Gwaii, British Columbia, using microsatellite markers. *Lessons from the islands: introduced species and what they tell us about how ecosystems work* (ed. by A.J. Gaston, T.E. Golumbia, J.-L. Martin and S.T. Sharpe). Proceedings from the Research Group on Introduced Species 2002 Conference. Queen Charlotte City, B.C. Canadian Wildlife Service Occasional Papers Series, Ottawa, ON.

Ricklefs, R.E. (1990) *Ecology*. W.H. Freeman and Co., New York, NY, USA.

Rose, C.L. (1982) *Deer responses to forest succession on Annete Island, southeast Alaska*. MSc. Thesis. University of Alaska, Fairbanks, AK, USA.

Rose, M.D. & Polis, G.A. (1998) The distribution and abundance of coyotes: the effects of allochthonous food subsidies from the sea. *Ecology*, **79,** 998–1007.

Saunders, D.A., Hobbs, R.J. & Margules, C.R. (1991) Biological consequences of ecosystem fragmentation: a review. *Conservation Biology*, **5,** 18–32.

Schneider, M.F. (2001) Habitat loss, fragmentation and predator impact: spatial implications for prey conservation. *Journal of Applied Ecology*, **38,** 720–735.

Schoen, J.W. & Kirchhoff, M.D. (1985) Seasonal distribution and home-range patterns of Sitka black-tailed deer on Admiralty Island, southeast Alaska. *Journal of Wildlife Management*, **49,** 96–103.

Schoen, J.W., Kirchhoff, M.D. & Wallmo, O.C. (1984) Sitka black-tailed deer/old-growth relationships in southeast Alaska: implications for management. *Proceedings of the symposium on fish and wildlife relationships in old growth forests* (ed. by W.R. Meacham, T.R. Merrell and T.A. Hanley), pp. 315–319. American Institute of Fisheries Research Biologists, Juneau, AK, USA.

Schoen, J.W., Kirchhoff, M.D. & Hughes, J.H. (1988) Wildlife and old-growth forests in southeastern Alaska. *Natural Areas Journal*, **8,** 138–145.

Scott, B.M.V. (1979) *The Vancouver Island Wolf (Canis lupus crassodon)*, an initial study of food habits and social organization. MSc Thesis, University of British Columbia, Vancouver, BC.

Scott, B.M.V. & Shackleton, D.M. (1980) Food habits of two Vancouver Island wolf packs: a preliminary study. *Canadian Journal of Zoology*, **58,** 1203–1207.

Soulé, M.E. & Simberloff, D. (1986) What do genetics and ecology tell us about the design of nature reserves? *Biological Conservation*, **35,** 19–40.

Stains, H.J. (1958) Field guide to guard hair of middle western furbearers. *Journal of Wildlife Management*, **22,** 95–97.

Szepanski, M.M., Ben-David, M. & Van Ballenberghe, V. (1999) Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. *Oecologia*, **120,** 327–335.

Taylor, R.J. (1984) *Predation: population and community biology*. Chapman and Hall, New York, NY, USA.

Terborgh, J., Lopez, L., Nunez, V.P., Rao, M., Shahabuddin, G., Orihuela, G., Riveros, M., Ascanio, R., Adler, G.H., Lambert, T.D. & Balbas, L. (2001) Ecological meltdown in predator-free forest fragments. *Science*, **294,** 1923–1926.

Thurber, J.M. & Peterson, R.O. (1993) Effects of population density and pack size on the foraging ecology of gray wolves. *Journal of Mammalogy*, **74,** 879–889.

Van Horne, B., Hanley, T.A., Cates, R.G., McKendrick, J.D. & Horner, J.D. (1988) Influence of seral stage and season on leaf chemistry of southeast Alaska deer forage. *Canadian Journal of Forest Research*, **18,** 90–99.

Voigt, D.R., Kolenosky, G.B. & Pimlott, D.H. (1976) Changes in summer foods of wolves in Central Ontario. *Journal of Wildlife Management*, **40,** 663–668.

Vucetich, J.A. & Peterson, R.O. (2004) The influence of top-down, bottom-up, and abiotic factors on the moose (*Alces alces*) population of Isle Royale. *Philosophical Transactions of the Royal Society of London, Series B. Biological Sciences*, **271,** 183–189.

Wallmo, O.C. (1981) *Mule and black-tailed deer of North America*. University of Nebraska Press, Lincoln, NE, USA.

Wallmo, O.C. & Schoen, J.W. (1980) Response of deer to secondary forest succession in southeast Alaska. *Forest Science*, **26,** 448–462.

Wallmo, O.C., Jackson, A.W., Hailey, T.L. & Carlisle, R.W. (1962) Influence of rain on the count of deer pellet groups. *Journal of Wildlife Management*, **26,** 50–55.

Weaver, J.L. (1993) Refining the equation for interpreting prey occurrence in gray wolf scats. *Journal of Wildlife Management*, **57,** 534–538.

Williamson, M. (1981) *Island populations*. Oxford University Press, New York, NY, USA.

Woodroffe, R. & Ginsberg, J.R. (1998) Edge effects and the extinction of populations inside protected areas. *Science*, **280,** 2126–2128.

## BIOSKETCHES

**Chris Darimont** is a PhD student at the University of Victoria, where his dissertation includes a subset of the study programs designed for the Rainforest Wolf Project. He is particularly interested in marine influences on terrestrial predator–prey systems and spatial ecology, as well as the role of predation risk in maintaining intra-population variability in foraging behaviour of prey.

**Michael Price** completed his Honours Biology at the University of Victoria on the work herein. He was supervised by **Neville Winchester**, Adjunct Assistant Professor, whose research focuses on the ecology and conservation of forest canopy invertebrate communiteis.

**Johanna Gordon-Walker** manages Rainforest Wolf Project field research, as well as collaborates on laboratory work.

**Paul Paquet** is an internationally recognized authority on the research and conservation of large carnivores and contributes to projects around the world. He is Senior Scientific Advisor for the Rainforest Wolf Project and the Raincoast Conservation Society, Senior Scientist at Conservation Science Inc., and Adjunct Professor at the University of Calgary.

# Genetic Structure and Migration in Native and Reintroduced Rocky Mountain Wolf Populations

STEPHEN H. FORBES* AND DIANE K. BOYD†

*Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A.
email sforbes@selway.umt.edu
†Wildlife Biology Program, School of Forestry, University of Montana, Missoula, MT 59812, U.S.A.

**Abstract:** *Gray wolf (*Canis lupus*) recovery in the Rocky Mountains of the U.S. is proceeding by both natural recolonization and managed reintroduction. We used DNA microsatellite analysis of wolves transplanted from Canada to two reintroduction sites in the U.S. to study population structure in native and reintroduced wolf populations. Gene flow due to migration between regions in Canada is substantial, and all three recovery populations in the U.S. had high genetic variation. The reintroduced founders were moderately genetically divergent from the naturally colonizing U.S. population. These findings corroborate that the reintroduction more than meets generally accepted genetic guidelines. Maintaining this variation, however, will depend on ample reproduction in the first few generations. In the long term genetic variation will best be retained if migration occurs among the recolonizing and the two transplanted populations. Evidence from field observation and genetic studies shows extensive dispersal by wolves, and we conclude that exchange among these groups due to natural dispersal is likely if public tolerance and legal protection are adequate outside lands designated for wolf recovery.*

Estructura Genética y Migración de Poblaciones Nativas y Reintroducidas del Lobo de las Montañas Rocallosas

**Resumen:** *La recuperacion del lobo gris (*Canis lupus*) en las montañas Rocallosas de los Estados Unidos ha procedido tanto de la recolonización natural, como de la reintroducción controlada. Para estudiar la estructura poblacional de lobos nativos y reintroducidos, utilizamos análisis de microsatélites de ADN de lobos transplantados de Canada hacia dos sitios de reintroducción en los Estados Unidos. El flujo de genes debido a la migración entre regiones del Canada es sustancial y las tres poblaciones en recuperación de Estados Unidos tuvieron una alta variación genética. Los fundadores de las reintroducciones fueron moderadamente divergentes de las poblaciones colonizadoras naturales desde el punto de vista genético. Estos resultados corroboran que la reintroducción concuerda mas que bien con los lineamientos genéticos generalmente aceptados. Sin embargo, mantener esta variación dependerá en gran medida de la reproducción de las primeras generaciones. En un largo plazo, la variación genética será retenida al máximo si ocurren migraciones entre las problaciones recolonizadoras y las transplantadas. Evidencias de campo y estudios de genética muestran una dispersión extensiva de los lobos y concluímos que el intercambio entre estos grupos debido a la dispersión natural es posible, siempre y cuando la tolerancia del público y la protección legal sean adecuadas fuera de lás tierras designadas como áreas de recuperación de los lobos.*

## Introduction

Wild canid populations worldwide vary in status from secure to fragmented, isolated, hybridized, or locally extinct. Canid conservation genetics has benefitted from the development of DNA microsatellite loci in the domestic dog (Ostrander et al. 1993; Gottelli et al. 1994; Roy et al. 1994; Garcia-Moreno et al. 1996; Forbes & Boyd 1996). The large number and high variability of these DNA markers make genetic studies of wild canids increasingly informative.

*Paper submitted August 29, 1996; revised manuscript accepted December 2, 1996.*

Wolves in the central Rocky Mountains have a history of persecution and tenuous recovery (Gunson 1992; Boyd et al. 1995; we consider the Rocky Mountains in their entirety: the central Rockies span the Canada-U.S. international boundary). Previously we used DNA microsatellites to study wolves that naturally recolonized western Montana from Canada from 1985–1995. High genetic variation in the U.S. wolves indicated that there was not a founding population bottleneck sufficient to diminish genetic variation during colonization (Forbes & Boyd 1996). All evidence from genetic and field data indicated that natural dispersal in wolves was adequate to preclude any concern about inbreeding in the colonizing population. The Montana population has grown to approximately 70 in at least seven breeding packs, occupying a region extending 350 km south of the Canadian border in Montana (Fritts et al. 1995; Fig. 1).

Wolves are endangered in the lower 48 states, but are numerous in large parts of Canada. The area of natural recolonization in Montana is one of three areas designated for wolf recovery in the western U.S. (U.S. Fish and Wildlife Service 1987, 1994). To further the recovery effort, during the winters of 1995 and 1996 wolves were trapped in central Alberta and northern British Columbia and transported to the other two recovery areas in Yellowstone National Park (YNP) and central Idaho, south and southwest of the naturally recolonizing population (Bangs & Fritts 1996; Fritts et al. 1997; Fig. 1).

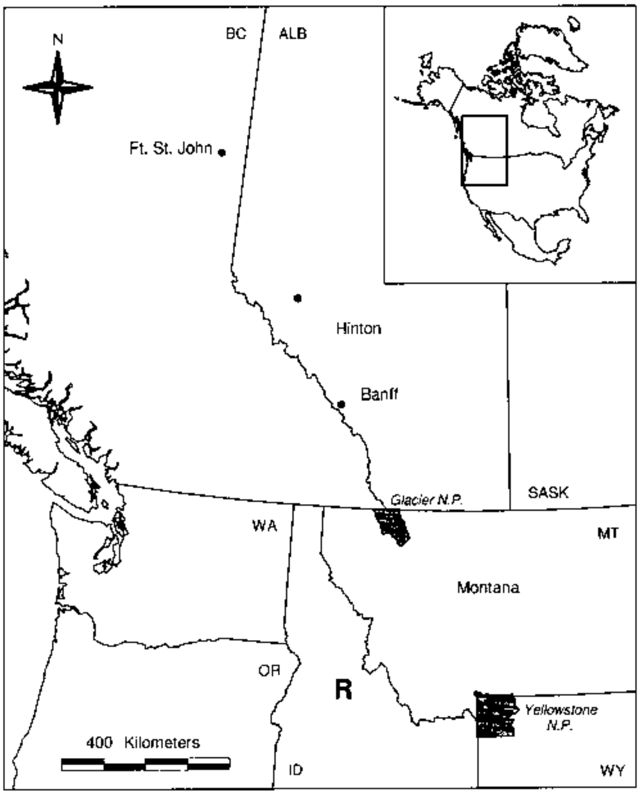


*Figure 1. Map of Rocky Mountain wolf range. Shaded areas in Canada indicate origins of the Banff sample, the 1995 Hinton transplants, and the 1996 Fort St. John transplants. The shaded area in Montana indicates the range of the recolonizing population. Indicated areas (R) in Yellowstone National Park and central Idaho are reintroduction sites used in both 1995 and 1996.*

We augmented the previous database with genotypes of all the transplanted wolves. The new data add to our knowledge of wolf population structure in Canada and provide a baseline for the initial genetic variation in U.S. wolf reintroduction areas. Our goal is to combine these genetic data with 15 years of field observation in Montana to better understand the genetic and demographic effects of both natural and managed wolf dispersal.

## Study Populations and Methods

Montana wolf recovery was monitored from the late 1970s to the present by the University of Montana Wolf Ecology Project (Ream et al. 1991) and the U.S. Fish and Wildlife Service (Fritts et al. 1995). Several animals from each pack were captured, blood sampled, and radiocollared (Boyd et al. 1995). The Banff Wolf Project concurrently monitored wolf populations in Banff, Yoho, and Kootenay National Parks of Canada (Paquet 1993).

The six Rocky Mountain wolf samples differ in population history and sampling structure. The four samples of naturally resident wolves (Fort St. John, Hinton, Banff, Montana) come from a 1350 km range in the northern and central Rockies (Fig. 1). The Fort St. John and Hinton animals are from resident populations where wolves were at times persecuted but never extirpated; Banff wolves were locally extirpated but recovered in the 1980s (Gunson 1983; 1992; Tompa 1983).

The Hinton and Fort St. John wolves were sampled when they were captured for translocation to the U.S. in 1995 and 1996, respectively (Bangs & Fritts 1996; Fritts et al. 1997). In each year approximately half of the wolves were released in YNP and half in central Idaho (Table 1). Thus, each introduced population is a mixture formed from the two Canadian sources. This reintroduction pattern means that population sampling differs among regions. The Fort St. John and Hinton samples were small subsets of large native populations, but these same animals are a complete sample of the reintroduced YNP and Idaho wolves. The Banff and Montana samples fall in between: they are not complete samples, but they do include members of all resident packs known to researchers. Allele frequencies for the Banff and Montana samples were previously reported (Forbes & Boyd 1996). The present dataset (Appendix) includes all the transplanted wolves and adds seven new wolves to the Montana sample. In both reintroduction years nine family

**Table 1. Genetic variation at 10 microsatellite loci in Rocky Mountain wolves.**[a]

| *Population* | N | A | $H_o$ | $H_e$ |
|---|---|---|---|---|
| Fort St. John (source) | 41.0 | 4.5 | 0.588 | 0.589 |
| Hinton (source) | 33.0 | 4.5 | 0.579 | 0.628 |
| Banff | 32.0 | 4.4 | 0.553 | 0.581 |
| Montana (recolonized) | 66.0 | 4.1 | 0.606 | 0.606 |
| Yellowstone (founders)[b] | 31.0 | 4.7 | 0.591 | 0.635 |
| Idaho (founders)[c] | 35.0 | 4.6 | 0.589 | 0.636 |
| Total[d] | 172.0 | 5.4 | 0.587 | 0.641 |

[a] N, *mean sample size per locus;* A, *mean number of alleles per locus;* $H_o$, *observed heterozygosity, and* $H_e$, *binomial (Hardy-Weinberg) expected heterozygosity (unbiased estimate). Eight wolves sampled at Fort St. John and Hinton were released and not transported to the U.S.*

[b] *Fourteen wolves from Hinton (1995) and 17 from Fort St. John (1996).*

[c] *Fifteen wolves from Hinton (1995) and 20 from Fort St. John (1996).*

[d] *One hundred six wolves from Canada and 66 from Montana.*

groups of wolves were collected. In YNP wolves were held in pens and released as family groups based on their pack membership in Canada, whereas in Idaho they were released immediately after transport (Bangs & Fritts 1996; Fritts et al. 1997; Table 1).

Blood samples were taken from live-trapped wolves (Ream et al. 1991; Boyd et al. 1995), and muscle samples were taken from wolves found dead. Laboratory methods were previously described (Forbes & Boyd 1996). The DNA microsatellite loci were amplified from purified DNA or from Chelex tissue preparations using the polymerase chain reaction (PCR). Ten dinucleotide repeat (AC)n loci characterized in the domestic dog (Ostrander et al. 1993) were chosen from those previously used in wolves (Roy et al. 1994; Forbes & Boyd 1996). Nine loci are the same in these two studies.

Population genetic parameters were calculated using BIOSYS-1 (Swofford & Selander 1989). Heterozygosity differences between samples were tested using a paired $t$ test on $H$ values at individual loci (Nei 1987; Leberg 1992). We estimated population differentiation using the $F_{ST}$ estimator θ (theta; Cockerham & Weir 1993) calculated by the program GENEPOP (Raymond & Rousset 1995). This program also estimates migration rate ($N_em$) based on genetic differentiation between subpopulations (Slatkin 1987; Slatkin & Barton 1989). Simulation studies showed that $\theta$ is the best choice of differentiation measure for estimating migration when a population is continuously distributed without discrete boundaries between demes (Slatkin & Barton 1989), a model that may be most appropriate for Canadian wolves (Nowak 1983). Tests for correlation between genetic differentiation and geographic distance (Slatkin 1993) were also calculated using programs in GENEPOP (DIST by M. Slatkin; and MANTEL by Raymond & Rousset). The Mantel matrix correlation tests are based on Spearman rank correlations ($R_S$).

## Results and Discussion

### Genetic Variation

Levels of genetic variation were high in all samples. Average heterozygosity ($H_e$) in the Canadian populations ranged from 0.581 to 0.628, and the recolonized Montana population fell within this range ($H_e$ = 0.606; Table 1). No two of these values were significantly different. Allelic diversity (the mean number of alleles per locus, $A$) ranged from 4.4 to 4.5 in Canada and was 4.1 in Montana. These levels of variation are comparable to those in wolves from across Canada and Alaska genotyped at 10 microsatellite loci by Roy et al. (1994). In that study only the sample from the Canadian Northwest Territories had significantly higher heterozygosity and more alleles than any of our six Rocky Mountain samples compared at the same nine loci (data not shown). The reintroduced YNP and Idaho groups are unusual population samples because both groups are nearly equal mixtures of animals from the same two sources (Fort St. John and Hinton). Observed heterozygosity is approximately the same in the source groups and in the mixed transplant groups (all $H_o$ = 0.579–0.591; Table 1). Expected heterozygosity ($H_e$) is higher in the introduced wolves than in the source populations, but this is expected in the combined groups because of allele frequency differences between the source populations (the Wahlund effect).

### Population Structure

Random mating (panmixia) is a proper null hypothesis for population structure. Realistically, however, we would not expect panmixia for most large mammals because they are frequently territorial and dispersal distances are generally limited (Chepko-Sade et al. 1987). The simplest indicator of departure from panmixia is allele frequency differentiation among geographically distant samples. Allele frequencies tested over all 10 loci differed significantly among the four native (non-reintroduced) Canadian and Montana samples in all pairwise tests (all $p < 0.001$ when combined over 10 loci). Significant allele frequency differences are compatible with substantial levels of gene flow, however (Wright 1931; 1969; Allendorf & Phelps 1981), and because of high allelic diversity, microsatellites are especially sensitive indicators of allele frequency differentiation. Significant allele frequency differences alone do not demonstrate biologically important isolation.

*F*-statistics provide more informative measures of population structure. The most important of these is $F_{ST}$, the proportion of total variation that is due to differences between subpopulations (if $F_{ST}$ = 1, subpopulations have no alleles in common; if $F_{ST}$ = 0, allele frequencies in all subpopulations are identical). Among the three Canadian populations and among all four native populations (including Montana colonizers) $F_{ST}$ (Nei 1977) was 0.074. This amount of differentiation is moderate for natural populations of animals in general (Nei 1987; Hartl & Clark 1989), and it agrees closely with other studies of wolves at similar geographic distances. Kennedy et al. (1991) also found an $F_{ST}$ of 0.074 in a group of eight wolf subpopulations from northwestern Canada assayed at five polymorphic allozyme loci. In another study using microsatellites, wolves from five populations sampled throughout North America had a predictably greater differentiation ($F_{ST} = 0.168$; Roy et al. 1994).

Tests for deviation from binomial expected (Hardy-Weinberg) genotype proportions in the four native populations (Fort St. John, Hinton, Banff, Montana) showed significant deviations only in the Hinton sample. In the Hinton wolves two individual loci had significant heterozygote deficits after correcting for the number of tests, and the randomization test combined over all 10 loci was also significant ($p < 0.01$; data not shown). This may be due to a moderate tendency of individuals to breed in or near their natal home range in this population or to a moderate, undetected dispersal barrier.

We also used Nei's standard genetic distance (*D*; Nei 1978) to measure pairwise population differences. Nei's *D*s among the native groups ranged from 0.093 between Banff and Montana to 0.223 between Fort St. John and Banff (Table 2; Fig. 2). Again, these distances are generally small compared to microsatellite *D*s among wolf populations spread throughout the continent, which ranged from 0.182 to 0.418 (Roy et al. 1994).

We tested for correlation between genetic differentiation and geographic distance between samples. In such tests positive correlations indicate isolation-by-distance, where gene flow between subpopulations results in greater similarity between neighboring sub-populations than between distant ones (Slatkin 1993). For these tests we combined our data with those of Roy et al. (1994), using the nine loci in common between the studies. For the four Rocky Mountain samples alone, genetic differentiation and geographic distance were significantly correlated ($R_S = 0.829$; $p < 0.05$; one-tailed test; Fig. 2, open circles). The 28 pairwise comparisons among all eight samples also showed positive correlation ($R_S = 0.652$; $p < 0.05$; Fig. 2, all symbols). This test was significant with the Vancouver Island population included, but the correlation was greater and the test more significant when the Vancouver Island sample was removed ($R_S = 0.837$; $p < 0.001$; Fig. 2, "V" points omitted). Vancouver Island falls markedly off the differentiation-by-distance curve at small distances. This population shows excess differentiation from the Rocky Mountain samples (dashed outline; Fig. 2), and this is attributable to genetic drift in a relatively isolated island population (Tompa 1983).

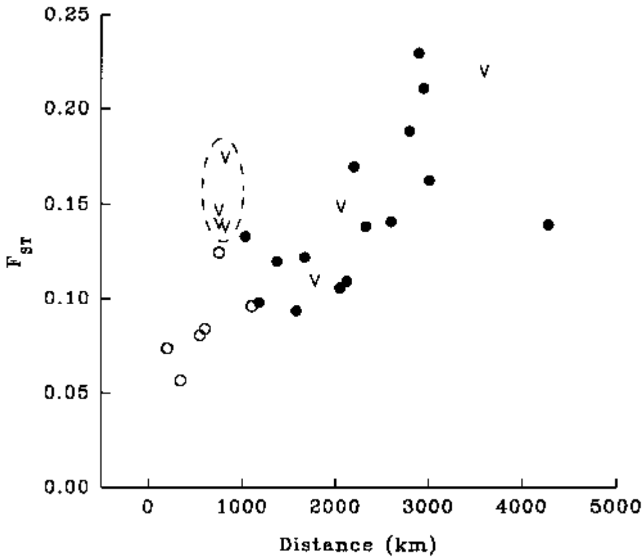


*Figure 2. Comparison of genetic differentiation (*$F_{ST}$ *estimator* $\theta$*) and geographic distance at nine microsatellite loci among wolf populations. There are 28 pairwise comparisons among eight populations (all symbols): four Rocky Mountain samples from the present study and four more distantly spaced populations (Vancouver Island, Kenai Peninsula, Northwest Territories, and Quebec; data from Roy et al. 1994). The open circles are the comparisons among the four Rocky Mountain samples. The "V" symbols are the comparisons with the Vancouver Island sample. The dashed line surrounds the points comparing Vancouver Island and its four nearest neighbors, which are the four Rocky Mountain samples.*

The high mutation rate and stepwise mutation mechanism at microsatellite loci make genetic distances such as Nei's *D* and $F_{ST}$ increasingly suspect as differentiation increases (Kimmel et al. 1996; Slatkin 1995; Nauta & Weissing 1996). In contiguous subpopulations, where gene flow is high or where separation is very recent, population processes will have a stronger effect than mutation and inferences based on these measures are reliable. The range within which this is true, however, is not well established and may vary among taxa. In the present case, positive correlations in the above tests indicate that isolation-by-distance is measurable between wolf subpopulations if samples are sufficiently numer-

**Table 2. Pairwise genetic distances among Rocky Mountain wolf populations.***

| *Population* | *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|---|
| Fort St. John | — | | | | | |
| Hinton | 0.150 | — | | | | |
| Banff | 0.223 | 0.127 | — | | | |
| Montana | 0.162 | 0.145 | 0.093 | — | | |
| Yellowstone founders | 0.023 | 0.028 | 0.164 | 0.133 | — | |
| Idaho founders | 0.016 | 0.037 | 0.137 | 0.118 | 0.005 | — |

**Unbiased standard genetic distance (Nei 1978).*

ous and large and if the tested populations cover a sufficient range of distances. The lack of differentiation-distance correlation found by Roy et al. (1994) may be due to absence of migration-drift equilibrium or to homoplasy accumulated due to back-mutation at large genetic divergences. However, lower statistical power due to smaller sample sizes, fewer populations, and a smaller range of geographic distances could also be responsible. Furthermore, inclusion of an island population may have obscured a pattern of migration-drift equilibrium on the rest of the continent.

## Genetic Estimates of Dispersal

Inferring reliable estimates of gene flow due to migration of individuals between populations is one of the most difficult problems in conservation biology (Varvio et al. 1986; Avise 1994). Because genetic estimates of migration are suspect where the evidence for migration-drift equilibrium is weak or lacking (Slatkin 1993), the most reliable estimates will be based on populations most likely to be in equilibrium based on independent information. For this purpose Fort St. John, Hinton, and the Northwest Territories are the best choices because (1) wolves were never fully extirpated from these areas (Gunson 1983; Heard 1983; Tompa 1983), so there is not a recent history of recolonization in these areas; (2) these populations are close enough together (600–1200 km) and in adequately continuous wolf habitat to provide potential gene flow by migration based on field data; and (3) divergence between contiguous populations will be least affected by high microsatellite mutation rates.

For the Fort St. John and Hinton samples (about 600 km apart), the migration estimate ($N_e m$) was 2.7 migrants per generation ($N_e$ is the effective population size, and $m$ is the proportion of the population that is migrants each generation; Slatkin 1987). Between the Northwest Territories and either Fort St. John or Hinton (about 1000 and 1200 km respectively) the estimates are correspondingly less: $N_e m = 1.6$ and 2.3 migrants per generation for the Northwest Territories/Fort St. John for the Northwest Territories/Hinton, respectively. Given that the error in genetically estimating migration may be 20 to 100% (Slatkin & Barton 1989), all the above results are in reasonable agreement. These estimates are expressed as the absolute number of migrants between populations, independent of population size. Thus, in a population of 100 packs (200 breeding adults) two migrants per generation would mean replacement of only 1% of the breeding adults each generation.

## Evidence of Dispersal from Field Studies

Because field and genetic data differ in their ability to estimate historical versus current gene flow, a combination of these approaches is advisable (Slatkin 1987; Avise 1994). Our field data corroborate that the genetically estimated rate of two or more migrants per generation is reasonable. The field evidence of migration rates in Rocky Mountain wolves comes from an intensive study of dispersal in the Glacier National Park (GNP) area recolonizing population, where high migration rates and migration distances ranging from 200 to over 800 km are reported (Ream et al. 1991; Boyd et al. 1995). These are comparable to reports of long-distance wolf dispersal in other areas such as Minnesota where human development of the landscape is substantial (Gese & Mech 1991; Mech et al. 1995). There is no cumulative evidence of sex bias in dispersal frequency or distance in these studies.

These large dispersal distances and rates suggest that movements among widely separated packs and among the three recovery areas are likely and that two migrants per generation between large, permanent wolf populations is possible. Distances between the population centers of the three recovery areas range from 370 km between YNP and central Idaho to 540 km between GNP and YNP (Fig. 1), and these distances are readily traversed by wolves when conditions are favorable. Southward breeding dispersal of wolves from GNP has already covered about half the distance from GNP to each of the two reintroduction sites (shaded area extending south of GNP; Fig. 1), and dispersal movements of Idaho wolves have already ranged near the natural colonization area (Fritts et al. 1997).

## Management for Wolf Migration

The mountainous character of the study area fragments the landscape into patches of suitable wolf habitat, usually centered around lower elevation valleys, in a matrix of unsuitable habitat. This precludes the existence of a continuous population of boundary-sharing packs, and it encourages dispersal and consequent gene flow among regions. If truly isolated in mountain valleys, these wolf packs might potentially suffer inbreeding depression. The long-distance movements described here, however, show that such isolation is very unlikely.

Generalizations drawn from studies of permanent populations in more homogeneous habitat (e.g., northern Minnesota, parts of Canada and Alaska) may not apply to expanding populations in heterogeneous, mountainous habitat. Patchy habitat distribution may make Rocky Mountain wolves more typical of wolves in human-affected landscapes, where populations become increasingly fragmented as development intrudes. Human interference (ranches, highways, poachers) rather than absolute distance will most likely limit migration between recovery areas. These obstructions, as well as political status and social attitudes, vary spatially and temporally throughout our international study area, but are nevertheless key factors in wolf conservation (Mech 1995; Fritts & Carbyn 1995).

Conservation planning includes enhancing genetic exchange among recovery areas by management for migration corridors. The effectiveness of corridors, however, depends on the needs and behaviors of individual species (Noss et al. 1996). Wolves disperse at much greater rates and over longer distances than other large carnivores, and they may be less prone to avoid human development when habitat quality is otherwise high (Mech 1995; Mech et al. 1995; Paquet et al. 1996). Neither do wolves necessarily choose designated recovery lands (U.S. Fish and Wildlife Service 1994) for habitation. Seven of the 15 breeding packs recorded during natural recolonization (Fortine, Marion, Ninemile, Boulder, Thompson River, Browning, and Choteau) were established both outside the recovery area and outside suggested wildlife migration corridors (U.S. Fish and Wildlife Service 1987). Because wolves disperse so effectively, planning for discrete corridors may be less important than management for wolf survival in the broad landscape linkages already in use by wolves (Fritts & Carbyn 1995; Noss et al. 1996). In the Rocky Mountains these connections are diminishing but apparently adequate at present.

### Genetic Aspects of Wolf Recovery

Reintroduced populations are generally small, and genetic principles must be considered in their management (Leberg 1990). The goal is to choose founders so as to avoid loss of genetic variation, which in general means using as many unrelated animals of both sexes as possible from a population with a high level of variation. In social animals, however, effects of management disruption on pair bonds and reproductive timing must also be considered. Prescriptions for wolf reintroduction call for use of animals from the closest thriving population to minimize outbreeding and loss of local adaptation and the transfer of extant packs to promote early reproduction (Shields 1983; U.S. Fish and Wildlife Service 1994).

The 1995 and 1996 reintroductions followed these guidelines (Fritts et al. 1997), and the result has been beneficial from a genetics perspective. The two genetically distinct source populations had high heterozygosity levels, and the mixing of these sources was additionally beneficial.

Genetic variation in the reintroduced populations is substantial and the initial population size is apparently adequate to prevent a small founding bottleneck ($N =$ 31 and 35 in YNP and Idaho, respectively). However, a founder effect is still inevitable in the first generations of reproduction. Heterozygosity is expected to be lost at a rate of $1/(2N_e)$ per generation where $N_e$ is the effective population size (Wright 1969), and in wolves $N_e$ is much less than the census population size due to the limitation of breeding to alpha pairs (Chepko-Sade et al. 1987). The severity of the founding bottleneck will depend on the initial rate of reproduction and ongoing survivorship. However, because the founding stock had high levels of genetic variation, the immediate concern is more about short-term demography than about genetics (Lande 1988). These demographic factors are difficult to predict and are confounded by the uncertainties of human-caused mortality.

The naturally recolonized Montana population potentially remains connected by migration with Canada. Thus, dispersal among the YNP and Idaho reintroduction areas and the recolonized Montana population could connect the U.S. and Canadian Rocky Mountain populations. Gene flow throughout the Rocky Mountains would ultimately connect the reintroduced U.S. populations to a large Canadian metapopulation that numbers in the tens of thousands. Artificial translocation is also seen as a viable option if natural migration is inadequate (U.S. Fish and Wildlife Service 1994).

## Conclusions

It appears that all Rocky Mountain wolves, whether they are in permanent, recovered, or reintroduced populations, have high heterozygosity ultimately because of the dispersal of genetically sufficient numbers of animals from stable population centers. We conclude that none of the three recovery populations in isolation would necessarily maintain a genetically viable population in the long run, but that the dispersal capabilities of wolves make such isolation unlikely if populations remain near recovery goals. A greater threat to wolf recovery is the possibility of chronically low numbers or minimal dispersal due to human-caused mortality. Broad landscape connections where wolves are not persecuted outside designated recovery areas are needed, and these can be enhanced through effective legal protection and public education.

A combination of field work and genetic analysis yields valuable knowledge of wolves that neither of these approaches alone can provide. The finding of high genetic variation obviates any immediate concerns about inbreeding in Rocky Mountain wolves. However, these same field and laboratory techniques will be needed in the future to assess population numbers and long-term effective population size and to identify dispersers as members of the natural population.

## Acknowledgments

We thank the University of Montana for institutional support and F. Allendorf for use of laboratory facilities. The Peter W. Busch Family Foundation, World Wildlife Fund Canada, and Parks Canada provided funding for the genetic analysis. Additional support for D. B. was provided by a McIntire-Stennis grant from the U.S. Department of Agriculture, the U.S. Fish and Wildlife Service, the U.S. Forest Service, and the National Park Service. P. Paquet, C. McTavish, C. Callaghan, S. Fritts, R. McClymont, M. Johnson, J. Fontaine, J. Till, C. Neimeyer, R. Branzell, S. Fain, M. Jimenez, and D. Hunter provided samples. We thank the British Columbia Wildlife Branch and the Alberta Fish and Wildlife Division for logistical support. F. Allendorf, S. Fritts, D. Mech, P. Paquet, and M. Slatkin made helpful comments on the manuscript. K. Pilgrim provided valuable technical assistance, and D. Pletscher, R. Ream, and M. Fairchild helped in many ways.

## Literature Cited

Allendorf, F. W., and S. R. Phelps. 1981. Use of allelic frequencies to describe population structure. Canadian Journal of Fisheries and Aquatic Sciences **38:**1507–1514.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York.

Bangs, E. E., and S. H. Fritts. 1996. Reintroducing the gray wolf to central Idaho and Yellowstone National Park. Wildlife Society Bulletin **24:**402–413.

Boyd, D. K., P. C. Paquet, S. Donelon, R. R. Ream, D. H. Pletscher, and C. C. White. 1995. Transboundary movements of a recolonizing wolf population in the Rocky Mountains. Pages 135–140 in L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, Alberta.

Chepko-Sade, B. D., W. M. Shields, J. Berger, Z. T. Halpin, W. T. Jones, L. L. Rogers, J. P. Rood, and A. T. Smith. 1987. The effects of dispersal and social structure on effective population size. Pages 287–321 in B. D. Chepko-Sade and Z. T. Halpin, editors. Mammalian dispersal patterns. University of Chicago Press, Chicago.

Cockerham, C. C., and B. S. Weir. 1993. Estimation of gene-flow from F-statistics. Evolution **47:**855–863.

Forbes, S. H., and D. K. Boyd. 1996. Genetic variation of naturally colonizing wolves in the Central Rocky Mountains. Conservation Biology **10:**1082–1090.

Fritts, S. H., and L. N. Carbyn. 1995. Population viability, nature reserves, and the outlook for gray wolf conservation in North America. Restoration Ecology **3:**26–38.

Fritts, S. H., E. E. Bangs, J. A. Fontaine, W. G. Brewster, and J. F. Gore. 1995. Restoring wolves to the northern Rocky Mountains of the United States. Pages 107–125 in L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, Alberta.

Fritts, S. H., E. E. Bangs, J. A. Fontaine, M. R. Johnson, M. K. Phillips, E. D. Koch, and J. R. Gunson. 1997. Planning and implementing a reintroduction of wolves to Yellowstone National Park and central Idaho. Restoration Ecology **5:**7–27.

Garcia-Moreno, J., M. D. Matocq, M. S. Roy, E. Geffen, and R. K. Wayne. 1996. Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. Conservation Biology **10:**376–389.

Gese, E. M., and L. D. Mech. 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. Canadian Journal of Zoology **69:**2946–2955.

Gottelli, D., C. Sillero-Zubiri, G. D. Applebaum, M. S. Roy, D. J. Girman, J. Garcia-Moreno, E. A. Ostrander, and R. K. Wayne. 1994. Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. Molecular Ecology **3:**310–312.

Gunson, J. R. 1983. Status and management of wolves in Alberta. Pages 25–29 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Gunson, J. R. 1992. Historical and present management of wolves in Alberta. Wildlife Society Bulletin **20:**330–339.

Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Heard, D. C. 1983. Historical and present status of wolves in the Northwest Territories. Pages 44–47 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Kennedy, P. K., M. L. Kennedy, P. L. Clarkson, and I. S. Liepins. 1991. Genetic variability in natural populations of the gray wolf, *Canis lupus*. Canadian Journal of Zoology **69:**1183–1188.

Kimmel, M., R. Chakraborty, D. N. Stivers, and R. Deka. 1996. Dynamics of repeat polymorphisms under a forward-backward mutation model: within- and between-population variability at microsatellite loci. Genetics **143:**549–555.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241:**1455–1460.

Leberg, P. L. 1990. Genetic considerations in the design of introduction programs. Transactions of the North American Wildlife and Natural Resources Conference **55:**609–619.

Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution **46:**477–494.

Mech, L. D. 1995. The challenge and opportunity of recovering wolf populations. Conservation Biology **9:**270–278.

Mech, L. D., S. H. Fritts, and D. Wagner. 1995. Minnesota wolf dispersal to Wisconsin and Michigan. American Midland Naturalist **133:**368–370.

Nauta, M. J., and F. J. Weissing. 1996. Constraints on Allele size at microsatellite loci: implications for genetic differentiation. Genetics **143:**1021–1032.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Annals of Human Genetics **41:**225–233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89:**583–590.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Noss, R. F., H. B. Quigley, M. G. Hornocker, T. Merrill, and P. C. Paquet. 1996. Conservation biology and carnivore conservation in the Rocky Mountains. Conservation Biology **10:**949–963.

Nowak, R. M. 1983. A perspective on the taxonomy of wolves in North America. Pages 10–19 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Ostrander, E. A., G. F. Sprague, and J. Rine. 1993. Identification and characterization of dinucleotide repeat (CA)n markers for genetic mapping in dog. Genomics **16:**207–213.

Paquet, P. C. 1993. Summary reference document - ecological studies of recolonizing wolves in the Central Canadian Rocky Mountains. Banff National Park Warden Service, Banff, Alberta.

Paquet, P. C., J. Wierchowski, and C. Callaghan. 1996. Effects of human activities on gray wolves in the Bow River Valley Banff National Park, Alberta—a summary. In J. Green, C. S. Pacas, L. Bayley, and L. Cornwell, editors. Ecological outlooks project: a cumulative effects assessment and futures outlook of the Banff Bow Valley. Department of Canadian Heritage, Ottawa, Ontario.

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86:**248–249.

Ream, R. R., M. W. Fairchild, D. K. Boyd, and D. H. Pletscher. 1991. Population dynamics and home range changes in a colonizing wolf population. Pages 349–366 in R. B. Keiter and M. S. Boyce, editors. The greater Yellowstone ecosystem: redefining America's wilderness heritage. Yale University Press, New Haven, Connecticut.

Roy, M. S., E. Geffen, D. Smith, E. A. Ostrander, and R. K. Wayne. 1994. Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution **11:**553–570.

Shields, W. M. 1983. Genetic considerations in the management of the wolf and other large vertebrates. Pages 90–92 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science **236:**787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution **47:**264–279.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:**457–462.

Slatkin, M., and N. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution **43:**1349–1368.

Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1. A computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, Champaign.

Tompa, F. S. 1983. Status and management of wolves in British Columbia. Pages 20–24 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service report series, number 45. Printing and Publishing, Supplies and Services, Ottawa.

U.S. Fish and Wildlife Service. 1987. Northern Rocky Mountain wolf recovery plan. U.S. Fish and Wildlife Service, Denver, Colorado.

U.S. Fish and Wildlife Service. 1994. Memorandum regarding a viable wolf population in the Northern Rocky Mountains. Pages 6–68 to 6–78 in Final environmental impact statement: the reintroduction of gray wolves to Yellowstone National Park and central Idaho. U.S. Fish and Wildlife Service, Denver, Colorado.

Varvio, S., R. Chakraborty, and M. Nei. 1986. Genetic variation in subdivided populations and conservation genetics. Heredity **57:**189–198.

Wright, S. 1931. Evolution in Mendelian populations. Genetics **16:** 97–259.

Wright, S. 1969. Evolution and the genetics of populations. Volume 2. The theory of gene frequencies. University of Chicago Press, Chicago.

## Appendix

**Allele frequencies at 10 microsatellite loci in Rocky Mountain wolves.**

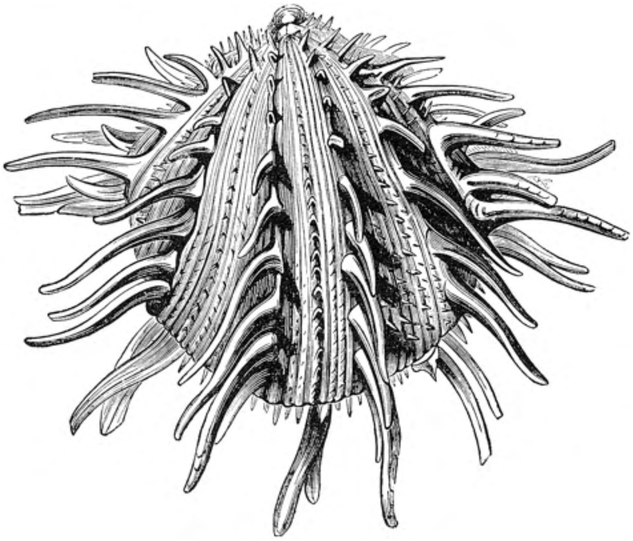
| | | *Population (sample size)* | | | | | |
|---|---|---|---|---|---|---|---|
| *Locus and allele*[a] | BP[b] | *Fort St. John* (41) | *Hinton* (33) | *Banff* (32) | *Montana* (66) | *Yellowstone* (31) | *Idaho* (35) |
| 2 | | | | | | | |
| D | 213 | 0.500 | 0.409 | 0.766 | 0.705 | 0.387 | 0.486 |
| E | 215 | 0.061 | 0.015 | 0.000 | 0.000 | 0.048 | 0.043 |
| F | 217 | 0.402 | 0.030 | 0.000 | 0.129 | 0.274 | 0.229 |
| H | 221 | 0.000 | 0.167 | 0.031 | 0.000 | 0.048 | 0.114 |
| I | 223 | 0.037 | 0.379 | 0.203 | 0.167 | 0.242 | 0.129 |
| 109 | | | | | | | |
| A | 143 | 0.012 | 0.348 | 0.094 | 0.030 | 0.226 | 0.129 |
| B | 145 | 0.427 | 0.136 | 0.203 | 0.212 | 0.274 | 0.314 |
| C | 147 | 0.024 | 0.227 | 0.281 | 0.159 | 0.161 | 0.086 |
| D | 149 | 0.305 | 0.106 | 0.031 | 0.250 | 0.161 | 0.271 |
| E | 151 | 0.122 | 0.061 | 0.375 | 0.303 | 0.048 | 0.086 |
| F | 153 | 0.012 | 0.000 | 0.000 | 0.000 | 0.016 | 0.000 |
| G | 155 | 0.098 | 0.121 | 0.016 | 0.045 | 0.113 | 0.114 |
| 123 | | | | | | | |
| E | 145 | 0.780 | 0.727 | 0.563 | 0.712 | 0.758 | 0.729 |
| F | 147 | 0.000 | 0.000 | 0.000 | 0.008 | 0.000 | 0.000 |
| G | 149 | 0.037 | 0.061 | 0.172 | 0.182 | 0.016 | 0.086 |
| H | 151 | 0.183 | 0.015 | 0.000 | 0.000 | 0.097 | 0.114 |
| I | 153 | 0.000 | 0.000 | 0.063 | 0.000 | 0.000 | 0.000 |
| J | 155 | 0.000 | 0.197 | 0.203 | 0.098 | 0.129 | 0.071 |
| 172 | | | | | | | |
| H | 155 | 0.488 | 0.485 | 0.141 | 0.288 | 0.516 | 0.457 |
| I | 157 | 0.512 | 0.515 | 0.859 | 0.712 | 0.484 | 0.543 |
| 200 | | | | | | | |
| E | 123 | 0.268 | 0.485 | 0.656 | 0.333 | 0.387 | 0.343 |
| I | 131 | 0.268 | 0.091 | 0.031 | 0.235 | 0.161 | 0.214 |
| J | 133 | 0.195 | 0.303 | 0.156 | 0.318 | 0.194 | 0.286 |
| K | 135 | 0.012 | 0.030 | 0.000 | 0.000 | 0.048 | 0.000 |
| L | 137 | 0.256 | 0.091 | 0.156 | 0.114 | 0.210 | 0.157 |
| 204 | | | | | | | |
| A | 202 | 0.049 | 0.197 | 0.281 | 0.318 | 0.097 | 0.143 |
| B | 204 | 0.085 | 0.242 | 0.344 | 0.129 | 0.177 | 0.157 |
| D | 208 | 0.317 | 0.333 | 0.344 | 0.318 | 0.339 | 0.300 |

**Appendix. Continued**

| | | *Population (sample size)* | | | | | |
|---|---|---|---|---|---|---|---|
| *Locus and allele*[a] | BP[b] | *Fort St. John (41)* | *Hinton (33)* | *Banff (32)* | *Montana (66)* | *Yellowstone (31)* | *Idaho (35)* |
| E | 210 | 0.549 | 0.227 | 0.031 | 0.235 | 0.387 | 0.400 |
| 225 | | | | | | | |
| B | 160 | 0.354 | 0.424 | 0.078 | 0.235 | 0.452 | 0.314 |
| C | 162 | 0.378 | 0.379 | 0.500 | 0.288 | 0.355 | 0.443 |
| D | 164 | 0.244 | 0.000 | 0.141 | 0.045 | 0.113 | 0.143 |
| E | 166 | 0.024 | 0.197 | 0.281 | 0.432 | 0.081 | 0.100 |
| 250 | | | | | | | |
| E | 134 | 0.000 | 0.000 | 0.063 | 0.000 | 0.000 | 0.000 |
| F | 136 | 0.244 | 0.197 | 0.250 | 0.182 | 0.258 | 0.171 |
| G | 138 | 0.183 | 0.273 | 0.047 | 0.053 | 0.290 | 0.200 |
| H | 140 | 0.232 | 0.348 | 0.313 | 0.076 | 0.161 | 0.386 |
| I | 142 | 0.000 | 0.015 | 0.063 | 0.205 | 0.000 | 0.000 |
| J | 144 | 0.232 | 0.136 | 0.266 | 0.485 | 0.226 | 0.143 |
| L | 148 | 0.110 | 0.030 | 0.000 | 0.000 | 0.065 | 0.100 |
| 344 | | | | | | | |
| A | 156 | 0.913 | 0.818 | 0.734 | 0.697 | 0.883 | 0.871 |
| D | 162 | 0.050 | 0.030 | 0.063 | 0.061 | 0.050 | 0.043 |
| E | 164 | 0.013 | 0.152 | 0.172 | 0.242 | 0.050 | 0.071 |
| F | 166 | 0.000 | 0.000 | 0.031 | 0.000 | 0.000 | 0.000 |
| G | 168 | 0.025 | 0.000 | 0.000 | 0.000 | 0.017 | 0.014 |
| 377 | | | | | | | |
| B | 146 | 0.073 | 0.076 | 0.000 | 0.023 | 0.000 | 0.129 |
| C | 148 | 0.098 | 0.136 | 0.141 | 0.500 | 0.145 | 0.114 |
| G | 156 | 0.049 | 0.000 | 0.094 | 0.000 | 0.032 | 0.029 |
| H | 158 | 0.037 | 0.045 | 0.016 | 0.045 | 0.048 | 0.014 |
| I | 160 | 0.000 | 0.045 | 0.000 | 0.000 | 0.032 | 0.014 |
| J | 162 | 0.134 | 0.439 | 0.203 | 0.144 | 0.242 | 0.314 |
| K | 164 | 0.122 | 0.061 | 0.063 | 0.045 | 0.129 | 0.086 |
| L | 166 | 0.488 | 0.197 | 0.438 | 0.242 | 0.371 | 0.300 |
| O | 172 | 0.000 | 0.000 | 0.047 | 0.000 | 0.000 | 0.000 |

[a] *Letter codes designate two-basepair allele size increments that match the codes in Roy et al. (1994).*

[b] BP *is the size of the PCR product for each allele in DNA basepairs.*



# Population Viability, Nature Reserves, and the Outlook for Gray Wolf Conservation in North America

Steven H. Fritts[1]
Ludwig N. Carbyn[2]

## Abstract

Theoretical work on population viability and extinction probabilities, empirical data from *Canis lupus* (gray wolf) populations, and expert opinion provide only general and conflicting conclusions about the number of wolves and the size of areas needed for conservation of wolf populations. There is no threshold population size or proven reserve design that guarantees long-term (century or more) survival for a gray wolf population. Most theoretical analyses of population viability have assumed a single, isolated population and lack of management intervention, neither of which is likely for wolves. Data on survival of actual wolf populations suggest greater resiliency than is indicated by theory. In our view, the previous theoretical treatments of population viability have not been appropriate to wolves, have contributed little to their conservation, and have created unnecessary dilemmas for wolf recovery programs by overstating the required population size. Nonetheless, viability as commonly understood may be problematic for small populations at the fringe of or outside the contiguous species range, unless they are part of a metapopulation. The capability of existing nature reserves to support viable wolf populations appears related to a variety of in situ circumstances, including size, shape and topography of the reserve; productivity, numbers, dispersion, and seasonal movement of prey; extent of poaching inside; degree of persecution outside; exposure to enzootica; attitudes of local people; and proximity to other wolf populations. We estimate that a population of 100 or more wolves and a reserve of several thousand square kilometers may be necessary to maintain a viable population in complete isolation, although 3000 km$^2$ or even 500–1000 km$^2$ may be adequate under favorable circumstances. In most cases, management intervention is probably necessary to assure the viability of relatively small, isolated populations. Because most reserves may be inadequate by themselves to ensure the long-term survival of wolf populations, favorable human attitudes toward the species and its management must be recognized as paramount, and cooperation of neighboring management jurisdictions will be increasingly important.

## Introduction

Any attempt to foretell the future is risky. Nonetheless, if history teaches us anything at all, it seems safe to predict that human attitudes and values will continue to be the ultimate factor limiting the number and distribution of wolves in North America. These factors, combined with conflicts over land-use priorities will shape the laws, policies, and human behavior that will determine the fate of wolves in different parts of the continent throughout the twenty-first century. The fate of the species clearly is in human hands; we could eradicate it if we decided to. Contemporary human manipulation of wolves ranges from annihilation as an undesirable predator of livestock and big game to reintroduction as an ecologically important "top consumer." Reintroduction programs (Fritts et al. 1994*a*; Parsons & Nicholopoulus 1994) and natural repopulation will re-establish wolves in parts of their former range and potentially create the situations that concern population viability theorists the most—that is, relatively small populations that are isolated or semi-isolated from other populations.

A viable wolf population requires an area of some minimum size with adequate prey and security from excessive human exploitation. There will always be a range of areas in which the spectrum passes from complete intolerance—such as areas of intensive agriculture—to tolerance in wildland areas to complete protection of wolves and their prey in some nature sanctuaries. In some regions, wolf populations may be able to survive only in parks, designated wilderness areas, wildlife refuges, and wildlife management areas, where conflict with human economic enterprise is minimal. Generally such areas may be useful but are not now essential to the survival of the species on a continent-wide basis. They are the most secure places, however, to which the range could collapse in future decades or centuries, facilitated by habitat fragmentation, and the result may be a complex subdivision of the species' range.

Accordingly, it becomes important to ask what minimum population levels and types of areas are necessary for wolf population survival. How much genetic exchange between and among populations is necessary to maintain genetic

---

[1] U.S. Fish and Wildlife Service, 100 N. Park, Suite 320, Helena, MT 59601, U.S.A.

[2] Canadian Wildlife Service, 4999–98 Avenue, Edmonton, Alberta, Canada T6B 2X3.

variability and vitality? What does it take to preserve wolves in special places, and what should be the size and characteristics of those areas? And is enough information available to begin to address these questions?

We synthesized information pertaining to the estimation of the minimum viable population (MVP) size for wolves, examined case histories of wolf populations, especially the performance of isolated or semi-isolated populations of various sizes, and solicited the opinions of wolf biologists to develop insight into the minimum population size and land-area requirements for wolf conservation. From this we discuss the concept of nature reserves for wolves and the characteristics of apparently adequate reserves. In doing so, we touch on what we think will be one of the major wolf conservation issues of the twenty-first century, as well as a general conservation issue. Although our treatment of this subject is preliminary, we hope to stimulate further thought on conservation biology issues regarding wolves, bring the issue into clearer focus, and provide hypotheses for future evaluations.

## What is the Potential Role of Nature Reserves in Wolf Conservation?

A nature reserve is an area that has been removed from development for the purpose of perpetuating natural conditions (Pyle 1980), but their characteristics, their management, and their capability for supporting large carnivores vary dramatically (Shafer 1990). Large carnivores are particularly vulnerable by virtue of their low numbers and wide-ranging movements. Often people living closest to wolves have negative perceptions about them. Therefore, it is especially challenging to protect these animals against anthropogenic effects that operate outside of nature reserves (Schonewald-Cox et al. 1992). In Canada and the U.S., national parks and preserves, national wildlife refuges, ecological reserves and wilderness areas are the best examples of such places, but none is free of human impacts. National parks are more likely to be free of practices such as livestock grazing and hunting that could conflict with the presence of wolves. Even within parks, factors such as tourism and roads may compromise the ability to protect certain wildlife, especially in temperate areas where human population is higher (Schonewald-Cox & Buechner 1992). National forests in the U.S. and military lands in both the U.S. and Canada might play a key long-term role in wolf conservation, but that role is less certain because of their multiple-use policies. Nature reserves may be buffered by surrounding lands that provide habitat (for example, Yellowstone National Park surrounded by national forests) or may have no buffer (for example, Riding Mountain National Park surrounded by agricultural lands). The adequacy of even the largest reserves to protect viable populations of carnivores over the long term is questionable (see Soulé 1987*a*; Grumbine 1990). To date, nature reserves have not played a critical role in gray wolf survival in the U.S. and Canada, although they have been important regionally. Most such reserves in the contiguous U.S. were established after the wolf was virtually extinct (Fritts et al. 1994*b*) and certainly not with wolf conservation in mind. In considering potential nature reserves for wolves, we need to recognize that places with minimal human influence are not necessarily required. Although people have come to associate wolves with undisturbed wilderness, wolves are able to survive in highly altered landscapes and near humans so long as food is available and they are not unduly persecuted (Mech 1995).

The availability of additional lands that could be occupied by wolves in combination with those currently occupied generally sets the upper limit to continent-wide population recovery. In Canada, wolves remain on about 86% of their former range (Carbyn 1983; Hummel & Pettigrew 1991). Within the 48 contiguous United States, the species was almost exterminated (except for Minnesota and Isle Royale) and currently occupies a small fraction of its former range comprised of Minnesota, Wisconsin, Michigan, and Montana (Thiel & Ream 1994). Wolves still occupy at least 85% of Alaska (Stephenson et al. 1994).

In the U.S., the areas with the greatest potential to afford protection to wolves and their prey are under federal ownership, including national parks, designated wilderness, national wildlife refuges, and possibly some tribal and military lands. Federal land in the U.S. totals 2,679,671 km$^2$, or 29% of the land area, with the percentage in most intermountain states ranging from 28% to 82%; federal land in Alaska totals 68% (U.S. Bureau of Land Management 1992). At the end of 1985, lands of the U.S. National Park System comprised 3.3% of the U.S. land base. At the end of 1992, the total area of national parks per se was about 193,400 km$^2$, with another 90,378 km$^2$ in national preserves (U.S. National Park Service 1993). It is heartening that 85% of lands adjacent to national parks is in public ownership (Newmark 1986). Nine units of the national park system exceed 10,000 km$^2$, and those, representing about 3% of all units, are in Alaska (Shafer 1990:98). Yellowstone National Park, at 8992 km$^2$, is the largest in the lower 48 states. Three units in the Wilderness Preservation System in Alaska (not part of the nine units previously mentioned) exceed 10,000 km$^2$, the largest being 53,365 km$^2$ (Wrangell–St. Elias National Park and Preserve). In July 1980, the Wilderness Preservation System in the U.S. included up to 3.8% of U.S. land base. On a global scale, 98% of the world's nature reserves are 10,000 km$^2$ or less and 85% are 1000 km$^2$ or less (Shafer 1990:63). Currently, less than 5% of wolves in the lower 48 states live within national parks (Isle Royale, Voyageurs, Glacier), where security is greatest.

Wildlands in the U.S. and Canada are owned or managed by a variety of agencies under widely differing mandates. Large carnivores, including wolves, are thought to require tracts of land larger than the jurisdiction of a single agency or landowner (Salwasser et al. 1987; Mattson & Reid 1991). Both in Canada and the U.S., cooperation between adjacent national parks, wildlife refuges, and other wildlands as conservation networks has been advocated as a means of greatly increasing the effective size of areas for large species (Salwasser et al. 1987; Hummel & Pettigrew 1991). Salwasser et al. (1987) identified 10 potential networks in the U.S., most in the western states, ranging from 925 $km^2$ to 12,373 $km^2$. Differences in the missions and legal mandates of government land-management agencies are the primary challenge to cooperative management of large carnivores (Salwasser et al. 1987).

National parks also provide the most secure areas for carnivore conservation in Canada. Canada's vast land mass (10 million $km^2$) is sparsely populated. In approximately 89% of its territory there are no major human settlements. The greatest north-south distance is some 4600 km, while east-west distance is about 5500 km. In 1993 Canada contained 36 national parks, of which 18 supported wolf populations (D. Poll, Canadian Parks Service, personal communication). National parks occupy about 180,000 $km^2$ of the land base. Thirty of the national parks within the Canadian Parks System are smaller than 10,000 $km^2$. The remaining six large national parks represent about 95% of the combined park area. Wood Buffalo National Park, at 44,800 $km^2$, is the largest in Canada and the second largest park in the world.

As is true for the U.S., the effectiveness of the Canadian National Parks System in protecting carnivores has been questioned, and one solution being proposed is to establish "Carnivore Conservation Areas" (Bath et al. 1988; Hummell & Pettigrew 1991). Five candidate areas were identified in western Canada, ranging in size from 6500 to 40,000 $km^2$. The concept was to identify protective core areas—critical areas with the highest protection—and secondary areas around the core in which resource extraction and use of renewable resources could occur in the most appropriate manner to minimize effects on wildlife populations in core areas. The biosphere reserve concept is very similar, as it promotes buffer areas adjacent to reserves, thus decreasing the probability of isolation (UNESCO 1974; Shafer 1990). The concept of Carnivore Conservation Areas has attracted little political support, although some progress is being made within the Canadian Rocky Mountain Parks complex (P. Paquet, World Wildlife Fund, personal communication).

## Concept of Minimum Viable Populations

The relationship between the size and design of nature reserves and long-term wolf conservation is inextricably related to the concept of minimum viable population. The estimation of MVP has not been widely applied to wolves, mainly because some 60,000 to 70,000 gray wolves survive over a broad area of the continent (Hayes & Gunson 1994; Stephenson et al. 1994; Thiel and Ream 1994).

Population viability analysis addresses the likelihood of a population's persistence over a specified period under specified conditions (Gilpin & Soulé 1986; Boyce 1992). The minimum viable population is a population large enough to permit "long-term" persistence despite genetic, demographic, and environmental uncertainties (Shaffer 1981). Discussions of MVPs are predicated on the availability of a minimum secure space, sometimes referred to as a minimum area requirement, that has a specified probability (such as 95% or 99%) of supporting a population for an arbitrarily chosen period (such as 100 or 200 years) (Soulé & Simberloff 1986; Belovsky 1987; Goodman 1987; Soulé 1987*a*; USFWS 1989; Boyce 1992). Parameters applicable to MVPs include genetic diversity, demographic stochasticity, environmental stochasticity, long-term stages in plant succession, natural catastrophes, and social dysfunction. Genetic heterozygosity is thought to be positively correlated with the reproductive vigor needed for population growth and adaptation to changing environments. Demographic factors affect population size and persistence and include such parameters as sex ratio, litter size, survival rates, age distribution, and age at first reproduction (Shaffer 1981). Variance in individual reproductive performance is a major component of both demographic and genetic stochasticity and thus is a major component of the analysis of population viability. Changes in climate and other unpredictable aspects of the environment affect the availability of key resources, especially vulnerable prey (Packard & Mech 1980) in the case of wolves. Similarly, long-term changes in plant succession can cause major shifts in prey availability, thus affecting a wolf population (Mech & Karns 1977; Thomas 1994).

The genetic variation of closed populations may be affected if the gene pool is small. Three types of variations are important: heterozygosity, gene diversity, and allelic diversity. Heterozygosity is genetic variation within an individual animal, and it diminishes with inbreeding. Gene diversity is directly related to the variation of core population representation in the living descendants of a population. Allelic diversity is a measure of the number of different alleles at a locus in the living descendants of a population. Debates have centered on the impact of inbreeding within a mammalian population. Small, isolated populations are theoretically more likely to become extinct within a specified period than large, contiguous populations because of loss of genetic variability, demographics, and unpredictable events. Small populations lose genetic variability much faster than larger populations, but the problem evidently can be offset with only one or a few migrants per generation (Allendorf 1983; Lacy 1987). Wolf dispersal can be very extensive, up to 886 km (Fritts 1983), and wolves can cross large expanses of open area and interstate highways (Licht & Fritts 1994), so complete isolation and problems of genetic variability would likely be rare. Actually, the effects of inbreeding and loss of genetic diversity on the persistence of wild populations in general has recently been questioned; no wild population has been demonstrated to have declined because of inbreeding (Caro & Laurenson 1994).

The development of the concept of minimal viable population has been highly theoretical and has generally followed two separate tracks. Community ecologists have focused on minimum areas for system viability, drawing on concepts from island biogeography, whereas population biologists have focused on the minimum population sizes or densities for target species (Soulé 1987*a*). The process of population viability analysis (PVA) was developed in the 1980s to examine how stochastic factors interact to influence the persistence of small populations (Gilpin & Soulé 1986). During the last decade, numerous papers have appeared on the genetic and demographic aspects of MVP, but development of an accurate model to predict population survival is still elusive. An MVP is yet to be confidently calculated for any species, according to Shafer (1990:102).

## Existing Information on the State of the Art

**Relevant Literature.** Calculations that consider genetic factors such as inbreeding generally involve an estimation of the ratio of the effective population size ($N_e$) to the total population ($N$). A 1% level of inbreeding per generation, which is often assumed to be the maximum acceptable for short-term viability, is said to require an $N_e$ of 50 (Soulé 1980:160). Accordingly, a wolf population would have to be well in excess of 50 just to meet the 1% rule (Theberge 1983). Bath et al. (1988), using the Reed et al. (1986) formula, calculated that an $N_e$ of 50 required 46–150 wolves (about the number that has been predicted to exist eventually in the Yellowstone ecosystem after reintroduction) (Yellowstone National Park et al. 1990). For long-term viability of animals, an $N_e$ of 500 has been suggested (Franklin 1980; Lande & Barrowclough 1987). For most species, $N$ must be about 3–4 times $N_e$. These rough figures for short-term and long-term survival have led to the so-called 50/500 rule for genetic fitness, which is often used despite a slim empirical basis (Shafer 1990:73; Foley 1992). Many writers have stressed that this number may only be within the right order of magnitude and that we do not have the ability to predict hard numbers (Grumbine 1990; Boyce 1992). From the information on reserve sizes presented earlier, it is clear that finding any totally protected reserves that could support an $N_e$ of 500 wolves in the lower 48 states or Canada would be very difficult—much less any additional such reserves.

Soulé (1980:162–163), using the 1% rule (and assuming that one-third of adults actually breed, that a certain amount of population fluctuation occurs, that generations overlap, and other assumptions), roughly calculated that an $N_e$ of 200 would be needed for wolves and that a total population of 600 or more would be needed to overcome loss of genetic variability. Theberge (1983:88–89) also anticipated loss of genetic variability in small or isolated populations of wolves and the loss of vigor and fecundity that could result. Based on the minimum $N_e$ of 50 wolves, he calculated that a minimum of 13,000 km$^2$ would be necessary to support a population in Canada, owing to the low densities of wolves, assumed at 1 wolf/260 km$^2$, in his calculations. He warned against actions that would create an increasingly patchy distribution, including regional prey depletion and long-term wolf control. Shields (1983), on the other hand, argued that wolves are organized into small, semi-isolated demes; a degree of inbreeding naturally characteristic of a fragmented population structure would allow maintenance of local adaptations that would enhance the species-wide genetic variability, allowing greater ability to respond to environmental change. Shields clearly underestimated the amount of gene flow normally occurring in wolf populations based on the dispersal behavior of the species (Brewster & Fritts 1994; Meier et al. 1994; Stephenson et al. 1994). Moreover, recent studies of genetic relationships within and among wolf populations have revealed substantial evidence of outbreeding (Kennedy et al. 1991; Lehman et al. 1992), with little opportunity for the development and maintenance of local adaptations. The reader is also referred to Chepko-Sade et al. (1987) for preliminary estimates of $N_e$ in wolves.

Estimating MVP from models is difficult for a variety of reasons, a major one being environmental stochasticity. Boyce (1992:500) reviewed five empirical studies on extinction for different vertebrate taxa on oceanic and habitat islands, which revealed that populations below 50 consistently show a high probability of extinction, whereas populations above 200 are often reasonably secure, given protected habitat. Soulé and Simberloff (1986:19) observed that thoughtful estimates of MVP for many animal species are rarely lower than $N_e$ of a few hundred, and this lower limit would often correspond to an actual population of about 1000. Soulé (1987*b*:175) guessed that MVPs (95% expectation of persistence for several centuries) would often be in the low thousands for vertebrates. If this were the case, long-term conservation for many wolf populations in a specific regional setting would be impossible.

Thomas (1990) used empirical data to estimate population sizes necessary for medium-long-term persistence and concluded that 10 is too few, 100 is usually inadequate, 1000 is adequate for species of normal variability, and 10,000 should permit the persistence of most birds and mammals. He pointed out, however, that populations that occupy habitat fragments far too small to hold thousands of individuals sometimes have strong conservation potential. If isolation is not complete, if variability in population size is low, and if the environment is stable, geometric mean values of

500 may allow long-term persistence (see Franklin 1980; Lande & Barrowclough 1987; Shafer 1990:73).

The only PVAs done on wolves to date have been on *Canis lupus baileyi*, the Mexican wolf (D. Parsons, U.S. Fish and Wildlife Service, personal communication), *Canis lupus* spp., the gray wolf in Italy (Ciucci & Boitani 1991), and *Canis rufus*, the red wolf (USFWS 1989). The red wolf analysis used a software program developed by J. Ballou, whereas the Mexican wolf and Italian wolf analyses employed the computer program VORTEX (Seal & Lacy 1989). The PVA for the Italian wolf was unique in that it examined an existing wild population (280–300 individuals). But field data on some of the population parameters needed for the analysis were not available, requiring the use of best guesses (Ciucci & Boitani 1991). This PVA depicted an extreme sensitivity of the Italian population to any increase in adult mortality. The analysis suggested a fairly high probability of extinction within both 100 and 60 years for the two presumably isolated subpopulations if any increase in adult mortality occurred, even in the absence of any environmental instability or inbreeding depression. However, a high probability of survival was predicted if adult mortality were held at 10%. Management intervention of the wild population(s) – translocation of animals or genetic material – and immediate establishment of a captive population were recommended. Problems for the Italian wolves are greatly exacerbated by the separation of the two main populations. Assessment of the accuracy of this model should consider that the population(s) recently rebounded from around 100 wolves for several decades to its present size, that it has been isolated for 100–200 years, and that it has been increasing.

The PVA for the red wolf concluded that it would not be possible to maintain a single, contiguous wild population of the hundreds or thousands that were determined necessary for viability. The chosen approach was to manage it as a metapopulation, with intensive migration management to preclude genetic and demographic problems. Establishment of 220 red wolves in the wild and 330 in captivity is the goal (USFWS 1989). The original founder population of the red wolf was 15 wild individuals.

The draft PVA for the Mexican wolf, which exists only in captivity, recommends that to recover the subspecies biologically a metapopulation of at least 1000 free-ranging wolves will be needed, although no justification for this number is provided. In addition, a population of 300–500 Mexican wolves in captivity was recommended to preserve 90% of the original genetic diversity for 200 years (D. Parsons, U.S. Fish and Wildlife Service, personal communication). Another analysis indicated that the maximum calculated potential level of genetic conservation for the captive population of the Mexican wolf would be 85%. A goal of retaining 75% of the original genetic diversity for 50 years was set. The entire population is based on four founders (P. Siminski, Arizona-Sonora Desert Museum, personal communication).

Discussion and studies about the reintroduction of wolves into Yellowstone National Park (8992 km$^2$) have addressed whether the park alone is an adequate reserve for wolves. Estimates of the number of wolves that would occupy the park range from 50 to 150, with the general conclusion that the park could support a self-sustaining population (Yellowstone National Park et al. 1990). Boyce (1990) developed a model that demonstrated an important relationship between the area (size) of the recovery zone for a hypothetical Yellowstone wolf population and its expected time to extinction. For example at $n = 40$, the estimated time to extinction was 20.8 years, and at $n = 60$ it was more than 350 years. Boyce emphasized the obvious: the security of a Yellowstone wolf population could be increased by expanding the recovery zone outside of Yellowstone National Park, which was assumed to increase population size.

Regarding reserve size, Belovsky (1987) concluded that the largest mammalian carnivores (10–100 kg) can be expected to persist 100 years in 0–22% of the world's current parks, but no park is large enough by itself to guarantee persistence for 1000 years. According to Shaffer (1987:81), larger mammalian species will require reserves on the order of 100,000–1,000,000 km$^2$ for a 95% probability of surviving even a century. Soulé (1987*b*:177) stated that "most MVPs will be so large that it will be impossible to contain this many individuals of a large animal in reserves and sanctuaries of modest size (up to thousands of km$^2$)." However, maintaining multiple populations can help offset this problem (Soulé 1987*b*).

There appears to be general consensus among conservation biologists that U.S. reserves are too small to support large vertebrate species for long periods (Schonewald-Cox 1983; Newmark 1985; Salwasser et al. 1987; Soulé 1987*a*; Grumbine 1990). Newmark (1985) examined eight parks and park assemblages in western North America and concluded that only one could support populations (MVP = 50) of wolves and other wide-ranging mammals. At a MVP of 500, even the largest preserve was one-sixth the necessary size. Similar conclusions were reached by Schonewald-Cox (1983), Salwasser et al. (1987), Grumbine (1990), and others. Simberloff (1988:504) suggested, however, that Newmark may have greatly overestimated the insularization of American parks and rates of mammalian extinction. Newmark (1985) pointed out the basic incongruence between legal and biotic boundaries of parks.

The size and spatial distribution and configuration of reserves has received much theoretical treatment. Goodman (1987) believed that a series of reserves, equal in total area to one large reserve, would have fewer extinctions from environmental perturbation than a large one, so long as there was migration among them (all other factors assumed to be equal). In fact, several writers have pointed out that relaxing the assumption of a single, totally isolated population alters persistence times dramatically (see Shaffer

1987). Many species, including wolves, in reality often exist as a population of populations, termed a metapopulation (Levins 1970). Allendorf's (1983) recommended management strategy of one successfully reproducing migrant per generation among isolated nature reserves would presumably occur naturally in a true metapopulation, maintaining genetic variability. Shaffer (1987) pointed out that metapopulation arrangements can aid significantly in reducing the size requirement of any particular reserve, so long as animals can move among reserve units. Wolves have a strong advantage in this regard because of their tendency to travel long distances (Ballard et al. 1983; Fritts 1983; Gese & Mech 1991; Ream et al. 1991). The extent to which environmental perturbations act in concert among the different patches of a metapopulation is a critical factor in determining the advantage of that arrangement (Gilpin 1987). For example, a catastrophe would probably affect only one patch, allowing repopulation from other patches. Actually, we are not aware of any examples of catastrophic effects to wolf populations except from human persecution.

Areas of 10,360–12,950 km$^2$ have been suggested as a minimum size for wolves (Mech, *vide* Henshaw 1979:430). Soulé (1980:163) calculated roughly that a wolf reserve would need to be at least 12,000 km$^2$ for short-term viability and an order of magnitude larger for long-term viability. American recovery plans for the gray wolf contain area and number requirements arrived at by consensus of recovery team members familiar with the species. The Northern Rocky Mountain Wolf Recovery Plan mentioned a minimum contiguous area of 7770 km$^2$, or a lesser area if adjacent available lands that could support wolves exceed 7770 km$^2$ in the aggregate of lands (USFWS 1987:22). The Mexican Wolf Recovery Plan recommended reestablishing a "viable, self-sustaining population of at least 100 Mexican wolves in the middle to high elevations of a 12,950 km$^2$ area within the Mexican wolf's historic range" (USFWS 1982:23). Areas being considered for release of Mexican wolves contain 2000–3000 km$^2$ and 13,000–18,000 km$^2$ of habitat thought to be suitable for wolves (D. Parsons, U.S. Fish and Wildlife Service, personal communication). One such area, the White Sands Missile Range, includes a total of 2580 km$^2$ considered as potential wolf habitat and was projected to support about 30 wolves (Bednarz 1989). An evaluation of areas suitable for wolves in Colorado identified a complex of 98,000 km$^2$ of public land (25,000 km$^2$ roadless) that is contiguous. The area was estimated to be capable of supporting 1128 wolves (Bennett 1994).

The revised Eastern Timber Wolf Recovery Plan (USFWS 1992) specified the need for two "viable" populations, Minnesota's plus one other. For a population outside of Minnesota to be considered viable, (1) if isolated, it must average at least one wolf per 128 km$^2$ (self-sustaining, $\geqslant 200$ wolves) distributed within a minimum of 25,600 km$^2$ over five consecutive years, or (2) if located within 160 km of a self-sustaining wolf population, it must average at least one wolf per 128 km$^2$ or consist of 100 wolves in an area of at least 12,800 km$^2$ over five consecutive years. The recovery team believed that a population of at least 200 wolves located more than 322 km from the Minnesota population (for example, in northern New York or northern Maine) was large enough to be viable, as well as to have sufficient genetic diversity to exist indefinitely in isolation. The team also believed that a smaller population (>100) in Wisconsin/Michigan would remain viable and retain necessary genetic diversity via immigration from Minnesota (USFWS 1992:25–26). Overall, the team believed that a healthy, self-sustaining population should include at least 100 interbreeding wolves.

**Survey of Biologists.** In November-December 1992, 43 American and Canadian biologists familiar with wolves were surveyed about whether a scenario as in the Northern Rocky Mountain Wolf Recovery Plan would equate to a viable population. Recovery goals set in the plan were 10 breeding pairs (assumed to be about 100 wolves) living in each of three separate areas for at least three consecutive years (USFWS 1987). The biologists were expected to apply their own understanding of the viability concept in their responses. Fourteen of the 23 who responded (61%) believed that 10 breeding pairs sustaining themselves for three consecutive years at least met the minimum standards of a viable population, whereas five believed this number was too low. An additional two (16 of 23) (70%) agreed that three such groups of 10 breeding pairs in a metapopulation for three consecutive years (about 300 wolves) met the definition of viable. Eleven volunteered the comment that an isolated single population would likely face future difficulty, but exchange among three subpopulations would greatly enhance viability of subpopulations. Relatedness of founders was another frequently mentioned consideration.

**Case Histories.** The use of empirical data lends the most valuable insight into the persistence of isolated or semi-isolated wolf populations of different size and in different types of areas, but such information is scarce.

*Isle Royale National Park, Biosphere Reserve, Michigan — 544 km$^2$.* The best information on the dynamics of a small, isolated wolf population is from Isle Royale in Lake Superior. The Isle Royale wolf population was probably founded in 1949 from a single gray wolf pair immigrating over the ice from mainland Minnesota, a distance of 24 km (Mech 1966). The population has numbered as high as 50, but from 1988 to 1993 it declined to about a dozen animals, and then increased to 17 (Peterson 1994). Wayne et al. (1991) calculated that the Isle Royale population may have only about 50% of the heterozygosity of mainland wolves. Both the founder effect and genetic drift were believed instrumental in reducing genetic variability, which may have been responsible for the population decline via poor reproduction. Despite development of these problems, it is important to note that the Isle Royale population thrived for 30 years and is still present 43 years later, although it is of questionable viability today (Wayne et al. 1991). In 1994, however, it increased significantly (Peterson 1994). An advantage of this island as a nature reserve is that it is completely surrounded by water and relatively free of most negative human influences. But absence, or near absence, of immigration is a clear disadvantage.

*Riding Mountain National Park, Man and Biosphere Reserve, Manitoba—2978 km²/6700 km².* This Biosphere Reserve consists of the park (2978 km²) and surrounding agricultural area. Wolves colonized the area during the early 1930s, probably from the Duck Mountains, a distance of about 35 km. The population has fluctuated between 40 and 120 wolves and has persisted some 60 years. About 70% of the packs are vulnerable to human exploitation, with considerable turnover in the population (Carbyn 1989). The surrounding agricultural zone affords no protection, so the effective zone for wolf conservation is the park area. A high prey biomass and lack of obvious clumping of prey seem to be important factors. The population appears secure in the short term, and the park probably provides a better sanctuary for wolves than is the case for the Jasper National Park complex, which is more than three times larger. This is because Jasper National Park includes suitable ungulate ranges, particularly winter ranges, which are clumped into long, narrow valley systems. Wolves in these valley systems are subjected to high vehicular and train mortality. Movements of packs along valley systems leads them outside the park, where they become vulnerable to human persecution.

*Jasper National Park, Alberta—10,878 km².* Wolf extermination and recolonization in this park has occurred twice in the 20th century (Boyd et al. 1994). The area has supported 40 to 80 wolves in recent years (Carbyn 1983; Dekker et al. 1994). As mentioned above, a large proportion of wolves leaves the park in winter. The population has undergone wide fluctuation due, at least in part, to predator control outside the park (J. Weaver, University of Montana, personal communication). Even though the Canadian Rocky Mountain National Parks cover large areas, the effective zones for wolf conservation are limited to areas of high ungulate concentrations. These areas are limited, often to valley bottoms. Areas that are also used as human transportation corridors render wolves vulnerable to vehicular and train mortality.

*Kenai Peninsula, Alaska—26,000 km².* This peninsula is essentially an island connected with the Alaska mainland by a narrow neck of land and ice only 16 km wide. About 14,278 km² on the Kenai are considered wolf habitat (Peterson et al. 1984). Wolves were extirpated by about 1915; natural recolonization began during the 1960s (Peterson & Woolington 1982). During the 1970s the population expanded rapidly and became established throughout the peninsula, with about 186 wolves present in 1981 (Peterson et al. 1984). Currently, the population is stable at 150–180 wolves, with substantial regulated human harvest.

*Alexander Archipelago, Alaska.* Wolves occupy a 540-km long complex of islands of various size and degree of isolation off the coast of southeastern Alaska. Island sizes range from less than 30 km² to more than 6700 km² (Prince of Wales Island), and distances between islands range from less than 1 km to 12 km. The overall population is estimated at about 750–1500 animals (M. Kirchoff, Alaska Department of Fish and Game, personal communication). The wolves occupying these islands, considered in some taxonomic treatments as *Canis lupus ligoni* (the Alexander Archipelago wolf), may have been isolated for centuries or even millennia by open water. The Alexander Archipelago wolf is currently being proposed by the U.S. Fish and Wildlife Service for listing as threatened under the Endangered Species Act of 1973, as amended (Lindell & Grossman 1994). Historical data suggest that only a few islands are sufficiently large to maintain persistent wolf populations completely within their boundaries (D. Person, University of Alaska, personal communication). Preliminary simulations of wolf population persistence indicated that an island size of 2500–3500 km² was needed for a population persistence of 250 years. Smaller islands may represent ephemeral sink populations maintained by dispersal from stable sources on larger islands (D. Person, University of Alaska, personal communication). Studies underway should provide additional information about the relationship between island size, wolf population size and persistence, and the actual degree of isolation from other wolf populations (D. Person, University of Alaska, personal communication). The smaller body size and skull dimensions of Alexander Archipelago wolves compared to Alaska mainland wolves (Pedersen 1982) supports the notion that they have indeed been isolated for a long period.

*Coronation Island, Alaska—73 km².* This island is part of the Alexander Archipelago discussed above and historically was not inhabited by wolves. Four wolves were introduced in 1960 by the Alaska Department of Fish and Game. Wolves increased to 13 (0.18 wolf/km²) in four years, declined to one wolf by 1968, and went extinct within 10 years of introduction. The conclusion from the experiment was that the island was too small to sustain a prey base for a population of wolves (Klein 1994) and that immigration of wolves was not possible.

*European wolf populations.* Various parts of Europe support wolves, also considered *Canis lupus ssp.*, and circumstances there shed light on the tenacity of wolf populations. Wolves on the Italian and Iberian peninsulas have survived long periods of isolation at greatly reduced population sizes.

In Italy, wolves were widespread until the second half of the nineteenth century. They have apparently been isolated from other European wolves for 100–200 years (Francisci & Guberti 1993) and are distributed in two apparently isolated populations in central and southern Italy (Boitani & Ciucci 1993). As a result of legal protection, populations have increased from 100–200 in 1971–1973 to 300–400 in 1990–1991, and distribution has expanded (Francisci & Guberti 1993). A study of 45 wolves by Randi reported by Francisci and Guberti (1993) suggested a panmictic population, polymorphic at average levels for nuclear genes and coding enzymes, and monomorphic at the mtDNA level. This population seems to be viable in every sense, appears limited only by killing by humans, and is believed to still be increasing as human attitudes toward the species become more positive.

The wolf population in Spain has been progressively reduced in number and distribution by human activities. Blanco et al. (1990) estimated a total population of 1500–2000 individuals, primarily in the northwest portion of the country, with small isolated groups of 40–56 individuals surviving in the south. Some 150 wolves remain in northeastern Portugal adjacent to the Spanish population (Promberger 1993). Wolves in Spain and Portugal have been isolated from continental populations for at least several decades and appear to be depressed only by human persecution.

The population in Poland numbered about 1000 wolves at the end of World War II, but subsequently they were considered pests and deliberately reduced to about 100 after the 1950s. After 1976, when their game-animal status was resumed, wolves increased steadily and were estimated at 858 by the spring of 1991 (Bobek et al. 1993).

At least 23 immigrant wolves, apparently from Poland, were killed in Germany between World War II and 1992. Reproduction occurred in the country in 1992 for the first time in 150 years (Promberger et al. 1993). The wolf was eliminated from France, but now a pack has become established in the French Alps, likely via dispersal from Italy (C. Promberger, Munich Wildlife Society, personal communication).

Wolves were widespread in Sweden and Norway until they were heavily persecuted and essentially eradicated by the beginning of the nineteenth century. Remnants survived for a long time with a surprisingly small population base in northern Scandinavia (Pulliainen 1993). The last wolf was killed in Sweden in 1966. Legal protection was granted in Sweden and Norway in 1973. Since then a population has become reestablished, probably via dispersal from Finland; although isolated from the nearest population by at least 500 km, this population has grown dramatically in the past two years, increasing to a total of 20–25 wolves in the two countries (Promberger et al. 1993). For years, Finland has represented the western margin of the European wolf population and has been the scene of several disappearances and attempts at recolonization by immigrants, all thwarted by humans.

Many other areas of Europe and Asia continue to support wolves, including small populations in Israel (Mendelssohn 1982) and Iran (Joslin 1982). Wayne et al. (1992) discovered greater genetic (mtDNA) subdivision of Old World wolf populations than in North American populations; they attributed the finding to smaller populations and greater isolation in Europe than in North America.

## Reserve Considerations

The overall size and configuration of nature reserves is important in providing adequate space for the protection of wolves, but prey biomass and prey distribution in the system are also critical. For example, the clumping of prey on winter range in mountain/valley systems tends to negate the value of large areas because the distribution of wolves is affected by the availability of prey. Areas may be large in absolute terms, but their effectiveness is reduced if they are long and narrow, thus increasing the probability that wolves will spend time outside the reserves and be exposed to harmful human activities. Ideally, relatively few wolf-pack territories should traverse or border the reserve edge, depending on conditions outside. However, in all of North America there are probably fewer than half a dozen wolf packs living exclusively within parks that are not potentially vulnerable to exploitation, or that would be vulnerable except for legal protection in the surrounding area. This is as true for the 24,400-km$^2$ Denali National Park and Preserve in Alaska (Meier et al. 1994) as for the 882-km$^2$ Voyagers National Park in Minnesota (P. Gogan, U.S. National Park Service, personal communication). Although several packs live well within the boundaries of the Gates of the Arctic National Park and Preserve in Alaska (34,300 km$^2$), they are subject to subsistence hunting (L. Adams, U.S. National Park Service, personal communication). Presently, all wolves within Minnesota's Management Zone I are totally protected (USFWS 1992), yet human-caused mortality is not uncommon along the periphery. Only Isle Royale National Park, being surrounded by water, affords absolute protection to all resident wolf packs.

The seasonal migration or movement of wolves in response to prey migration is well known, primarily from tundra and mountainous areas. In some U.S. and Canadian national parks, prey populations regularly emigrate across park boundaries to areas not under park jurisdiction (Banfield 1949; Houston 1971). Forbes and Theberge (1994) reported that extensive excursions by several Algonquin Park wolf packs to a deer yard outside the park resulted in a high rate of human-caused mortality. Consequently, wolves from half of the packs in one of the few officially protected wolf populations in Canada were vulnerable. Seven of nine Yellowstone elk herds migrate out of the park in winter (40% of Yellowstone elk leave the park), causing concern that some wolf packs may follow them and be vulnerable to human taking, although most wolves are expected to remain on the high-density elk winter range within the park (Singer 1991). The availability of ungulate winter habitat appears to limit the abundance of wolves in Glacier National Park. The 2–3 wolf packs that have used the park occupy the western edge because of prey distribution (Ream et al. 1991). The farthest-ranging Alaskan caribou herd (the Western Arctic herd), migrates over an area of 350,000 km$^2$, which includes several units of the National Park System (P. Valkenburg, Alaska Department of Fish and Game, personal communication).

Productivity manifested as prey biomass per unit area is also important. This factor affects wolf density, which affects total wolves supported within a given area (Keith 1983; Fuller 1989). A 5000-km$^2$ area in the Rocky Mountain chain, with 30% of its area in high elevation rock outcrops, ice, and snow, will not have the same carrying capacity for wolves as an area of equal size of lower elevation in a large river valley system. Riding Mountain National Park in Manitoba (2978 km$^2$) is a highly productive system where three ungulate species exist in moderate-to-high densities year round. Early indications in the northern Rockies of the U.S. are that more remote and pristine areas will often be avoided by wolves because of high elevation, steep terrain, deep snow, and poorer habitat quality for seasonal use by ungulates (Fritts et al. 1994*b*; P. Paquet, World Wildlife Fund, personal communication). Elsewhere, areas remaining for conservation of wolves and other species are by and large not the places where wolves originally existed in highest densities (such as the Great Plains), and in some cases they may include some of the poorest habitat (Fritts et al. 1994*b*). Generally, more northerly reserves will have to be larger because overall prey biomass density is lower.

Appropriate legal protection is required for wolf populations within nature reserves, and flexible but meaningful legislation is necessary to reduce the loss of wolves from reserves when they leave those areas. Poaching must not be a significant problem, both within and outside of the reserves.

Canid diseases can be important mortality factors in wolf populations (Mech & Goyal 1993; Brand et al. 1994). Therefore, the presence of other related species can influence persistence of enzootica in the system. Infection from domestic dogs may be a significant factor. If severe enough, disease outbreaks could have political repercussions if the transmission from domestic to wild and from wild to domestic animals becomes a concern to people living in the area. In some cases, hybridization between wild and domestic canids could occur and become a threat to maintenance of the wild wolf gene pool (see Boitani 1984; Ciucci & Boitani 1991).

Of primary importance to wolf conservation within nature reserves are the attitudes and density of humans living around the reserves. For example, rural populations living in high densities within third-world countries may not have as great an impact on wolves as the use of areas adjacent to reserves by a mobile population of hunters on a network of roads using all-terrain vehicles and sophisticated high-powered rifles. A high density of roads in and near the reserve could contribute to human-caused mortality (Mech et al. 1988). The presence or absence of livestock adjacent to nature reserves and the degree of hunting of large ungulates are important factors in the human response to wolves when they leave a reserve. The overall attitude of local society toward wolves and the tolerance shown toward the species underlies each of these considerations (Kellert 1985). This is one of the most important factors of all, because deliberate killing of wolves by humans can reduce or eliminate populations (Young 1944). Whether or not poison might be used is critical, because poison is far more effective in extirpating wolves than other human means, especially in a forested landscape. The inadvertent killing of wolves within nature reserves can result from automobile and train strikes. For example, cars striking wolves on the Trans-Canada highway in Banff National Park, Alberta, is a significant cause of mortality (P. Paquet, World Wildlife Fund, personal communication).

Wolf populations should not be totally isolated. Dispersal corridors connecting populations for occasional or regular gene flow theoretically would allow greater persistence of the populations, although we have previously pointed out that wolves are excellent dispersers and that the presence of discrete dispersal corridors per se seems to be less important to them than to most other species. Nonetheless, connectivity between reserves can enhance wolf colonization of adjacent areas and movement between reserves. The colonization pattern and movements of wolves within the Rocky Mountain chain in southwestern Canada and Montana are examples (Boyd et al. 1994). At population lows and where exchange of individuals among populations does not occur, loss of genetic variability is possible and may in the long run be detrimental to wolves living in isolation. But this result remains to be documented for wild wolves.

## Discussion and Conclusions

From a theoretical standpoint, the calculations of minimum viable populations for wolves suggest that it may be impossible to contain enough individuals in a single reserve to maintain a self-sustaining, viable population. Riding Mountain National Park, Manitoba, appears to be physically large enough at about 3000 km$^2$ to accomplish the objective of total ecosystem protection, and some exchange of wolves between this area and populations to the north likely occurs. We conclude, given the appropriate prey base and types of land uses around the area, that 3000 km$^2$ may be an adequate area to protect a core wolf population. It should be noted that farming around Riding Mountain National Park is largely for grain production. In areas with cattle ranching, the human pressures are greater (Fritts et al. 1992), particularly if areas surrounding nature reserves contain mosaics of forest and open range land.

In the absence of experimentation (opportunities are limited, but some work is possible), the next best approach to learning about wolf population viability is long-term monitoring of isolated or semi-isolated wolf populations to determine their persistence times (Peek et al. 1991). The upcoming re-establishment and long-term monitoring of wolves in Yellowstone National Park and central Idaho (Fritts et al. 1994*a*) and in the Southwestern U.S. (Parsons & Nicholopoulus 1994), as well as long-term data from the red wolf program in North Carolina (Phillips et al. 1994), will significantly increase understanding of these processes and enhance wolf conservation. Similarly, natural re-establishment of wolf populations in certain areas of Europe (France, Germany, Sweden, and Norway) will enhance the understanding of wolf population survival in a modern and changed human landscape (Promberger & Schroeder 1993). The large amount of environmental stochasticity characteristic of Yellowstone National Park (Boyce 1990) will make study of a population there especially interesting during the early stages of restoration.

Clearly, no one really knows the MVP of wolves or the size and design of reserve that can guarantee long-term survival. Theories about the viability of populations and extinction probabilities need to be tailored more appropriately to wolves and continually refined. While we encourage this endeavor, we will continue to insist on extreme caution in interpreting the results. Efforts to build on existing theory should benefit from data derived from the long-term monitoring of key populations over long periods. To date, most theoretical treatments of this subject have assumed a single, isolated population, but wolves move long distances and through various habitat types. The extraordinary dispersal capability of the species – hence a large degree of "metapopulation connectedness"– may be a major reason that small, local populations can survive and why simple theoretical models are not adequate for wolves. Our skepticism about existing theory notwithstanding, we believe that the persistence of small wolf populations for a century or more may be difficult at the fringe of or outside of the contiguous species range unless human-caused mortality is extremely low.

According to existing theory, few individual U.S. and Canadian parks would be able by themselves to support viable populations over long periods if extermination took place outside the park areas. The field data available, however, suggest that wolf populations may be considerably more resilient than suggested by the theoretical MVP calculations to date. Furthermore, most wolf biologists replying to our survey view the wolf as less vulnerable than indicated by existing theory, at least if not extirpated by humans. We see the fate of wolves in most of North America as more a function of human tolerance than of nature reserve availability, although reserves may provide insurance of some base population level.

Even under the bleakest of fragmentation scenarios, we view the long-term future of wolves over much of the continent to be as a metapopulation in which one unit can augment or even repopulate others via dispersal. A wolf population that was truly in danger of extinction could be artificially augmented with wolves from another area, unless introducing genetic material from another area is not desirable (Bednarz 1989). Overall, very little regional genetic differentiation has been found in gray wolves in North America (Wayne et al. 1992). Metapopulation arrangements can aid in reducing the size requirement for any particular reserve, so long as wolves can move or be moved among reserve units (Lacy 1987; Shaffer 1987:79).

In conclusion, we believe that wolf conservationists should not be daunted by theoretical treatments of population viability to date. Moreover, the lack of wolf reserves of sufficient size to satisfy population viability theorists should not alone negate restoration efforts. Common sense dictates that conservation and restoration programs should always seek the largest reserves possible yet not be thwarted by lack of large or otherwise suitable areas. Situations in which small size would lead to frequent conflicts and routinely require intensive management may be an exception. Even then, a system of small-scale zoning could be helpful (Mech 1995). For the foreseeable future, the most challenging wolf conservation efforts will involve fringe populations – especially those located in the southern part of the species' range – and rare subspecies, such as the Mexican wolf. Since single jurisdictions will rarely support the number of wolves necessary for theoretical viability, joint efforts of different jurisdictions should be stressed. We strongly agree with others, including Soulé (1987*a*:7–8), who argue that the conservation of relatively small numbers of a species in small reserves may be worthwhile for ecological or social reasons, even though these populations may add little or nothing to the theoretical viability of the species as a whole. It will have to be recognized, however, that the establishment and maintenance of such populations will require some management intervention at times, compared to larger reserves where little or no intervention should be necessary to maintain self-perpetuating wolf populations.

## Acknowledgments

We thank the many students of wolves who responded to the survey regarding the definition of a viable wolf population, and the following individuals for their helpful review of the manuscript: F. Allendorf, E. Bangs, L. Bennett, M. Boyce, J. Fontaine, S. Forbes, D. Huff, L. D. Mech, T. Meier, D. Parsons, D. Pletscher, H. Salwasser, and C. Schonewald-Cox. We greatly appreciate the assistance of K. Pennington and C. Tenney in manuscript preparation. The statements made and conclusions reached do not necessarily reflect the opinions or policies of the U.S. Fish and Wildlife Service, the Canadian Wild-

life Service, or any of the reviewers, but are the sole responsibility of the authors.

## LITERATURE CITED

Allendorf, F. W. 1983. Isolation, gene flow, and genetic differentiation among populations. Pages 51–65 in C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas, editors. Genetics and conservation. Benjamin/Cummings Publishing Company, Menlo Park, California.

Ballard, W. B., R. Farnell, and R. O. Stephenson. 1983. Long distance movement by a group of wolves. Canadian Field-Naturalist 97:333.

Banfield, A. W. F. 1949. An irruption of elk in Riding Mountain National Park. Journal of Wildlife Management 13:127–134.

Bath, A. J., H. A. Dueck, and S. Herrero. 1988. Carnivore conservation areas: a potential for comprehensive, integrated management. Unpublished Report to World Wildlife Fund, Canada.

Bednarz, J. C. 1989. An evaluation of the ecological potential of White Sands Missile Range to support a reintroduced population of Mexican wolves. Endangered Species Report 19. U.S. Fish and Wildlife Service, Albuquerque, New Mexico.

Belovsky, G. E. 1987. Extinction models and mammalian persistence. Pages 35–57 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Bennett, L. E. 1994. Colorado gray wolf recovery: a biological feasibility study. Final report to the U.S. Fish and Wildlife Service, University of Wyoming Fish and Wildlife Cooperative Research Unit, Laramie, Wyoming.

Blanco, J. C., L. Cuesta, and S. Reig, editors. 1990. El Lobo (*Canis lupus*) en espana situacion, problematica y apuntes sobre su ecologia. Ministero de Agricultura Pesca y Alimentacion; Icona; Publicaciones del Instituto Nacional para la Conservacion de la Naturaleza; Gran Via de San Francisco, 35; 28005 Madrid, Spain.

Bobek, B., M. Kosobucka, K. Perzanowski, and K. Plodzien. 1993. Distribution and wolf numbers in Poland. Pages 27–29 in C. Promberger and W. Schroeder, editors. Wolves in Europe: status and perspectives. Munich Wildlife Society, Ettal, Germany.

Boitani, L. 1984. Genetic considerations on wolf conservation in Italy. Bollettino di Zoologia 51:367–373.

Boitani, L., and P. Ciucci. 1993. Wolves in Italy: critical issues for their conservation. Pages 75–90 in C. Promberger and W. Schroder, editors. Wolves in Europe: status and perspectives. Munich Wildlife Society, Ettal, Germany.

Boyce, M. S. 1990. Wolf recovery for Yellowstone National Park. Pages 3-4 to 3-58 in Yellowstone National Park, U.S. Fish and Wildlife Service, University of Wyoming, University of Idaho, Interagency Grizzly Bear Study Team, University of Minnesota Cooperative Park Studies Unit, editors. Wolves for Yellowstone? A Report to the United States Congress, vol. II, Research and Analysis. National Park Service, Yellowstone National Park, Wyoming.

Boyce, M. S. 1992. Population viability analysis. Annual Review of Ecology and Systematics 23:481–506.

Boyd, D. K., P. C. Paquet, S. Donelon, R. R. Ream, D. H. Pletscher, and C. C. White. 1994. Dispersal characteristics of a recolonizing wolf population in the Rocky Mountains. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Brand, C. J., M. J. Pybus, W. B. Ballard, and R. O. Peterson. 1994. Infectious and parasitic diseases of the gray wolf and their potential effects on wolf populations in North America. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Brewster, W. G., and S. H. Fritts. 1994. Taxonomy and genetics of the gray wolf in western North America: a review. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Carbyn, L. N. 1983. Management of non-endangered wolf populations in Canada. Acta Zoologica Fennica 174:239–243.

Carbyn, L. N. 1989. Wolves in Riding Mountain National Park: ecosystem protection and thoughts on holistic conservation strategy. Pages 82–96 in D. Seip, S. Pittigrew, and R. Archibald, editors. Wolf-prey dynamics and management. Proceedings of the Wolf Symposium, May 10–11, 1988. B.C. Ministry of Environment Working Report No. WR-40. University of British Columbia, Vancouver, British Columbia.

Caro, T. M., and M. K. Laurenson. 1994. Ecological and genetic factors in conservation: a cautionary note. Science 263:485–486.

Chepko-Sade, B. D., W. H. Shields, J. Berger, Z. T. Halpin, W. T. Jones, L. L. Rogers, J. P. Rood, and A. T. Smith. 1987. The effects of dispersal and social structure on effective population size. Pages 287–321 in B. D. Chepko-Sade and Z. T. Halpin, editors. Mammalian dispersal patterns. University of Chicago Press, Chicago, Illinois.

Ciucci, P., and L. Boitani. 1991. Viability assessment of the Italian wolf and guidelines for the management of the wild and a captive population. Ricerche di Biologia della' Selvaggina No. 89.

Dekker, D. W., W. Bradford, and J. R. Gunson. 1994. Historic and contemporary populations of elk and wolves in Jasper National Park, Alberta. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Foley, P. 1992. Small population genetic variation at loci under stabilizing selection. Evolution 46:763–774.

Forbes, G. J., and J. B. Theberge. 1994. Influences of a migratory deer herd on wolf movements and mortality in and near Algonquin Park, Canada. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Francisci, F., and V. Guberti. 1993. Recent trends of wolves in Italy as apparent from kill figures and specimens. Pages 91–102 in C. Promberger and W. Schroeder, editors. Wolves in Europe: status and perspectives. Munich Wildlife Society, Ettal, Germany.

Franklin, I. A. 1980. Evolutionary change in small populations. Pages 135–149 in M. E. Soulé and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Fritts, S. H. 1983. Record dispersal by a wolf from Minnesota. Journal of Mammalogy 64:166–167.

Fritts, S. H., W. J. Paul, L. D. Mech, and D. P. Scott. 1992. Trends and management of wolf-livestock conflicts in Minnesota. Resource Publication 181. U.S. Fish and Wildlife Service, Washington, D. C.

Fritts, S. H., E. E. Bangs, J. A. Fontaine, W. G. Brewster, and J. F. Gore. 1994*a*. Restoring wolves to the Northern Rocky Mountains of the United States. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Fritts, S. H., E. E. Bangs, and J. F. Gore. 1994*b*. The relationship of wolf recovery to habitat conservation and biodiversity in the northwestern United States. Landscape and Urban Planning 28:23–32.

Fuller, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs 105:1–41.

Gese, E. M., and L. D. Mech. 1991. Dispersal of wolves in northeastern Minnesota, 1969–1989. Canadian Journal of Zoology 69:2946–

2955.

Gilpin, M. E. 1987. Spatial structure and population vulnerability. Pages 126–139 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Gilpin, M. E., and M. E. Soulé. 1986. Minimum viable populations: process of species extinction. Pages 19–34 in M. E. Soulé, editor. Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, Massachusetts.

Goodman, G. E. 1987. The demography of chance extinction. Pages 11–34 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Grumbine, R. E. 1990. Viable populations, reserve size, and federal lands management: a critique. Biological Conservation 4:127–134.

Hayes, R. D., and J. R. Gunson. 1994. Status and management of wolves in Canada. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Henshaw, R. E. 1979. Workshop: reintroduction of wolves into the wild. Pages 420–457 in E. Klinghammer, editor. The behavior and ecology of wolves. Garland STPM Press, New York.

Houston, D. B. 1971. Ecosystems of national parks. Science **172**:648–651.

Hummel, M., and S. Pettigrew. 1991. Wild hunters. Key Porter Books, Toronto, Ontario.

Joslin, P. 1982. Status, growth and other facets of the Iranian wolf. Pages 196–203 in F. H. Harrington and P. C. Paquet, editors. Wolves of the world. Noyes Publications, Park Ridge, New Jersey.

Keith, L. B. 1983. Population dynamics of wolves. Pages 66–77 in L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology and management. Report Series 45. Canadian Wildlife Service, Ottawa, Ontario.

Kellert. 1985. Public perception of predators, particularly the wolf and coyote. Biological Conservation **31**:167–189.

Kennedy, P. K., M. L. Kennedy, P. L. Clarkson, and I. S. Liepins. 1991. Genetic variability in natural populations of the gray wolf, *Canis lupus*. Canadian Journal of Zoology **69**:1183–1188.

Klein, D. R. 1994. The introduction, increase and demise of wolves on Coronation Island, Alaska. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Lacy, R. C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology **1**:143–158.

Lande, R., and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pages 87–123 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Lehman, N. P., P. Clarkson, L. D. Mech, T. J. Meier, and R. K. Wayne. 1992. A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. Behavioral Ecology and Sociobiology **30**:83–94.

Levins, R. 1970. Extinction. Pages 77–107 in M. Gustenhaver, editor. Some mathematical questions in biology, vol 2. American Mathematical Society, Providence, Rhode Island.

Licht, D. S., and S. H. Fritts. 1994. Gray wolf (*Canis lupus*) occurrences in the Dakotas. American Midland Naturalist **132**:74–81.

Lindell, J., and E. Grossman. 1994. 90-day finding and commencement of status review for a petition to list the Alexander Archipelago wolf. Federal Register **59**:26476–26477.

Mattson, D. J., and M. W. Reid. 1991. Conservation of the Yellowstone grizzly bear. Conservation Biology **5**:364–372.

Mech, L. D. 1966. The wolves of Isle Royale. National Park Service Fauna Series 7. United States Government Printing Office, Washington, D.C.

Mech, L. D. 1995. The challenge and opportunity of recovering wolf populations. Conservation Biology **9**:1–9.

Mech, L. D. 1966. The wolves of Isle Royale. National Park Service Fauna Series 7. United States Government Printing Office, Washington, D.C.

Mech, L. D., and S. M. Goyal. 1993. Canine parvovirus effect on wolf population change and pup survival. Journal of Wildlife Diseases **29**:330–333.

Mech, L. D., and P. D. Karns. 1977. Role of the wolf in a deer decline in the Superior National Forest. Research Paper NC-148. U.S. Department of Agriculture, St. Paul, Minnesota.

Mech, L. D., S. H. Fritts, G. L. Radde, and W. J. Paul. 1988. Wolf distribution and road density in Minnesota. Wildlife Society Bulletin **16**:85–87.

Meier, T. J., J. W. Burch, L. D. Mech, and L. G. Adams. 1994. Pack structure and genetic relatedness among wolf packs in a naturally-regulated population. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Mendelssohn, H. 1982. Wolves in Israel. Pages 173–195 in F. H. Harrington and P. C. Paquet, editors. Wolves of the world. Noyes Publications, Park Ridge, New Jersey.

Newmark, W. D. 1985. Legal and biotic boundaries of western North American national parks: a problem of congruence. Biological Conservation **33**:197–208.

Newmark, W. D. 1986. Species-area relationship and its determinants for mammals in western North American national parks. Biological Journal of the Linnean Society **28**:83–98.

Packard, J. M., and L. D. Mech. 1980. Population regulation in wolves. Pages 135–150 in M. N. Cohen, R. S. Malpass, and H. G. Klein, editors. Biological mechanisms of population regulation. Yale University Press, New Haven, Connecticut.

Parsons, D. R., and J. E. Nicholopoulus. 1994. An update on the status of the Mexican wolf recovery program. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Pedersen, S. 1982. Geographical variation in Alaska wolves. Pages 345–361 in F. H. Harrington and P. C. Paquet, editors. Wolves of the world. Noyes Publications, Park Ridge, New Jersey.

Peek, J. M., E. E. Brown, S. R. Kellert, L. D. Mech, J. H. Shaw, and V. Van Ballenberghe. 1991. Restoration of wolves in North America. Technical Review 91-1. Wildlife Society, Bethesda, Maryland.

Peterson, R. O. 1994. Out of the doldrums for Isle Royale wolves? International Wolf **4**:19.

Peterson, R. O., and J. D. Woolington. 1982. The apparent extinction and reappearance of wolves on the Kenai Peninsula, Alaska. Pages 334–344 in F. H. Harrington and P. C. Paquet, editors. Wolves of the world. Noyes Publications, Park Ridge, New Jersey.

Peterson, R. O., J. D. Woolington, and T. N. Bailey. 1984. Wolves of the Kenai Peninsula, Alaska. Wildlife Monographs **88**:1–52.

Phillips, M. K., R. Smith, V. G. Henry, and C. Lucash. 1994. Red wolf reintroduction program. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Promberger, C. 1993. Population trends of wolves in Portugal. Pages 109–112 in C. Promberger and W. Schroeder, editors. Wolves in Europe: current status and perspectives. Munich Wildlife Society, Ettal, Germany.

Promberger, C., and W. Schroeder, editors. 1993. Wolves in Europe: current status and perspectives. Munich Wildlife Society, Ettal, Germany.

Promberger, C., C. Vogel, and M. Von Loeper. 1993. Wolves in Germany. Pages 31–34 in C. Promberger and W. Schroeder, editors. Wolves in Europe: current status and perspectives. Munich Wildlife Society, Ettal, Germany.

Pulliainen, E. 1993. The wolf in Finland. Pages 15–20 in C. Promberger and W. Schroeder, editors. Wolves in Europe: status and perspectives. Munich Wildlife Society, Ettal, Germany.

Pyle, R. M. 1980. management of nature preserves. Pages 319–327 in M. E. Soulé, and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Ream, R. R., M. W. Fairchild, D. K. Boyd, and D. H. Pletscher. 1991. Population dynamics and home range changes in a colonizing wolf population. Pages 349–366 in R. B. Keiter and M. S. Boyce, editors. The greater Yellowstone ecosystem: redefining America's wilderness heritage. Yale University Press, New Haven, Connecticut.

Reed, J. M., P. D. Doerr, and J. R. Walters. 1986. Determining minimum viable populations for birds and mammals. Wildlife Society Bulletin 14:255–261.

Salwasser, H., C. Schonewald-Cox, and R. Baker. 1987. The role of interagency cooperation in managing for viable populations. Pages 159–173 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Schonewald-Cox, C. M. 1983. Conclusions: guidelines to management: a beginning attempt. Pages 414–445 in C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and W. L. Thomas. Genetics and conservation. Benjamin/Cummings, Menlo Park, California.

Schonewald-Cox, C., and M. Buechner. 1992. Park protection and public roads. Pages 374–395 in P. L. Fiedler and S. K. Jain, editors. Conservation Biology. Chapman and Hall, New York.

Schonewald-Cox, C., M. Buechner, R. Sauvajot, B. A. Wilcox. 1992. Cross-boundary management between national parks and surrounding lands: a review and discussion. Environmental Management 16:273–282.

Seal, U. S., and R. C. Lacy. 1989. Florida panther (*Felis concolor coryi*) population viability analysis and species survival plan. Report to United States Fish and Wildlife Service Captive Breeding Specialist Group. Species Survival Commission, International Union for the Conservation of Nature and Natural Resources, Apple Valley, Minnesota.

Shafer, C. L. 1990. Nature reserves: island theory and conservation practice. Smithsonian Institution Press, Washington, D.C.

Shaffer, M. L. 1981. Minimum population sizes for species conservation. Bioscience 31:131–134.

Shaffer, M. 1987. Minimum viable populations: coping with uncertainty. Pages 69–86 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Shields, W. M. 1983. Genetic considerations in the management of the wolf and other large vertebrates: an alternative view. Pages 90–92 in L. N. Carbyn, editor. Wolves in Canada and Alaska. Report Series 45. Canadian Wildlife Service, Ottawa, Ontario.

Simberloff, D. 1988. The contribution of population and community biology to conservation science. Annual Review of Ecology and Systematics 19:473–511.

Singer, F. J. 1991. Some predictions concerning a wolf recovery into Yellowstone National Park: how wolf recovery may affect park visitors, ungulates, and other predators. Transactions of the North American Wildlife and Natural Resources Conference 56:567–583.

Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151–169 in M. E. Soulé and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Soulé, M.E. 1987*a*. Viable populations for conservation. Cambridge University Press, New York.

Soulé, M. E. 1987*b*. Where do we go from here? Pages 175–189 in M. E. Soulé, editor. Viable populations for conservation. Cambridge University Press, New York.

Soulé, M. E., and D. Simberloff. 1986. What do genetics and ecology tell us about the design of nature reserves? Biological Conservation 35:19–40.

Stephenson, R. O., W. Ballard, C. Smith, and K. Richardson. 1994. Wolf biology and management in Alaska – update to 1991. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Theberge, J. B. 1983. Considerations in wolf management related to genetic variability and adaptive change. Pages 86–89 in L. N. Carbyn, editor. Wolves in Canada and Alaska. Report Series 45. Canadian Wildlife Service, Ottawa, Ontario.

Thiel, R. P., and R. R. Ream. 1994. Status of the gray wolf in the lower 48 states, 1992. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

Thomas, C. D. 1990. What do real population dynamics tell us about minimum viable population sizes? Conservation Biology 4:324–327.

Thomas, D. C. 1994. Wolf-caribou relationships in Canada. In L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and management of wolves in a changing world. Canadian Circumpolar Institute, University of Alberta, Edmonton. (In press.)

UNESCO (United Nations). 1974. Task force on criteria and guidelines for the choice and establishment of biosphere reserves. Final Report Series No. 22. Paris, France.

U.S. Bureau of Land Management. 1992. Public land statistics 1991. United States Department of the Interior, Bureau of Land Management, Washington, D.C.

USFWS (U.S. Fish and Wildlife Service). 1982. Mexican wolf recovery plan. Albuquerque, New Mexico.

USFWS (U.S. Fish and Wildlife Service). 1987. Northern Rocky Mountain Wolf Recovery Plan. Denver, Colorado.

USFWS (U.S. Fish and Wildlife Service). 1989. Red wolf recovery plan. Atlanta, Georgia.

USFWS (U.S. Fish and Wildlife Service). 1992. Recovery plan for the eastern timber wolf. Twin Cities, Minnesota.

U.S. National Park Service. 1993. The National Parks: index 1993. Department of the Interior, Washington, D.C.

Wayne, R. K., N. Lehman, D. Gorman, P. J. P. Gogan, D. A. Gilbert, K. Hansen, R. O. Peterson, U. S. Seal, A. Eisenhawer, L. D. Mech, and R. J. Krumenaker. 1991. Conservation genetics of the endangered Isle Royale gray wolf. Conservation Biology 5:41–51.

Wayne, R. K., N. Lehman, M. W. Allard, and R. L. Honeycutt. 1992. Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. Conservation Biology 6:599–569.

Yellowstone National Park, U.S. Fish and Wildlife Service, University of Wyoming, University of Idaho, Interagency Grizzly Bear Study Team, and University of Minnesota Cooperative Park Studies Unit, editors. 1990. Wolves for Yellowstone? A Report to the United States Congress, Vol. II, research and analysis. National Park Service, Yellowstone National Park, Wyoming.

Young, S. P. 1994. The wolves of North America, Part I. American Wildlife Institute, Washington, D.C.

# Improving conservation policy with genomics: a guide to integrating adaptive potential into U.S. Endangered Species Act decisions for conservation practitioners and geneticists

**Article** *in* Conservation Genetics · February 2019

DOI: 10.1007/s10592-018-1096-1

**5 authors**, including:

W. Chris Funk
Colorado State University
**310** PUBLICATIONS **9,445** CITATIONS
SEE PROFILE

Brenna R Forester
U.S. Fish and Wildlife Service
**61** PUBLICATIONS **2,469** CITATIONS
SEE PROFILE

Sarah J Converse
University of Washington
**127** PUBLICATIONS **3,788** CITATIONS
SEE PROFILE

Steve Morey
U.S. Fish and Wildlife Service
**64** PUBLICATIONS **962** CITATIONS
SEE PROFILE

Conservation Genetics
https://doi.org/10.1007/s10592-018-1096-1

REVIEW ARTICLE

# Improving conservation policy with genomics: a guide to integrating adaptive potential into U.S. Endangered Species Act decisions for conservation practitioners and geneticists

W. C. Funk[1,2] · Brenna R. Forester[1] · Sarah J. Converse[3] · Catherine Darst[4] · Steve Morey[5]

Received: 3 December 2017 / Accepted: 4 August 2018

**Abstract**

Rapid environmental change makes adaptive potential—the capacity of populations to evolve genetically based changes in response to selection—more important than ever for long-term persistence of at-risk species. At the same time, advances in genomics provide unprecedented power to test for and quantify adaptive potential, enabling consideration of adaptive potential in estimates of extinction risk and laws protecting endangered species. The U.S. Endangered Species Act (ESA) is one of the most powerful environmental laws in the world, but so far, the full potential of genomics in ESA listing and recovery decisions has not been realized by the federal agencies responsible for implementing the ESA or by conservation geneticists. The goal of our paper is to chart a path forward for integrating genomics into ESA decision making to facilitate full consideration of adaptive potential in evaluating long-term risk of extinction. For policy makers, managers, and other conservation practitioners, we outline why adaptive potential is important for population persistence and what genomic tools are available for quantifying it. For conservation geneticists, we discuss how federal agencies can integrate information on the effect of adaptive potential on extinction risk—and the related uncertainty—into decisions, and suggest next steps for advancing understanding of the effect of adaptive potential on extinction risk. The mechanisms and consequences of adaptation are incredibly complex, and we may never have a complete understanding of adaptive potential for any organism. Nevertheless, we argue that the best available evidence regarding adaptive potential can now be incorporated by federal agencies into modeling and decision making processes, while at the same time conserving genome-wide variation and striving for a deeper understanding of adaptive potential and its effects on population persistence to improve decision making into the future.

**Keywords** Adaptation · Genomics · U.S. Endangered Species Act · Conservation policy · Extinction risk

## Introduction

The unprecedented rate of global environmental change means that many species and populations will have to **adapt** (see Box 1 for glossary of genetic terms in bold) to this change, or go extinct (Bell and Collins 2008; Hoffmann and Sgro 2011). The dramatic increase in human population size and associated rapid environmental change has been termed the “Great Acceleration” by the International Geosphere–Biosphere Programme (Steffen et al. 2015). Human-caused climate change, in particular, has already impacted biodiversity at all levels of biological organization and on every continent, including extinction of many species, with many more extinctions projected (Penuelas et al. 2013; Scheffers et al. 2016). Three primary mechanisms allow persistence in the face of this rapid environmental change:

---

✉ W. C. Funk
Chris.Funk@colostate.edu

[1] Department of Biology, Colorado State University, Fort Collins, CO 80523, USA

[2] Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO 80523, USA

[3] U.S. Geological Survey, Washington Cooperative Fish and Wildlife Research Unit, School of Environmental and Forest Sciences (SEFS) & School of Aquatic and Fishery Sciences (SAFS), University of Washington, Seattle, WA 98195, USA

[4] U.S. Fish and Wildlife Service, Ventura, CA 93003, USA

[5] U.S. Fish and Wildlife Service, Portland, OR 97232, USA

dispersal, **phenotypic plasticity**, and genetically-based adaptation to changing conditions (Dawson et al. 2011; O'Connor et al. 2012; Nicotra et al. 2015). In addition, **epigenetic** variation may play a role in buffering populations from environmental change (Bernatchez 2016; Verhoeven et al. 2016). Thus, species with greater **adaptive potential**—the capacity to evolve genetically-based changes in traits in response to changing environmental conditions—will be more resilient to climate and other environmental change.

Despite recognition of the critical importance of adaptive potential for persistence in the face of environmental change, it has been difficult or impossible to quantify for the vast majority of species. Adaptive potential is ultimately determined by the amount of **additive genetic variation** for adaptive traits within and among populations (see section below on "What determines adaptive potential of a species?"). Thus, genetic differences among individuals within populations, as well as genetic differences among populations, contribute to the overall adaptive potential of a species. The traditional approach for quantifying additive genetic variation within populations is to estimate the proportion of variance in a trait that is heritable using controlled breeding (Falconer and MacKay 1996). The gold standard for testing for adaptive differences among populations is a reciprocal transplant experiment. In these experiments, individuals from two different populations are transplanted to the environment of the other population to test whether individuals have greater fitness in their native environment compared to the foreign environment, demonstrating local adaptation (Clausen et al. 1948). Controlled breeding designs and reciprocal transplant experiments, however, are not feasible for most species of conservation concern, especially mobile or large endangered animals with small population sizes.

Fortuitously, the **genomics** revolution provides more power than ever to test for and quantify adaptation and adaptive potential to improve implementation of conservation policy (Black et al. 2001; Luikart et al. 2003; Beaumont and Balding 2004; Allendorf et al. 2010). For the first time, **population genomics** provides a means of testing for adaptation in species for which controlled breeding and reciprocal transplant experiments are impractical or impossible. Population genomics is the use of genome-wide data (e.g., **single-nucleotide polymorphisms [SNPs]**) at thousands to millions of **loci** across the genome of a sample of organisms to make inferences about micro-evolutionary processes (**gene flow, genetic drift, selection**, and **mutation**; Black et al. 2001; Luikart et al. 2003). The field has been enabled by rapid advances in **next-generation sequencing (NGS)** technology and computational power (Glenn 2011; Catchen et al. 2013; Hohenlohe et al. 2013). Due to the huge number of loci included in population genomic studies (typically thousands to hundreds of thousands), various statistical approaches can be used to identify putatively **adaptive loci** (Beaumont and Balding 2004; Joost et al. 2007; Coop et al. 2010; Frichot et al. 2013; Forester et al. 2018). By contrast, traditional **population genetic** approaches, which use a much smaller number of loci (e.g., 10–20), have much less power to identify adaptive loci because they evaluate insufficient numbers of molecular **markers**.

In particular, genomics has tremendous potential to improve our ability to incorporate information on adaptive potential into laws protecting endangered species. Several countries have enacted such laws, including the Endangered Species Act in the United States (ESA; passed in 1973), the Biodiversity Law of Costa Rica (passed in 1992), the Endangered Species Protection Act of Australia (passed in 2002), Canada's Species at Risk Act (passed in 2002), and the South African National Environmental Management Biodiversity Act (passed in 2004; Waples et al. 2013). Here, we focus on application of genomics in listing and recovery decisions under the ESA as a case study of how information on adaptive potential inferred from genomics and other approaches can be integrated into risk assessments to improve implementation of endangered species laws. The ESA is one of the most powerful environmental laws in the world, providing the statutory basis for listing and legal protection of species and subspecific units determined to be threatened or endangered (Carroll et al. 1996; Waples et al. 2013). Since the law was signed in 1973, 2318 species—with ranges inside and outside the U.S.—have been listed as threatened or endangered, and 53 of these species have recovered to the point where they could be delisted (https://ecos.fws.gov/ecp0/reports/delisting-report), indicating it can be an effective law for improving the conservation status of at-risk species.

Many factors are considered in ESA listing and recovery decisions, including information on the capacity of species to adapt to cope with new environmental stressors (Carroll et al. 1996; Shaffer and Stein 2000; Vucetich et al. 2006; see Box 2). However, given the past difficulty of quantifying adaptive potential, little information has typically been available for most species that are candidates for listing. Because genomics greatly increases the feasibility of characterizing adaptive potential in non-model species, it can improve ESA listing and recovery decisions by allowing estimation and integration of adaptive potential into models of extinction risk. Due to the nascency of genomics, it has had limited application to the ESA so far. Compared to evolution and ecology where the use of genomics is widespread, its application to conservation questions has lagged behind due to several previously discussed obstacles (Shafer et al. 2015). However, this is changing rapidly as genomic tools are increasingly applied to conservation problems (Garner et al. 2016). Now is a critical time to determine how best to use genomics to directly inform conservation policy, including ESA decisions.

The goal of this paper is to provide guidance on how genomics can be integrated into ESA decision making to facilitate full consideration of adaptive potential in evaluating long-term extinction risk. This paper is geared towards both conservation practitioners (e.g., policy makers and managers) as well as conservation geneticists. For conservation practitioners, our objectives are to explain: (1) what determines adaptive potential; (2) why adaptive potential is important to conservation; and (3) what genomic tools are available for quantifying adaptive potential. For conservation geneticists, our objectives are to: (1) explain how federal agencies make ESA listing and recovery decisions, and how information on adaptive potential can be incorporated into these decisions; (2) explain how these decisions can be made in the face of uncertainty about the effects of adaptive potential on extinction risk; and (3) suggest next steps for advancing understanding of the effect of adaptive potential on extinction risk. Although we focus on the ESA here, our discussion of ways to apply genomics to ESA decision making should be applicable to similar laws in other countries.

## Box 1 Glossary of genetic and evolutionary terms

### Adapt

When a population adapts, it is showing a genetically-based change in a trait in response to natural selection that increases fitness within the population. As used throughout this paper, an evolutionary process.

### Adaptive loci

Regions of DNA that have been identified as under selection. These loci may be candidates when genetic markers are anonymous, or may be validated if they are linked to or inside a gene (identified by using an annotated reference genome or transcriptome).

### Adaptive potential

The capacity to evolve genetically-based changes in traits in response to changing environmental conditions. Also known as “evolutionary potential”.

### Additive genetic variation

The amount of the total genetic variation that responds to natural selection. The phenotypic similarity between parents and offspring is a product of additive genetic variation.

### Allele

Alternative forms of a gene or molecular marker.

### Divergent selection

When different alleles are favored in different environments at a genetic locus. Reduces genetic variation within populations and increases genetic divergence among populations.

### Effective population size ($N_e$)

The size of an ideal population that would experience the same amount of genetic drift as in the focal population. Typically smaller than the census (observed) population size.

### Epigenetics

Heritable changes in gene expression that do not involve changes in the DNA sequence. For example, environmentally-induced variation in DNA methylation can cause differential gene expression.

### Evolutionary rescue

An increase in population growth and avoidance of extinction through adaptation from standing genetic variation, mutation, or gene flow.

### Gene expression

The transcription of information encoded in a gene into a messenger RNA, which then is translated into a protein.

### Gene flow

The movement of alleles among populations resulting from dispersal and successful reproduction. Increases genetic variation within populations and reduces genetic divergence among populations.

### Genetic drift

Random changes in allele frequencies due to the finite sampling of alleles in each generation. Reduces genetic variation within populations and increases genetic divergence among populations. Is more important in small populations than large populations.

### Genomic data

Any large set of molecular markers (e.g., hundreds to millions) that can be used to address questions related to neutral and adaptive variation.

### Genomics

Genotyping large sets of genetic markers to whole genome sequencing. In all cases the goal is to study the function and/or structure of these markers.

### Heritability

Most commonly refers to the proportion of phenotypic variation among individuals that is due to additive genetic variation (also known as "narrow-sense heritability"). This form of heritability provides a measure of the evolvability of the trait. By contrast, "broad-sense heritability" is the proportion of phenotypic variation among individuals that is due to all genetic differences, including additive, dominance, and epistatic genetic variation.

### Inbreeding depression

A reduction in the fitness of offspring resulting from matings between closely related individuals.

### Linkage disequilibrium (LD)

The non-random association of alleles at different loci. Influenced by many factors, including recombination, genetic drift, selection, and effective population size. LD is generally higher in populations with smaller effective sizes.

### Locus

The location of a region of DNA on a chromosome. Plural is "loci."

### Markers

Genetic sequence information used to differentiate individuals, populations, and species. Examples include microsatellites, single nucleotide polymorphisms, and DNA sequences.

### Mutation

Change in the genetic sequence and the ultimate source of genetic variation. Variation generated by mutation is acted upon by genetic drift, gene flow, and selection to determine the genetic variation within and among populations.

### Neutral processes

Microevolutionary processes that are neutral (i.e., without selection). Includes mutation, genetic drift, and gene flow.

### Next-generation sequencing (NGS)

Nucleotide sequencing technologies that produce millions of DNA or RNA sequence reads in a single run.

### Phenotypic plasticity

The capacity of a single genotype to produce multiple phenotypes in response to different environmental conditions.

### Population genetics

The use of a small set (tens) of molecular markers from a sample of organisms to make inferences about neutral microevolutionary processes.

### Population genomics

The use of a large set (thousands to millions) of genome-wide molecular markers from a sample of organisms to make inferences about neutral and adaptive microevolutionary processes.

### Selection

Differences in survival and reproduction of different genotypes that result in differential contributions to subsequent generations.

### Single nucleotide polymorphism (SNP)

A one base pair difference in DNA sequence between individuals in a population. A common molecular marker used in genomic studies.

## Box 2 Adaptive potential and ESA listing and recovery decisions: history, present, and future

Until recently, adaptive potential was not explicitly considered in U.S. Endangered Species Act (ESA) listing and recovery decision making. Over the past several years, the U.S. Fish and Wildlife Service (USFWS) has included evaluation of a species' resiliency, redundancy, and representation in listing and recovery efforts (Shaffer and Stein 2000; USFWS 2016; Smith et al. 2018). Resiliency is the ability to sustain populations in the face of environmental variation and stochasticity. Redundancy is the ability to withstand catastrophic events, protecting a species against unpredictable and highly consequential events for which adaptation is unlikely. Representation is the ability to adapt to changing environmental conditions; it is the species' evolutionary capacity or flexibility. It is under this latter category that agency scientists have started to include assessments of adaptive potential.

For example, the 2017 listing of the rusty patched bumble bee (*Bombus affinis*; Fig. 1) as endangered used a proxy for adaptive potential when evaluating this species' representation (USFWS 2017). Agency scientists assessed the past, present, and future occupancy of ecoregions (areas of unique climatic conditions) and found a decline from 15 to 6 occupied ecoregions from historical to current time, with only two ecoregions remaining occupied under three future risk scenarios (Symanski et al. 2016a). This ongoing loss of ecological representation influenced the species listing decision, since these declines have "…greatly reduced the rusty patched bumble bee's ability to adapt to changing environmental conditions and to guard against further losses of adaptive diversity…" (USFWS 2017).

Another recent listing decision for the eastern massasauga rattlesnake (*Sistrurus catenatus*; Fig. 1) used a similar approach, but incorporated a wider range of available information, including ecological, phenotypic, and genetic data, to identify ecotypes that were representative of adaptive diversity (Symanski et al. 2016a, b; USFWS 2016). Drawing on published studies using microsatellite loci and mitochondrial DNA, agency scientists identified three distinct analysis units that represented the genetic and ecological diversity needed to maintain adaptive potential. Changes in the extent of occurrence within these units over time were then used to support a threatened listing status for this species, since "…losses of geographical areas among and within the analysis units may equate to irreplaceable losses of adaptive diversity…" (Symanski et al. 2016a, b).

These cases illustrate the effective use of proxies for adaptive potential, when direct evaluations are not available from genomic data. While genomic assessments of adaptive potential have not yet been used (to our knowledge) in an ESA listing or recovery decision, these data are increasingly available in many species of conservation concern. For example, the Southwestern willow flycatcher (*Empidonax traillii extimus*; Fig. 1) was listed as endangered in 1995, prior to consideration of adaptive potential or representation (USFWS 1995). Even at the time of listing, there was debate about the validity of this subspecies classification, which has continued over the past 20 years (Zink 2015; Theimer et al. 2016). However, a recent genomic analysis of the willow flycatcher complex has demonstrated the presence of local adaptation across the four subspecies related to temperature extremes (Ruegg et al. 2018), supporting the established subspecies designations. In addition, based on adaptive genotype-environment relationships, this study found evidence that the Southwestern subspecies is at the greatest risk for climate-mediated extinction due to high levels of genomic vulnerability (a measure of the mismatch between adaptive genotypes and future environmental conditions). These data not only support ongoing recovery efforts, but also provide direction for targeted recovery work in populations with low genomic vulnerability, where the implementation of recovery actions is likely to be most effective (Ruegg et al. 2018).

These examples illustrate how adaptive potential is increasingly being recognized as an important factor to consider when evaluating a species' extinction risk and capacity for recovery. Genomic data can play an important role in these efforts by allowing adaptive potential to be quantified. With the increasing application of genomic methods to species of conservation concern, we expect an increase in the use of genomics-informed assessments of adaptive potential in listing and recovery decisions.

**Fig. 1** Rusty patched bumblebee (*Bombus affinis*; photo credit: Johanna James-Heinz), eastern massasauga rattlesnake (*Sistrurus catenatus*; photo credit: Nick Cairns), and willow flycatcher (*Empidonax traillii*; photo credit: Kelly Colgan Azar)

## Adaptation and conservation

### What determines adaptive potential of a species?

Adaptation is genetically-based change in traits that increases fitness. Adaptation results from selection on heritable phenotypic variation. The rate of adaptation in a given trait is proportional to the amount of additive genetic variation underlying that trait (Falconer and Mackay 1996). The adaptive potential of a species is determined by the amount of additive genetic variation within and among populations in fitness-related traits (Fig. 2). The ultimate source of all genetic variation is mutation (Dobzhansky and Wright 1941). Genetic drift, gene flow, and selection then act on variation generated by mutation to structure variation within and among populations. Genetic drift—random changes in **allele** frequencies due to the finite number of alleles passed on to the next generation—causes a reduction in genetic variation within populations and divergence in allele frequencies among populations. The **effective size of a population (*$N_e$*)**, which is typically smaller than the observed number of individuals in a population, determines the rate of genetic drift (Wright 1938; Kimura and Crow 1963). Isolated populations with smaller $N_e$ have more genetic drift and less adaptive potential. Gene flow is the movement of alleles among populations caused by dispersal and reproduction (Wright 1943; Slatkin 1987). Gene flow is predicted to cause an increase in genetic variation within populations, but a reduction in genetic divergence among populations. Finally, selection is caused by differences in fitness among genotypes (Fisher 1930; Haldane 1930; Wright 1931). Depending on the form of selection, it can decrease or increase genetic variation within and among populations. **Divergent selection** occurs when different alleles are favored in different environments at a given locus, and results in a reduction in within-population genetic variation and an increase in genetic differences among populations at the loci under selection. Ultimately, to conserve adaptive potential, it is necessary to conserve multiple large populations with minimal genetic drift, and allow gene flow among these populations so that they can exchange adaptive variants (Garant et al. 2007; Allendorf et al. 2013). In species or conservation units that exist as single populations, management should be focused on reducing threats and maximizing population size to minimize the probability of extinction.

### Why is adaptation relevant to conservation?

Population genetic theory predicts that maintenance of additive genetic variation, the source of adaptive potential, is important for long term persistence (Burger and Lynch 1995). One well-known guideline for avoiding **inbreeding depression** and maintaining additive genetic variation is the "50/500 rule" (Franklin 1980). It states that a

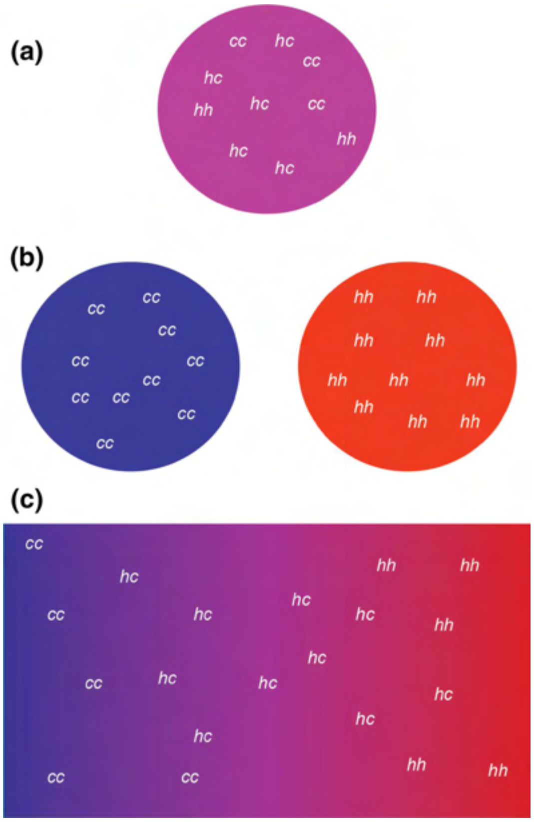


**Fig. 2** Potential distribution of adaptive potential within and among populations, and genomic approaches for characterizing adaptive potential under these different scenarios. Although heat tolerance is most likely a polygenic trait, here, we show a single locus contributing to heat tolerance for the sake of simplicity. Each two-letter genotype represents a single diploid individual. If, for example, hot (denoted by *h*) and cold (denoted by *c*) tolerant alleles are segregating at a given locus within a population, then adaptive potential for heat tolerance allows the evolution of this trait within this population (**a**). In this case, genomic approaches could be used to estimate the heritability of heat tolerance or to identify loci underlying heat tolerance using a genome-wide association study (GWAS). Alternatively, if two different populations are fixed for alternative alleles at this locus due to strong divergent selection in hot (red) vs. cold (blue) environments, then there is no adaptive potential within populations, but there is adaptive potential in the entire metapopulation or species as a whole (**b**). In this case, heat tolerance could evolve in the population fixed for the *c* allele as long as immigration (natural or mediated by humans) into this population from the population fixed for the *h* allele occurs. Here, genomics could be used to test for loci with signatures of divergent selection and adaptive divergence using $F_{ST}$ outlier tests. Finally, if a continuously distributed species is locally adapted to a climatic gradient, then an allele frequency cline should evolve at loci underlying thermal tolerance (**c**). In this case, genotype-by-environment associations (GEA) can be used to identify loci that are putatively involved in adaptation along this climatic gradient. (Color figure online)

minimum $N_e$ of 50 is needed to avoid significant inbreeding depression over the short-term, and a minimum $N_e$ of 500 is needed to maintain adaptive potential over the long-term. This rule of thumb has been the subject of much debate (Jamieson and Allendorf 2012, 2013; Frankham et al. 2013), but the general idea that large $N_e$ is needed to maintain adaptive potential, especially with rapid environmental change, is well accepted (Bell and Collins 2008; Hoffmann et al. 2017).

Some of the best evidence for the importance of adaptive potential in conservation comes from controlled laboratory experiments in model species. For example, Frankham et al. (1999, 2002) have demonstrated that small laboratory populations of *Drosophila melanogaster* are unable to evolve and persist in response to stressful environmental conditions. Populations that went through extreme bottlenecks of two individuals for one to three generations went extinct in high salt environments more frequently than outbred control populations (Frankham et al. 1999). Similarly, populations with low genetic variation maintained for 50 generations went extinct more often than populations with higher genetic variation (Frankham et al. 2002). Controlled laboratory experiments in flower beetles (*Tribolium castaneum*) yield similar results. Agashe et al. (2011) found that genetically diverse wheat-adapted flour beetle populations exposed to a novel suboptimal corn resource were less likely to go extinct than genetically depauperate populations. Moreover, genetically diverse populations recovered more quickly. Thus, laboratory studies support theoretical expectations that populations with more genetic variation are more likely to adapt and persist in novel stressful environments.

We also know that some populations have evolved in response to rapid environmental change (Hoffmann and Sgro 2011; Hansen et al. 2012; Penuelas et al. 2013; Scheffers et al. 2016; Cattau et al. 2017). Kovach et al. (2012) found evidence for the evolution of earlier run timing in a population of pink salmon (*Oncorhynchus gorbuscha*) in Auke Creek, Alaska. Late-migration phenotypes decreased from 30% to less than 10% in this population over a 40-year period during which temperatures in Auke Creek increased significantly. Moreover, a genetic marker for late migration timing decreased threefold during the same timeframe. In another example, Franks et al. (2016) found rapidly evolved changes in multiple phenotypic traits, including flowering time, in *Brassica rapa* in response to a multiyear drought in California. By comparing genome-wide differences between individuals sampled before vs. after the drought, they uncovered shifts in allele frequencies in many genes, some of which are related to drought stress and flowering time. These and many other examples demonstrate that rapid environmental change has already resulted in evolved adaptations (Hoffmann and Sgro 2011; Savage and Zamudio 2011; Hansen et al. 2012; Bataille et al. 2015; Scheffers et al. 2016; Cattau et al. 2017).

In contrast, other populations lack adaptive potential or have failed to evolve in response to environmental change. An example of the lack of adaptive potential comes from the intertidal copepod, *Tigriopus californicus* (Kelly et al. 2012). This species is highly adapted to local temperatures across a latitudinal gradient of 17° along the Pacific coast of North America. Less than one percent of quantitative variance in thermal tolerance is partitioned within populations; most variance in this trait is due to differences among populations. Moreover, heat tolerant phenotypes observed in low latitude populations did not evolve in high latitude populations after 10 generations of strong selection, indicating a lack of adaptive potential within populations. In chinook salmon (*Oncorhynchus tshawytscha*), Muñoz et al. (2015) found a lack of additive genetic variation for arrhythmic temperature of the heart, which constrains the thermal limit to a maximum of 24.5 °C. Based on projected increases in river temperatures, the authors estimated a 17% chance of catastrophic population decline by 2100, assuming behavioral and phenological changes do not buffer the population from increasing temperature. These examples suggest that many populations will likely lack sufficient additive genetic variation to adapt quickly enough to the pace of climate change, at least without aggressive between-population translocation efforts. Moreover, we know based on theory and lab studies, such as those described above, that reduction in population sizes and isolation caused by habitat loss, fragmentation, and other anthropogenic stressors will reduce the capacity of populations to adapt in response to climate or other environmental change. Thus, we need to be particularly cognizant of the erosion of adaptive potential for at-risk species with small population sizes. To determine the adaptive potential of a species, we first need to quantify heritable variation within populations and adaptive differences among them (Fig. 2).

## Quantifying adaptive potential

### Traditional approaches for quantifying adaptive potential

Within a population, adaptive potential for a given trait is determined by the **heritability** of that trait. Heritability can be thought of as the proportion of variance in a trait that is caused by genetic factors. The greater the heritability of a trait, the more it will evolve across generations in response to selection. In contrast, if heritability of a trait is zero, then that trait cannot evolve in response to selection. Traditionally, heritability is estimated using methods that rely on controlled breeding experiments, so that the identities of

**Table 1** Traditional (non-genomic) and genomic approaches for quantifying adaptive potential, including data and logistical requirements, limitations, and the strength of inference provided by each method

| Approach | Minimum data needed, logistical requirements | Limitations | Strength of evidence |
|---|---|---|---|
| Non-genomic methods | | | |
| Heritability | Fitness or fitness-relevant trait data; pedigree (relatedness) data; best case, controlled conditions | Difficult or impossible for at-risk species, large vertebrates without long-term studies of marked individuals or controlled breeding experiments. Estimates are population-specific | Medium to high |
| Environmental heterogeneity | Environmental data | Correlative; environmental differences represent an untested hypothesis of local adaptation | Very low |
| Phenotypic variation | Phenotypic data | Correlative; if phenotypic and environmental data are correlated, provides stronger support but is confounded by plasticity | Very low to low |
| Common garden experiments | Fitness or fitness-relevant trait data; controlled conditions; best case, reared multiple generations | Difficult or impossible for at-risk species, large vertebrates. Potentially confounded by genotype-by-environment interactions (alleviated by replicated experiments across environmental gradient) | High to very high |
| Reciprocal transplant experiments | Fitness or fitness-relevant trait data; controlled conditions; best case, reared multiple generations | Difficult or impossible for at-risk species, large vertebrates. Requires multiple generations to control for potential maternal effects | Very high |
| Genomic methods | | | |
| Heritability (pedigree-free) | Fitness or fitness-relevant trait data; genomic data (for inferring relatedness) | Requires large sample sizes of phenotyped individuals. Estimates are population-specific | Medium to high |
| Genome-wide association study | Genomic data, phenotypic data | Correlative, requires dense genotyping and large sample size of phenotyped individuals; stronger inference when combined with complementary data (e.g., from GEA) or when tied to a well-annotated reference genome | Medium to high |
| Differentiation-based methods | Genomic data | Sensitive to violation of underlying population genetic models, requires population-based sampling, only identifies strong divergent selection; stronger inference when combined with complementary data (e.g., from GWAS) or when tied to a well-annotated reference genome | Medium to medium–high |
| Genotype-environment associations | Genomic data, environmental data | Correlative, though stronger inference possible when combined with complementary data (e.g. from GWAS) or when tied to a well-annotated reference genome | Medium to high |
| Transcriptomics | Transcriptomic data; at minimum, replicated field-based study; best case, controlled experimental treatments | Usually requires destructive sampling; correlative, robust inference requires carefully controlled experimental designs; inference from field-based studies are limited | Low to medium–high |

parents and offspring are known. For example, heritability can be estimated as the regression coefficient in a regression of family means against midparent values (the average trait value of both parents) for a given trait (Falconer and Mackay 1996). In addition to controlled breeding, these experiments often used controlled laboratory conditions (often termed a "common garden," as early studies of heritability were typically done on plants) to control for the influence of the environment on phenotypic variation, making them difficult or impossible to implement in many species (Table 1).

Adaptive differences among populations can also increase the adaptive potential of a species. This is why several authors have emphasized the importance of delineating and maintaining adaptively divergent populations for long-term persistence of species (Ryder 1986; Waples 1991; Crandall et al. 2000; Fraser and Bernatchez 2001; Funk et al. 2012). Even when a trait is not heritable within populations, the trait can evolve if genetic differences among populations in that trait are moved among populations via gene flow (Fig. 2). Thus, it is important to quantify adaptive differences among populations in addition to heritable variation within populations. Three main lines of evidence are traditionally used to demonstrate adaptive differentiation among populations (Table 1). First, environmental differentiation that is accompanied by phenotypic divergence across sites or populations can provide support for local adaptation (Hanson et al. 2017). For example, tortoise populations in the Galapagos Archipelago can be found in two habitat types: mesic and xeric. Tortoises in mesic habitats have a dome-shaped shell, while tortoises in xeric habitats have a saddleback-shaped shell, indicating that tortoises may be locally adapted (Fritts 1984). This hypothesis is supported by data indicating that competition for resources is more intense and agonistic behaviors more common in xeric habitats, where the saddleback phenotype is at a competitive advantage (Fritts 1984). Saddleback tortoises have longer necks and forelimbs, and a carapace shape that allows for higher vertical reach, characteristics that increase both vertical feeding range and dominance during agonistic encounters.

The second traditional line of evidence for adaptive differences among populations comes from common garden experiments (Claussen et al. 1948; McKay et al. 2001; Thorpe et al. 2005). While environmental and phenotypic differences among populations can be useful for hypothesis generation, inferences based on these patterns alone can be confounded by phenotypic plasticity, which is the capacity of a single genotype to produce multiple phenotypes in response to different environmental conditions. A common garden experiment can be used to test whether phenotypic variation is genetically determined or due to plasticity. If phenotypic differences between individuals from different environments are maintained when they are reared in a common environment, it indicates the phenotype is genetically based, rather than plastic. Most variation in traits is due to a combination of genetic variation and plasticity.

The third traditional line of evidence for adaptive differences among populations comes from reciprocal transplant experiments (Berven 1982; Nagy and Rice 1997; Sork 2018). In these experiments, individuals are swapped between two different environments to test whether individuals are adapted to their local environment. If individuals do better in their native environment compared to the foreign environment, this suggests they are locally adapted to their native environment. However, in addition to being difficult or impossible to implement for many at-risk species, the use of controlled breeding and reciprocal transplant experiments to gauge overall adaptive potential within and between populations can be problematic (Hendry et al. 2011; Hoffmann et al. 2017). In particular, results of these experiments are only relevant to the trait, environment, and population that is studied, and can overestimate or underestimate adaptive potential (Harrisson et al. 2014).

Research on the threatened Italian agile frog (*Rana latastei*) provides an example of a combined, non-genomic (traditional) approach to testing for local adaptation and quantifying adaptive potential. This species shows differences in larval growth over short geographic distances as a function of temperature: tadpoles in colder environments take approximately 1 month longer for metamorphosis compared to tadpoles in warmer environments, which led Ficetola and De Bernardi (2005) to hypothesize that these frogs are adapted to their local temperature regime. Interestingly, a common garden experiment confirmed a genetically based difference in developmental rates among these populations, but in the opposite direction to that observed in the wild. Cold-adapted tadpoles developed faster than warm-adapted tadpoles held at the same temperature. This indicates selection for faster development in cold climates, despite slower growth in the field (Ficetola and De Bernardi 2005). These results were used to provide support for in situ conservation of cold-adapted populations, rather than transferring individuals between cold and warm populations, which could potentially disrupt local adaptation (Ficetola and De Bernardi 2005).

## Genomic approaches for quantifying adaptive potential

While traditional methods for investigating adaptive potential can be useful in certain species, the necessity of controlled breeding and experiments means they will not be applicable for most at-risk populations and species. Fortunately, technological advances in next-generation sequencing provide novel opportunities to estimate adaptive potential in wild populations, providing stronger evidence than simple correlative approaches and avoiding the need for manipulative experiments (Table 1). Cost-effective genomic sequencing methods can be used in any species, even in cases where no prior genomic information is available (Andrews et al. 2016). These sequencing approaches produce large genomic datasets, for example, genotypes at thousands of SNPs for hundreds of individuals (Lowry et al. 2016; McKinney et al. 2017; Catchen et al. 2017). These **genomic data** can then be used in downstream analyses to estimate adaptive potential within and among populations. While many of these methods do not require a reference genome (i.e., an assembled genome), a high-quality genome assembly and subsequent gene annotation can improve both data quality (e.g., genotyping accuracy) and downstream inferences, such as the identification of candidate genes and calculation of **linkage disequilibrium** as a function of physical distance (Davey et al. 2011; Manel et al. 2016).

Within populations, genomic data can facilitate the estimation of adaptive potential by allowing for pedigree-free estimation of heritability, eliminating the need for controlled breeding or long-term field-based data collection. Instead, genomic data can be used directly to estimate the relatedness among all pairs of individuals in a focal population (Gienapp et al. 2017). Heritability is then estimated by testing the relationship between trait similarity and relatedness among individuals (Visscher et al. 2008; Sillanpää 2011). Sampling requirements can be high for these studies (e.g., 150–200 individuals and ~ 25,000 SNPs; Stanton-Geddes et al. 2013), and can be difficult to generalize since the number of individuals and markers needed can vary across species and populations due to differences in effective population sizes and linkage disequilibrium. However, since many species of conservation concern have small effective sizes, they can be good candidates for genomic-based heritability studies since the estimation error for heritability is proportional to the effective size (Visscher and Goddard 2015), and increased linkage disequilibrium among loci will reduce the number of SNPs required for robust estimates. For example, a recent study estimated heritability for four quantitative (continuously varying) traits in Corsican blue tits (*Cyanistes caeruleus ogliastrae*) using both genomic and pedigree-based approaches (Perrier et al. 2018). The authors found that 15,000 SNPs genotyped across 494 individuals was sufficient to match or surpass the accuracy of heritability estimates provided by a 7-year pedigree-based study of > 1600 individuals.

Genomic data can also be used within populations to identify the specific loci that underlie variation in fitness-related traits using genome-wide association studies (GWAS; Korte and Farlow 2013). Similar to heritability, GWAS generally requires large sample sizes (e.g. Hong and Park 2012), including the measurement of fitness-relevant traits in many individuals and dense genomic sampling (many genetic markers [e.g., SNP loci] across the genome so that most genes are physically linked to at least one marker). Again, GWAS in populations with small effective sizes will have the advantage of increased linkage disequilibrium, which will reduce the number of markers required to sample the genome effectively (McKinney et al. 2017). For example, Hess et al. (2016) used univariate and multivariate GWAS analyses to identify the genetic basis of the adult migration-timing phenotype in threatened Klickitat River steelhead (*Oncorhynchus mykiss*), part of the Middle Columbia River steelhead distinct population segment. Using a genomic data set of 15,239 SNPs genotyped in 237 individuals, this study identified 18 SNPs that explained ~ 60% of the variation in the adult migration-timing phenotype, information that can be used to inform conservation at pre-adult life stages and better assign adults to summer or winter-run phenotypes. In a similar study, Prince et al. (2017) used association mapping to determine the genetic basis of premature migration. They found that premature migration is associated with the same locus in multiple populations of steelhead and Chinook salmon (*O. tshawytscha*).

Estimating heritability and identifying loci that underlie trait variation using GWAS both require choosing the trait or traits to analyze. Given that adaptation to changing environmental conditions will likely involve many traits, a well-grounded understanding of the biology and natural history of the study species is essential to predict which traits will have the most important effects on fitness and population persistence in the face of environmental change and that should therefore be the focus of efforts to quantify adaptive potential. Researchers must also acknowledge uncertainty regarding which traits will be most important, and should include this uncertainty in models of extinction risk (see "Incorporating adaptive potential into Endangered Species Act decisions in the face of uncertainty" below).

Genomic data can also inform estimates of among-population adaptive potential. For these analyses, presumably adaptive loci with genetic signatures of divergent selection are identified, and then these loci are used to quantify adaptive potential (e.g., Bonin et al. 2007). Major approaches for identifying candidate adaptive loci include differentiation-based methods, genotype-environment associations, and transcriptomics (Table 1). Differentiation-based methods detect adaptive markers by distinguishing locus-specific patterns (caused by selection) from genome-wide patterns (caused by **neutral processes** such as genetic drift and gene flow; Luikart et al. 2003). These methods only require genomic data (they do not use environmental or phenotypic data) and are useful for detecting strong selection between populations, but do not uncover the potential environmental drivers of adaptation and are generally less useful for detecting contemporary selection (de Villemereuil et al. 2014). Additionally, these methods are typically dependent on theoretical population genetic models, which are violated in many empirical systems (Bierne et al. 2013).

Genotype-environment association (GEA) methods provide an alternative that does not require population genetic models and can be used with individual or population-based sampling designs. GEAs identify adaptive variation using associations between allele distributions and environmental variables hypothesized to drive selection, identified as a pattern of selected alleles at higher frequency in certain environments. The inclusion of environmental predictors improves power over differentiation-based methods, allows better detection of signals of contemporary selection, and identifies the environmental factor(s) underlying adaptation (Rellstab et al. 2015; Forester et al. 2018). When phenotypic data are available across multiple populations in different home environments, GEA can be combined with GWAS to link loci under selection to phenotypes, improve power to detect adaptive loci, and further strengthen inference of local adaptation (Berg and Coop 2014; Lasky et al. 2015). Although differentiation-based methods, GEA, and GWAS can identify different adaptive loci, the results of these different statistical tests can be combined in a common framework to increase power to infer patterns of local adaptation (François et al. 2016).

Finally, transcriptomics is another method of genomic data acquisition that quantifies **gene expression** in response to the environment and its effect on phenotypes (Alvarez et al. 2015). While this can be a more efficient means of surveying adaptive variation, since all RNA transcripts are by definition functional, transcriptomics is not easily applied in wild populations or at-risk species. Tissue requirements are more stringent than for genomic sequencing methods, and often require destructive sampling, for example to access internal organs. Additionally, robust transcriptomic studies require controlled, experimental treatments to reduce environmental effects and high-quality gene annotations so the function of transcripts is known. This limits the utility of field-based transcriptomic studies, which require careful design and execution since gene expression is highly sensitive to environmental conditions (Todd et al. 2016). Currently, field-based transcriptomic studies are most valuable for generating hypotheses for future research, limiting their utility for estimating adaptive potential in wild populations of at-risk species. Despite these limitations, field-based transcriptomic studies can be used effectively in species with adequate genomic resources to address conservation-relevant questions. For example, Thomas and Palumbi (2017) used temporal transcriptomic sampling of a reef-building coral (*Acropora hyacinthus*, which has extensive genomic resources available) subjected to a bleaching event to document long-term (> 12 month) disruption of the coral transcriptome despite the apparent recovery of the coral symbiont population. These lasting effects on species long after the return of normal environmental conditions has implications for ecosystem resiliency in the face of increasing extreme environmental events associated with climate change. In summary, despite the limitations of individual genomic methods, when combined, these methods finally allow the possibility of quantifying adaptive potential in species of conservation concern, providing the exciting opportunity of integrating this information into ESA decision making.

## Endangered Species Act decisions and adaptive potential

### How are Endangered Species Act listing and recovery decisions made?

Decision makers at the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS), the two agencies responsible for administering the U.S. Endangered Species Act (ESA 1973, as amended), make decisions using the most up-to-date scientific information to evaluate extinction risk to species and consider that evaluation within the context of society's willingness to tolerate risks (Doremus 1997; Waples et al. 2013). They face the difficult task of balancing species protection against the burden of regulations. For these decision makers, addressing the values of diverse stakeholders can be an uphill battle (Rohlf 1991; Ruhl 2004). In this section, we outline the important listing and recovery decisions faced by the conservation practitioners responsible for administering the ESA. Figure 3 details the steps involved in both listing and recovery decisions.

A species can be listed as either an endangered or threatened species under the ESA depending on the degree of threat it faces (ESA 1973, Sect. 3, 4a). An endangered species is one that is *in danger of extinction* throughout all or a significant portion of its range. A threatened species is one that is *likely to become endangered in the foreseeable future* throughout all or a significant portion of its range. Thus, ultimately, the decision to list a species is based on its extinction risk. The ESA defines species broadly to include species, subspecies, varieties, and, for vertebrates, distinct population segments (DPS), defined as a population or group of populations that is discrete and significant in relation to the entire species. The USFWS and NMFS rely increasingly on genetics in defining species (USFWS and NMFS 1996). Accordingly, scientists' conclusions about whether populations are genetically distinct have become extremely important in decision making (Brosi and Biber 2009). However, debate continues about how best to use increasingly detailed genomic information to identify species and how to determine if the entity meets the definition of threatened or

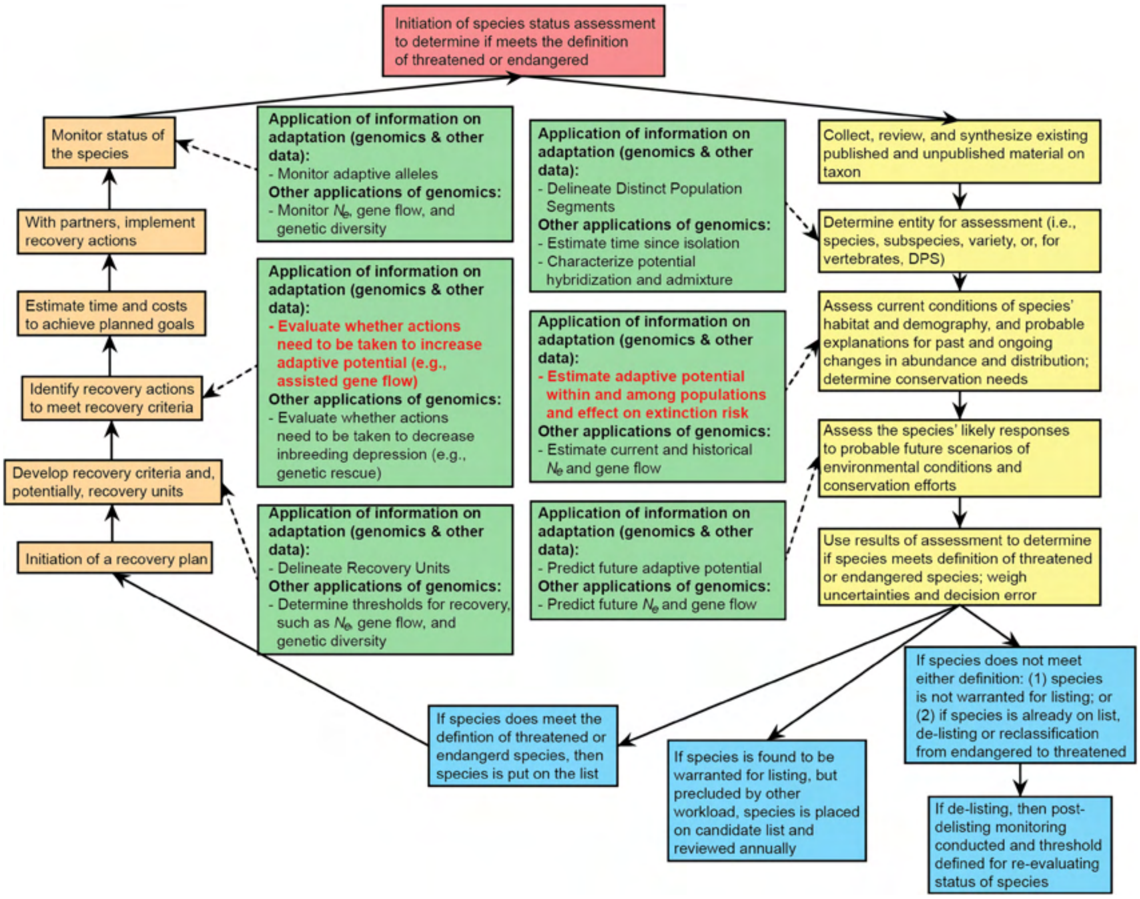


**Fig. 3** Flow chart showing steps involved in U.S. Endangered Species Act (ESA) listing decisions (yellow boxes) and recovery decisions (orange boxes). Blue boxes show possible outcomes of listing decisions. Green boxes show examples of ways in which information on adaptive potential (based on genomics and other data) and other inferences from genomics can inform different steps of listing and recovery decision workflows. In this paper, we focus on how information on adaptive potential can be incorporated into models of extinction risk to improve ESA listing and recovery decisions (shown in bold red font). See text for details on how genomics and other approaches can be used to infer adaptive potential and how uncertainty in these inferences can be included in decision making. (Color figure online)

endangered (e.g., Haig and D'Elia 2010; Regan et al. 2013; Keith et al. 2015; Boyd et al. 2017).

The ESA's ultimate goal is to recover species so they no longer need protection under the ESA. Recovery plans describe the biological state at which protection is no longer needed, called recovery criteria, and the recommended steps to get there (Taylor et al. 2005; Neel et al. 2012). The ESA stipulates that recovery criteria be measurable and objective and that listing and delisting decisions be based on the best science. Both requirements inject a primary role for science, although how recovery criteria are set is not defined in the ESA (Doak et al. 2015). Defining recovery units, management sub-units of the listed entity, is optional, but, where used, sub-units should collectively encompass the entire listed entity and should each have recovery criteria (NMFS and USFWS 2010). Every recovery unit must be recovered before the species can be delisted.

Recovery is not likely to be a fast process; it takes time to address threats that were years in the making. The first milestone in recovery is halting the decline of the species. Next is stabilizing the species, followed by increasing numbers and distribution—finally to the point that it is secure in the wild and the intent of the recovery criteria is met. If the threats have been sufficiently reduced, delisting the species may be considered. The analysis to determine if a species no longer meets the definition of threatened or endangered is analogous to the status assessment the USFWS and NMFS undertake when first determining whether a species should be added to the endangered species list. The ESA requires monitoring of delisted species for at least 5 years to assess their ability to sustain themselves without the protective measures of the ESA. If threats to the species change or unforeseen events change the stability of the population, USFWS or NMFS may extend the monitoring period or re-list the species.

ESA biologists and decision makers, scientists, and other stakeholders often express concern about insufficient information for a particular ESA decision. Indeed, perfect information might lead to different, even better decisions, but the ESA has a strict policy on the luxury of perfect information. The ESA makes it clear that listing decisions are to be based on the "…best *available* scientific and commercial information…" (italics ours) (ESA 1973, Sect. 4b). Several statutory deadlines assure that ESA decisions are not postponed in favor of additional research. In particular, the 12-month finding, which is the bulk of the status assessment, dictates that USFWS and NMFS have 1 year to make their listing determinations. Thus, ESA decision makers almost always find themselves in the position of making tough decisions under high uncertainty. Given these rigid legal constraints, conservation geneticists need to understand how information on adaptive potential can improve ESA decision making so that they know what information is most important to provide to USFWS and NMFS decision makers.

## How can information on adaptive potential improve Endangered Species Act decisions?

Incorporating information on adaptive potential into models of extinction risk can improve ESA listing and recovery decisions by increasing the accuracy of these models. On the one hand, if models do not allow for the possibility of adaptation in response to novel environmental stressors, then extinction estimates might be biased high, which could result in ESA listing of a species that actually has the capacity to evolve and persist in the face of environmental change. On the other hand, if the possibility that threats (e.g., habitat loss, invasive species, overexploitation, etc.) have decreased or are decreasing adaptive potential is not considered, then extinction estimates may be biased low, resulting in not listing a species for which listing is warranted. In the context of recovery decisions, an understanding of how to exploit available adaptive potential, or be conservative in the face of a lack of adaptive potential, could be beneficial in choosing optimal actions. Since ESA decisions are ultimately based on extinction risk, the quantification of adaptive potential with genomics—so that this information can be incorporated into models that predict extinction risk—is arguably the most important application of genomics in ESA decision making. Genomics has numerous other important applications in the ESA listing and recovery workflow, including delineating conservation units (Funk et al. 2012), inferring evolutionary history (Lemmon et al. 2012), quantifying hybridization (Payseur and Rieseberg 2016), and estimating $N_e$ and gene flow (Waples et al. 2016; Fig. 3). However, as these applications of genomics to conservation policy have already been discussed in detail elsewhere, here we focus on discussing how incorporating information on adaptive potential, based on genomics and other approaches, can improve estimates of extinction risk and, therefore, ESA listing and recovery decision making.

In the context of ESA listing decisions, models can be developed to investigate how adaptive responses in specific traits may allow species to avoid extinction under rapid environmental change. A recent example in a population of sockeye salmon (*Oncorhynchus nerka*) from the Fraser River, Canada, used empirical data to parameterize an individual-based model to determine how evolution of migration timing impacted species persistence under a range of climate change projections (Reed et al. 2011). They found that, with evolution of earlier migration timing, the risk of extinction by 2100 was predicted to be only 17% of that faced by the population with no adaptive potential. This scenario simulated a heritability of 0.5 for migration timing and a 2 °C increase during this time frame (resulting in a 9% and 53% probability of extinction with vs. without adaptive potential, respectively). The authors concluded that the rates of evolution included in their models are plausible given estimated heritabilities and rates of microevolution in migration timing in salmon. A similar modeling approach could be used to assess the effect of adaptive potential on extinction risk in species status assessments of ESA candidate species.

Models have also been used to predict how gene flow of adaptive alleles among populations could improve adaptive potential and mitigate extinction in entire metapopulations or species. Creech et al. (2017) used simulations to investigate the spread of adaptive genotypes in desert bighorn sheep (*Ovis canadensis nelsoni*), a habitat specialist threatened by habitat loss and fragmentation due to climate change and other anthropogenic effects. They found that adaptation from standing genetic variation already present within populations had a much higher chance of spread and likelihood of persistence than adaptive variation arising from a new mutation, especially when landscapes were more highly connected. These results highlighted the importance of retaining high levels of genetic variation within populations, while maintaining the metapopulation structure that is characteristic of the subspecies across its range. Metapopulation models such as this and others (Converse et al. 2017) provide a means of assessing how spatial variation in adaptive alleles can influence extinction risk in an entire species, subspecies, or DPS.

If the listing process identifies a species as threatened or endangered, genomic data related to adaptive potential can inform specific recovery actions that mitigate extinction risk through the directional movement of "pre-adapted" individuals between populations to facilitate adaptation to changing conditions (Aitken and Whitlock 2013). This action, called assisted gene flow, has been advocated for long-lived, sessile species such as trees (e.g., Steane et al. 2014), and species that have a limited ability to track climate conditions to which they are adapted (Sgro et al. 2011). While such interventions include risks, for many populations and species that either lack the capacity for long-distance movement or have no available suitable habitats to disperse into, introduction of adaptive genetic variation may be the only possible path to persistence. In these cases, consideration of potentially far-reaching benefits and careful evaluation to minimize the risks of assisted gene flow can provide an important option for the management of vulnerable populations (Weeks et al. 2011; Aitken and Whitlock 2013). Thus, despite uncertainty, information on adaptive potential based on genomics and other sources can help inform ESA listing and recovery decisions.

## Incorporating adaptive potential into Endangered Species Act decisions in the face of uncertainty

Decisions about endangered species management always will be made in the face of uncertainty, making recognition and quantification of uncertainty associated with information used in decision making as important as the information itself (Runge et al. 2011). However, uncertainty need not be paralyzing. Although the best available information may not be perfect, it can only lead to poor decision making if uncertainty associated with it is not recognized. Decision analysis is the application of decision science to render decisions that are more likely to achieve management objectives, are more robust to uncertainty, and are more transparent to those outside the decision-making process (Keeney 1992; Gregory et al. 2012; Converse et al. 2013; Garrard et al. 2017). All decisions are composed of a consistent set of components including: the decision to be made, the management objectives of the decision maker, the alternative management actions under consideration, models designed to predict the consequences of each alternative on the management objectives, and some approach to solving the decision (frequently known as optimization). In decision analysis, we break the decision into these components to identify and tackle impediments to the decision.

Decision making under uncertainty is the impetus for a large set of methods in decision analysis. General approaches to dealing with uncertainty in decision making include: (1) characterizing uncertainty and deciding in the face of that uncertainty; (2) characterizing uncertainty and choosing to delay a decision while further information is gathered; or (3) characterizing uncertainty and choosing to decide while simultaneously learning. The last of these can only occur for iterated decisions, and is known as adaptive management (Walters 1986; Williams et al. 2007; Runge 2011).

In the context of this paper, we are interested in decisions—either listing or recovery decisions—to maximize the long-term viability of some taxon. To predict viability, we may need to predict how adaptive potential affects extinction risk. Including information on adaptive potential will require recognizing the substantial uncertainty around it, although ignoring it has the potential to introduce bias and underrepresent uncertainty.

With listing decisions, we are interested in whether the adaptive potential of the species could change extinction risk. Based on observed survival and birth rates, we can predict probability of persistence as well as uncertainty around that prediction, and a manager can decide based on that information. However, if we consider that survival or birth rates might improve due to adaptation, our predicted probability of persistence will increase, while our uncertainty will now reflect uncertainty about the degree to which adaptation might increase these rates. As discussed above (see section on "How can information on adaptive potential improve Endangered Species Act decisions?"), integrating adaptive potential can move decisions away or towards listing.

In recovery decisions, we are interested in considering which management actions might improve the status of a listed species. In these cases, the role of adaptive potential is likely to be more nuanced. For example, perhaps a translocation (e.g., assisted gene flow) is contemplated because of changing climate in the species' range, and uncertainty about whether the species has the capacity to adapt. But a translocation will reduce the viability of the species in its existing range because some individuals will be removed, and establishment in the new location is uncertain. Should a manager do the translocation or not? Here, it could result in greater danger to the species to ignore adaptive potential.

For these reasons, it is critical to contemplate how uncertainty, including uncertainty about the effect of adaptive potential, can be integrated into decisions. For one-time decisions, a manager can decide immediately or can delay the decision to learn. Two issues must be considered here: first, is it legally or politically feasible to delay, and second, is it worthwhile to delay? Answering the first question will require analysis of the social aspects of the decision. Answering the second question will require analysis of the value of information (Runge et al. 2011; Williams et al. 2011; Johnson et al. 2014; Canessa et al. 2015). Value of information is a set of methods for evaluating the expected increase in management performance associated with learning. We anticipate how much management outcomes might improve if we had additional information. Calculating the value of information often will require elicitation of expert judgment, because we are anticipating the value of something that we do not yet know, and so the analysis does not lend itself to empirical approaches. Runge et al. (2011) provide an overview, an example, and a comprehensive review of value of information.

Whether we do delay decisions to learn, or plan to learn as we manage, uncertainty will remain. Therefore, we will ultimately need to make decisions in the face of uncertainty about how management actions will affect extinction risk. When we consider that the species we manage may be undergoing adaptation, uncertainty is likely to be substantial. When making decisions under uncertainty, we are primarily concerned with characterizing that uncertainty and understanding how the risk attitude of the decision maker should be accounted for in the analysis. Characterizing the uncertainty involves estimating the probability of various outcomes, given a management alternative, via some predictive model. Integrating the risk attitude of the decision maker involves recognizing that a manager may have a non-linear utility function, whereby, for example, an action resulting in a relatively high predicted probability of persistence but relatively high uncertainty may be less preferred than an action resulting in a lower predicted probability of persistence but with relatively low uncertainty, such that the risk of particularly poor outcomes is overall lower under the preferred action. This is akin to preferring an investment portfolio that is lower return but also lower risk. A thorough analysis of the uncertainty around probability of persistence is critical in allowing us to integrate uncertainty, and risk tolerance, into our decision making. And accounting for as many factors as possible that influence risk, including adaptive potential, will allow us to produce the most thorough analysis of the state of our population under the actions considered.

## Advancing understanding of the effect of adaptive potential on extinction risk

One of the main challenges to improving models of extinction risk that incorporate adaptive potential is estimating adaptive potential in traits important for fitness in the face of environmental change (e.g., thermal tolerance, disease resistance, susceptibility to environmental contaminants, resistance to or tolerance of invasive species, etc.). To parameterize extinction risk models that allow evolution, at a minimum, modelers need to know, or at least hypothesize: (1) how traits affect survival and birth rates (which is both a measure of selection on these traits and necessary to parameterize demographic models); and (2) the heritability of these traits. Mark-recapture analysis can be used to test how traits affect survival and birth rates (White and Burnham 1999). Genomic and other approaches are necessary to quantify the heritability of these traits. As described above, genomics can be used to infer relatedness among individuals, and thereby allow estimation of heritability of traits within a population. However, more research is needed to figure out how to integrate inferences from multiple genomic analyses to inform models of extinction risk, since alone, most of these analyses do not provide all necessary information for parameterizing these models. For example, genome-wide association studies (GWAS) identify loci related to variation in a trait of interest, but they do not test whether the trait is related to fitness or is adaptive. Genotype-environment association (GEA) approaches, in contrast, identify loci that are related to specific environmental features and presumably adaptive, but they do not determine which phenotypic traits mediate the fitness effects of these loci. It will clearly be necessary to integrate these different types of genomic analyses to identify traits that increase fitness in response to specific environmental stressors and that are heritable, so that this information can be incorporated into models of extinction risk. This is an important frontier in conservation genomics to make genomics more useful for informing extinction risk.

Controlled experiments, while impractical for most species of conservation concern, will remain important for testing under what conditions adaptation can rescue populations from extinction. Although these experiments do not necessarily directly inform extinction risk for specific species of conservation concern, they are nonetheless often the only means of rigorously testing evolutionary theory on the potential of adaptation to reduce extinction probabilities. For example, research on the adaptive potential of two rainforest-restricted fruit fly species demonstrated very low additive genetic variation (the substrate for adaptation) for desiccation resistance, even though other traits maintained high levels of genetic variation (Hoffmann et al. 2003; Kellermann et al. 2006). This result calls into question the generalization that most traits will maintain sufficient additive genetic variation to ensure adaptive potential (Blows and Hoffmann 2005), and also illustrates that trait-specific measures of genetic variance are not necessarily indicative of overall adaptive capacity. In addition, experimental studies of model species allow testing management strategies as a proof of concept in the lab. For example, experimental evolution in yeast populations has provided evidence not only for the efficacy of **evolutionary rescue** (an increase in population growth and avoidance of extinction through adaptation from standing genetic variation, mutation, or gene flow), but also for the environmental, demographic, and selective conditions under which it is most likely to occur (Bell and Gonzalez 2009, 2011). Additional experimental studies such as these are needed to better characterize thresholds related to adaptive potential, including levels of additive genetic variance required for adaptive responses to different rates and magnitudes of environmental change, and to provide guidelines for management actions such as assisted gene flow.

Finally, ongoing studies of wild populations that leverage the power of genomics to inform adaptive potential are needed to better characterize evolutionary responses to climate and other environmental change. As genomic studies become more common, comparative genomics will be one avenue for investigating the mechanisms underlying loss of adaptive potential in threatened species and taxonomic groups and the resulting implications for extinction risk. For example, a comparative study of the genomes of 43 bird species, including eight species recovering from endangered or vulnerable status, showed loss of adaptive variation related to agrochemical pollution (Li et al. 2014). Meta-analyses will also be essential for developing a more general understanding of the genomic and environmental landscape of adaptive potential, including under what circumstances populations may adapt, or fail to adapt, to changing conditions (Merilä and Hendry 2014). The increasing use of genomic approaches to characterize adaptive potential in wild populations will facilitate these efforts.

## Conclusions

Genomics has the potential to improve ESA listing and recovery decisions—and similar decisions in other countries—by providing information on adaptive potential for wild populations for which it is difficult or impossible to characterize adaptation using traditional approaches like controlled breeding or reciprocal transplant experiments. Incorporating this information into population models will lead to more accurate estimates of extinction risk, improving decision making and allocation of scarce conservation resources. In this paper, we provide specific guidelines on where in the listing and recovery decision making workflows this information is most pertinent. Although genomics, like any scientific tool, is imperfect, we cannot afford to be paralyzed by uncertainty in using this information to make decisions. A rich decision theoretic framework has already been developed for making management decisions in the face of uncertainty, which can readily be applied to decisions involving inference about adaptive potential. At the same time, conservation geneticists should continue striving to improve our understanding of the effects of adaptive potential on extinction risk using modeling, controlled experimental studies of model species, and case studies of wild populations. This will help reduce uncertainty to improve future management decisions. Finally, we urge conservation geneticists to develop partnerships with conservation practitioners charged with making tough decisions regarding the conservation management of small, at-risk populations to facilitate integration of the best science on the effects of adaptive potential on extinction risk into these decisions.

**Acknowledgements** We first thank A. Rus Hoelzel for inviting WCF to the Discussion Meeting at Durham University in September 2017 on "Conserving Adaptive Potential and Functional Diversity." Without Rus' initiative and perseverance in organizing this meeting, we would not have been spurred to assemble the diverse team of coauthors needed to bring together the ideas outlined here. We also thank Robin Waples and Gordon Luikart for excellent suggestions for improving the manuscript. We acknowledge funding from Colorado State University and National Science Foundation Ecology of Infectious Diseases Grant (DEB 1413925). The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service.

## References

Agashe D, Falk JJ, Bolnick DI (2011) Effects of founding genetic variation on adaptation to a novel resource. Evolution 65:2481–2491

Aitken SN, Whitlock MC (2013) Assisted gene flow to facilitate local adaptation to climate change. Annu Rev Ecol Evol Syst 44:367–388. https://doi.org/10.1146/annurev-ecolsys-110512-135747

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nat Rev Genet 11:697–709

Allendorf FW, Luikart G, Aitken SN (2013) Conservation and the genetics of populations, 2nd edn. Wiley, Oxford

Alvarez M, Schrey AW, Richards CL (2015) Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? Mol Ecol 24:710–725

Andrews KR, Good JM, Miller MR et al (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. Nat Rev Genet 17:81–92. https://doi.org/10.1038/nrg.2015.28

Bataille A, Cashins SD, Grogan L, Skerratt LF, Hunter D, McFadden M, Scheele B, Brannelly LA, Macris A, Harlow PS, Bell S, Berger L, Waldman B (2015) Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. Proc R Soc B 282:20143127

Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. Mol Ecol 13:969–980

Bell G, Collins S (2008) Adaptation, extinction and global change. Evol Appl 1:3–16

Bell G, Gonzalez A (2009) Evolutionary rescue can prevent extinction following environmental change. Ecol Lett 12:942–948. https://doi.org/10.1111/j.1461-0248.2009.01350.x

Bell G, Gonzalez A (2011) Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. Science 332:1327–1330. https://doi.org/10.1126/science.1203105

Berg JJ, Coop G (2014) A population genetic signal of polygenic adaptation. PLOS Genet 10:e1004412. https://doi.org/10.1371/journal.pgen.1004412

Bernatchez L (2016) On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. J Fish Biol 89:2519–2556

Berven KA (1982) The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. Evolution 36:962–983

Bierne N, Roze D, Welch JJ (2013) Pervasive selection or is it… why are FST outliers sometimes so frequent? Mol Ecol 22:2061–2064. https://doi.org/10.1111/mec.12241

Black WCIV, Baer CF, Antolin MF, DuTeau NM (2001) Population genomics: genome-wide sampling of insect populations. Annu Rev Entomol 46:441–469

Blows MW, Hoffmann AA (2005) A reassessment of genetic limits to evolutionary change. Ecology 86:1371–1384. https://doi.org/10.1890/04-1209

Bonin A, Nicole F, Pompanon F et al (2007) Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conserv Biol 21:697–708. https://doi.org/10.1111/j.1523-1739.2007.00685.x

Boyd C, DeMaster DP, Waples RS, Ward EJ, Taylor BL (2017) Consistent extinction risk assessment under the U.S. Endangered

Species Act. Conserv Lett 10:328–336. https://doi.org/10.1111/conl.12269

Brosi BJ, Biber EG (2009) Statistical inference, Type II error, and decision making under the US Endangered Species Act. Front Ecol Environ 7:487–494

Burger R, Lynch M (1995) Evolution and extinction in a changing environment—a quantitative-genetic analysis. Evolution 49:151–163

Canessa S, Guillera-Arroita G, Lahoz-Monfort JJ, Southwell DM, Armstrong DP, Chadès I, Lacy RC, Converse SJ (2015) When do we need more data? A primer on calculating the value of information for applied ecologists. Methods Ecol Evol 6:1219–1228

Carroll R, Augspurger C, Dobson A, Franklin J, Orians G, Reid W, Tracy R, Wilcove D, Wilson J (1996) Strengthening the use of science in achieving the goals of the endangered species act: an assessment by the Ecological Society of America. Ecol Appl 6:1–11

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. Mol Ecol 22:3124–3140

Catchen JM, Hohenlohe PA, Bernatchez L et al (2017) Unbroken: RADseq remains a powerful tool for understanding the genetics of adaptation in natural populations. Mol Ecol Resour 17:362–365. https://doi.org/10.1111/1755-0998.12669

Cattau CE, Fletcher RJ Jr, Kimball RT, Miller CW, Kitchens WM (2017) Rapid morphological change of a top predator with the invasion of a novel prey. Nature Ecol Evol 2(1):108

Claussen J, Keck DD, Hiesey WM (1948) Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. Carnegie Institution of Washington Publication, Washington

Converse SJ, Moore CT, Armstrong DP (2013) Demographics of reintroduced populations: estimation, modeling, and decision analysis. J Wildl Manag 77:1081–1093

Converse SJ, Bailey LL, Mosher BA, Funk WC, Gerber BD, Muths E (2017) A model to inform management actions as a response to chytridiomycosis-associated decline. Ecohealth 14:S144–S155

Coop G, Witonsky D, Di Rienzo A, Pritchard JK (2010) Using environmental correlations to identify loci underlying local adaptation. Genetics 185:1411–1423

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. Trends Ecol Evol 15:290–295

Creech TG, Epps CW, Landguth EL et al (2017) Simulating the spread of selection-driven genotypes using landscape resistance models for desert bighorn sheep. PLoS ONE 12:e0176960. https://doi.org/10.1371/journal.pone.0176960

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12:499–510

Dawson TP, Jackson ST, House JI et al (2011) Beyond predictions: biodiversity conservation in a changing climate. Science 332:53–58. https://doi.org/10.1126/science.1200303

de Villemereuil P, Frichot É, Bazin É et al (2014) Genome scan methods against more complex models: when and how much should we trust them? Mol Ecol 23:2006–2019. https://doi.org/10.1111/mec.12705

Doak DF, Himes Boor GK, Bakker VJ, Morris WF, Louthan A, Morrison SA, Stanley S, Crowder LB (2015) Recommendations for improving recovery criteria under the US Endangered Species Act. Bioscience 65:189–199

Dobzhansky T, Wright S (1941) Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*. Genetics 26:23–51

Doremus H (1997) Listing decisions under the Endangered Species Act: why better science isn't always better policy. Wash Univ Law Q 75:1029–1153

[ESA] US Endangered Species Act of 1973, as amended, Pub. L. No. 93-205, 87 Stat. 884 (28 Dec 1973). http://www.fws.gov/endangered/esa-library/pdf/ESAall.pdf

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Longman, Harlow

Ficetola GF, De Bernardi F (2005) Supplementation or in situ conservation? Evidence of local adaptation in the Italian agile frog *Rana latastei* and consequences for the management of populations. Anim Conserv 8:33–40. https://doi.org/10.1017/S1367943004001805

Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford

Forester BR, Lasky JR, Wagner HH, Urban DL (2018) Comparing methods for detecting multilocus adaptation with multivariate genotype-environment associations. Mol Ecol 27:2215–2233

François O, Martins H, Caye K, Schoville SD (2016) Controlling false discoveries in genome scans for selection. Mol Ecol 25:454–469

Frankham R, Lees K, Montgomery ME, England PR, Lowe EH, Briscoe DA (1999) Do population size bottlenecks reduce evolutionary potential? Anim Conserv 2:255–260

Frankham R, Ballou JD, Briscoe AD (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge

Frankham R, Brook BW, Bradshaw CJA, Traill LW, Spielman D (2013) 50/500 rule and minimum viable populations: response to Jamieson and Allendorf. Trends Ecol Evol 28:187–188

Franklin IR (1980) Evolutionary change in small populations. In: Soulé ME, Wilcox BA (eds) Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland

Franks SJ, Kane NC, O'Hara NB, Tittes S, Rest JS (2016) Rapid genome-wide evolution in *Brassica rapa* populations following drought revealed by sequencing of ancestral and descendant gene pools. Mol Ecol 25:3622–3631

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741–2752

Frichot E, Schoville SD, Bouchard G, Francois O (2013) Testing for associations between loci and environmental gradients using latent factor mixed models. Mol Biol Evol 30:1687–1699

Fritts TH (1984) Evolutionary divergence of giant tortoises in Galapagos. Biol J Linn Soc 21:165–176. https://doi.org/10.1111/j.1095-8312.1984.tb02059.x

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. Trends Ecol Evol 27:489–496. https://doi.org/10.1016/j.tree.2012.05.012

Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. Funct Ecol 21:434–443

Garner BA, Hand BK, Amish SJ, Bernatchez L, Foster JT, Miller KM, Morin PA, Narum SR, O'Brien SJ, Roffler G, Templin WD, Sunnucks P, Strait J, Warheit KI, Seamons TR, Wenburg J, Olsen J, Luikart G (2016) Genomics in conservation: case studies and bridging the gap between data and application. Trends Ecol Evol 31:81–83

Garrard GE, Rumpff L, Runge MC, Converse SJ (2017) Rapid prototyping for decision structuring: an efficient approach to conservation decision analysis. In: Bunnefeld N, Nicholson E, Milner-Gulland E (eds) Decision-making in conservation and natural resource management: models for interdisciplinary approaches. Cambridge University Press, Cambridge

Gienapp P, Fior S, Guillaume F, Lasky JR, Sork VL, Csillery K (2017) Genomic quantitative genetics to study evolution in the wild. Trends Ecol Evol 32:897–908

Glenn TC (2011) Field guide to next-generation DNA sequencers. Mol Ecol Resour 11:759–769

Gregory R, Failing L, Harstone M, Long G, McDaniels T, Ohlson D (2012) Structured decision making: a practical guide to environmental management choices. Wiley, Oxford

Haig SM, D'Elia J (2010) Avian subspecies and the U.S. Endangered Species Act. Ornithol Monogr 67:24–34

Haldane JBS (1930) A mathematical theory of natural and artificial selection (VI Isolation). Proc Cambridge Philos Soc 26:220–230

Hansen MM, Olivieri I, Waller DM, Nielsen EE, Grp GW (2012) Monitoring adaptive genetic responses to environmental change. Mol Ecol 21:1311–1329

Hanson JO, Rhodes JR, Riginos C, Fuller RA (2017) Environmental and geographic variables are effective surrogates for genetic variation in conservation planning. PNAS 114:12755–12760

Harrisson KA, Pavlova A, Telonis-Scott M, Sunnucks P (2014) Using genomics to characterize evolutionary potential for conservation of wild populations. Evol Appl 7:1008–1025. https://doi.org/10.1111/eva.12149

Hendry AP, Kinnison MT, Heino M et al (2011) Evolutionary principles and their practical application. Evol Appl 4:159–183. https://doi.org/10.1111/j.1752-4571.2010.00165.x

Hess JE, Zendt JS, Matala AR, Narum SR (2016) Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. Proc R Soc B 283. https://doi.org/10.1098/rspb.2015.3064

Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. Nature 470:479–485

Hoffmann AA, Hallas RJ, Dean JA, Schiffer M (2003) Low potential for climatic stress adaptation in a rainforest *Drosophila* species. Science 301:100–102. https://doi.org/10.1126/science.1084296

Hoffmann AA, Sgro CM, Kristensen TN (2017) Revisting adaptive potential, population size, and conservation. Trends Ecol Evol 32:506–517. https://doi.org/10.1016/j.tree.2017.03.012

Hohenlohe PA, Day MD, Amish SJ, Miller MR, Kamps-Hughes N, Boyer MC, Muhlfeld CC, Allendorf FW, Johnson EA, Luikart G (2013) Genomic patterns of introgression in rainbow and westslope cutthroat trout illuminated by overlapping paired-end RAD sequencing. Mol Ecol 22:3002–3013

Hong EP, Park JW (2012) Sample size and statistical power calculation in genetic association studies. Genomics Inform 10:117–122. https://doi.org/10.5808/GI.2012.10.2.117

Jamieson IG, Allendorf FW (2012) How does the 50/500 rule apply to MVPs? Trends Ecol Evol 27:578–584

Jamieson IG, Allendorf FW (2013) A school of red herring: reply to Frankham et al. Trends Ecol Evol 28:188–189

Johnson FA, Hagan G, Palmer WE, Kemmerer M (2014) Uncertainty, robustness, and the value of information in managing a population of northern bobwhites. J Wildl Manag 78:531–539

Joost S, Bonin A, Bruford MW, Despres L, Conord C, Erhardt G, Taberlet P (2007) A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. Mol Ecol 16:3955–3969

Keeney RL (1992) Value-focused thinking: a path to creative decision making. Harvard University Press, Cambridge

Keith D, Akçakaya HR, Butchart SHM, Collen B, Dulvy NK, Homes EE, Hutchings JA, Keinath D, Schwartz MK, Shelton AO, Wapes RS (2015) Temporal correlations in population trends: conservation implications from time-series analysis of diverse animal taxa. Biol Conserv 192:247–257

Kellermann VM, Heerwaarden B van, Hoffmann AA, Sgrò CM (2006) Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest *Drosophila* species. Evolution 60:1104–1108. https://doi.org/10.2307/4095411

Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proc R Soc B 279:349–356

Kimura M, Crow JF (1963) The measurement of the effective population number. Evolution 17:279–288

Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:29

Kovach RP, Gharrett AJ, Tallmon DA (2012) Genetic change for earlier migration timing in a pink salmon population. Proc R Soc B 279:3870–3878

Lasky JR, Upadhyaya HD, Ramu P et al (2015) Genome-environment associations in sorghum landraces predict adaptive traits. Sci Adv 1:e1400218. https://doi.org/10.1126/sciadv.1400218

Lemmon AR, Emme SA, Lemmon EM (2012) Anchored hybrid enrichment for massively high-throughput phylogenomics. Syst Biol 61:727–744

Li S, Li B, Cheng C et al (2014) Genomic signatures of near-extinction and rebirth of the crested ibis and other endangered bird species. Genome Biol 15:557. https://doi.org/10.1186/s13059-014-0557-1

Lowry DB, Hoban S, Kelley JL et al (2016) Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. Mol Ecol Resour 17:142–152. https://doi.org/10.1111/1755-0998.12635

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet 4:981–994

Manel S, Perrier C, Pratlong M, Abi-Rached L, Paganini J, Pontarotti P, Aurelle D (2016) Genomic resources and their influence on the detection of the signal of positive selection in genome scans. Mol Ecol 25:170–184

McKay JK, Bishop JG, Lin JZ, Richards JH, Sala A, Mitchell-Olds T (2001) Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. Proc R Soc B 268:1715–1721

McKinney GJ, Larson WA, Seeb LW, Seeb JE (2017) RADseq provides unprecedented insights into molecular ecology and evolutionary genetics: comment on Breaking RAD by Lowry et al. (2016). Mol Ecol Resour 17:356–361. https://doi.org/10.1111/1755-0998.12649

Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol Appl 7:1–14. https://doi.org/10.1111/eva.12137

Muñoz NJ, Farrell AP, Heath JW, Neff BD (2015) Adaptive potential of a Pacific salmon challenged by climate change. Nat Clim Change 5:163–166

Nagy ES, Rice KJ (1997) Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. Evolution 51:1079–1089

[NMFS and USFWS] National Marine Fisheries Serivice and U.S. Fish and Wildlife Service (2010) Interim endangered and threatened species recover planning guidance, version 1.3. http://www.nmfs.noaa.gov/pr/recovery/

Neel MC, Leidner AK, Haines A, Goble DD, Scott JM (2012) By the numbers: how is recovery defined by the US Endangered. Species Act? BioScience 62:646–657

Nicotra AB, Beever EA, Robertson AL et al (2015) Assessing the components of adaptive capacity to improve conservation and management efforts under global change. Conserv Biol 29:1268–1278. https://doi.org/10.1111/cobi.12522

O'Connor MI, Selig ER, Pinsky ML, Altermatt F (2012) Toward a conceptual synthesis for climate change responses. Glob Ecol Biogeogr 21:693–703

Payseur BA, Rieseberg LH (2016) A genomic perspective on hybridization and speciation. Mol Ecol 25:2337–2360

Penuelas J, Sardans J, Estiarte M, Ogaya R, Carnicer J, Coll M, Barbeta A, Rivas-Ubach A, Llusia J, Garbulsky M, Filella I, Jump AS (2013) Evidence of current impact of climate change on life: a walk from genes to the biosphere. Glob Chang Biol 19:2303–2338

Perrier C, Delahaie B, Charmantier A (2018) Heritability estimates from genomewide relatedness matrices in wild populations: application to a passerine, using a small sample size. Mol Ecol Resour (**in press**)

Prince DJ, O'Rourke SM, Thompson TQ, Ali OA, Lyman HS, Saglam IK, Hotaling TJ, Spidle AP, Miller MR (2017) The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. Sci Adv 3:e1603198

Reed TE, Schindler DE, Hague MJ, Patterson DA, Meir E, Waples RS, Hinch SG (2011) Time to evolve? Potential evolutionary responses of Fraser River sockeye salmon to climate change and effects on persistence. PLoS ONE 6:e20380

Regan TJ, Taylor BL, Thompson GG, Cochrane JF, Ralls K, Runge MC, Merrick R (2013) Testing decision rules for categorizing species' extinction risk to help develop quantitative listing criteria for the U.S. Endangered Species Act. Conserv Biol 27:821–831

Rellstab C, Gugerli F, Eckert AJ et al (2015) A practical guide to environmental association analysis in landscape genomics. Mol Ecol 24:4348–4370. https://doi.org/10.1111/mec.13322

Rohlf DJ (1991) Six biological reasons the Endangered Species Act doesn't work—and what to do about it. Conserv Biol 5:273–282

Ruegg K, Bay RA, Anderson EC, Saracco JF, Harrigan RJ, Whitfield M, Paxton EH, Smith TB (2018) Ecological genomics predicts climate vulnerability in an endangered southwestern songbird. Ecol Lett (**in press**)

Ruhl JB (2004) The battle over the Endangered Species Act methodology. FSU College of Law, Public Law Research Paper No. 99

Runge MC (2011) An introduction to adaptive management for threatened and endangered species. J Fish Wildl Manag 2:220–233

Runge MC, Converse SJ, Lyons JE (2011) Which uncertainty? Using expert elicitation and expected value of information to design an adaptive program. Biol Conserv 144:1214–1223

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. Trends Ecol Evol 1:9–10

Savage AE, Zamudio KR (2011) MHC genotypes associate with resistance to a frog-killing fungus. Proc Natl Acad Sci USA 108:16705–16710

Scheffers BR, De Meester L, Bridge TCL, Hoffmann AA, Pandolfi JM, Corlett RT, Butchart SHM, Pearce-Kelly P, Kovacs KM, Dudgeon D, Pacifici M, Rondinini C, Foden WB, Martin TG, Mora C, Bickford D, Watson JEM (2016) The broad footprint of climate change from genes to biomes to people. Science 354:aaf7671

Sgro C, Lowe A, Hoffmann A (2011) Building evolutionary resilience for conserving biodiversity under climate change. Evol Appl 4:326–337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Shafer ABA, Wolf JBW, Alves PC, Bergstrom L, Bruford MW, Brannstrom I, Colling G, Dalen L, De Meester L, Ekblom R, Fawcett KD, Fior S, Hajibabaei M, Hill JA, Hoezel AR, Hoglund J, Jensen EL, Krause J, Kristensen TN, Krutzen M, McKay JK, Norman AJ, Ogden R, Ouml;sterling EM, Ouborg NJ, Piccolo J, Popovic D, Primmer CR, Reed FA, Roumet M, Salmona J, Schenekar T, Schwartz MK, Segelbacher G, Senn H, Thaulow J, Valtonen M, Veale A, Vergeer P, Vijay N, Vila C, Weissensteiner M, Wennerstrom L, Wheat CW, Zielinski P (2015) Genomics and the challenging translation into conservation practice. Trends Ecol Evol 30:78–87

Shaffer ML, Stein MA (2000) Safeguarding our precious heritage. In: Stein BA, Kutner LS, Adams JS (eds) Precious heritage: the status of biodiversity in the United States. Oxford University Press, New York, pp 301–321

Sillanpää MJ (2011) On statistical methods for estimating heritability in wild populations. Mol Ecol 20:1324–1332

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Smith DR, Allan NL, McGowan CP, Szymanski JA, Oetker SR, Bell HM (2018) Development of a Species Status Assessment process for decisions under the U.S. Endangered Species Act. J Fish Wildl Manag 9:302–320

Sork VL (2018) Genomic studies of local adaptation in natural plant populations. J Hered 109:3–15

Stanton-Geddes J, Yoder JB, Briskine R, Young ND, Tiffin P (2013) Estimating heritability using genomic data. Methods Ecol Evol 4:1151–1158

Steane DA, Potts BM, McLean E et al (2014) Genome-wide scans detect adaptation to aridity in a widespread forest tree species. Mol Ecol 23:2500–2513. https://doi.org/10.1111/mec.12751

Steffen W, Broadgate W, Deutsch L, Gaffney O, Ludwig C (2015) The trajectory of the Anthropocene: the Great Acceleration. Anthropocene Rev 2:81–98

Szymanski J, Smith T, Horton A, Parkin M, Ragan L, Masson G, Olson E, Gifford K, Hill L (2016a) Rusty patched bumble bee (*Bombus affinis*) species status assessment. U.S. Fish and Wildlife Service, Final Report, Version 1

Szymanski J, Pollack C, Ragan L, Redmer M, Clemency L, Voorhies K, JaKa J (2016b) Species status assessment for the eastern massasauga rattlesnake (*Sistrurus catenatus*). U.S. Fish and Wildlife Service, SSA Report Version 2

Taylor MFJ, Suckling KF, Rachlinski JJ (2005) The effectiveness of the Endangered Species Act: a quantitative analysis. Bioscience 55:360–367

Theimer TC, Smith AD, Mahoney SM, Ironside KE (2016) Available data support protection of the Southwestern willow flycatcher under the Endangered Species Act. Condor 118:289–299

Thomas L, Palumbi SR (2017) The genomics of recovery from coral bleaching. Proc R Soc B https://doi.org/10.1098/rspb.2017.1790

Thorpe RS, Reardon JT, Malhotra A (2005) Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). Am Nat 165:495–504

Todd EV, Black MA, Gemmell NJ (2016) The power and promise of RNA-seq in ecology and evolution. Mol Ecol 25:1224–1241. https://doi.org/10.1111/mec.13526

U.S. Fish and Wildlife Service (1995) Endangered and threatened wildlife and plants; final rule determining endangered status for the Southwestern Willow Flycatcher. Fed Reg 60:10694–10715

[USFWS and NMFS] U.S. Fish and Wildlife Service and National Marine Fisheries Service (1996) Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Fed Reg 61:4722–4725

U.S. Fish and Wildlife Service (2016) Endangered and threatened wildlife and plants; threatened species status for rusty patched bumble bee. Fed Reg 82:3186–3208

U.S. Fish and Wildlife Service (2017) Endangered and threatened wildlife and plants; endangered species status for the eastern massasauga rattlesnake. Fed Reg 81:667214–671936

Verhoeven KJF, Vonholdt BM, Sork VL (2016) Epigenetics in ecology and evolution: what we know and what we need to know. Mol Ecol 25:1631–1638

Visscher PM, Goddard ME (2015) A general unified framework to assess the sampling variance of heritability estimates using pedigree or marker-based relationships. Genetics 199:223–232

Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era—concepts and misconceptions. Nat Rev Genet 9:255–266

Vucetich JA, Nelson MP, Phillips MK (2006) The normative dimension and legal meaning of endangered and recovery in the US Endangered Species Act. Conserv Biol 20:1383–1390

Walters C (1986) Adaptive management of renewable resources. MacMillan, New York

Waples RS (1991) Pacific salmon, *Oncorhynchus* spp., and the definition of 'species' under the Endangered Species Act. Act Mar Fish Rev 53:11–22

Waples RS, Nammack M, Cochrane JF, Hutchings JA (2013) A tale of two acts: endangered species listing practices in Canada and the United States. Bioscience 63:723–734

Waples RK, Larson WA, Waples RS (2016) Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. Heredity 117:233–240

Weeks AR, Sgro CM, Young AG et al (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol Appl 4:709–725. https://doi.org/10.1111/j.1752-4571.2011.00192.x

White GC, Burnham KP (1999) Program MARK: survival estimation from populations of marked animals. Bird Study Suppl 46:120–138

Williams BK, Szaro RC, Shapiro CD (2007) Adaptive management: the U.S. Department of the Interior Technical Guide. Adaptive Management Working Group, U.S. Department of the Interior, Washington DC

Williams BK, Eaton MJ, Breininger DR (2011) Adaptive resource management and the value of information. Ecol Modell 222:3429–3436

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159

Wright S (1938) Size of population and breeding structure in relation to evolution. Science 87:430–431

Wright S (1943) Isolation by distance. Genetics 28:114–138

Zink RM (2015) Genetics, morphology, and ecological niche modeling do not support the subspecies status of the endangered willow flycatcher (*Empidonax traillii extimus*). Condor 117:78–86

# Demography of a recovering wolf population in the Yukon

**Article** *in* Canadian Journal of Zoology · February 2000

DOI: 10.1139/cjz-78-1-36

**2 authors**, including:

Robert D. Hayes
Bob Hayes Consulting

# Demography of a recovering wolf population in the Yukon

**R.D. Hayes and A.S. Harestad**

**Abstract**: We studied the dynamics of a wolf (*Canis lupus*) population recovering from intensive reduction in the Finlayson Lake area, Yukon, Canada. Within 6 years, numbers increased from 29 wolves, then stabilized at 245. The colonization of vacant territories by young wolf pairs was the primary mechanism of early population recovery. Reproduction and a low dispersal rate increased pack size in later years, and pack splitting allowed dispersing wolves to remain near natal packs. The rate of increase in the wolf population was density-dependent and related to wolf density, but was also related to the dispersal rate. The dispersal rate was density-independent and related to mean pack size and prey biomass : wolf index. The survival rate was age-dependent and not related to wolf density. In the early years of recovery, the rate of increase was supported by high survival rates and low dispersal rates. In later years, dispersal rates increased, stabilizing mean pack size and wolf density. Wolf density stabilized at levels predicted by the prey supply, but whether the wolf population is regulated by the availability of prey resources remains unresolved. Wolf density, pack density, and mean pack size were similar in 1983 and 1996, despite a 2- to 3-fold difference in prey biomass. We suggest that the interaction of wolf density and mean pack size in stable prey systems needs to be studied to determine the roles played by food supply and wolf social behavior in regulating wolf abundance.

**Résumé** : Nous avons étudié la dynamique d'une population du Loup gris (*Canis lupus*) en voie de rétablissement après une réduction importante de ses effectifs dans la région du lac Finlayson, Yukon, Canada. Après 6 ans, le nombre de loups est passé de 29 à 245 et s'est stabilisé à ce niveau. La colonisation des territoires vacants par de jeunes couples a été le coup d'envoi du rétablissement de la population. Au cours des années subséquentes, la reproduction et un faible taux de dispersion ont donné lieu à une augmentation du nombre d'individus dans les meutes et la division des meutes a permis aux loups qui se sont dispersés de rester près de leurs meutes d'origine. Les taux d'augmentation étaient fonction de la densité des loups, mais étaient aussi fonction des taux de dispersion, qui eux ne dépendaient pas de la densité, mais étaient reliés au nombre moyen de loups dans une meute et à la valeur de l'indice biomasse des proies : nombre de loups. Le taux de survie était fonction de l'âge et ne dépendait pas de la densité des loups. Au cours des 1$^{\text{ères}}$ années du rétablissement, le taux d'augmentation du nombre de loups s'est trouvé consolidé par des taux de survie élevés et des taux de dispersion faibles. Au cours des années subséquentes, les taux de dispersion ont augmenté, ce qui a donné lieu à une stabilisation du nombre moyen de loups par meute et de la densité. La densité des loups s'est stabilisée aux niveaux prévus en fonction de la disponibilité des proies, mais le contrôle de la population en fonction de la disponibilité des proies reste à démontrer. La densité de la population, la densité des meutes et le nombre moyen de loups par meute on été semblables en 1983 et en 1996 en dépit d'une différence importante dans la biomasse de proies (par un facteur de 2 à 3). Il nous apparaît essentiel d'étudier les interactions entre la densité des loups et le nombre moyen de loups dans une meute dans des systèmes ou la ressource proies est stable, de façon à pouvoir déterminer l'influence de la quantité de nourriture et du comportement social des loups sur leur abondance.

[Traduit par la Rédaction]

## Introduction

In this paper we describe the population dynamics of an increasing wolf (*Canis lupus*) population that was recovering after 7 years of intensive aerial reduction. We also examine the nature of the numerical response of wolves to increasing ungulate densities. From 1983 through 1989, the Yukon Fish and Wildlife Branch annually reduced wolf density to less than 20% of the pre-reduction level in the 23 000-km$^2$ range of the Finlayson woodland caribou (*Rangifer tarandus*) herd (Farnell and McDonald 1988; R. Farnell, Yukon Fish and Wildlife Branch, Box 2703, Whitehorse, YT Y1A 2C6, Canada, unpublished data). The initial density of wolves was 10.3/1000 km$^2$ in February 1983. Density was reduced to 1.5 wolves/1000 km$^2$ by 1 April of each year. Wolves annually recovered to an average of 3.7/1000 km$^2$ by the following February (R. Farnell, unpublished data). During wolf reduction, and for a few years afterwards, caribou and moose (*Alces alces*) numbers increased rapidly (Jingfors 1988; Larsen and Ward 1995; R. Farnell, unpublished data).

In past studies in which wolves were reduced in order to increase prey numbers, the wolf response was not adequately monitored afterwards (Gasaway et al. 1983, 1992; Bergerud and Elliot 1998), wolves were not followed until their numbers stabilized (Hayes et al. 1991), or a continued wolf harvest reduced rates of increase (Hayes et al. 1991; Boertje et al. 1996). In our study, wolf harvest during recovery was negligible. We were able to radio-collar wolves in most

Received February 1, 1999. Accepted August 31, 1999.

**R.D. Hayes.** Yukon Fish and Wildlife Branch, Box 5429, Haines Junction, YT Y0B 1L0, Canada.
**A.S. Harestad.** Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

packs to examine the roles played by recruitment, survival, ingress, and egress in the dynamics of a recovering wolf population until it reached stability.

Biologists have been interested in determining what regulates the growth of wolf populations and the density at which they stabilize in relation to prey abundance. Early studies (Murie 1944; Cowan 1947; Rausch 1967) showed that wolf populations increased more slowly than was thought to be theoretically possible (Packard and Mech 1980). Pimlott (1967) hypothesized that wolf density was regulated somewhere below their ungulate food supply through biosocial mechanisms.

Previous studies (Fritts and Mech 1981; Peterson et al. 1984; Ballard et al. 1987; Hayes et al. 1991; Boertje et al. 1996) suggested that ungulate food resources regulate increasing wolf populations. In each study, harvest caused substantial wolf mortality, depressing the numerical response of wolves. We followed changes in wolf, moose, and caribou abundance until wolf numbers stabilized, providing conditions that allowed us to test the hypothesis that wolf numbers are regulated by ungulate food resources (Keith 1983; Fuller 1989).

We tested 4 hypotheses about the nature of the wolf-population response:

$H_0$1: the rate of increase is density-dependent and inversely related to pack density and wolf density; $H_a$1: the rate of increase is density-independent and inversely related to mean pack size and dispersal rate;

$H_0$2: the survival rate is density-dependent and inversely related to wolf density; $H_a$2: the survival rate is age-dependent;

$H_0$3: the dispersal rate is density-dependent and positively related to pack density and wolf density; $H_a$3: the dispersal rate is density-independent and negatively related to mean pack size.

$H_0$4: the numerical response is tightly regulated by the availability of prey resources.

We examined the process of wolf recovery to determine the relative importance of reproduction, survival, ingress, and egress in the formation and growth of wolf packs to equilibrium.

## Methods

### Study area

The 23-000 km$^2$ Finlayson Study Area (FSA; Fig. 1) is located in the east-central Yukon (62°N, 128°W) and is bounded by the annual home range of the Finlayson caribou herd (Farnell and McDonald 1988). The study area is bordered by the Ross River valley to the west, the Pelly Mountains to the south, and the Logan Mountains to the north and east (Fig. 1). The central study area is part of the Pelly Plateau, a complex of small mountains, forested rolling hills, and plateaus that are separated by broad U-shaped valleys. Detailed physiographic and vegetation descriptions are found in Oswald and Senyk (1977).

Other ungulate prey in the study area included about 100 Dall sheep (*Ovis dalli dalli*) in the Pelly Mountains and 200–300 mountain goats (*Oreamnus americanus*) in the Logan Mountains (J. Carey, Yukon Fish and Wildlife Branch, Box 2703, Whitehorse, YT Y1A 2C6, Canada, unpublished data). A small number of mule deer (*Odocoileus hemionus*) also live on open slopes along the Pelly River (R. Hayes, personal observation).

Small-mammal prey include the snowshoe hare (*Lepus americanus*), beaver (*Castor canadensis*), and arctic ground squirrel (*Spermophilus parryi*). Snowshoe hares were abundant from 1989 until 1991, when the hare population in the Yukon crashed (Krebs et al. 1995).

Other carnivores include the grizzly bear (*Ursus arctos*), black bear (*Ursus americanus*), wolverine (*Gulo gulo*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), and lynx (*Lynx canadensis*). Ravens (*Corvus corax*) scavenged wolf kills.

### Estimating wolf numbers

We estimated annual wolf numbers in the 23 000-km$^2$ study area by means of aerial counts in February and March. From 1990 through 1994, we used both radiotelemetry (Mech and Karns 1977; Peterson et al. 1984; Ballard et al. 1987; Messier and Crête 1985; Fuller 1989; Hayes et al. 1991) and aerial snow-tracking methods (Stephenson 1978). In 1996, we used aerial snow-tracking methods alone to determine wolf population size.

We believe that a total count is appropriate for wolves because most live in packs with minimal spatial overlap (Mech 1970), and wolves make extensive snow trails that can be followed by trained observers (Stephenson 1978). Two requirements of the total-count method are (1) that the complete area is searched, and (2) that groups have not been missed or counted twice (Norton-Griffiths 1978). We believe that annual wolf counts were accurate for the following reasons: (*i*) study-area packs occupied discrete home ranges; (*ii*) packs traveled in predictable areas (e.g., rivers, creeks, lakes) where prey wintered; (*iii*) wolf trails were extensive, highly visible, and easily recognized by experienced observers; (*iv*) wolf habitat was searched between territories until packs were located or observers were confident that no wolves were present; and (*v*) pack duplication was minimal because most FSA packs were radio-collared each winter and their locations were known during snow-tracking surveys.

Crews of two fixed-wing aircraft and one helicopter searched for wolves. Routes mainly followed watercourses and riparian habitats where ungulates wintered. We searched alpine areas at least once each winter. In forests, we flew 10–15 km wide transects, making more extensive searches of meadows, lake margins, and open forests, where the probability of seeing wolf trails was greatest.

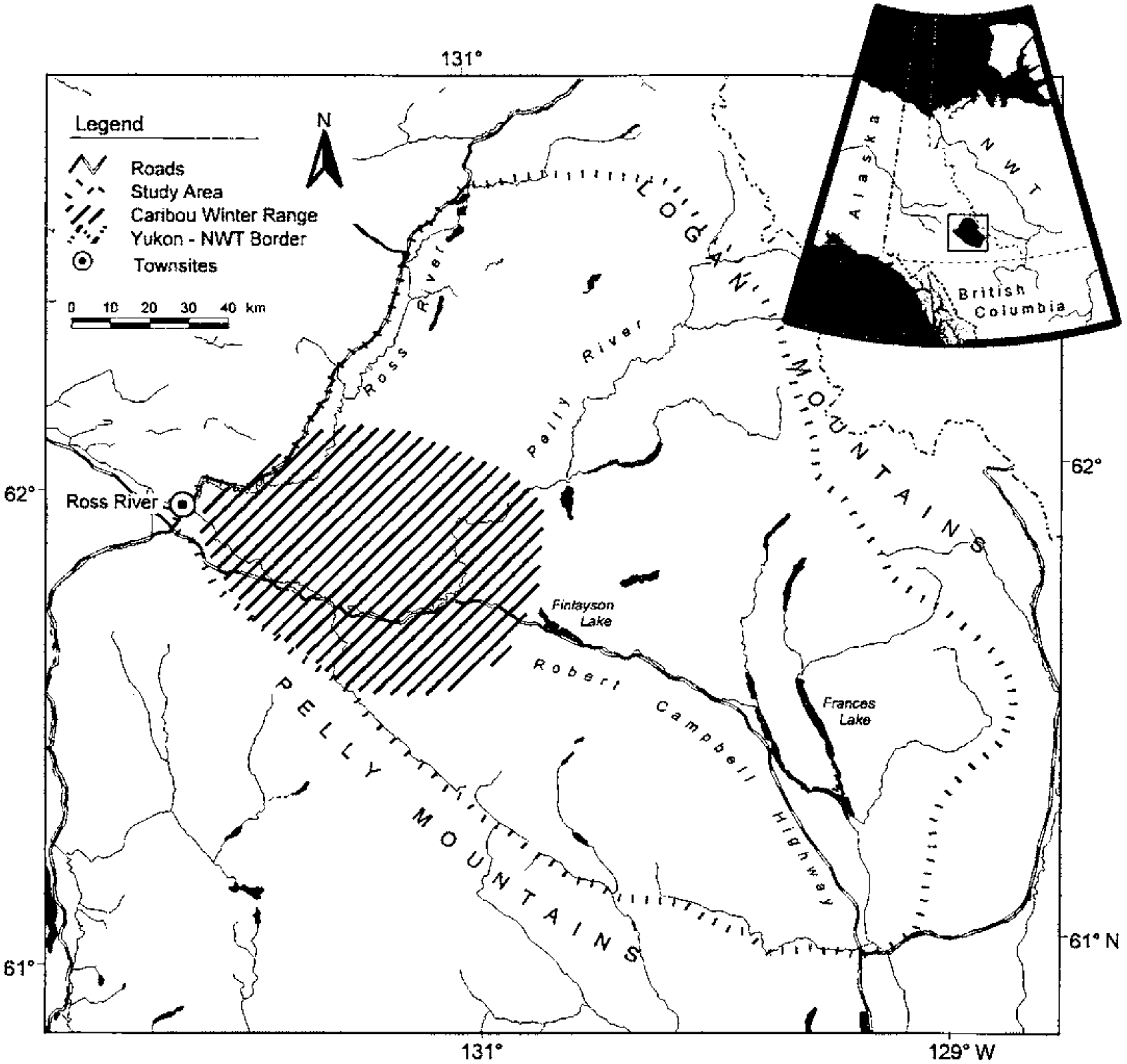
We followed trails until wolves were seen or we could estimate the number from separate track counts. Wherever possible, we back-tracked wolf trails to determine activities and travel routes. In the core caribou winter range (Fig. 1), we could not rely on aerial snow-tracking because wolf trails were obscured by caribou trails and snow craters. In these areas we searched for wolf trails by truck and snow machine for up to 15 km on each side of a 160-km section of the Robert Campbell Highway (Fig. 1).

We used the finite rate of increase ($\lambda$; number of wolves in March of year$_{n+1}$ / number of wolves in March of year$_n$) to determine annual rates of change. The biological year for wolves began on 1 May. We defined a pack as a group of two or more wolves that traveled together for more than 1 month (Messier 1994). We assumed that single wolves represented 10% of the annual winter wolf populations (Mech 1973).

### Radiotelemetry and home ranges

We radio-collared wolves in all new wolf packs from 1990 through 1993. We radio-collared both members of newly formed wolf pairs, and in larger packs we selected adult wolves for capture according to their differences in appearance and behavior from subadults (Hayes et al. 1991). Helicopter crews immobilized wolves with 2-cc Capchur darts (Palmer Chemical and Equipment Company, Douglasville, Ga.). Wolves received Telazol (Fort Dodge Laboratories Inc., Fort Dodge, Iowa) at a dosage of 8.0 ± 3.0 (mean ± SD) mg/kg (range 4.4–23.4 mg/kg). Wolves were sexed and classified as pup, yearling, 2–3 years old, or older, based on tooth coloration,

**Fig. 1.** The Finlayson Study Area (FSA).



wear, and canine length and eruption patterns (Van Ballenberge et al. 1975). We fixed wolves with Telonics MOD 500 radio collars equipped with mortality sensors in order to document behavior, survival, and home-range use from fixed-wing aircraft (Mech 1974).

We did not locate radio-collared wolves frequently enough to describe annual home ranges. We located wolves a few times in summer and autumn, and collected nearly all winter locations at daily intervals during predation studies (Hayes et al. 2000). We used 95% area convex polygons (Ackerman et al. 1990) to estimate the total area used in all years by wolf packs with an aggregate of 30 or more location points.

## Reproduction, survival, mortality causes, and dispersal

We estimated litter size at birth from corpora lutea counts from 19 reproducing females killed in 1985 through 1989 in the study area (R. Hayes, unpublished data). The same method was used for assessing in utero productivity in other studies (Fritts and Mech 1981; Peterson et al. 1984; Boertje and Stephenson 1992). We counted pups during autumn, based their small size and subordinate behavior (Harrington et al. 1983; Peterson and Page 1988).

We estimated annual survival rates using a Kaplan–Meier (K–M) procedure modified for staggered entry of radio-collared individuals (Pollock et al. 1989*a*, 1989*b*). We assumed that newly collared wolves of a given age-class had the same probability of survival as previously collared animals in that age-class. We calculated bounds on survival estimates by censoring wolves with which we lost radio contact because of either dispersal or transmitter failure. We compared survival-rate differences using log-rank $\chi^2$ tests (Pollock et al. 1989*a*).

We separated mortalities of radio-collared wolves into those that were human-caused and those that probably occurred from natural causes. We assumed that a wolf died from natural causes if it was found a long distance from town or roads. A wolf was regarded as dispersed if it permanently left its original pack and either formed a new pack or joined an existing one (Messier 1985*b*).

### Estimating prey biomass : wolf indices

We estimated annual moose and caribou densities by interpolating from population estimates conducted before (Farnell and McDonald 1988; Jingfors 1988) and during our study (Larsen and Ward 1995; R. Ward, unpublished data; R Farnell, unpublished data). Annual estimates of moose and caribou population sizes are presented in a companion paper (see Appendix, Table A1 in Hayes et al. 2000).

We calculated the expected wolf density in 1996 using the ungulate biomass equation of Fuller (1989). We estimated the relative biomass contribution of each prey species using values of 6 for moose, 2 for caribou, and 1 for sheep, mountain goats, and mule deer from other studies (Keith 1983; Ballard et al. 1987; Fuller 1989). To calculate annual biomass indices, we estimated ungulate population sizes, multiplied by each biomass value, then divided the product by wolf density (Fuller 1989).

## Results

### Radiotelemetry

We radio-collared 78 wolves (40 females, 38 males) including 3 lone wolves. Of the 75 pack wolves, 57 were collared once, 16 were collared twice, and two were collared 3 times in order to maintain radio contact. We radio-collared 45 adults (59%), 24 yearlings (32%), and 9 pups (9%). No wolves suffered serious injury from being captured. We collared wolves in 26 of the 39 (66%) packs that established during our study (Table 1, Fig. 2). We established radio contact with 71% of packs each winter (range 46–88%). We collared 21 small packs in the first year they established territories in the FSA, 4 packs in their second year, and 1 pack in its third year. We monitored the activities of 22 collared wolves in 11 packs in 1990, 38 in 18 packs in 1991, 39 in 22 packs in 1992, 44 in 18 packs in 1993, and 24 in 12 packs in 1994 (Table 1). By 1994, we had lost radio contact with 14 of the 26 packs because of wolf deaths, dispersals, or transmitter failures. By 1996, no radio collars were transmitting.

We located radio-collared wolves from fixed-wing aircraft 2017 times between 8 February 1990 and 31 March 1994: 85% of locations were made in winter, 8% in summer, and 6% in autumn. We monitored collared wolves for a total of 1374 wolf-months and individuals for 18.6 ± 1.7 (mean ± SE) months (range 1–49 months). We followed packs for 73 ± 7.4 (mean ± SE) months and located pack members on 13 ± 1.1 (mean ± SE) days each year (range 4–19 days).

### Annual changes in wolf abundance

Table 2 summarizes annual rates of increase and changes in wolf and pack numbers, and mean pack size. Wolf numbers rapidly increased from 29 known survivors at the end of the wolf reduction (15 March 1989) to a maximum of 245 wolves in March 1996. The finite rate of increase ($\lambda$) was greatest during the first year of recolonization, then declined as the population apparently approached stability by 1994. The annual rate of increase was negatively correlated with the number of wolf packs ($r^2 = 0.82$, df = 6, $P = 0.01$) and mean pack size ($r^2 = 0.84$, df = 6, $P = 0.01$), but wolf density (the product of both) was the best fitting slope ($r^2 = 0.97$, df = 6, $P < 0.001$). However, the rate of increase was also strongly related to the dispersal rate ($r^2 = 0.99$, df = 6, $P = 0.006$). Thus, we have evidence for accepting both $H_0 1$ and $H_a 1$.

The number of packs increased from 14 in 1990 to between 23 and 28 after 1991 (Table 2). Mean pack size increased from 4.4 wolves in 1990 to 7.8 in 1994 ($t = -2.3$, df = 36, $P = 0.025$) and to 9 by 1996 (Table 2).

Figure 3 shows the general distribution of wolf packs from 1990 through 1996. Home ranges of radio-collared packs were exclusive in the first 2 years of recovery, but overlaps developed after 1991, when territorial space became limited. Perimeters of some pack territories were unstable from year to year, although activity centers remained stable except for those of 6 packs that all shifted their home ranges substantially in some years. We determined the 95% convex polygon areas for 17 wolf packs that we located on more than 30 days (range 38–86 days) (Fig. 4). The multiyear home-range area was 1478 ± 203 (mean ± SE) km$^2$, ranging from 722 to 3800 km$^2$.

### Reproduction and survival rates

We estimated that the wolf litter size at birth was 5.7 ± 0.4 (mean ± SE) pups. We found no packs with more than one female producing litters each year. The percentage of packs that contained pups increased each year from 35% in 1990 to 93% by 1994 (Table 2). Ten colonizing pairs (53%) raised pups through their first breeding period, 5 (26%) failed to reproduce because a mate died, and 4 (21%) failed for unknown reasons. One pair remained in the same territory for 4 years but was never seen with pups. Another pair remained together for 3 years before successfully raising pups. Three females died before giving birth and two died shortly afterwards (all their pups died before autumn). Mate mortality caused reproductive failure of pairs at least 9 times during our study.

Annual survival rates of all radio-collared wolves did not vary (Pearson's correlation coefficient, $\chi^2 = 0.4$, df = 3, $P = 0.94$) and remained high at 0.84 ± 0.02 (mean ± SE). There was no difference in survival rates ($\chi^2 = 0.08$, df = 1, $P > 0.75$) between early-recovery years (March 1990 through February 1992) and later years (March 1992 through April 1994). Seasonal survival rates also did not differ between periods ($\chi^2 = 0.16$, df = 2, $P > 0.90$). Therefore, we have evidence for rejecting $H_0 2$: the survival rate is density-dependent and inversely related to wolf density.

The mean number of pups alive in March was 4.3, significantly smaller ($t = -2.2$, df = 39, $P = 0.04$) than the mean litter size at birth (5.7 pups). From this difference, we estimated that the pup survival rate was 0.75. Age-specific survival rates did not vary among subadults (pups and yearlings), young adults (2 and 3 years old), and older adults (Pearson's correlation coefficient, $\chi^2 = 1.5$, df = 2, $P = 0.47$). Wolves less than 3 years old had significantly lower survival rates (Table 3) than older wolves ($\chi^2 = 4.7$, $P < 0.05$). Mean annual survival rates were 0.81 for yearlings and 0.89 for adults. Therefore, we had evidence supporting $H_a 2$: the survival rate is age-dependent.

Fifteen radio-collared females and 10 males died during our study. Most wolves were between 1 and 5 years old (Fig. 5). Twenty-one deaths occurred from unknown natural

**Table 1.** Annual sizes of wolf packs in the study area, February 1990 to March 1996.

| Pack | Origin | 1990 | 1991 | 1992 | 1993 | 1994 | 1996 |
|---|---|---|---|---|---|---|---|
| Seven Wolf L. | C | 2 (1) | 7 (2) | 10 (1) | 11 (3) | 5 (1) | 16 |
| Yusezyu R. | C | 2 (2) | 8 (2) | 11 (2) | 11 (2) | 13 (1) | 1 |
| Jackfish L. | C | 2 (2) | 7 (3) | 11 (4) | 13 (6) | 10 (2) | 14 |
| Tyers R. | C | 2 (2) | 2 (2) | 2 (2) | 2 (2) | Dead$^a$ | |
| Ketza R. | C | 2 (1) | 2 (2) | 2 (1) | 3 (2) | 4 (3) | |
| Wolverine L. | C | 2 (1) | 2 (2) | 2 (1) | 2 (2) | 4 (1) | 4 |
| Finlayson L. | C | $2^b$ | 2 (2) | 2 (2) | Dispersed | | |
| Mink L. | C | $2^b$ | 4 (2) | 8 (2) | 7 (2) | 9(3) | |
| Woodside R. | R | 4 (3) | 7 (2) | 11 (3) | 7 (3) | $10^c$ | 12 |
| Prevost R. | R | 6 (2) | 11 (3) | 10 (2) | 11 (1) | 6 (1) | 9 |
| Tuchitua R. | IS | 11 (3) | 6 (2) | 10 (2) | 9 (3) | SO | |
| Frances L. | IS | 17 (2) | 9 (2) | 15 (3) | $13^b$ | $15^b$ | 15 |
| Otter Creek | C | $2^b$ | $2^b$ | 2 (2) | 2 (2) | 6 (2) | 13 |
| Weasel L. | R | 6 (2) | 13 (3) | 4 (2) | 12t | ? | |
| Upper Pelly R. | C | Lone (1) | 2 (2) | 2 (1) | 2 (2) | 5 (2) | 16 |
| Big Campbell East | C | NP | $3^b$ | 14 (1) | 7 (3) | $20^d$ (4) | |
| Tuchitua R.East | SP | NP | $14^b$ | SO | | | |
| Light Creek | C | NP | 2 (2) | 6 (2) | 8 (4) | 11 (1) | 11 |
| McEvoy L. | C | NP | 2 (2) | BU | | | |
| Ketza R. II | IS | NP | $4^b$ | $6^b$ | $5^b$ | ? | |
| Gonzo L. | C | NP | $3^b$ | BU | | | |
| One Island L. | C | NP | 2 (1) | 4 (1) | $4^b$ | SO | |
| East Arm | C | NP | 2 (2) | Dispersed | | | |
| Dragon L. | C | NP | NP | 2 (1) | $10^b$ | $8^c$ | |
| Lobster L. | SP | NP | NP | 7 (1) | 6 (2) | ?$^e$ | |
| Fire Creek | C | NP | NP | 3 (2) | 4 (2) | 11 (2) | 11 |
| Needle L. | C | NP | NP | $2^b$ | $2^b$ | $9^c$ | 10 |
| Nipple Mt. | SP | NP | NP | $6^b$ | 4 (2) | 2 (1) | 12 |
| Weasel L. II | SP | NP | NP | 6 (1) | 11 | ?$^e$ | |
| Hoole R. | C | NP | NP | 3 (1) | $6^c$ | $5^c$ | 10 |
| Big Campell West | SP | NP | NP | NP | $10^c$ | $0^d$ | 6 |
| McEvoy L. II | SP | NP | NP | NP | $6^b$ | $7^c$ | 11 |
| Furniss L. | UNK | NP | NP | NP | NP | $6^c$ | 12 |
| Hegsted | SP | NP | NP | NP | NP | $6^c$ | 10 |
| Whitefish L. | C | NP | NP | NP | NP | $2^b$ | 4 |
| Hyland–Tyers R. | IS | NP | NP | NP | NP | $8^f$ | |
| Pike Mt. | NP | NP | NP | NP | NP | NP | 2 |
| Donk L. | NP | NP | NP | NP | NP | NP | 8 |
| Total | | 62 (22) | 116 (38) | 168 (39) | 188 (43) | 218 (24) | 207 (0) |

**Note:** Numbers in parentheses are numbers of radio-collared wolves. Packs designated with II indicate a second pack formed in or near another pack's home range of the same name. BU, pack broke up; C, colonizing pack; R, resident pack; IS, in-shifter; NP, pack not present; SO, pack shifted out; SP, pack formed by splitting; UNK, unknown origin.

[a] Both wolves died.

[b] Pack size was estimated from track counts only.

[c] Pack was seen during the census.

[d] The Big Campbell East and West packs joined again in 1994 after splitting in 1993.

[e] Pack was not observed in 1994. It was assumed to be present and its size was estimated to be 7.8 wolves, based on the average size of 19 other packs seen in 1994.

[f] Pack was tracked in the former range of the Tyers River pack but was seen outside the Finlayson Study Area boundary.

cause, 1 death was of a breeding female killed by a bear, and 3 were human-caused. Age at death was 3.4 ± 0.4 (mean ± SE) years and there was no difference between the sexes ($t$ = –0.13, df = 23, $P$ = 0.90).

## Dispersal

Twenty-five (33%) radio-collared wolves dispersed permanently during our study, including 7 that remained in the FSA, and 18 censored wolves probably emigrated. Of the seven wolves that dispersed inside the FSA, four formed new packs, one dispersed into a neighboring pack, and two older males remained within their former pack territories. Censored wolves apparently emigrated outside the FSA, based on their ages, behavior, and censorship schedules. Five censored wolves were alone the last time they were seen in their territories. Predispersing wolves temporarily separated

**Fig. 2.** Radiotelemetry history of 26 wolf packs in the FSA from February 1990 to March 1994. Solid lines indicate periods and broken lines indicate that radio contact was lost but the pack was seen or wolf trails indicated that the pack was present.

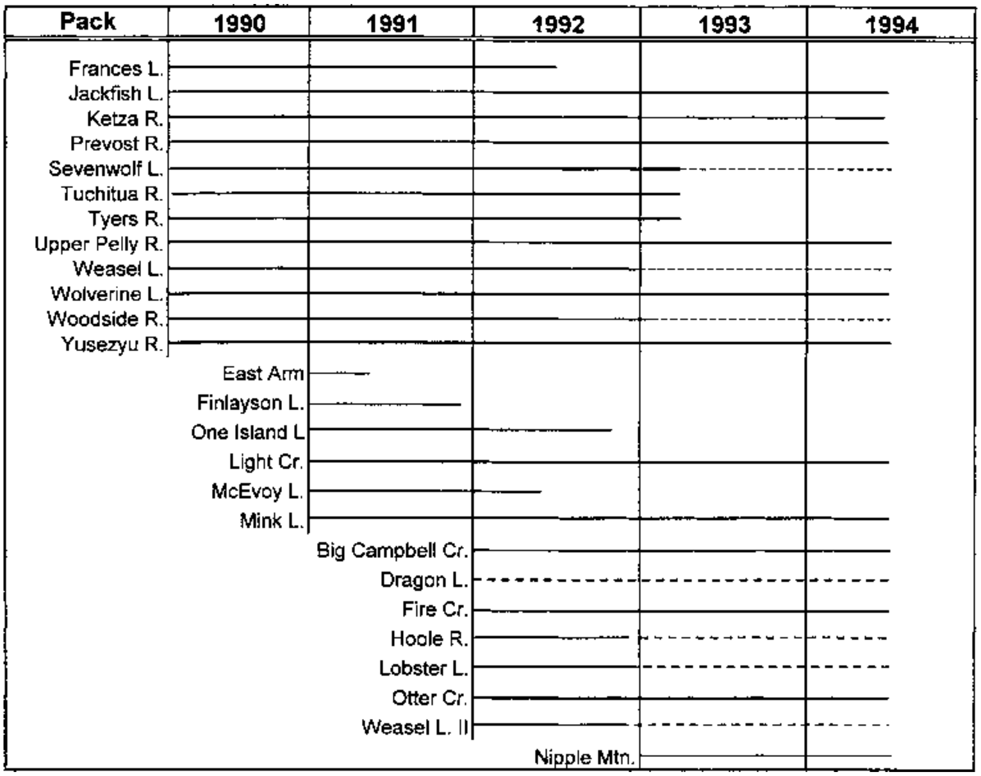


**Table 2.** Annual changes in wolf population sizes, March 1989 through March 1996.

| Year | No. of wolves alive in late winter | Percentage of pre-reduction no.[a] | No. of packs | Percentage of packs reproducing | Pack size (mean ± SE) | Wolf density (no./1000 km$^2$) | $\lambda$ |
|---|---|---|---|---|---|---|---|
| 1989[b] | 29 | 0.12 | 7 | | | 1.4 | |
| 1990 | 69 | 0.28 | 14 | 35 | 4.4±1.2 | 3.0 | 2.38 |
| 1991 | 128 | 0.52 | 23 | 52 | 5.0±0.8 | 5.6 | 1.85 |
| 1992 | 185 | 0.76 | 26 | 71 | 6.0±0.8 | 8.0 | 1.44 |
| 1993 | 207 | 0.84 | 27 | 81 | 7.1±0.7 | 9.0 | 1.12 |
| 1994 | 240 | 0.98 | 28 | 93 | 7.8±0.8 | 10.4 | 1.16 |
| 1996 | 245 | 1.00 | 25 | Unknown | 9.0±1.0 | 10.6 | 1.01 |

[a] The pre-reduction population size was 245 wolves in March 1983 (A. Baer, unpublished data).

[b] Data from 1989 were obtained after the last year of wolf reduction was completed (R. Farnell, unpublished data).

from their packs before permanently leaving their natal territories (Messier 1985*b*). Two pairs established temporary territories and then disappeared.

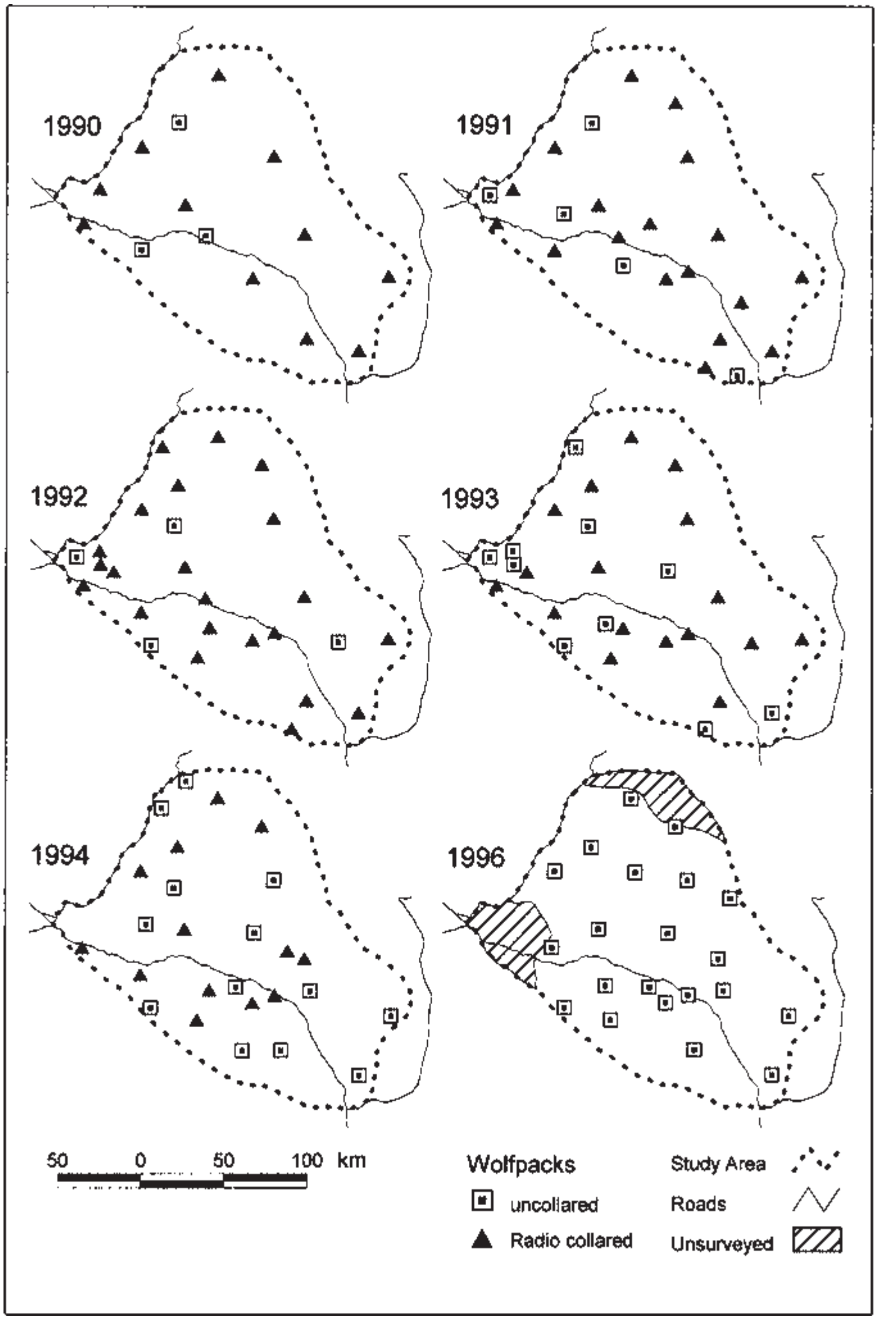
Wolves between 2 and 4 years old accounted for 77% of the wolves that dispersed or were censored (Fig. 6). Seven of 10 radio-collared wolves were censored between April and June, when natal dispersal from wolf packs is highest (Zimen 1976, 1982; Fuller 1989; Gese and Mech 1991).

Age of dispersers was 2.9 ± 0.3 (mean ± SE) years; there was no difference between the sexes (Mann–Whitney *U* test, $U = 51$, df = 1, $P = 0.13$). Dispersal rates were 0% in 1991, 17% in 1992, 33% in 1993, and 50% in 1994. Dispersal rates increased (Pearson's correlation coefficient, $\chi^2 = 6.9$, df = 1, $P < 0.01$) in late-recovery years (0.45, 1992–1994) compared with earlier years (0.09, 1990–1992). Dispersal rate was strongly related to annual mean pack size ($r^2 = 0.95$, df = 3, $P < 0.03$). Dispersal was not related to number of packs ($r^2 = 0.59$, df = 3, $P < 0.23$) or wolf density ($r^2 = 0.83$, df = 3, $P = 0.860$). Therefore, we have evidence for rejecting $H_03$ and accepting $H_a3$: the dispersal rate is density-independent and negatively related to mean pack size. The dispersal rate was also related to the ungulate biomass : wolf biomass index each year ($r^2 = 0.95$, df = 3, $P < 0.03$).

### Ungulate biomass : wolf index

We estimated the theoretical wolf density in the FSA in 1996 on the basis of ungulate biomass, following Fuller (1989). Based on total available prey biomass ($x = 1.95$), the expected density was 10.6 wolves/1000 km$^2$. Our estimates showed densities of 10.4 and 10.6/1000 km$^2$ in 1994 and

**Fig. 3.** General locations of wolf packs each winter from 1990 to 1994 and in 1996.



1996, respectively, indicating that wolf numbers continued to increase slightly, and were stabilizing at the density predicted from the ungulate biomass : wolf index.

### Dynamics of wolf-pack formations

We classified wolf packs as residents, colonizing pairs, inshifters, or splitters, based on their probable origins in the FSA. We did not radio-collar resident survivors in 1989, therefore we could not assess their contributions to the formation of colonizing packs. In 1990, we believed that 3 resident packs of 4 to 6 wolves (Table 1) accounted, in total, for 16 of the 62 wolves (26%) which were present that winter. The 3 resident packs were well inside the study-area boundary. Each pack contained pups, indicating that breeding was not disturbed by the last year of reduction. Two resident packs remained in their original territories until 1996 (Table 1). The Weasel Lake pack remained until 1993, but we could not locate it thereafter.

During our study, a total of 22 colonizing pairs (or trios) established territories in vacant areas, accounting for 57% of all territory formations. Early colonizing pairs either came from resident packs that survived the 1989 reduction or were wolves that immigrated from outside the FSA. Colonizers were the foundation of the reestablished wolf population during early recovery (Fig. 7). Nine pairs colonized the area in 1990, seven in 1991, four in 1992, none in 1993, and one each in 1994 and 1996. Eighteen pairs were radio-collared and their pack histories were documented for up to 49 con-

**Fig. 4.** Total 95% minimum convex area polygons for 17 wolf packs in the FSA.

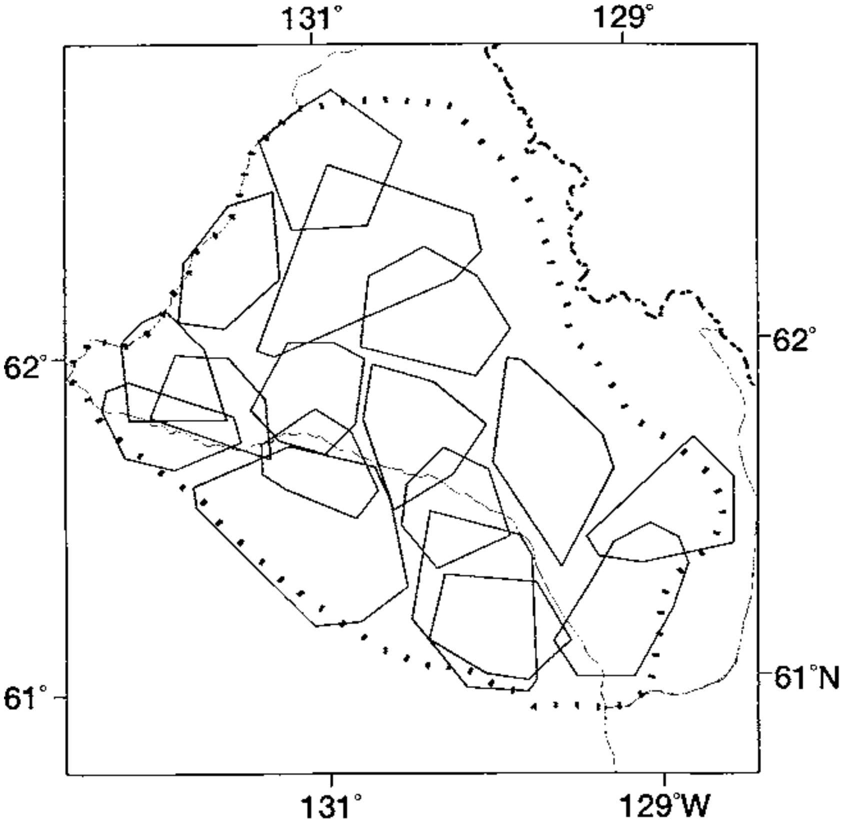


**Table 3.** Kaplan–Meier survival probabilities by wolf age-class, 1990–1994.

| Age-class | No. of wolves at risk | No. of deaths | Survival | Variance | 95% confidence interval Lower | 95% confidence interval Upper |
|---|---|---|---|---|---|---|
| Pup | 8 | 3 | 0.63 | 0.0183 | 0.34 | 0.89 |
| Yearling | 36 | 7 | 0.81 | 0.0035 | 0.69 | 0.92 |
| Two-year-olds | 45 | 8 | 0.82 | 0.0027 | 0.72 | 0.92 |
| Three-year-olds | 32 | 1 | 0.97 | 0.0009 | 0.91 | 1.00 |
| Four-year-olds | 33 | 4 | 0.88 | 0.0028 | 0.77 | 0.98 |
| Five-year-olds and older | 51 | 6 | 0.88 | 0.0018 | 0.80 | 0.97 |

secutive months (Figs. 2 and 7). By 1994, 13 colonizing pairs (72%) had successfully bred and remained in the FSA, 1 pair reproduced but shifted outside the FSA, 2 pairs dispersed before breeding, and 2 pairs separated for unknown reasons before reproducing (Table 1). By 1994, packs originally formed by colonizing pairs represented 46% of all packs and 51% of the 218 pack wolves. After 1994 we lost radio contact with all wolves so we could not follow their specific pack histories. Colonizing pairs all contained a male and female wolf. The age of 28 wolves captured in pairs was 3.20 ± 0.38 (mean ± SE) years.

Four packs shifted home ranges into the FSA from boundary areas. In 1990, 2 large packs shifted in (Table 1), accounting for 45% (28 wolves) of all wolves during that winter. The Tuchitua River pack remained in the study area until 1994, then for unknown reasons shifted its range outside. The Frances Lake pack remained in its territory during all winters. Three wolf packs shifted out of the FSA during our study (Table 1).

Four radio-collared packs increased to a large size, then split into a total of 9 smaller groups by 1994 (Table 4). At the time of splitting, packs consisted of 14 ± 1.5 (mean ± SE) wolves. The Frances pack split 3 times. By 1994, 39% of pack wolves originated from pack splits. All groups that split from their original pack apparently established territories in nearby areas.

## Discussion

We found evidence that the rate of increase in wolf population size was density-dependent and negatively related to the number of wolves, indicating that competition ultimately limited population size. Dispersal was a key factor limiting population growth. Dispersal was density-independent, but

**Table 4.** Chronology of large wolf packs that split between 1990 and 1994.

| Original pack | 1990 | 1991 | 1992 | 1993 | 1994 | Split packs |
|---|---|---|---|---|---|---|
| Frances R. | 17[a] | 9 | 15[a] | 13[a] | 15 | Frances R. |
| | | 12[b] | | | 6 | Hegsted |
| | | | | 4 | 2 | Nipple Mt. |
| Big Campbell | | 3 | 14[a] | 7 | 20[c] | Big Campbell |
| | | | | 10 | | |
| Weasel L. | 6 | 13[a] | 4 | 12 | —[d] | Weasel L. |
| | | | 7 | 6 | —[d] | Lobster L. |
| | | | 6 | 11 | —[d] | Weasel L. II |
| Woodside R. | 4 | 7 | 11[a] | 7 | 10 | Woodside R. |
| | | | | 6 | 7 | MacPherson L. |
| Total | 27 | 44 | 57 | 76 | 84 | |

[a] Size of pack that split.

[b] Tuchitua R. East pack. Pack shifted out of the study area in 1992.

[c] The Big Campbell packs rejoined in 1994.

[d] No survey of pack. Each pack was estimated at eight wolves, based on the average size of other packs in 1994.

**Fig. 5.** Numbers of radio-collared wolves that died as yearlings or older during the study.

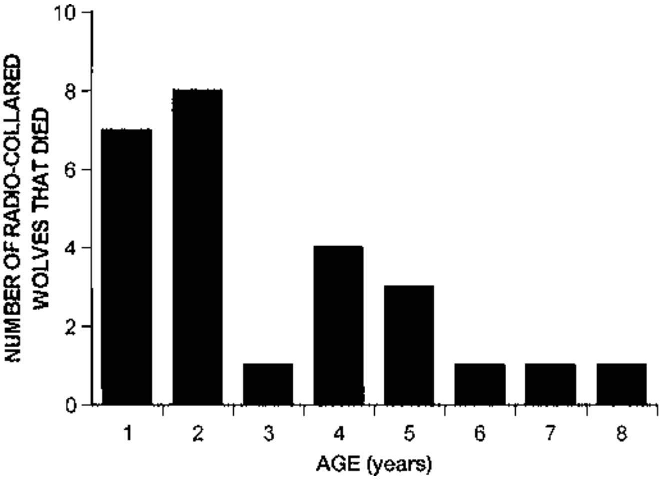
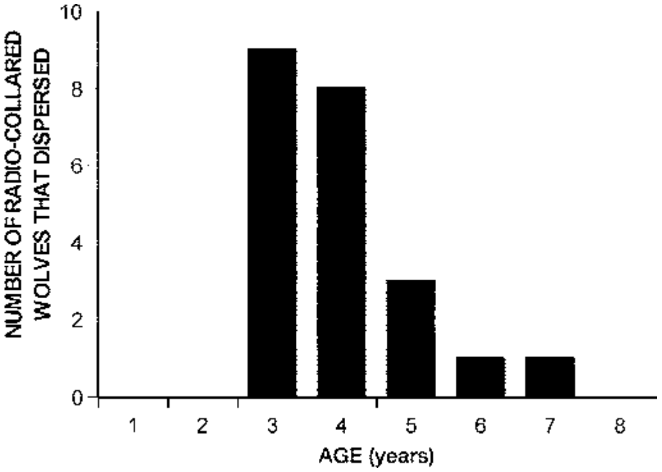


**Fig. 6.** Ages of radio-collared wolves that dispersed or were censored from packs during the study.



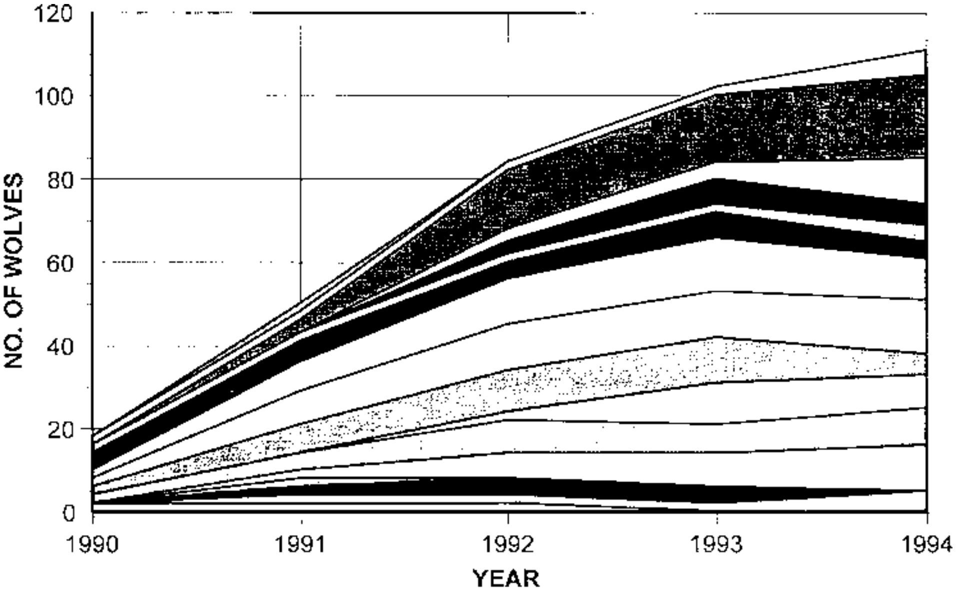
was strongly related to both pack size and the ungulate biomass available to wolves each year (Fuller 1989). Rate of increase was also negatively related to dispersal rate, indicating that the limiting effect of dispersal on the size of wolf packs had a strong influence on population growth. The survival rate of wolves was density-independent, but it was related to their age-class.

Wolves reached pre-reduction density within 5 years after reduction ended. The number of packs in the area recovered to the 1983 pre-reduction level of 25 (R. Farnell, unpublished data) within 3 years. Hayes et al. (1991) observed a similar high rate of increase in wolves in the southern Yukon after reduction; the population reached 88% of its pre-reduction size in 4 years.

Colonization by pairs was the factor that most affected wolves' rate of increase in the early years of recovery in Minnesota (Fritts and Mech 1981), Alaska (Peterson et al. 1984), southern Yukon (Hayes et al. 1991), and British Columbia (Bergerud and Elliot 1998). We observed the same general pattern of population increase in the FSA. The highest rate of increase occurred between 1990 and 1991 ($\lambda$ = 2.38), when most packs consisted of pairs (64%) breeding for the first time. R. Farnell (unpublished data) also found high annual rates of increase in our study area during the reduction period ($\lambda$ = 2.06–2.53), when pairs composed 30% of the packs, which is similar to findings by Bergerud and Elliot (1998). In our study, high rates of increase in early years were supported by high wolf survival rates and low dispersal rates from small packs.

After wolf pairs established territories in the FSA, reproduction rapidly caused pack sizes to increase. The percentage of packs that contained pups increased each year until 1994, when more than 90% of packs were reproducing. Wolf productivity and pup survival rates ultimately depend upon the availability of ungulates (Zimen 1976; Keith 1983; Messier 1985*a*; Boertje and Stephenson 1992). Both moose and caribou numbers increased during our study and wolf litter size was similar to that in other studies where food availability was high (Harrington et al. 1983; Fuller 1989; Boertje and Stephenson 1992). The juvenile wolf survival rate was among the highest reported in the literature. Harrington et al. (1983) believed that the presence of more "helpers" in a pack

**Fig. 7.** Annual changes in sizes of wolf packs that were first radio-tagged as pairs or trios between March 1990 and March 1994.



increased pup survival rates. We found no evidence that pup survival rates increased with the size of packs. Similar to observations by Peterson et al. (1984), first-time breeding pairs were apparently as capable of raising pups as those that had bred before.

All radio-collared packs produced single litters, similar to observations by Peterson et al. (1984). In a heavily exploited population in Alaska, Ballard et al. (1987) found that 7–10% of wolf packs produced more than one litter. Social constraints usually limit breeding to a single dominant female (Medjo and Mech 1976; Zimen 1976). The killing of dominant pack members can lead to breeding instability by allowing subordinate females to be bred (Woolpy 1968). During reduction, most packs were completely removed, leaving few fragmented groups (R. Farnell, unpublished data). Exploitation was very low during recovery. This allowed for high breeding stability and, hence, the production of single litters.

The survival rates that we observed could represent the maximum possible for wild wolves. Survival rates were lower in six other studies where harvest rates were considerably higher than in the FSA (Fritts and Mech 1981; Peterson et al. 1984; Messier 1985*a*; Ballard et al. 1987; Fuller 1989; Hayes et al. 1991). The mean pup survival rate in those studies was 27% lower (0.48) than the rate we observed, yearling survival was 20% lower (0.61), and adult survival was 30% lower (0.59). We had little information on the cause of death of most wolves. All deaths of young wolves occurred in summer and fall, when wolves are most likely to disperse from natal packs in response to intrapack aggression (Fuller 1989; Gese and Mech 1991). Most deaths of adult wolves occurred during winter, when pack territories are most vigorously defended by adults (Mech 1970).

By 1994, dispersal increased to the level found in a stable wolf population in Minnesota (49%; Fuller 1989). Messier (1985*a*) and Peterson and Page (1988) showed that intrapack competition for food determined whether young wolves stayed or were ejected in favor of new pups (Zimen 1976; Harrington et al. 1983). The age and social position of wolves influence dispersal rates, which increase rapidly with the onset of sexual maturity (Packard and Mech 1980; Messier 1985*b*; Gese and Mech 1991). As our study packs increased to a more normal size, dispersal rates of young wolves increased, which tended to stabilize pack size.

Wolf packs have a social-capacity limit of about 13 wolves, which is independent of food supply (Mech 1970; Zimen 1976). When packs reached this size in the FSA, they tended to split. Packs split when subordinate wolves disperse as a group in response to social stimuli from dominant members (Zimen 1976). Wolves are strongly philopatric, as is shown by recent mitochondrial DNA studies (Lehman et al. 1992). Colonizing near the edge of parental territory allows dispersers long-term use of familiar areas, and minimizes the survival cost of dispersing to a new location where food resources are unknown and the chance of being killed by conspecifics is higher (Cooch et al. 1993). Pack splitting was particularly advantageous in the FSA because space and ungulate resources in adjacent areas were sufficient to allow related wolves to establish new territories.

### Regulation of wolves by prey supply

Mech (1986), Gasaway et al. (1983), and Peterson and Page (1983) showed that the numerical response of wolves was loosely regulated by diminishing food resources through a weak negative feedback that enabled wolf numbers to lag behind prey declines for long periods. If the numerical response is equally loose when prey availability increases, then wolves could exceed the densities at which prey:wolf ratios should stabilize, causing prey numbers to decline. If wolves' numerical response is sensitive to prey abundance, then wolf populations should stabilize at a density that does not exceed the prey-biomass supply (Pimlott 1967; Keith 1983; Fuller 1989; Messier 1994).

We found supporting evidence for $H_0 4$: wolves' numerical response is tightly regulated by prey resources; however, we also found evidence that wolves' social behavior could have been as important in limiting population size. The wolf density observed in 1996 (10.4–10.6) closely matched the expected stable density (10.6), based on the ungulate biomass :

wolf index (Fuller 1989). There is evidence that wolves' numerical response is closely regulated by the availability of food resources (Keith 1983; Messier and Crete 1985; Fuller 1989; Messier 1994; Bergerud and Elliot 1998), but it is not clear whether prey abundance or wolf sociobiology is the cause/effect. Packard and Mech (1980) proposed that wolf numbers are regulated by a synergistic, two-way feedback with their prey. They argued that changes in food resources ultimately cause changes in wolves' social behavior that adjust wolf reproduction, dispersal, and survival rates in order to balance wolf numbers and food supply. Social behavior is also thought to influence the lag time, i.e, how rapidly wolf numbers adjust to changing food resources (Packard and Mech 1980).

Our data, and those of Bergerud and Elliot (1998), support Fuller's (1989) view that dispersal is the primary mechanism determining how wolves adjust their numbers to the prey supply. We found other evidence that dispersal rates were density-independent and strongly related to wolf-pack size. Therefore, dispersal was apparently linked to both intrapack wolf sociobiology, which regulated the maximum pack size, and per capita prey availability.

We compared long-term wolf densities in the study area and found that wolf abundance was not sensitively regulated by food resources. Despite a 2- to 3-fold increase in ungulate biomass, there was no difference in mean pack size before wolf reduction began in 1983 (9.0 ± 1.0 (mean ± SE) wolves; A. Baer, Yukon Fish and Wildlife Branch, Box 2703, Whitehorse, YT Y1A 3C6, unpublished data) and after wolf numbers stabilized in 1996 (9.2 ± 1.0 (mean ± SE)). Similarly, pack density did not differ in 1983 and 1996 (1.04 packs/1000 km$^2$), nor did overall wolf density in the study area (10.3–10.6 wolves/1000 km$^2$). It is possible that the pre-reduction data reflect the tendency of wolves to lag behind prey declines, but we could not test this. However, the similarities in pack size are evidence that dispersal was not tightly linked to per capita prey abundance.

Average pack size for moose-hunting wolves is about 10 wolves in North America (Mech 1970; Zimen 1976). In most Yukon areas, moose-hunting packs range in size between 6 and 10 wolves (Hayes and Baer 1987; Hayes and Bowers 1987; Hayes et al. 1991). The stabilization of the FSA wolf population could be explained by a tight functional response to prey availability (i.e., the individual or group kill rate determining the physical condition and productivity of breeders), or by social interactions that limit packs in an area to some predetermined maximum number and size, that are only loosely related to food supply. Food supply per wolf was inversely related to pack size in our study area and elsewhere (Thurber and Peterson 1993), with pairs showing much higher kill rates. Schmidt and Mech (1997) proposed that wolves live in larger packs not because food acquisition increases as wolf numbers increase, but because adult pairs can share surplus food with their offspring for kin-selection reasons. Therefore, we should not expect pack size to be sensitively linked to prey availability.

That the mean size of wolf packs in the FSA is consistent with that of other Yukon moose-hunting packs does not tell us whether social interactions or prey availability is more important. To fully test $H_0 4$ and determine the nature of wolves' numerical response, pack densities and mean pack size will have to be measured over a range of steady-state prey populations to assess which variables are controlled by social interactions or prey resources. The best evidence for regulation by prey availability would be a strong relation between both wolf-pack size and pack density and a range of stable moose densities. Our evidence suggests that food supply and social behavior interact, wolves' numerical response being a relatively loose correlation of function of prey availability with some socially limited maximum pack size, which is an assumption of current "predator pit" models (Messier 1994).

### Data quality

Our study of wolf-recovery dynamics was limited by certain methods that we used. Because of infrequent year-round monitoring of wolves, we could not accurately measure mortality causes or true dispersal rates. We inferred most pack splits from coincidental sharp declines in the size of a large pack of radio-collared wolves and the presence of newly formed packs nearby. Wolf survival rates were probably biased during the early years of our study. The K–M procedure assumes that animals are sampled randomly, which did not happen in the early years of our study, when most (60%) radio-collared wolves were young adults in pairs. Early-colonizing wolves had a clear survival advantage over later colonizing wolves that entered the population, because they established territories and reproduced without competition.

Nevertheless, 6 years of study appeared to be sufficient for observing a wolf population increase from very low abundance and reach a state of equilibrium.

## Acknowledgements

The Yukon Fish and Wildlife Branch provided generous financial and logistic support for this study. We sincerely thank A. Baer for conducting a major part of the field studies, including capture, snow-tracking, and radiotelemetry of wolves. Other field staff who assisting in the study include P. Maltais, P. Koser, C. Promberger, U. Wotschikowsky, P. Kaczensky, R. Florkiewicz, D. Bakica, D. Anderson, and D. Thiel. D. Denison, T. Hudgin, and D. Drinnan flew fixed-wing aircraft with great skill and interest. Helicopter pilots J. Witham and J. Henderson made wolf-capture flights seem easy. All pilots were invaluable members of the study team. G. Sharam, M. Oakley, F.L. Bunnell, E. Cooch, and R. Ydenberg made positive criticisms of the manuscript. We also thank Dr. A. Angerbjorn and an anonymous reviewer for improving the paper. We especially thank B. Gasaway for his friendship and valuable insights into wolf–prey systems, and for his constructive advice on the manuscript before his untimely death.

## References

Ackerman, B.B., Leban, F.A., Garton, E.O., and Samuel, M.D. 1990. User's manual for program HOME RANGE. 2nd ed. Forest, Wildlife, and Range Experiment Station Tech. Rep. No. 15, University of Idaho, Moscow.

Ballard, W.B., Whitman, J.S., and Gardiner, C.L. 1987. Ecology of an exploited wolf population in south-central Alaska. Wildl. Monogr. No. 98.

Bergerud, A.T., and Elliot, J.P. 1998. Wolf predation in a multiple-ungulate system in northern British Columbia. Can. J. Zool. **76**: 1551–1569.

Boertje, R.D., and Stephenson, R.O. 1992. Effects of ungulate availability on wolf reproductive potential in Alaska. Can. J. Zool. **70**: 2441–2443.

Boertje, R.D., Valkenburg, P., and McNay, M. 1996. Increase in moose, caribou, and wolves following wolf control in Alaska. J. Wildl. Manage. **60**: 474–489.

Cooch, E.G., Jefferies, R.L., Rockwell, R.F., and Cooke, F. 1993. Environmental change and the cost of philopatry: an example in the lesser snow goose. Oecologia, **93**: 128–138.

Cowan, I.M. 1947. The timber wolf in the Rocky Mountain National Parks of Canada. Can. J. Res. **25**: 139–174.

Farnell, R., and McDonald, J. 1988. The demography of Yukon's Finlayson caribou herd, 1982–87. Yukon Fish and Wildlife Branch Rep., Whitehorse.

Fritts, S.H., and Mech, L.D. 1981. Dynamics, movements, and feeding ecology of a newly protected wolf population in northwestern Minnesota. Wildl. Monogr. No. 80.

Fuller, T.K. 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. No. 105.

Gasaway, W.C., Stephenson, R.O., Davis, J.L., Shepherd, P.E.K., and Burris, O.E. 1983. Interrelationships of wolves, prey, and man in interior Alaska. Wildl. Monogr. No. 84.

Gasaway, W.C., Boertje, R.D., Grangaard, D.V., Kelleyhouse, D.G., Stephenson, R.O., and Larsen, D.G. 1992. The role of predation in limiting moose at low densities in Alaska and Yukon and implications for conservation. Wildl. Monogr. No. 120.

Gese, E.M., and Mech, L.D. 1991. Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969–1989. Can. J. Zool. **69**: 2946–2955.

Harrington, F.H., Mech, L.D., and Fritts, S.H. 1983. Pack size and wolf pup survival: their relationship under varying ecological conditions. Behav. Ecol. Sociobiol. **13**: 19–26.

Hayes, R.D., and Baer, A.M. 1987. Wolf inventory, Nisutlin River area. *In* Wolf population research and management studies in the Yukon: population inventories 1985–1987. *Edited by* R. Hayes. Yukon Fish and Wildlfe Branch Rep., Whitehorse.

Hayes, R.D., and Bowers, K. 1987. Wolf inventory, Wolf Lake area. *In* Wolf population research and management studies in the Yukon: population inventories 1985–1987. *Edited by* R. Hayes. Yukon Fish and Wildlfe Branch Rep., Whitehorse.

Hayes, R.D., Baer, A.M., and Larsen, D.G. 1991. Population dynamics and prey relationships of an exploited and recovering wolf population in the southern Yukon. Yukon Fish and Wildlife Branch Rep. TR 91-1, Whitehorse.

Hayes, R.D., Baer, A.M., Wotschikowsky, U., and Harestad, A.S. 2000. Kill rate by wolves on moose in the Yukon. Can. J. Zool. **78**: 49–59.

Jingfors, K. 1988. Moose population characteristics in the North Canol and Frances Lake areas, November 1987. Yukon Fish and Wildlfe Branch Rep., Whitehorse.

Keith, L.B. 1983. Population dynamics of wolves. Can. Wildl. Serv. Rep. Ser. 45. pp. 66–77.

Krebs, C.J., Boutin, S., Boonstra, R., Sinclair, A.R.E., Smith, J.N.M., Dale, M.R.T., Martin, K., and Turkington, R. 1995. Impact of food and predation on the snowshoe hare cycle. Science (Washington, D.C.), **269**: 1112–1115.

Larsen, D.G., and Ward, R.M.P. 1995. Moose population characteristics in the Frances Lake and North Canol areas. Yukon Fish and Wildlife Branch Rep. PR 95-1, Whitehorse.

Lehman, N., Clarkson, P., Mech, L.D., Meier, T.J., and Wayne, R.K. 1992. A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. Behav. Ecol. Sociobiol. **30**: 83–94.

Mech, L.D. 1970. The wolf: ecological and behavior of an endangered species. Natural History Press, New York.

Mech, L.D. 1973. Wolf numbers in the Superior National Forest of Minnesota. U.S. For. Serv. Res. Rep. NC-07.

Mech, L.D. 1974. Current techniques in the study of elusive wilderness carnivores. *In* Proceedings of 11th Congress of the International Union of Game Biologists, Stockholm, Sweden, 3–7 September 1973. *Edited by* I. Kjerner and P. Bjurholm. Swedish National Environment Protection Board, Stockholm. pp. 315–322.

Mech, L.D. 1986. Wolf population in the central Superior National Forest, 1967–1985. U.S. For. Serv. Res. Pap. NC-270.

Mech, L.D., and Karns, P.D. 1977. Role of the wolf in a deer decline in the Superior National Forest. U.S. For. Serv. Res. Rep. NC-148.

Medjo, D.C., and Mech, L.D. 1976. Reproductive activity in nine- and ten-month-old wolves. J. Mammal. **57**: 406–408.

Messier, F. 1985*a*. Social organization, spatial distribution, and population density of wolves in relation to moose density. Can. J. Zool. **63**: 1068–1077.

Messier, F. 1985*b*. Solitary living and extraterritorial movements of wolves in relation to social status and prey abundance. Can. J. Zool. **63**: 239–245.

Messier, F. 1994. Ungulate population models with predation: a case study with North American moose. Ecology, **75**: 478–488.

Messier, F., and Crête, M. 1985. Moose–wolf dynamics and the natural regulation of moose populations. Oecologia, **65**: 503–512.

Murie, A. 1944. The wolves of Mount McKinley. U.S. Natl. Park Serv. Fauna Natl. Parks U.S. Fauna Ser. No. 5.

Norton-Griffiths, M. 1978. Counting animals. African Wildlife Leadership Foundation, Nairobi, Kenya.

Oswald, E.T., and Senyk, J.P. 1977. Ecoregions of the Yukon Territory. Fisheries and Environment, Ottawa, Ont.

Packard, J.M., and Mech, L.D. 1980. Population regulation in wolves. *In* Biosocial mechanisms of population regulation. *Edited by* M.N. Cohen, R.S. Malpass, and H.G. Klein. Yale University Press, New Haven, Conn. pp. 135–150.

Peterson, R.O., and Page, R.E. 1983. Cyclic fluctuations of wolves and moose at Isle Royale National Park, U.S.A. Acta Zool. Fenn. **174**: 252–254.

Peterson, R.O., and Page, R.E. 1988. The rise and fall of Isle Royale wolves, 1975–1986. J. Mammal. **69**: 89–99.

Peterson, R.O., Woolington, J.D., and Bailey, T.N. 1984. Wolves of the Kenai Peninsula, Alaska. Wildl. Monogr. No. 88.

Pimlott, D.H. 1967. Wolf predation and ungulate populations. Am. Zool. **7**: 267–278.

Pollock, K.H., Winterstein, S.R., Bunck, C.M., and Curtis, P.D. 1989*a*. Survival analysis in telemetry studies: the staggered entry design. J. Wildl. Manage. **53**: 7–15.

Pollock, K.H., Winterstein, S.R., and Conroy, M.J. 1989*b*. Estimation and analysis of survival distributions for radio-tagged animals. Biometrics, **45**: 99–109.

Rausch, R.A. 1967. Some aspects of the population ecology of wolves, Alaska. Am. Zool. **7**: 253–265.

Schmidt, P.A., and Mech, L.D. 1997. Wolf pack size and food acquisition. Am. Nat. **150**: 513–517.

Stephenson, R.O. 1978. Characteristics of exploited wolf populations. Alaska Department of Fish and Game Federal Aid in Wildlife Restoration, Final Rep., Projects W-17-3 through W-17-8, Fairbanks.

Thurber, J.M., and Peterson, R.O. 1993. Effects of population density and pack size on the foraging ecology of gray wolves. J. Mammal. **74**: 879–889.

Van Ballenberge, V., Erickson, A.W., and Byman, D. 1975. Ecology of the timber wolf in northeastern Minnesota. Wildl. Monogr. No. 43.

Woolpy, J.H. 1968. The social organization of wolves. Nat. Hist. **77**: 46–55.

Zimen, E. 1976. On the regulation of pack size in wolves. Z. Tierpsychol. **40**: 300–341.

Zimen, E. 1982. A wolf pack sociogram. *In* Wolves of the world: perspectives of behavior, ecology, and conservation. *Edited by* F.H. Harrington and P.C. Paquet, Noyes Data Corp., Park Ridge, N.J. pp. 282–322.

Conserv Genet (2014) 15:1111–1121
DOI 10.1007/s10592-014-0604-1

RESEARCH ARTICLE

# Genetic rescue in Isle Royale wolves: genetic analysis and the collapse of the population

**Philip W. Hedrick · Rolf O. Peterson · Leah M. Vucetich · Jennifer R. Adams · John A. Vucetich**

Received: 1 February 2014 / Accepted: 28 March 2014 / Published online: 9 April 2014

**Abstract** While genetic rescue is known to benefit population viability, the duration of that benefit is poorly understood. We document what appears to be the waning benefit of genetic rescue after approximately 2–3 generations for the wolf population in Isle Royale National Park. The fitness benefit of genetic rescue declined because of inbreeding and population abundance declined when the inbred individuals exhibited low reproduction and survival. Only detailed studies of other cases will reveal what aspects of these dynamics represent general features of genetic rescue. We also present evidence indicating that numerous past immigration events have likely gone undetected. This finding is of particular significance because the Isle Royale wolf population has maintained good population viability for decades even though it was small and thought to be isolated from the mainland population of wolves. Past gene flow also suggests that human-assisted gene flow is necessary to conserve the ecosystem services associated with predation, since climate warming has reduced the frequency of ice bridges and with it the only opportunity for unassisted gene flow.

**Keywords** Ancestry · Gene flow · Heterozygosity · Inbreeding · Pedigree · Relatedness

P. W. Hedrick (✉)
School of Life Sciences, Arizona State University, Tempe, AZ 85192, USA
e-mail: philip.hedrick@asu.edu

R. O. Peterson · L. M. Vucetich · J. A. Vucetich
School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA

J. R. Adams
Department of Fish and Wildlife Resources, University of Idaho, Moscow, ID 83844, USA

## Introduction

Endangered species often face significant genetically related threats, including lowered fitness due to loss of genetic variation, increase of detrimental variants, and inbreeding. One escape from the impact of lowered fitness is genetic rescue in which the natural or artificial introduction of individuals from outside the population provides genetic variation that subsequently results in higher fitness (Tallmon et al. 2004). Genetic rescue has resulted in dramatic population recovery when Florida panthers (Johnson et al. 2010), adders (Madsen et al. 1999), bighorn sheep (Hogg et al. 2006) and prairie chickens (Westemeier et al. 1998) from other populations were introduced into low fitness populations. The natural introduction of one male wolf had important beneficial effects in Scandinavian wolves (Vilà et al. 2003) and wolves on Isle Royale in Lake Superior (Adams et al. 2011).

Because many populations have become small and isolated in recent decades due to human impacts, genetic rescue will probably become a natural phenomenon or management action of great significance. However, because many of the known examples are from very recent years, the longer term impact of genetic rescue has not been documented or examined. In fact, Hedrick and Fredrickson (2010) advocated thorough monitoring after the introduction of outside individuals to determine how long the initial positive effect remained and if any possible negative side effects, such as lower effective population size or inbreeding and subsequent lowered fitness, resulted.

The Isle Royale wolf population has already offered some very important lessons and insights about genetic rescue. In particular, genetic rescue from the immigration of single male resulted in a “genomic sweep” and the ancestry of this individual increased quickly to over 50 %

in the population (Adams et al. 2011). Also, because genetic rescue took place during a period of low prey (moose) numbers, the wolf population size did not increase over that before the genetic rescue (Hedrick et al. 2011).

Here we present further analysis of this case study that helps better understand what may be some general features of genetic rescue over time. In Adams et al. (2011), we concluded the immigration of a single male increased the fitness in the population and implied that this would be beneficial for the population. However, that benefit might have been temporary because in the last several years the population has greatly declined and the extraordinary initial success appears to have subsequently diminished in its favorable impact. Here, we conduct a more thorough analysis of the genetic impact of this genetic rescue event and extend that analysis forward in time to the present day to provide a more complete understanding of genetic rescue as an example for other studies.

## Study system

The Isle Royale population was founded about 1950 by wolves from the mainland population (about 20 km away at the nearest point) living northwest of Isle Royale and it has been assumed that the Isle Royale wolf population was completely isolated from this population. For over 50 years on Isle Royale, both wolves and moose, their main prey, have been monitored and studied (Peterson et al. 1998; Vucetich and Peterson 2004). From genetic examination of scats, it was discovered in 2009 that a male wolf known as M93 (M indicates male) migrated from nearby mainland Ontario, Canada in 1997, probably across the ice bridge present that year (Adams et al. 2011). He was behaviorally dominant over resident wolves and his pack and first mate and their descendants quickly dominated the genetic ancestry of the population. By 2008, 59.4 % of the genetic ancestry in the population was from him (see below), resulting in a genomic sweep of the population. Although this success was a strong indication of increased fitness from genetic rescue, the environment during this period was poor because of deteriorating prey base of moose and population numbers did not increase substantially (Hedrick et al. 2011). From 2005 on, all the ancestry in the Isle Royale population was descended from three individuals, the male immigrant M93), and two females, F99 (F indicates female) his first mate, and F67, another female population resident.

In the last few years, the population numbers of Isle Royale wolves have declined and in 2012 and 2013, there were only 9 and 8 wolves, respectively, the lowest numbers ever recorded. The year 2012 was also remarkable for being the first year since records were kept (1971) that no reproduction was detected.

## Methods

We constructed a pedigree for the years, 1998–2013, based on 18 microsatellite loci that were derived from samples of feces and blood of wolves. For methodological details, see Adams et al. (2011). Our analysis here is based on temporal trends in a variety of genetic indicators derived either from this pedigree or from microsatellite heterozygosity. In particular, we calculated the proportion of ancestry ($a_i$) from each of the $i$ wolves with known descendants from 1999 on. To do this for each individual offspring, half the ancestry was assigned to the known male parent and half to the known female parent. The inbreeding coefficient was calculated using the additive approach (Ballou 1983). In addition, the proportion of the inbreeding coefficient attributable to each of the known ancestors for the pedigreed population was determined using gene-drop simulation (MacCluer et al. 1986).

The observed individual heterozygosity ($H_{Oi}$) for 18 microsatellite loci was calculated for nearly all of the 99 individuals in the pedigree (four individuals had genotypes for less than 18 microsatellite loci and two individuals first seen in 2012 were not genotyped for these same loci). The expected individual heterozygosity for a given year was calculated as the product of the ancestry from founder $i$ in that year, the observed heterozygosity of that founder, and the complement of the inbreeding coefficient (1–$f$) in that year as

$$H_E = (1 - f) \sum a_i H_{O.i} \tag{1}$$

To better understand why there was no reproduction in 2012, we analyzed the relatedness between potential mates. A new measure of relatedness that takes into account past inbreeding (Hedrick and Lacy 2014), can be calculated using the different modes of identity-by-descent of four homologous genes possessed by two individuals (Jaquard 1971; Lynch and Walsh 1998). Given the probability of the nine different identity-by-descent modes $\Delta_i$, the new measure of relatedness between individuals $x$ and $y$ is

$$r_{xy} = \Delta_1 + \Delta_7 + \frac{3}{4}(\Delta_3 + \Delta_5) + \frac{1}{2}\Delta_8 \tag{2}$$

We compare this to another measure of relatedness (Wright 1922) that also takes into account past inbreeding, generally known as the coefficient of relationship (Crow and Kimura 1970)

$$r^*_{xy} = \frac{2f_{xy}}{\left[(1 + f_x)(1 + f_y)\right]^{1/2}} \tag{3}$$

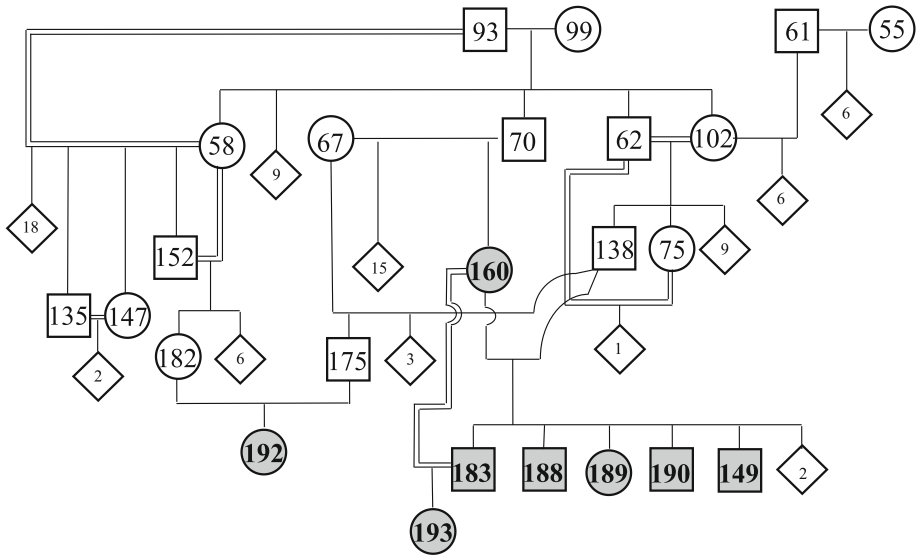


**Fig. 1** Pedigree for Isle Royale wolves where *squares* indicate males, *circles* indicate females, and the number in them are the identification number of an individual. *Diamonds* indicate other progeny with the number in them indicating the number of progeny. *Shaded* individuals indicate the ones known alive in 2012 and *double lines* indicate matings between first-degree relatives

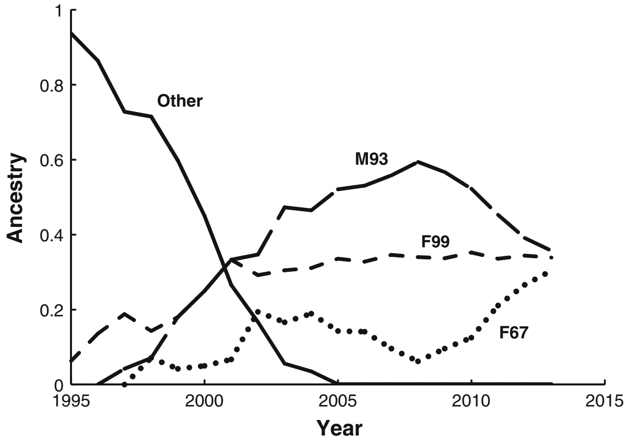


**Fig. 2** Ancestry over years in the Isle Royale wolf population descended from M93, F99, F67, and Other wolves

where $f_x$, and $f_y$, are the inbreeding coefficients in individuals $x$ and $y$, and $f_{xy}$ is the inbreeding coefficient of an offspring from individuals $x$ and $y$.

In an isolated population, the expected heterozygosity in generation $t$ ($H_t$) is predicted to decline as a function of the effective population size $N_e$ and the number of generations from an initial value of $H_0$ as

$$H_t = H_0\left(1 - \frac{1}{2N_e}\right)^t \tag{4}$$

(Hedrick 2011). The influence of gene flow from outside the population can be incorporated using the island model of Wright (1940) as

$$H_t = 1 - \left[\frac{1}{2N_e} + \left(1 - \frac{1}{2N_e}\right)(1 - H_{t-1})\right](1 - m)^2 \tag{5}$$

given that the rate of gene flow per generation into the populations is $m$.

## Results

### Pedigree analysis and recent genetic change

Figure 1 gives the updated pedigree through 2013 and some salient, context-providing features of that pedigree are the following. In 2012, there were nine individuals alive and eight were identified genetically (indicated by shaded symbols in Fig. 1). The four males and female F189 present in 2012 are full sibs. Ancestry from female F67 continues in the population because F160 is the daughter of F67. Eight individuals were identified from aerial surveys in 2013 and seven of them were detected through their fecal DNA. These wolves were the same as those detected in 2012, except M183 was not detected.

In the early years of the pedigreed population, ancestry from three wolves (male immigrant M93, his first mate F99, and female F67) generally rose, while the ancestry from other wolves (including M61 and F55) declined to zero by 2005 (Fig. 2). Within a decade (~2.5 generations) of the arrival of M93, his ancestry had risen to 59.4 % in 2008, greater than the ancestry of all other individuals combined. His ancestry rose to such a high level in part because he mated with his own daughter (F58) to produce 21 offspring. Between 2008 and 2013, the ancestry of M93 declined, while the ancestry of F67 increased and the ancestry of F99 remained about constant. By 2013, the ancestry of those three wolves had become approximately equal.

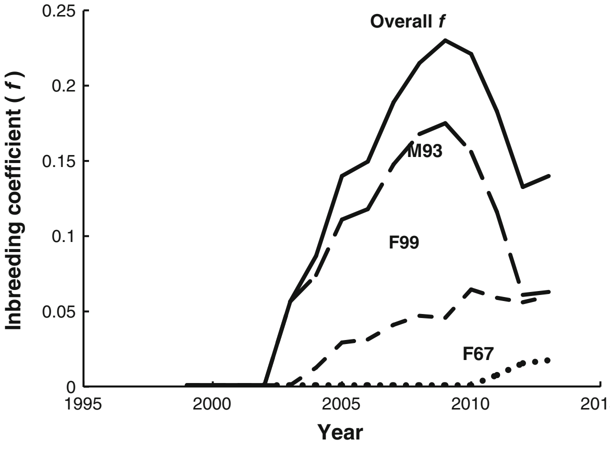


**Fig. 3** The overall level of inbreeding (*f*) in the Isle Royale wolf population over time and the identity-by-descent attributable to wolves M93, F99, and F67

**Table 1** The matings between first-degree relatives, the inbreeding coefficient of their progeny (some of these parents were already inbred), and the number of progeny produced (seen in the annual survey in January and February) from each mating

| Father | Mother | Type of first-degree mating | Inbreeding coefficient | Number progeny |
|---|---|---|---|---|
| M93 | F58 | Father–daughter | 0.25 | 21 |
| M152 | F58 | Mother–son | 0.375 | 6 |
| M183 | F160 | Mother–son | 0.3125 | 1 |
| M135 | F147 | Brother–sister | 0.375 | 2 |
| M62 | F75 | Father–daughter | 0.375 | 1 |
| M62 | F102 | Brother–sister | 0.25 | 11 |

The level of inbreeding in the pedigreed population started to increase in 2003 and reached a maximum in 2009 of 0.230 (Fig. 3). In the past several years, the level of inbreeding has declined, reaching levels of 0.133 and 0.140 in 2012 and 2013, respectively. This decline has resulted primarily from the short lives and low reproductive contribution of the highly inbred wolves in the population (see below).

The inbreeding (identity-by-descent) experienced by the population can be attributed to either M93, F99, or F67 (Fig. 3). A very large proportion of the inbreeding in the early years is attributable to the immigrant M93, primarily because of the highly successful mating of M93 with his daughter F58. This father-daughter mating resulted in identity-by-descent from M93 but not from F99. For example, in 2009, 76 % of the inbreeding was from M93 and only 24 % from F99. Recently in 2012 and 2013, the level of inbreeding from M93 and F99 was nearly identical. Only since 2011 was there any inbreeding attributable to F67.

**Table 2** The nine wolves with an inbreeding coefficient of 0.375 due to two consecutive generations of matings between first-degree relatives, the year they were first and last seen in the annual count in January and February (birth and death years here, respectively), and the matings in these two generations

| Wolf | Generation 1 | Generation 2 | Birth | Death | Life span |
|---|---|---|---|---|---|
| M177 | Brother (M62)–sister (F102) | Father (M62)–daughter (F75) | 2007 | 2008 | 2 |
| M181 | Father (M93)–daughter(F58) | Mother (F58)–son (M152) | 2008 | 2008 | 1 |
| F182 | Father (M93)–daughter(F58) | Mother (F58)–son (M152) | 2008 | 2010 | 3 |
| M179 | Father (M93)–daughter(F58) | Brother (M135)–sister(F147) | 2008 | 2010 | 3 |
| F180 | Father (M93)–daughter(F58) | Brother (M135)–sister(F147) | 2008 | 2009 | 2 |
| M141 | Father (M93)–daughter(F58) | Mother (F58)–son (M152) | 2009 | 2010 | 2 |
| F184 | Father (M93)–daughter(F58) | Mother (F58)–son (M152) | 2009 | 2011 | 3 |
| F185 | Father (M93)–daughter(F58) | Mother (F58)–son (M152) | 2009 | 2011 | 3 |
| M187 | Father (M93)–daughter(F58) | Mother (F58)–son (M152) | 2010 | 2011 | 2 |

The basis for using years of detected in samples of fecal DNA as a basis for estimating lifespan is given in Marucco et al. (2012)

Detailed inspection of the pedigree reveals additional insight. In particular, there have been six matings between first-degree relatives, two father-daughter matings, two mother-son matings, and two brother-sister matings, which have produced progeny (Table 1). Overall these matings between close relatives produced 42 offspring, 44.7 % of the known 94 progeny produced in the pedigreed population.

Much of the decline in overall inbreeding level from 2009 to 2013 (Fig. 3) is explained by the death of nine wolves with an inbreeding coefficient of 0.375, the result of two consecutive generations of close inbreeding (Table 2). All of these wolves had short lifespans (mean of 2.33 years) and all of them had died by 2011. Specifically, of the wolves recruited into the pedigree between 2009 and 2011, there is a two-fold difference in *f* between the seven alive in 2012 (0.152) and the six not alive in 2012 (0.292). None of these highly inbred wolves reproduced.

The increase in the ancestry of F67 and concomitant decrease in the ancestry of M93 is explained by inspecting the pattern of inbreeding that occurred between 2008 and 2013. Because the male immigrant M93 is unrelated to resident females F99 and F67, any offspring between them have an inbreeding coefficient of zero. With the recent increase of the ancestry of F67, some matings between individuals with only F67 ancestry and with only M93 and

**Table 3** The inbreeding coefficient $f_{xy}$ of an offspring from a mating between male $x$ and female $y$, the inbreeding coefficient $f_y$ of the female (the males all have $f_x = 0.125$), the relatedness, $r_{xy}$, of Hedrick and Lacy (2014), and the coefficient of relationship, $r_{xy}$*, of Wright (1922) for the four males and four females known to be present in 2012

| Male ($x$) | Female ($y$) | $f_y$ | $f_{xy}$ | $r_{xy}$ | $r_{xy}$* |
|---|---|---|---|---|---|
| M149, M183, M188, M190 | F160 | 0.000 | 0.312 | 0.595 | 0.589 |
| M149, M183, M188, M190 | F189 | 0.125 | 0.344 | 0.617 | 0.611 |
| M149, M183, M188, M190 | F192 | 0.125 | 0.219 | 0.396 | 0.389 |
| M149, M188, M190 | F193 | 0.312 | 0.328 | 0.567 | 0.540 |
| M183 | F193 | 0.312 | 0.438 | 0.734 | 0.720 |

F99 ancestry have resulted in offspring with inbreeding coefficients of zero, consequently resulting in a decline in the average inbreeding in recent years.

Insight about mating behavior and reproduction in 2012 depends on understanding the relationships among individuals that were alive in 2012. In particular, the four males present in 2012 were full sibs (Table 3). The four females were F160 (mother of the full sibs), F189 (a full sib of the males), and two other females F192 and F193. All four full-sib males are equivalent when mating with females F160, F189, and F192. However, because M183 was the father of F193, a potential mating between them requires separate evaluation (see below).

If one of the four males were to have mated with F160 or F189, or if one of the three males excluding M183 were to have mated with F193, then the inbreeding coefficient of their offspring ($f_{xy}$) would be greater than 0.3 (Table 3). The high $f_{xy}$ value from F160 would be because she is the mother of the four full-sib males, and the high $f_{xy}$ value from F189 would be because she is a full sib of the four males. If F192 were to have mated with any of the four males, their progeny would have a lower inbreeding coefficient ($f_{xy} = 0.219$). The lower value of $f_{xy}$ for an offspring of F192 is because she is not a descendent of F160 and is the granddaughter of F67. Finally, if M183 were to have mated with his daughter F193, their progeny would have a higher inbreeding coefficient ($f_{xy} = 0.438$).

Of the 16 possible matings that could have occurred in 2012, four represent matings between the least related wolves and matings that would have produced offspring with the lowest $f$ (matings between F192 and any of the males, see Table 3). In fact, the F192 and M190 pair ($r_{xy} = 0.396$ and $r_{xy}* = 0.389$) were the only pair of wolves to display signs of courtship and mating behavior during the 2012 mating season (February). Nevertheless, we were unable to detect any sign of any pups having been born in 2012. However, aerial surveys conducted in January, 2014 suggested that this pair had given birth to two or three pups that had survived at least to 9 months of age (Vucetich and Peterson 2014).

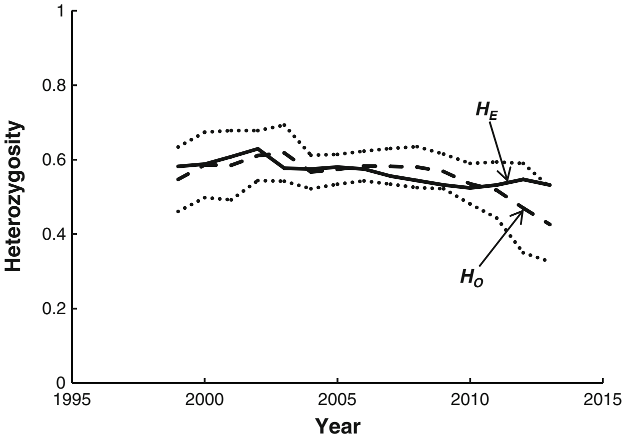


**Fig. 4** The observed heterozygosity ($H_O$) (*broken line*) for 18 loci for Isle Royale wolves from 1999 to present and 95 % confidence limits (*dotted lines*) and the expected heterozygosity ($H_E$) (*solid line*) based on the observed heterozygosity of the five ancestors, their ancestry, and level of inbreeding for each year

### Observed and expected microsatellite heterozygosity

The observed heterozygosity is relatively stable for the early years, 1999–2006, with a mean value of 0.589 (Fig. 4). More recently, however, $H_O$ declined to 0.426. The variance and standard errors of the observed heterozygosity among individuals was influenced both by the variance in inbreeding among individuals and the variance in observed heterozygosity among the five ancestors of the pedigreed population (the observed individual heterozygosities of the ancestors were $H_{O93} = 0.833$, $H_{O99} = 0.500$, $H_{O67} = 0.500$, $H_{O61} = 0.444$, and $H_{O55} = 0.389$). Expected heterozygosity was a good predictor of $H_O$ throughout most of the study period. However, by 2013, $H_O$ had become significantly lower than $H_E$ (Fig. 4).

### High heterozygosity in the Isle Royale population

The Isle Royale population would have been expected to have lost most of its original heterozygosity if it had been isolated throughout the approximate 12 generations (assuming the generation length is 4 years, see Peterson et al. 1998 for justification) that passed from the time of its founding around 1950 until the arrival of M93 in 1997. More precisely, 81.7 % of the original heterozygosity is expected to have been lost (Fig. 5), according to the estimate for $N_e$ of 3.8 (Peterson et al. 1998) based on Eq. (4).

By contrast, the population appears to have lost only 32.2 % of its heterozygosity. This estimated loss is based on

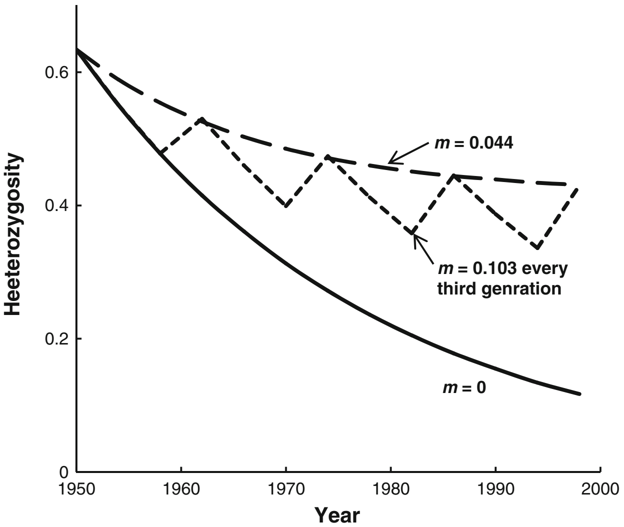


**Fig. 5** The expected change in heterozygosity when there is no gene flow ($m = 0$), there is a constant rate of gene flow each generation ($m = 0.044$), and there is gene flow every third generation of $m = 0.103$

comparing the mean observed heterozygosity for 20 wolves on Isle Royale before 1998 (0.430 ± 0.022) to the mean observed heterozygosity for 35 mainland wolves from near the Minnesota–Canadian border (0.634 ± 0.020), which represents the source population of Isle Royale wolves.

A possible explanation for the discrepancy is undetected gene flow. For example, if the rate of gene flow had been 0.044 each generation, then heterozygosity is expected to have declined to the value observed in 1998 (Fig. 5). This rate of gene flow represents about 1 in 23 individuals being a migrant, which corresponds to about one migrant per generation, because the mean population size on Isle Royale for the period 1959–1998 is 23.4 individuals. The observed heterozygosity could also have resulted from higher levels of gene flow occurring less frequently, such as $m = 0.103$ every third generation which corresponds to about 2.3 migrants every third generation (Fig. 5).

The possibility of undetected gene flow prompted us to review field observations from the four decades prior to the arrival of M93 for clues suggesting the occurrence of such events (Table 4). First, several wolves, including four that were black, arrived over an ice bridge in 1967. One of the black wolves was observed living in 1968 in the single pack that comprised the population at that time. Behavioral observations indicated that he subsequently became the alpha male in 1971 and 1972 (Wolfe and Allen 1973). Other field observations indicated that two pups had been recruited into the population in 1971 and another four in 1972 (Peterson et al. 1998). Because no black pups were observed, the black wolf was presumed, at the time, not to be the father. Contemporary understanding for the inheritance of coat color in wolves (Anderson et al. 2009), however, indicates that it would not be unexpected to observe only gray pups from the mating between a gray mother and a black father, if that father had been a heterozygote for the gene determining coat color. About two generations later, in the summer of 1980, a National Park Service employee photographed a wolf pup that also might have been black. After this photograph is retrieved from the Park's archives and inspected, we are likely to know more about this event.

**Table 4** Movements of wolves and other canids between Isle Royale and the mainland by year from the 1940s to 2013, characteristics of the migrants and whether they reproduced

| Date | Description of animals | Reproduction | Citation |
|---|---|---|---|
| Late 1940s | Founding pair (or more) | Yes | Mech (1966) |
| 1967 | Four black wolves | Yes | Wolfe and Allen (1973) |
| 1977 | Wolf | Chased off island | Peterson (1979) |
| 1980 | Wolf (black? pup) | Yes (implied) | This study |
| 1996–1998 | Coyote-like canid | No (lone animal) | This study |
| 1997 | Wolf (M93) | Yes | Adams et al. (2011) |

These field observations of black wolves are noteworthy because the arrival of M93, a gray-colored wolf, was only detected from genetic observations made more than a decade after his arrival (Adams et al. 2011). Because of his gray appearance, his arrival would have been undetectable from field observations alone. Black wolves are uncommon in the Great Lakes region, representing approximately only approximately 3 % of the population (Mech and Frenzel 1971, Mech and Paul 2008). The rarity of black wolves and the detection of two, and possibly three, black wolves in the four decades of observation prior to 1998 suggests the plausibility of additional undetected gene flow involving gray-colored wolves.

Gene flow is possible only in the presence of ice bridges that form during some winters. These bridges are sometimes present for a few days and other times they persist for several weeks. Travelling at a typical speed (Mech 1994), a wolf could cross an ice bridge in three to 6 hours. In 2008, two radio-collared wolves disappeared shortly after an ice bridge had formed (unpubl. field notes). Similarly in early 2014, female F189 disappeared after an ice bridge had formed and was subsequently was found dead on the mainland. A pack of wolves was also observed chasing a pack mate onto an ice bridge in 1977 and airborne observers followed the egress event halfway to the mainland (Peterson 1979).

During 3 years (1996–1998) following a period when ice bridges had been present in 1994, 1996, and 1997, a lone animal that appeared to be a coyote was observed on Isle Royale (unpubl. field notes). Populations of fox and coyote are thought to have been established on Isle Royale early in the 20th century by migrants that would have crossed an ice bridge but the coyote population was driven to extinction shortly after the wolf population was established in the late 1940s. Mech (1966) recounts several instances that likely involved individual wolves crossing ice bridges during the first half of the 20th century. Collectively, these observations further suggest the plausibility of undetected movement and consequent gene flow for wolves (or other canid species).

Inbreeding measures the level of identity by descent and if migrant and resident wolves did not have recent common ancestors, then their progeny could have an inbreeding level of zero. Further, it would potentially take some time for inbreeding to accumulate because the closest mating in wolves to produce inbred offspring is either a parent—offspring or a mating of siblings, both of which would take at least two generations (8 years). If there was an earlier unrelated migrant (like M93), then the inbreeding level could potentially decline back to low levels.

As a result, undetected gene flow should also influence the level of inbreeding the population had exhibited just prior to the arrival of M93. For that time, $f$ had been estimated as 0.801, using an indirect estimator that assumed the absence of gene flow (Peterson et al. 1998; Adams et al. 2011). Another indirect, but useful, basis for estimating $f$ before the arrival of M93 that accounts for having observed higher than expected heterozygosity resulting from undetected gene flow is

$$f_t = 1 - \frac{H_t}{H_0} \tag{6}$$

Replacing $H_t$ in this equation with the heterozygosity for Isle Royale wolves prior to 1998 (0.430) and $H_0$ with the heterozygosity for 35 wolves living near the Minnesota–Canadian border (0.634), yields $f_t = 0.332$ for the time prior to the arrival of M93. The expected pattern change in $f$ given these two initial inbreeding points, and the known remainder composition of the population is given in Fig. 6.

## Discussion

### General conclusions

The success of immigrant M93 and his influence on the Isle Royale wolf population was remarkable. Within a generation of his arrival, the inbreeding coefficient had declined from 0.332 to 0.036 (Fig. 6). He sired 34 offspring, 21 of which were with his daughter and within 2.5 generations, and his ancestry had risen to 59.4 % (Fig. 2). This high ancestry level is further supported by molecular genetic data showing that the only variant of the Y chromosome present in the population today was inherited from M93 (Adams et al. 2011). In addition, he was a heterozygote for two new alleles (allele 224 at locus FH2226 and allele 146 at locus C05.377) that increased to frequencies of 33 and 27 % in the population by 2009, bracketing the expected value of 28 % (= 56 %/2) expected from the pedigree for that year (Adams et al. 2011). This rise in ancestry is, to our knowledge, faster and greater than any previously documented for a wild vertebrate population and as a result, the impact of the immigrant represents a genomic sweep of the population.

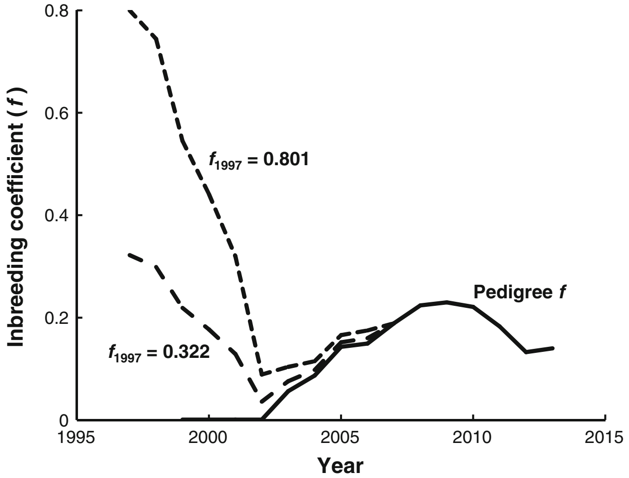


**Fig. 6** The mean inbreeding coefficient ($f$) over time from the individuals in the known pedigree and that expected given that in 1997 $f = 0.801$ (as assumed in Peterson et al. 1998; Adams et al. 2011), and assuming that $f = 0.322$ as estimated from observed heterozygosity on Isle Royale and the mainland in 1997

Demographic benefits, such as increased rates of recruitment or survival that might have resulted from the arrival of M93 were masked by a severe collapse in food supply that coincided with his arrival (Adams et al. 2011). As a result, his impact on population fitness was indicated by the dramatic rise in his ancestry (Hedrick et al. 2011). In recent years, however, the ancestry of M93 has declined to 33.9 % and became approximately co-equal with two other individuals. That decline in ancestry coincided with a decline in mean inbreeding level ($f$) and observed heterozygosity ($H_O$) (Figs. 3, 4). Ordinarily, a decline in $f$ is associated with an increase in $H_O$. However, if $f$ declines as a result of increased ancestry from an unrelated individual with lower $H_O$, then the pattern we observed would be expected. Specifically, ancestry from F67 increased as ancestry from M93 decreased (Fig. 2), and F67 had a lower

$H_O$ than did M93 ($H_{O67} = 0.50$ and $H_{O93} = 0.833$). This observation represents another possible mechanism by which $f$ and $H_O$ might not be strongly associated (Balloux et al. 2004).

Figure 5 and the estimated microsatellite heterozygosity of the population indicate that it had been highly inbred before the arrival of M93. The genomic sweep and rapid rise in ancestry of M93 in years following his arrival also indicated the population had low fitness. The more recent decline in his ancestry was associated with selection against his inbred descendants and resulted in an increase in ancestry from the individuals with lower fitness which had been present before his immigration (F99 and F67). That dynamic is likely associated with the decline in demographic performance for both wolf abundance and recruitment that occurred during the same period.

Ordinarily, one would not expect a decline in mean $f$ (as occurred between 2008 and 2013) to be associated with a decline in demographic performance. Specifically, mean $f$ rose prior to 2008 because a large portion of the individuals alive at that time were the result of two successive generations of close inbreeding ($f = 0.375$) but they also had high ancestry from M93. Those wolves had short lifespans, did not reproduce, and died between 2008 and 2011 (Table 2). Their deaths caused mean $f$ to decline and contributed importantly to the decline in population abundance and recruitment rate.

The low fitness of those inbred wolves might have been attributable to the expression of recessive deleterious alleles inherited from M93. Any migrant from a large outbred population (like the Canadian wolf population) is expected to carry a number of such detrimental alleles. Given successive generations of close inbreeding, these alleles would have contributed to the decline of the population because those inbred wolves with high ancestry from M93 represented a large portion of the population. As examples in other wolf populations, both the Swedish wolf (Liberg et al. 2005) and the Mexican wolf (Fredrickson et al. 2007) populations were segregating for multiple detrimental variants that greatly reduced the fitness for inbred individuals. Bijlsma et al. (2010) observed similar dynamics of deleterious alleles in experimental populations of *Drosophila*.

That those inbred wolves represented a large portion of the population is a legacy of the success of M93. Being so successful and bringing about a genomic sweep, allowed for the possibility of recessive deleterious alleles to increase in frequency with little chance of being purged (Hedrick 1994). Ultimately, the apparently short-lived benefit of migrant M93 might have been associated with how very successful his lineage had become. His success was largely attributable to the degree the Isle Royale population had, at the time of the arrival of M93, been suffering from low fitness. In any event, the beneficial effect of M93 appears to have begun waning about two or three generations after his arrival. The details of this short-lived benefit illustrate potential predictions about the nature of genetic rescue (Hedrick and Fredrickson 2010).

The apparent failure of the Isle Royale wolf population to reproduce in 2012 is remarkable for being the first time since such records have been kept (1971) that no signs of reproduction were detected. Recent genetic analyses offer new insight on that failure. In 2012, the population was organized into two social groups: the West-end Duo was a newly formed and young pair and failed to produce pups. Because those wolves are closely related ($r = 0.39$) and the inbreeding coefficient of their offspring would have been relatively high ($f = 0.22$), one would not be surprised if their reproductive output is lackluster. Nevertheless, that pair is still alive and their lifetime reproductive output remains to be seen.

The other wolves present formed the Chippewa Harbor Group, which was comprised of five individuals. In February, 2012, those wolves had not shown any signs of courtship or even signs of hierarchy that accompany courtship. We now know that this group was comprised of a mother (F160) and her four offspring. One might expect inbreeding avoidance given that every pairing of wolves in that group was a first-order relationship (full siblings or parent-offspring). Moreover, the alpha male of Chippewa Harbor Pack (M138) had died just 2–3 months prior to the 2012 mating season and the pairing of wolves M138 and F160 had successfully reproduced in previous years. M138 died prematurely along with two other wolves when they drowned in a flooded mine shaft. If M138 had not died (along with two other wolves), then the survival and recruitment rates in 2011–2013 would almost certainly have been higher, and the population would not have declined from 16 to 8 wolves. That mine shaft tragedy—an artifact of 19th century mining and consequently an anthropogenic influence on the population—appears to have been an important contributor to the current high risk of population extinction.

## High heterozygosity

The higher than expected heterozygosity that we observed appears as though it can be explained by previously undetected gene flow (Fig. 5; Table 4). While inbreeding avoidance and selection for heterozygotes (against homozygotes) might, in principle, also explain higher than expected $H$, additional considerations suggest otherwise. First, prior analyses indicated that inbreeding avoidance is relatively weak in the Isle Royale population (Geffen et al. 2011). Moreover, matings between first-degree relatives represent 6 of the 11 reproductively successful pairings that we observed in the pedigree, and 45 % of the 94 progeny that survived long enough to be recruited into the population were the result of first-degree matings. Much of the inbreeding resulted from immigrant M93 mating with his daughter (F58) and F58 mating with her son (M152). Overall, inbreeding avoidance appears not to have been strong enough to overcome the impact of small population size. One potential exception is the recent pairing of M190 and F192, involving the least related wolves present (Table 3).

Selection appears not to have consistently favored heterozygotes. For example, observed and expected heterozygosity were similar during the early years of the study period, and $H_O$ declined relative to $H_E$ during the most recent years (Fig. 4). Additional insight rises from considering temporal trends in mean inbreeding level, which increased as the ancestry of M93 increased; then both began to decline in 2008 (Figs. 2, 3). Because M93 had relatively high heterozygosity, those trends are consistent with selection for higher heterozygosity followed by selection against individuals with higher heterozygosity, but happened to be more inbred. Overall, selection appears to have been against individuals with high $f$. Moreover, $f$ is not significantly correlated with microsatellite heterozygosity ($r = 0.105$, $P = 0.51$, for individuals with $f > 0$), in large part because M93 who was responsible for a large portion of inbreeding (Fig. 3) had relatively high heterozygosity. Because the impact of M93 appears unprecedented, these kinds of dynamics may be unusual. Selection for heterozygosity was reported in a study of 31 microsatellite loci for the inbred population of Swedish wolves (Bensch et al. 2006), but not in a subsequent analysis of 237 loci for the same population (Hagenblad et al. 2009).

## Climate change

The genetic health of the Isle Royale population has very likely been maintained by periodic gene flow, which is only possible during winters in which an ice bridge has formed. Moreover, the frequency of ice bridges has steadily declined throughout the past five decades. The mean expected probability of observing an ice bridge during the 1960s was 0.67 while for the past decade, the mean expected frequency is only 0.16 (Vucetich and Peterson 2014). Lake Superior is expected to be largely ice free by 2040 (Austin and Colman 2007). The effect of anthropogenic climate change on the health of the Isle Royale wolf population has significant policy implications because Isle Royale is a National Park and one of the few places on earth inhabited by an un-persecuted top predator, an un-hunted large ungulate population, and a forest that is not commercially logged. Whether the National Park Service decides to actively conserve the wolves of Isle Royale through genetic rescue will set an important precedent for whether they will mitigate the threat of climate change in instances where doing so is feasible (Vucetich et al. 2012, a, b).

## Comparisons to other organisms

As we suggested in the introduction, because many populations have become small and isolated due to human impacts, genetic rescue will probably become of greater importance for rare and/or endangered species. In most other well-known examples of genetic rescue, the population numbers have increased and in general the populations appear to have higher viability than before the natural or artificial genetic rescue. For example, the translocations of prairie chickens (Westemeier et al. 1998) appears to have been effective in increasing fitness and genetic variation and the population initially increased, although now the population is struggling and appears to be limited by suitable habitat (Bouzat et al. 2009). In the isolated Swedish population of adders (Madsen et al. 1999), translocation initially resulted in enhanced population growth (Madsen et al. 2004). However, the recent construction of a house and a brick wall, unrelated to genetic rescue, have dramatically reduced population numbers (Madsen and Ujvari 2011). The introduction of Texas pumas into the Florida panther population resulted in dramatic population recovery (Hostetler et al. 2010; Johnson et al. 2010) although now the population appears potentially limited by available habitat.

The natural introduction of one male wolf initially had important beneficial effects in Scandinavian wolves (Vilà et al. 2003) although a subsequent analysis documented significant inbreeding depression (Liberg et al. 2005). Since then, two male wolves naturally migrated into the population and three wolves were translocated into the population from northern Sweden, resulting in both a lower inbreeding coefficient and higher reproductive success (O. Liberg, personal comm.). Crosses between lineages in Mexican wolves resulted in higher fitness (Fredrickson et al. 2007) and recently numbers in the reintroduced population have increased. However, as yet there has been no detailed analysis determining how much genetic rescue or other factors have influenced this population increase. Genetic rescue in the population of bighorn sheep living in the National Bison Range resulted in significant population increase (Hogg et al. 2006) and recent genomic analysis has examined what loci might have been involved (Miller et al. 2012).

These examples of genetic rescue suggest that its benefits can be mediated by other ecological factors. In particular, beneficial effects of genetic rescue were straightforward for the Swedish wolf population where habitat was not limiting and where genetic rescue occurred on multiple occasions. However, the effects of genetic rescue appear to have been limited in several other cases where habitat was limited or had become degraded. The Isle Royale case represents another kind of complexity. Here, the beneficial effects of genetic rescue were not detectable because of a sudden diminishment in food supply that coincided with genetic rescue (Adams et al. 2011; Hedrick et al. 2011) and ultimately limited by the geographical constraints on the size of the population.

The case study reported here is significant because few documented instances of genetic rescue have been observed long enough or in sufficient detail to understand how long one can expect the beneficial effects of genetic rescue to persist. Clearly, additional case studies will be required before an adequate understanding is developed.

**Acknowledgments** PWH was partially supported by the Ullman Professorship and ROP was partially supported by the Robbins Chair. This work was also supported in part by the US National Science Foundation (DEB-0918247), Isle Royale National Park (CESU Task Agreement No. P11AC90808), and a McIntyre-Stennis Grant (USDA-Nifa #224870).

## References

Adams JR, Vucetich LM, Hedrick PW, Peterson RO, Vucetich JA (2011) Genomic sweep and potential genetic rescue during limiting environmental conditions in an isolated wolf population. Proc R Soc B 278:3336–3344

Anderson TM, vonHoldt BM, Candille SI, Musiani M, Greco C, Stahler DR, Smith DW, Padhukasahasran B, Randi E, Leonard JA, Bustamante CD, Ostrander EA, Tang H, Wayne RK, Barsh GS (2009) Molecular and evolutionary history of melanism in North American gray wolves. Science 323:1339–1343

Austin JA, Colman SM (2007) Lake Superior summer water temperatures are increasing more rapidly than regional air temperatures: a positive ice-albedo feedback. Geophys Res Lett 34:L06604. doi:10.1029/2006GL029021

Ballou JD (1983) Calculating inbreeding coefficients from pedigrees. In: Schonewald-Cox M, Chambers SM, MacBryde B, Thomas L (eds) Genetics and conservation. Benjamin/Cummings Publishing, Menlo Park, pp 509–523

Balloux F, Amos W, Coulson T (2004) Does heterozygosity estimate inbreeding in real populations? Mol Ecol 13:3021–3031

Bensch S, Andrén H, Hansson B, Pedersen HC, Sand H, Sejberg D, Wabakken P, Äkesson M, Liberg O (2006) Selection for heterozygosity gives hope to a wild population of inbred wolves. PLoS One 1:e72

Bijlsma R, Westerhof DD, Roekx LP, Pen I (2010) Dynamics of genetic rescue in inbred *Drosophila melanogaster* populations. Conserv Genet 11:449–462

Bouzat JL, Johnson JA, Toepfer JE, Simpson SA, Esker TL, Westemeier RL (2009) Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. Conserv Genet 10:191–201

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and Row, New York

Fredrickson RJ, Siminski P, Woolf M, Hedrick PW (2007) Genetic rescue and inbreeding depression in Mexican wolves. Proc R Soc B 274:2365–2371

Geffen E, Kam M, Hefner R, Hersteinsson P, Angerbjörn A, Dalèn L, Fuglei E, Norèn K, Adams JR, Vucetich J, Meier TJ, Mech LD, vonHoldt BM, Stahler DR, Wayne RK (2011) Kin encounter rate and inbreeding avoidance in canids. Mol Ecol 20:5348–5358

Hagenblad J, Olsson M, Parker HG, Ostrander EA, Ellegren H (2009) Population genomics of the inbred Scandinavian wolf. Mol Ecol 18:1341–1351

Hedrick PW (1994) Purging inbreeding depression. Heredity 73:363–372

Hedrick PW (2011) Genetics of populations, 4th edn. Jones and Bartlett, Boston

Hedrick PW, Fredrickson R (2010) Guidelines for genetic rescue: examples from Mexican wolves and Florida panthers. Conserv Genet 11:615–626

Hedrick PW, Lacy RC (2014) Measuring relatedness between inbred individuals

Hedrick PW, Adams JR, Vucetich JA (2011) Reevaluating and broadening the definition of genetic rescue. Conserv Biol 25:1069–1070

Hogg JT, Forbes SH, Steele BM, Luikart G (2006) Genetic rescue of an insular population of large mammals. Proc R Soc B 273:1491–1499

Hostetler JA, Onorato DP, Nichols JD, Johnson WE, Roelke ME, O'Brien SJ, Jansen D, Oki MK (2010) Genetic introgression and the survival of Florida panther kittens. Biol Conserv 143:2789–2796

Jaquard A (1971) Genetic information given by a relative. Biometrics 28:1101–1114

Johnson WE, Onorato DP, Roelke ME, Land ED, Cunnningham M, Beldon RC, McBride R, Jansen D, Lotz M, Shindle D, Howard J, Wildt DE, Penfold LM, Hostetler JA, Oli MK, O'Brien SJ (2010) Genetic restoration of the Florida panther. Science 329:1641–1645

Liberg O, Andrén H, Pederson H-C, Sand H, Sejbeg D, Wabakken P, Åkesson M, Bensch S (2005) Severe inbreeding depression in a wild wolf (*Canis lupus*) population. Biol Lett 1:17–20

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland

MacCluer JW, VandeBerg JL, Read B, Ryder OA (1986) Pedigree analysis by computer simulation. Zoo Biol 5:147–160

Madsen T, Ujvari B (2011) The potential demise of a population of adders (*Vipera berus*) in Smygehuk, Sweden. Herpetol Conserv Biol 6:72–74

Madsen T, Shine R, Olsson M, Wittzell H (1999) Restoration of an inbred adder population. Nature 402:34–35

Madsen T, Ujvari B, Olsson M (2004) Novel genes continue to enhance population growth in adders (*Vipera berus*). Biol Conserv 120:145–147

Marucco F, Vucetich LM, Peterson RO, Adams JR, Vucetich JA (2012) Evaluating the efficacy of non-invasive genetic methods and estimating wolf survival during a ten-year period. Conserv Genet 13:1611–1622

Mech LD (1966) The wolves of Isle Royale. US National Park Service Fauna Series No. 7 Government Printing Office, p 210

Mech LD (1994) Regular and homeward travel speeds of Arctic wolves. J Mammal 75:741–742

Mech LD, Frenzel LD (Eds) (1971) Ecological studies of the timber wolf in northeastern Minnesota (vol 52). US North Central Forest Experiment Station

Mech LD, Paul WJ (2008) Wolf body mass cline across Minnesota related to taxonomy? Canad J Zool 86:933–936

Miller JM, Poissant J, Hogg JT, Coltman DW (2012) Genomic consequences of genetic rescue in an insular population of bighorn sheep (*Ovis canadensis*). Mol Ecol 21(1583):1596

Peterson RO (1979) Social rejection following mating of a subordinate wolf. J Mammal 60:219–221

Peterson RO, Thomas NJ, Thurber JM, Vucetich JA, Waite TA (1998) Population limitation and the wolves of Isle Royale. J Mammal 79:828–841

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. Trends Ecol Evol 19:489–496

Vilà C, Sundqvist A-K, Flagstad Ø, Seddon J, Björnerfledt S, Kojola I, Casulli A, Sand H, Wabakken P, Ellegren H (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. Proc R Soc B 270:91–97

Vucetich JA, Peterson RO (2004) The influence of prey consumption and demographic stochasticity on population growth rate of Isle Royale wolves (*Canis lupus*). Oikos 107:309–320

Vucetich JA, Peterson RO (2014) The wolves of Isle Royale, 2013–2014, annual report. Michigan Technological Univ. p 16

Vucetich JA, Nelson MP, Peterson RO (2012) Should Isle Royale wolves be reintroduced? A case study on wilderness management in a changing world. In: George Wright Forum (vol 29, No. 1, pp 126–147)

Vucetich JA, Peterson RO, Nelson MP (2013a) Discernment and Precaution: A Response to Cochrane and Mech. In: George Wright Forum (vol 30, No. 3, pp 333–340)

Vucetich JA, Peterson RO, Nelson MP (2013b) Response to Gostomski. In: George wright forum (vol 30, No. 1, pp 101–102)

Westemeier RL, Brawn LD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. Science 282:1695–1698

Wolfe MW, Allen DL (1973) Continued studies of the status, socialization, and relationships of Isle Royale wolves, 1967–1970. J Mammal 54:611–635

PROF. PHILIP HEDRICK (Orcid ID : 0000-0002-4525-4923)

Article type : Original Manuscript

# Genetics and extinction and the example of Isle Royale wolves

Philip W. Hedrick[1], Jacqueline A. Robinson[2], Rolf O. Peterson[3], and John A. Vucetich[3]

[1]School of Life Sciences, Arizona State University, Tempe, AZ 85287 USA
[2]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095, USA and Institute for Human Genetics, University of California, San Francisco, CA 94143, USA (present address).
[3]School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931 USA.

**Keywords**: immigration, inbreeding, inbreeding depression, pedigree, runs of homozygosity, SNPs, extinction, *Canis lupus*

**Correspondence**
Phil Hedrick, School of Life Sciences, Arizona State University, Tempe, AZ 85287 USA.
Email: philip.hedrick@asu.edu

## Abstract

Genetic factors have long been a concern in the extinction and viability of species with the short-term effects focusing on inbreeding depression. Genetic rescue has been suggested as a means to overcome the detrimental effects of inbreeding depression. However, it has been difficult to document the genetic dynamics over time of genetic rescue, inbreeding depression, and other genetic relationships in endangered species. We show here using a detailed pedigree and genomic data that genetic rescue in the gray wolf (*Canis lupus*) population on Isle Royale had only a temporary positive effect reducing inbreeding depression and then the genetic changes from the immigration event resulted in a population decline and now imminent extinction of the population. Examining the genetic details of this situation shows how genetic dynamics after the initial positive effects of genetic rescue have passed can return a small population to a path toward extinction. Thus, the successful conservation of critically small populations would likely depend on alleviating the cause of having become critically small, such as habitat restoration, or periodic re-application of genetic rescue in a manner that does not result in negative genetic dynamics.

## Introduction

For nearly four decades, conservation biologists have been concerned with genetic impacts on the extinction of populations and species (Soule & Wilcox, 1980). These concerns focused on two main genetic issues, a short-term concern of avoiding inbreeding depression and a long-term concern of maintaining genetic variation so that future adaptation would be possible (Franklin, 1980).

There is substantial and increasing evidence of inbreeding depression, that is, inbred individuals have lowered fitness compared to non-inbred individuals (Hedrick & Garcia-Dorado, 2016). The increase in documented cases of large inbreeding depression appears partly due to the examination of other fitness components besides viability, such as fecundity and mating success, and partly due to measuring inbreeding depression in more natural environments. In addition, new genomic approaches to estimate inbreeding have provided greater resolution for quantifying inbreeding, facilitating studies of inbreeding depression in the wild (Kardos *et al.*, 2016). For example in red deer (*Cervus elaphus*), given an inbreeding level equivalent to that in progeny from a mating between half sibs, the predicted lifetime breeding success was only about 10% that when there was no inbreeding (Huisman *et al.*, 2016).

One approach to overcome the impact of inbreeding depression is genetic rescue (Tallmon *et al.*, 2004; Whiteley *et al.*, 2015), that is, the introduction of genetic variation from unrelated individuals to an inbred population. Hedrick & Fredrickson (2010) provided guidelines about when genetic rescue might be beneficial. They also suggested that the effects of genetic rescue could be short-lived and in some cases even result in genetic swamping of the target population by ancestry from the immigrants potentially resulting in subsequent low effective population size. These guidelines were primarily developed for endangered species like the Mexican wolves (*C. l. baileyi*) and Florida panthers (*Puma concolor coryi*) when potentially different management options, such as releases from captivity and translocation, are available. However, they are also useful to provide an understanding when genetic rescue occurs because of natural migration as in the Isle Royale wolf population.

Recently, examples of genetic rescue have been documented in organisms as diverse as butterflies (Roitman *et al.*, 2017), dogs (Stronen *et al.*, 2017), marsupials (Weeks *et al.*, 2017), pines (Hamilton *et al.*, 2017), fish (Robinson *et al.*, 2017), and rodents (La Haye *et al.*, 2017). In addition, meta-analysis has suggested that genetic rescue provided benefits in a very high proportion of the cases examined (Frankham, 2015) and that these benefits persist for several generations (Frankham, 2016).

In spite of these developments, the connection that genetics is a primary cause of extinction in wild populations is generally difficult to document and even downplayed by some conservation biologists. However, if there is detailed genetic information about the relationships of individuals in a wild population, then documentation of genetic problems is potentially possible. In addition, recent developments in genomic analysis now provide insight into genetic changes that were previously not known (Kardos *et al.*, 2018). Here we present and synthesize current genetic data on the gray wolf population in Isle Royale National Park, Michigan, USA in an effort to understand its imminent extinction using both pedigree and genomic data.

## Methods

## Background on the Isle Royale Wolf Population

Isle Royale National Park is on an island in Lake Superior about 20 km from the nearest mainland point in Ontario, Canada. The wolf population there was founded about 1950, probably by two (or three) wolves from the mainland population in Ontario, Canada and Minnesota, USA (Adams *et al.*, 2011). For 60 years, the Isle Royale wolf population and the population of moose, their main prey, have been monitored and studied (Peterson *et al.*, 1998; Peterson & Vucetich, 2016). It has been generally assumed that the Isle Royale wolf population during this period was nearly or completely isolated from the mainland population. However, there is evidence that additional immigration to Isle Royale has periodically taken place from the mainland population (Hedrick *et al.*, 2014).

Most importantly, it was discovered in 2008 from genetic examination of scats that a male wolf known as M93 (M indicates male), or Old Grey Guy, migrated to Isle Royale from nearby mainland Ontario, Canada, probably in 1997, across the ice bridge present that year (Adams *et al.*, 2011). He was behaviorally dominant over resident wolves, mated with a native female for several years, and their descendants quickly dominated the genetic ancestry of the population. This genetic rescue event plausibly prevented the population from going extinct in the late 1990s and early 2000s because it occurred when wolf demography was poor due to inbreeding and a collapse in the moose population food supply.

However, in the past few years the numbers of Isle Royale wolves have dramatically declined from 19 in 2010 to only two from 2016 to 2018. These last two wolves are closely related and do not appear reproductive. In the five years during which these two wolves have been together, they only produced a single pup that died in less than a year. The physical appearance of the offspring was aberrant (Vucetich & Peterson 2015) and its expected inbreeding coefficient was 0.438 (Hedrick *et al.*, 2017).

Figure 1 is a photo of the two wolves observed in 2017, the adult female F193 below and the adult male M183 above. This photo shows the female snarling at the male and other photos taken over an hour of observation also show intense aggressive displays by the female with no change in her receptivity. This behavior strongly suggests that the female would not mate with this male. Given the history and circumstances of this pair, there is no reason to expect them to successfully reproduce before they die. The decline, and now imminent extinction, of the Isle Royale wolf population provides an example of how genetic changes can result in extinction of a population.

[insert Figure 1]

## Molecular techniques and pedigree construction

We collected samples of DNA from blood from wolves that were live-captured and radio-collared, skeletal remains, and fecal samples at sites where wolves had fed on moose carcasses (the same protocol and microsatellite loci used by Adams *et al.*, 2011 were used here). The microsatellite genotype from each fecal sample was assigned to one of the wolf packs, based upon the pack territory where the kill site was located and the genotypes of other wolves in the pack detected at the same kill site. We determined the genetic identity of alpha wolves from direct observations and genetic exclusion. For example, alpha wolves can be identified in the field by their behavioral interactions with subordinate wolves. The genetic identity of some alpha wolves was determined when a sample of their feces was collected immediately after observing them defecate (see Adams et al. 2011 for more details). All family relationships assigned from field observations were tested genetically using exclusion.

We used the microsatellite genotypes and field observations to construct a pedigree of the Isle Royale wolf population for the years 1998-2018. We also determined, by direct observation of movement and behavior, territorial boundaries of the packs during these aerial surveys. The accuracy of observed numbers of genotypes representing offspring in each pack for each year was checked by comparing those numbers with the number of offspring observed during winter field season. All pedigree relationships assigned from field observations were confirmed using genetic exclusion. The pedigree was trimmed here to show primarily only the wolves from which the population is believed to have descended since the late 1990s and the two remaining wolves.

Using the relationship,

$$f = \frac{H_0 - H_t}{H_0} \tag{1}$$

where $H_0$ is the heterozygosity from the ancestral Minnesota-Canadian population and $H_t$ is the heterozygosity in the Isle Royale population, this expression gives an estimate of inbreeding $f$ resulting from genetic drift (e.g. Hedrick, 2011).

Estimates of genome-wide heterozygosity and $F_{ROH}$ were derived from whole genome sequences generated by Robinson et al. (2018). Briefly, DNA from Isle Royale wolves was extracted from blood samples archived at Michigan Technological University and sequenced on the Illumina HiSeq 4000, generating paired reads 100 base pairs in length. The pipeline used to convert raw sequence data into high quality genotypes is described in more detail in Robinson *et al.* (2018). Briefly, raw reads were aligned to the domestic dog genome using bwa MEM (Li, 2013) before removal of PCR duplicates and low quality reads. Base quality score recalibration and genotyping were performed with the Genome Analysis Toolkit (GATK, McKenna *et al.*, 2010) and genotypes were filtered for quality and depth, leaving only high quality biallelic SNPs.

Genomic heterozygosity was defined as the total number of heterozygous genotypes divided by the total number of called genotypes. Runs of homozygosity (ROH) were identified using VCFtools (Danecek *et al.*, 2011) and ROH spanning regions with fewer than 50 variant sites were excluded. The proportion of the genome that consists of ROH can then be estimated, giving another measure of inbreeding, $F_{ROH}$.

## Results

### Before immigration

Genetic variation in Isle Royale wolves before the immigration of M93 was estimated using both microsatellite loci and genomic SNP markers. First, the mean observed microsatellite heterozygosity for 20 wolves on Isle Royale before 1998 (0.430) was much lower than the mean observed heterozygosity for 35 mainland wolves from near the Minnesota–Canadian border (0.634), which represents the source population of Isle Royale wolves (Hedrick *et al.*, 2014)(Table 1). From equation (1), the estimate of the inbreeding coefficient is $f$ = 0.322 (Table 1). In other words, the significant loss in heterozygosity from the source population resulted in the sizable inbreeding level of 0.322, indicating that the population was quite inbred before M93 immigrated.

[insert Table 1]

Similarly, the mean per-site heterozygosity from the Minnesota-Canadian population is 0.00160 and is significantly higher than that observed in the Isle Royale population in the animals born before 1998 of 0.00116 (Table 1)(Robinson *et al*., 2018). Using these values from genomic markers and expression (1), then $f = 0.275$, further evidence that genetic drift had a quite high impact genetic variation and the inbreeding estimate.

Also using SNPs, the proportion of the genome that consists of ROH can be estimated, giving another measure of the inbreeding, $F_{ROH}$. In this case, $F_{ROH} = 0.158$ in the Minnesota-Canada population and $F_{ROH} = 0.370$ in the Isle Royale population before 1998 (Robinson *et al*., 2018). The relatively high value in the Minnesota-Canada is influenced by a high value for one of the wolves sampled, perhaps suggesting that she had some history of recent inbreeding. The difference between $F_{ROH}$ for the Isle Royale population and putative ancestral population of 0.212 gives a general estimate of the inbreeding accumulated on Isle Royale before 1998. These three different high estimates of inbreeding before the immigration suggests that the population fitness was low and that the population was a good candidate for genetic rescue but also susceptible to a genomic sweep.

Another indication of the low fitness in the Isle Royale wolf population, presumably the result of inbreeding depression, is the high rate of individuals with bone malformations (58%) (Räikkönen *et al*., 2009; see also Robinson *et al.*, 2018), a level that has increased over time. For comparison, the incidence of similar malformations in outbred wolf populations in historic Scandinavia was 0%, contemporary Finland was 1%, and only 10% in modern inbred Scandinavian wolves (Räikkönen *et al.*, 2009). The fitness impacts of these malformations are not clear, but in dogs they have been implicated in quite debilitating syndromes (Morgan *et al.*, 1993).

## After immigration

After M93 immigrated on to the island in 1997, his genetic dominance emerged very quickly. To understand the genetic dynamics of this change, the pedigree in Figure 2 can be used which shows the two remaining wolves, M183 and F193, as shaded and their known ancestors, M93, F99, and F67.

[insert Figure 2]

This successful reproduction of M93 resulted in a  rapid increase in the proportion of genetic ancestry from him and a  “genomic sweep” where the proportion of all genes in the population that can be traced back to him increased quickly to an expected value of 59.4% of the population in 2008 (Hedrick *et al.*, 2014). From 2005 on, all the ancestry in the Isle Royale population has been descended from only three individuals; the male immigrant M93, F99, and F67, another female population resident. In other words, genetic rescue had a strong beneficial influence for about a decade (about 2.5 wolf generations) after the arrival of M93, followed by a return of genetic problems attributable this time to the reduction of diversity in the gene pool due to the elimination of ancestry from other individuals except his initial mate and one other female.

A major factor causing the very high proportion of M93 ancestry was the result of him, after his first mate F99 died, mating with his daughter F58. This father-daughter mating produced 21 progeny total in five litters from 2003 to 2007. Ordinarily only 50% of the ancestry in progeny would be from one parent of a pair but in this case, any progeny from the father-daughter mating would be expected to have 75% of their ancestry from M93. In 2008, 9 of 24 individuals were progeny from the father-daughter mating, resulting in the particularly high M93 ancestry that year.

After the immigration of M93, the level of inbreeding plummeted (Fig. 2a of Adams *et al.*, 2011) and then rose quickly from  2003 to 2012 (Fig. 3 of Hedrick *et al.*, 2014).  This was mainly due to inbreeding from M93 and again from the large number of progeny from the mating of M93 with his daughter F58 that resulted in identity-by-descent from M93 but not from the founder F99. For example, in 2009, 76% of the inbreeding in the population was from M93 (see Fig. 3 in Hedrick *et al.*, 2014).

After this, the most striking change was that by 2012 none of the 21 descendants of the father-daughter mating were alive and none of them had any surviving descendants. As a result, both the ancestry and inbreeding from M93 greatly declined. Presumably, this change was the result of lower fitness of these descendants because they were homozygous due to inbreeding for detrimental variation originally brought into the population by M93. The loss of these 21 individuals also greatly reduced the population size.

Much of the decline in overall inbreeding level from 0.230 in 2009 to 0.140 in 2013 is explained by the death of nine wolves with an inbreeding coefficient of 0.375, the result of two consecutive generations of close (first-degree) inbreeding (Hedrick *et al.*, 2014). All of these wolves had short lifespans (mean of 2.33 years compared to about 6 years for other unexploited wolf populations as indicated by the data in Fuller et al. 2003) and all of them had died by 2011. Specifically, of the wolves recruited into the pedigree between 2009 and 2011, there is a two-fold difference in inbreeding between the seven alive in 2012 (0.152) and the six not alive in 2012 (0.292). None of these highly inbred wolves reproduced.

In the last few years, the population numbers of Isle Royale wolves have declined dramatically and there are only two wolves remaining, a male (M183) and a female (F193), in early 2018. These two adults are very closely related and are both father and daughter and half siblings because they have the same mother F160. They are in fact the most closely related pair of the four males and four females that were present in the population in 2013 (Hedrick *et al.*, 2014). The expected inbreeding coefficient of an offspring from them was the highest of any possible pair at 0.438 and the expected relatedness between them is 0.734 (Hedrick *et al.*, 2017). Reflecting the reduction in M93 ancestry from the peak discussed above, the expected M93 ancestry for M183 is 0.375 and for F193 is 0.3125.

## Discussion

### Genetics of the Isle Royale wolf population

The decline of the Isle Royale wolf population, and now for all intents and purposes its imminent extinction, provides a detailed case study of how genetic changes can result in the extinction of a population. Because of the detailed examination of the Isle Royale wolf population, important genetic factors resulting in its imminent extinction have been documented and discussed here. First, because of the relative isolation of the population from immigrants and its relative small size, the amount of genetic variation was significantly reduced compared to its source population and estimates of inbreeding levels were large, $f = 0.322$ from microsatellites, and $f = 0.278$ and $F_{ROH} = 0.370$ from two approaches using estimates from genomic data. In addition, the documented very high rate of bone malformations indicated inbreeding depression.

Second, this lowered fitness contributed to the great genetic success of male M93 who migrated in to the population in 1997. His immigration resulted in a short-lived genetic rescue and then in a genomic sweep in which in 2008 his ancestry was 59.4% of the population. Finally, this great genetic success resulted in a substantial cost because he produced 21 inbred progeny with a daughter, all of whom died without contributing any surviving descendants. This, and other close inbreeding, resulted in individuals with low fitness and a subsequent decline in the population size. In other words, a series of genetically related events likely ultimately resulted in a great reduction in the Isle Royale wolf population and now its imminent extinction.

Further, the initial progeny from M93 and his mate F99, such as his daughter F58, might have had higher fitness than other wolves on Isle Royale because some recessive detrimental alleles accumulated in the Isle Royale population were covered up as heterozygotes in these initial offspring. In fact, M93 mated with daughter F58 even though unrelated females were present in the population, suggesting that she was more fit than the other resident females without M93 ancestry. The success of these offspring could have initially increased the frequency of detrimental variants brought in by M93 but then with inbreeding these detrimental alleles were subsequently expressed as homozygotes and resulted in lowered fitness. In sum, the genetic benefit of M93 was dramatic but short-lived.

The putative pup of M183 and F193 seen in 2015, had an expected inbreeding coefficient of 0.438, but its actual inbreeding coefficient could have been considerably higher (or lower) because of the large 95% confidence limits around this pedigree estimate (0.311 to 0.565) (Hedrick *et al.*, 2017). This pup had an abnormal phenotypic appearance with a quite unusual, short tightly curled tail, appeared to have an unusual posture, and was relatively small. Further, field observations suggest that this offspring was short lived and died as a pup (Peterson & Vucetich, 2016). These malformations and the pup's short life suggest the negative impact of inbreeding depression on its phenotype and survival.

Genomic estimates of inbreeding based on analysis of runs of homozygosity identified with many thousands of SNPs are expected to be higher than inbreeding predicted from our pedigree because of common ancestry of founders F99 and F67 in resident ancestors that are not included in the pedigree. For example, for the seven animals in which there is both a pedigree estimate of inbreeding $f$ and $F_{ROH}$, the mean $f$ is 0.091 and the mean $F_{ROH}$ is 0.332 (Robinson *et al.*, 2018). The difference, 0.241, gives an estimate of the increase expected in inbreeding from unknown identity-by-descent found by genomic analysis over that predicted from the known pedigree. In other words, pedigree-based measures of inbreeding can provide basic guideposts, but realized values are likely higher due to unknown common ancestry.

## General relevance

It has been generally difficult to document the impact of genetic factors on extinction in natural populations. However, endangered species often face significant genetically related threats, including lowered fitness due to loss of genetic variation, increase of detrimental variants, and inbreeding. One way to overcome the impact of lowered fitness is genetic rescue in which the natural or artificial introduction of individuals from outside the population provides genetic variation that subsequently results in higher fitness (Tallmon *et al.*, 2004; Whitely *et al.*, 2015; Hedrick & Garcia-Dorado, 2016). Because many populations have become small and isolated in recent decades due to human impacts, genetic rescue is likely to become a management action or natural event of great importance for rare or endangered species in the future. Also because many of the known examples are from recent years, the longer term impact of genetic rescue has not been documented or examined.

There have been a number of important cases where genetic rescue has resulted in substantial population recovery. In most of the well-known examples of genetic rescue, the population numbers initially increased and in general the populations appear to have higher viability than before the natural or artificial genetic rescue but other factors now appear to be limiting population numbers in some cases. For example, the translocations of prairie chickens (Westemeier *et al.*, 1998) appears to have been effective in increasing fitness and genetic variation and the population initially increased. However, the population is now limited by suitable habitat (Bouzat *et al.*, 2009) and the outlook is not as good. In the isolated Swedish population of adders (Madsen *et al.*, 1999), translocation initially resulted in enhanced population growth (Madsen *et al.*, 2004). However, the construction of human obstacles, a house and a brick wall, have dramatically reduced population numbers (Madsen & Ujvari, 2011). Genetic rescue in the population of bighorn sheep living in the National Bison Range resulted in significant population increase (Hogg *et al.,* 2006) and recent genomic analysis has examined what loci might have been involved (Miller *et al.*, 2012) although recently up to 70% of the population has been lost due to pneumonia (Heyler 2018).

The introduction of Texas pumas into the Florida panther population resulted in dramatic population recovery (Hostetler *et al.*, 2010; Johnson *et al.*, 2010) although now the population appears potentially limited by available habitat and human-caused mortality. The outcome of the Florida panther genetic rescue might also be threatened by inbreeding and low effective population size in current and future generations (Hedrick, 2010). The natural introduction of one male wolf initially had important beneficial effects in Scandinavian wolves (Vilà *et al.*, 2003) although a subsequent analysis documented significant inbreeding depression (Liberg *et al.*, 2005). Since then, two male wolves naturally migrated into the population and three wolves were translocated into the population from northern Sweden, resulting in both a lower inbreeding coefficient and higher reproductive success (O. Liberg, personal communication). Detailed examination of genetic rescue in Scandinavian wolves demonstrated that offspring of immigrants had higher breeding success than resident inbred individuals (Akesson *et al.,* 2016). Crosses between lineages in Mexican wolves resulted in higher fitness (Fredrickson *et al.*, 2007), numbers in the reintroduced population increased, but have plateaued in recent years. As yet there has been no detailed analysis determining how much genetic rescue or other factors, such as supplemental feeding of denning females, influenced this initial population increase and recent plateau.

The general positive evaluation of genetic rescue, along with the increasing likelihood of lowered fitness in small, isolated populations make detailed evaluation of genetic rescue particularly important. Only in populations where individuals are identified and their genetic relationship to others in the population is known can the reasons for the success or failure of genetic rescue be evaluated in detail. Two such examples besides the Isle Royale wolf population are that of the translocation of Texas pumas in to the Florida panther population and the natural immigration of wolves from the north into the Scandinavian wolf population. In both of these populations continued monitoring of the genetic success or failure of individuals with known relationships appears fundamental to understanding the outcome of genetic rescue.

The discussion of the genetic impacts on extinction in Isle Royale wolves illustrates a potential problem relying on a single genetic rescue event as an approach to overcoming inbreeding depression. Moreover, persistence of the Isle Royale population throughout its seven-decade existence was likely supported by multiple genetic rescue events (Hedrick *et al.*, 2014). In other words, although genetic rescue can alleviate problems associated with inbreeding depression over the short term, it can also generate problems itself, ones that in the case of the Isle Royale wolf population has resulted in its imminent extinction. Thus, the successful conservation of critically small populations would likely depend on alleviating the cause of having become critically small, such as habitat restoration, or periodic re-application of genetic rescue. The case study reported here is particularly significant because few documented instances of genetic rescue and the ensuing changes have been observed long enough or in sufficient detail to understand both the beneficial and detrimental effects of genetic rescue. Clearly, many additional case studies will be required before an overall perspective is developed.

During the final review of this manuscript, the National Park Service has begun relocating wolves from the mainland to Isle Royale. Their plan allows for translocating up to 20 to 30 wolves from the mainland over the next three to five years to re-establish a breeding wolf population on Isle Royale. At this point, it is not clear how, or if, the translocated wolves will interact with the two remaining closely related wolves.

## Acknowledgements

We appreciate the suggestion to write this article by Erik Olson and comments by Chris Funk, Bob Wayne, and several anonymour reviewers. We acknowledge support from the National Science Foundation (DEB-1453041), National Institute of Health (R35GM119856), National Park Service (CESU Task Agreement No. P11AC90808), National Geographic Society and a McIntyre-Stennis Grant (USDA-Nifa #1004363). R.O.P thanks the Robbins Chair in Sustainable Management of the Environment for partial support.

## References

Adams, J.R.,Vucetich, L.M., Hedrick, P.W., Peterson, R.O. & Vucetich, J.A.  (2011). Genetic rescue and genomic sweep of the wolves of Isle Royale. *Proc. R. Soc. Biol*. **278**, 3336-3344.

Akesson, M., Liberg, O., Sand, H.A., Wabakken, P., Bensch, S. & Flagstad, Ø. (2016). Genetic rescue in a severely inbred wolf population. *Molec. Ecol*. **25**, 4745–4756.

Bouzat, J.L., Johnson, J.A., Toepfer, J.E., Simpson, S.A., Esker, T.L. &Westemeier, R.L. (2009). Beyond the beneficial effects of translocations as an effective tool for the genetic restoration of isolated populations. *Cons, Genet*. **10**, 191–201.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T. & McVean, G. (2011). The variant call format and VCFtools. *Bioinformatics* **27**, 2156-2158.

Frankham, R. (2015). Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. *Molec. Ecol*. **24**, 2610–2618.

Frankham, R. (2016). Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. *Biol. Cons*. **195,** 33–36.

Franklin, I.R. (1980). Evolutionary change in small populations. Pages 135-149 in Soule ME, Wilcox BA, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland MA.

Fredrickson, R.J., Siminski, P., Woolf, M. & Hedrick, P.W. (2007). Genetic rescue and inbreeding depression in Mexican wolves. *Proc. R. Soc. Biol*. **274**, 2365–2371.

Fuller T.K., Mech L.D., & Cochrane J.F. (2003) Wolf population dynamics. Pages 161-191 in Mech LD, Boitani L, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago.

Hamilton, J.A., Royauté, R., Wright, J.W., Hodgskiss, P. &Ledig, F.T. (2017). Genetic conservation and management of the California endemic, Torrey pine (*Pinus torreyana* Parry): Implications of genetic rescue in a genetically depauperate species. *Ecol. Evol*. **7**, 7370–7381.

Hedrick, P.W. (2010). Genetic future for Florida panthers. *Science* **330**, 1744.

Hedrick, P.W. (2011). Genetics of populations (4th Edition). Jones and Bartlett, Boston.

Hedrick, P.W. & Fredrickson, R.J. (2010). Genetic rescue: guidelines an example from Mexican wolves and Florida panthers. *Cons. Genet*. **11**, 615-626.

Hedrick, P.W. & Garcia-Dorado, A. (2016). Understanding inbreeding depression, purging, and genetic rescue. *Trends Ecol. Evol*. **31**, 940-952.

Hedrick, P.W., Peterson, R.O., Vucetich, L.M, Adams, J.R. & Vucetich, J.A. (2014). Genetic rescue in Isle Royale wolves: genetic rescue and the collapse of the population. *Cons. Genet.* **15**, 1111-1121.

Hedrick, P.W., Kardos, M., Vucetich, L., Peterson, R. & Vucetich, J. (2017). Genomic variation of inbreeding and ancestry in the remaining two Isle Royale wolves. *J. Hered*. **108**, 120-126.

Heyler, P.B. 2018. National Bison Range in serious peril (August 24, 2018). *Missoulian*, Missoula, Montana.

Hogg, J.T., Forbes, S.H., Steele, B.M. & Luikart, G. (2006). Genetic rescue of an insular population of large mammals. *Proc. R. Soc. Biol.* **273**, 1491–1499.

Hostetler, J.A., Onorato, D.P., Nichols, J.D., Johnson, W.E., Roelke, M.E., O'Brien, S.J., Jansen, D. & Oki, M.K. (2010). Genetic introgression and the survival of Florida panther kittens. *Biol. Cons*. **143**, 2789–2796.

Huisman, J., Kruuk, L.E.B., Ellis, P.A., Clutton-Brock, T. & Pemberton, J.M. (2016). Inbreeding depression across the lifespan in a wild mammal population. *Proc. Nat. Acad. Sci. USA*. **113**, 3585–3590.

Johnson, W.E., Onorato, D.P., Roelke, M.E., Land, E.D., Cunnningham, M., Beldon, R.C., McBride, R., Jansen, D., Lotz, M., Shindle, D., Howard, J., Wildt, D.E., Penfold, L.M., Hostetler, J.A., Oli, M.K. & O'Brien, S.J. (2010). Genetic restoration of the Florida panther. *Science* **329**, 1641–1645.

Kardos, M., Taylor, H.R., Ellegren, H., Luikart, G. & Allendorf, F.W. (2016). Genomics advances the study of inbreeding depression in the wild. *Evol. Appl.* **9**, 1205-1218.

Kardos, M., Äkesson, M., Fountain, T., Flagstad, Ø., Liberg, O., Olason, O., Sand, H., Wabakken, P., Wikenros, C. & Ellegren, H. (2018). Genomic consequences of intensive inbreeding in an isolated wolf population. *Nat. Ecol. Evol*. **2**, 124-131.

La Haye, M.J.J., Reiners, T.E., Raedts, R., Verbist, V. & Koelewijn, H.P. (2017). Genetic monitoring to evaluate reintroduction attempts of a highly endangered rodent. *Cons. Genet*. **18**, 877–892.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv preprint arXiv*:1303.3997.

Liberg, O., Andrén, H., Pederson, H.-C., Sand, H., Sejbeg, D., Wabakken, P., Åkesson, M. & Bensch, S. (2005). Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biol. Letters* **1**, 17–20.

Madsen, T., Shine, R., Olsson, M. & Wittzell, H. (1999). Restoration of an inbred adder population. *Nature* **402**, 34–35.

Madsen, T., Ujvari, B. & Olsson, M. (2004). Novel genes continue to enhance population growth in adders (Vipera berus). *Biol. Cons.* **120**, 145–147.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M. & DePristo, M.A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. **20**, 1297-1303.

Miller, J.M., Poissant, J., Hogg, J.T. & Coltman, D.W. (2012). Genomic consequences of genetic rescue in an insular population of bighorn sheep (*Ovis canadensis*). *Molec. Ecol*. **21**, 1583-1596.

Morgan, J.P., Bahr, A., Franti, C.E. & Bailey, C.S. (1993). Lumbosacral transitional vertebrae as a predisposing cause of cauda equina syndrome in German shepherd dogs: 161 cases (1987–1990). *J. Amer. Vet. Med. Assoc*. **202**, 1877–1882.

Peterson, R.O., Thomas, N.J., Thurber, J.M,, Vucetich, J.A. & Waite, T.A. (1998). Population limitation and the wolves of Isle Royale. *J. Mammal.* **79**, 828–841.

Peterson, R.O. & Vucetich, J.A. (2016). Ecological studies of wolves on Isle Royale. Annual Report 2015-2016. Michigan Technological University. Houghton, MI.

Räikkönen, J., Vucetich, J.A., Peterson, R.O, & Nelson, M.P. (2009). Congenital bone deformities and the inbred wolves (*Canis lupus*) of Isle Royale. *Biol. Cons*. **142**, 1025-1031.

Robinson, J.A., Ortega-Del Vecchyo, D., Fan, Z., Kim, B.Y., Marsden, C.D., Lohmueller, K.E. & Wayne, R.K. (2016). Genomic flatlining in the endangered island fox. *Curr. Biol*. **26**, 1183-1189.

Robinson, J.A., Räikkönen, J., Vucetich, L.M., Vucetich, J.A., Peterson, R.O., Lohmueller, K.E. & Wayne, R.K. (2018). Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. bioRxiv, 440511. doi:10.1101/440511.

Robinson, Z.L., Coombs, J.A., Hudy, M., Nislow, K.H., Letcher, B.H. & Whiteley, A.R. (2017). Experimental test of genetic rescue in isolated populations of brook trout. *Molec. Ecol*. **26**, 4418–4433.

Roitman, M., Gardner, M.G., New, T.R., Nguyen, T.T.T., Roycroft, E.J., Sunnucks, P., Yen, A.L. & Harrisson, K.A. (2017). Assessing the scope for genetic rescue of an endangered butterfly: the case of the Eltham copper. *Insect Cons. Diver*. **10**, 399-414.

Soule, M.E. & Wilcox, B.A. (eds.). (1980). Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland MA.

Stronen, A.V., Salmela, E., Baldursdóttir, B.K., Berg, P., Espelien, I.S., Järvi, K., Jensen, H., Kristensen, T.N., Melis, C., Manenti, T., Lohi, H. & Pertoldi, C. (2017). Genetic rescue of an endangered domestic animal through outcrossing with closely related breeds: A case study of the Norwegian Lundehund. *PloS ONE* **12**(6), e0177429.

Tallmon, D.A., Luikart, G. & Waples, R. (2004). The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol*. **19**, 489-496.

Vilà, C., Sundqvist, A.-K., Flagstad, Ø., Seddon, J., Björnerfledt, S., Kojola, I., Casulli, A., Sand, H., Wabakken, P. & Ellegren, H. (2003). Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proc. R. Soc. Biol*. **270**, 91–97.

Vucetich, J.A. & Peterson, R.O. (2015). Ecological Studies of Wolves on Isle Royale, Annual Report 2014–2015. Michigan Technological University, Houghton, MI.

Weeks, A.R., Heinze, D., Perrin, L., Stoklosa, J., Hoffmann, A.A., van Rooyen, A., Kelly, T., Mansergh, I. (2017). Genetic rescue increases fitness and aids rapid recovery of an endangered marsupial population. *Nature Commun*. **8**, 1071.

Westemeier, R.L., Brawn, L.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L. & Paige, K.N. (1998). Tracking the long-term decline and recovery of an isolated population. *Science* **282**, 1695–1698.

Whiteley, A.R., Fitzpatrick, S.W., Funk W.C. & Tallmon, D.A. (2015). Genetic rescue to the rescue. *Trends Ecol. Evol*. **30**, 42-49.

**Table 1.** The heterozygosity of microsatellites and SNPs in a sample of wolves from Minnesota and from Isle Royale before 1998 and the estimated inbreeding coefficient *f* before 1998 and on the bottom row is the estimated inbreeding coefficient from ROH (runs of homozygosity) for these two groups and difference between them in the rightmost numerical column.

| | Minnesota | Before 1998 | *f* |
|---|---|---|---|
| Heterozygosity* (microsatellites) | 0.634 | 0.430 | 0.322 |
| Heterozygosity** (SNPs) | 0.00160 | 0.00116 | 0.278 |
| $F_{ROH}$ ** | 0.158 | 0.370 | 0.212*** |

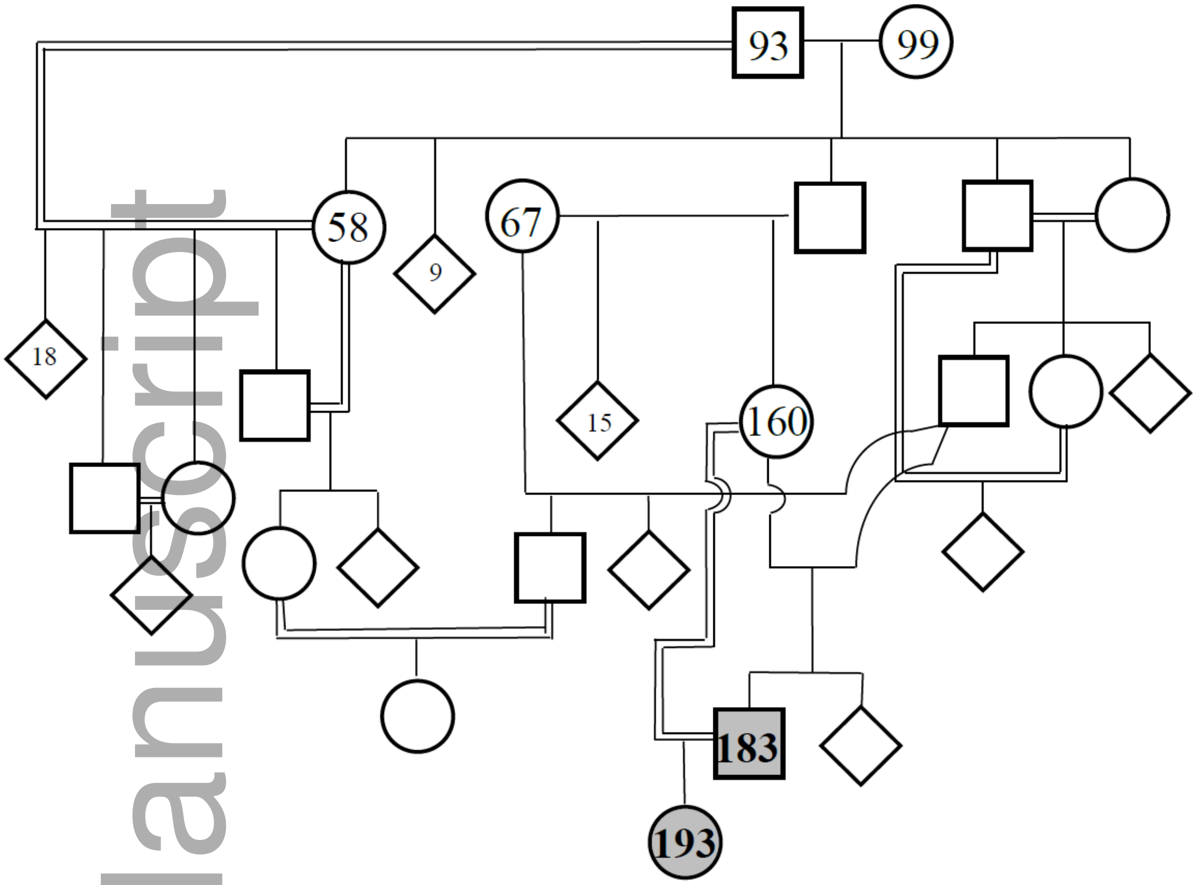
*These values are from Hedrick et al. (2014)

**These values are calculated from the raw data in Robinson *et al*. (2018).

***This *f* value is the difference in $F_{ROH}$ between the Isle Royale sample prior to 1998 and the Minnesota sample.

**Figure 1** Photo of the two remaining wolves on Isle Royale observed in 2017 with the female F193 below snarling at the male M183 above in response to his courtship advances.

**Figure 2** Pedigree showing the remaining two wolves, M183 and F193, as shaded and their known ancestors, M93, F99, and F67, in the Isle Royale population. Double lines indicate matings between relatives, squares indicate males, and circles indicate females. Notice that F160 is the mother of M183 and F193 and that M183 is also the father of F193. Diamonds indicate multiple progeny, for example, the diamond on the left indicates 18 of the progeny from M93 and his daughter F58.


93
99
58
9
67
18
15
160
183
193

biology
letters

# Severe inbreeding depression in a wild wolf *Canis lupus* population

Olof Liberg, Henrik Andrén, Hans-Christian Pedersen, Håkan Sand, Douglas Sejberg, Petter Wabakken, Mikael Åkesson and Staffan Bensch

*Biol. Lett.* 2005 **1**, 17-20
doi: 10.1098/rsbl.2004.0266

biology
letters

*Biol. Lett.* (xxxx)
doi:10.1098/rsbl.2004.0266

# Severe inbreeding depression in a wild wolf *Canis lupus* population

**Olof Liberg[1,*], Henrik Andrén[1], Hans-Christian Pedersen[2], Håkan Sand[1], Douglas Sejberg[3], Petter Wabakken[4], Mikael Åkesson[3] and Staffan Bensch[3]**

[1]*Grimsö Wildlife Research Station, Department of Conservation Biology, Swedish University of Agricultural Science, SE-73091 Riddarhyttan, Sweden*
[2]*Norwegian Institute for Nature Research, Tungasletta 2, NO-7485 Trondheim, Norway*
[3]*Department of Ecology, University of Lund, SE-22362 Lund, Sweden*
[4]*Hedmark University College, Faculty of Forestry and Wildlife Management, N-2480 Koppang, Norway*
**Author for correspondence* (*olof.liberg@nvb.slu.se*)

**The difficulty of obtaining pedigrees for wild populations has hampered the possibility of demonstrating inbreeding depression in nature. In a small, naturally restored, wild population of grey wolves in Scandinavia, founded in 1983, we constructed a pedigree for 24 of the 28 breeding pairs established in the period 1983–2002. Ancestry for the breeding animals was determined through a combination of field data (snow tracking and radio telemetry) and DNA microsatellite analysis. The population was founded by only three individuals. The inbreeding coefficient $F$ varied between 0.00 and 0.41 for wolves born during the study period. The number of surviving pups per litter during their first winter after birth was strongly correlated with inbreeding coefficients of pups ($R^2 = 0.39$, $p < 0.001$). This inbreeding depression was recalculated to match standard estimates of lethal equivalents (2B), corresponding to 6.04 (2.58–9.48, 95% CI) litter-size-reducing equivalents in this wolf population.**

**Keywords:** inbreeding depression; lethal equivalents; pedigree; conservation biology; wolf

## 1. INTRODUCTION

Inbreeding depression is assumed to be a serious problem for the conservation of small populations (Gilpin & Soulé 1986), but has been difficult to demonstrate in nature (Caughley 1994). The main obstacle has been the construction of pedigrees necessary for calculating inbreeding coefficients. Recently, modern molecular techniques have allowed indirect genetic measurement of inbreeding depression in wild animals, including mammals (e.g. Coltman *et al.* 1999; Slate *et al.* 2000). Inbreeding measured as genetic similarity between individuals does not directly translate to inbreeding coefficients. This often prevents one from establishing the level of inbreeding responsible for the decreased fitness, as well as direct comparisons with other studies. These problems are circumvented when using pedigree analysis; however, this has rarely been done in wild populations, with some notable exceptions (e.g. Keller 1998; Loeske *et al.* 2002). By combining DNA techniques with ecological field data, we have constructed a complete pedigree and demonstrated severe inbreeding depression in the wild Scandinavian wolf, *Canis lupus*, population. The wolf became extinct in Scandinavia (Norway and Sweden) at the end of 1960s. Around 1980, at least two wolves immigrated and founded a new population in south-central Scandinavia, 900 km from the edge of the large Finnish/Russian source population (Wabakken *et al.* 2001; Vila *et al.* 2003). The first reproduction occurred in 1983, and by 2002 the population included approximately 100 wolves.

## 2. MATERIALS AND METHODS

### (a) *Field data*

The wolf population has been monitored since 1980, based on snow tracking and, from 1998, also on radio telemetry. Territorial pairs were distinguished and the number of animals in packs counted (Wabakken *et al.* 2001). A 'pair' is two breeding adults producing offspring together, while a 'pack' is the total number of individuals in a family, for example, the pair and its dependent offspring. The 'territory' is the geographical area where the pair is living. As a fitness measure, we used the number of pups per litter surviving until the first winter after birth ('winter litter size'). We used data for first-born litters of each breeding pair only, because for subsequent litters, tracks from pups of the year could not be separated from those of yearlings and older philopatric siblings (Mech 1970). In darted wolves, ageing was based on the growth zone in the tibia for pups and tooth wear for adults, and in retrieved dead wolves annual tooth cementum layers (C1) were counted.

### (b) *Genetic analyses*

Samples were derived from the blood of captured wolves, the muscle of dead wolves ('tissue'), from oestrus blood on snow and from scats. Genomic DNA from tissue was isolated using standard phenol/chloroform–isoamylalcohol extraction protocols. Two isolates were extracted from faecal samples with a Qiamp DNA stool mini kit (Qiagen, Valencia, CA, USA). Faecal and oestrus blood samples were extracted in a separate workspace treated with ultraviolet light to avoid contamination (Sarkar & Sommer 1990). Negative extraction controls were used throughout.

We scored tissue samples for allelic variation at 32 autosomal microsatellite loci, and faecal samples on a subset of 16 (for details see Electronic Appendix). To minimize scoring errors associated Q1 with low quality DNA (Taberlet *et al.* 1999), faecal samples were amplified a minimum of four times (twice per isolate). Heterozygotes were accepted if both alleles were present in two amplifications and homozygotes if four positive amplifications showed only one allele. If neither condition was met, samples were re-amplified. Problematic samples were amplified up to 10 times. Q2 In the few samples, where an ambiguous result still occurred, we recorded a half-locus (Miller *et al.* 2002).

The pedigree was determined by parentage analysis. We used material from 163 wolf individuals; 113 of these were based on muscle from dead wolves or blood from anaesthetized wolves, the rest from faeces and/or from oestrus blood found in snow. A missing genotype of one parent was reconstructed from genotypes of the known parent and pups of that pair. Of the 48 breeding wolves in the pedigree used in the analysis, genotypes of 16 were reconstructed, 25 were based on tissue (muscle or blood drawn directly from the animal) and seven were based on faeces/oestrus blood. The three incestuous pairs in the period 1987–1990 were completely reconstructed from genotypes of 10 wolves born during this period. Here several alternatives were possible. We chose the most parsimonious alternative, but tested all possible alternatives, and none changed the results of this study other than marginally.

---

The supplementary Electronic Appendix is available at http://dx.doi.org/10.1098/rsbl.2004.0266 or via http://www.journals.royalsoc.ac.uk.

*Received* 27 September 2004
*Accepted* 21 October 2004

Inbreeding coefficients were calculated with the software PEDIGREE VIEWER 5.0 (© Brian and Sandy Kinghorn).

### (c) *Statistical analyses*

We used parametric statistics (ANCOVA) in the analyses of inbreeding effects, including the interaction terms between the independent variables in the initial model. Ages of breeding females were treated as a two state variable: young (2–3 years) and old (4 years or older). Genetic load is expressed in terms of lethal Q3 equivalents, based on viability data (Kalinowski & Hedrick 1998). We calculated an analogous parameter, litter-reducing equivalents, by regressing litter size ($W_i$) against the inbreeding coefficient ($f_i$) using the relationship $\ln W_i = \ln W_0 - Bf_i$, where $W_0$ is the litter size for outbred litters ($f_0$). Inbreeding effects on population growth rate ($\lambda$) were tested using a Leslie matrix with five age classes. We used data from our study population for survival and reproduction, adjusted to give a baseline growth rate similar to the one observed in the period 1991–2000 of $\lambda = 1.29$ (Wabakken *et al.* 2001).

For further details on Material and methods, see online Electronic Appendix.

## 3. RESULTS AND DISCUSSION

### (a) *Pedigree, inbreeding coefficients and litter sizes*

We traced the complete ancestry for both male and female in 24 of the 28 breeding wolf pairs registered during the period 1983–2002, constructing the first complete pedigree back to its founders that has been published for a wild mammal population (Keller & Q4 Waller 2002), and calculated inbreeding coefficients ($F$; figure 1). The first founding wolf pair reproduced for 3 years, (1983–1985) until the female was shot in 1985, but offspring from this pair continued to breed within the same territory until 1994 through incestuous matings (figure 1). In 1991, an immigrant male mated with a daughter of the first breeding pair and contributed to the large variation in the inbreeding coefficient $F$ in the population (0.00–0.41). Apart from the early incestuous matings, we recorded only two later cases of full sibling pairings (pairs O and U in figure 1). Nevertheless, most animals born after 1997 have inbreeding coefficients close to or higher than 0.25, a level corresponding to full sibling mating (figure 1).

The sizes of winter litters for first breeding pairs were strongly affected by the inbreeding coefficient of the pups ($n = 24$, $R^2 = 0.39$, $p < 0.001$; figure 2), while the inbreeding coefficients of the mother (partial $R^2 = 0.04$, $p = 0.23$), of the father (partial $R^2 = 0.07$, $p = 0.11$), the age of the mother (partial $R^2 = 0.09$, $p = 0.076$) and time (partial $R^2 = 0.10$, $p = 0.058$) did not contribute significantly to the same model. After removing offspring inbreeding coefficients, there was indeed an effect of the mother's inbreeding coefficient ($n = 24$, $R^2 = 0.27$, $p = 0.01$), but not from the father's (partial $R^2 = 0.02$, $p = 0.41$), nor from age of the mother (partial $R^2 = 0.08$, $p = 0.13$) or time (partial $R^2 = 0.10$, $p = 0.075$). Inbreeding of the father ($R^2 = 0.06$, $p = 0.25$), or time ($R^2 = 0.001$, $p = 0.88$) had no effect alone. The inbreeding coefficient increased over the years for pups and mothers ($r = 0.49$, $p = 0.016$ and $r = 0.58$, $p = 0.003$).

We are confident that the demonstrated inbreeding effect was not a by-product of association with coincidental trends in the environment, for example

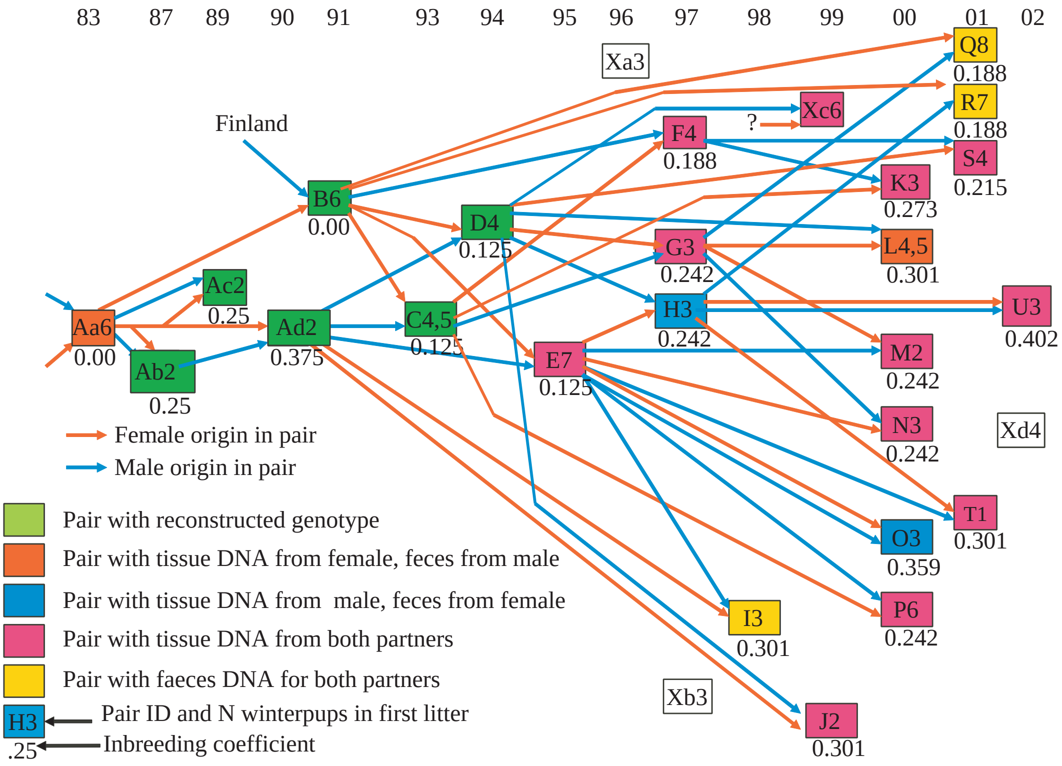


Figure 1. Pedigree of the Scandinavian wolf population. Boxes indicate breeding pairs and arrows trace the ancestry of male (blue) and female (red) in each pair. Colours of boxes indicate how the genotype of the wolves in the pair was determined. Unfilled boxes indicate pairs with missing genotype. Pairs are fitted to the time-scale on top according to their year of first reproduction. The number of winter pups in first litters and inbreeding coefficients for offspring, are indicated. If number of pups was determined as a range (e.g. 4–5) we used the mean value (e.g. 4.5).

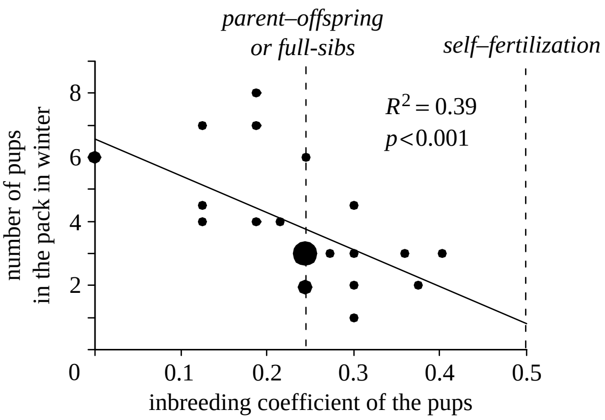


Figure 2. The number of pups that survived to winter for first-born litters in relation to the inbreeding coefficient of the pups. Small dots refer to one data point each, medium sized to two data points and large to four data points. Inbreeding levels corresponding to parent–offspring or full-sibling mating, and self-fertilization are indicated.

weather or food, as time itself had no effect on litter size. Change in prey availability can also be discarded considering that the number of moose (*Alces alces*), the most important prey for wolves in Scandinavia (Sand *et al.* in press), stayed high (greater than 1 moose per $km^2$ in all wolf territories) during the study period (Hörnberg 2001). It was well above the threshold (0.5 moose per $km^2$) under which wolf populations are reported to be affected (Messier, 1994).

### (b) *Effects of inbreeding on demography*

The quantitative inbreeding effect was a reduction of 1.15 winter pup per litter for each increase of 0.1 in the $F$ for pups (winter litter size$=(6.54–11.51)F$; figure 2). In our population model, an increase of offspring inbreeding coefficient $F$ of 0.1 reduced the growth rate $\lambda$ from 1.29 to 1.21, assuming all litters were affected equally by inbreeding. Zero population growth ($\lambda = 1$) would be reached at an average $F$ of 0.48. Our chosen fitness measure, winter litter size, actually represents a combination of fecundity and early survival. It is possible that more fitness components, for example, yearling or adult survival, could be affected, which would make the demographic consequences even more severe. The Scandinavian wolf population thus may have a gloomy future unless it can be purged of its genetic load through natural selection, or receives new genetic variation from outside. However, the effectiveness of purging in small populations has been questioned (Hedrick & Kalinowski 2000), and the probability of natural immigration also seems low, as no new immigrants have appeared in the last 13 years. In an earlier report concerning this population, it was claimed that the male immigrating in 1991 'rescued' the population (Vila *et al.* 2003). Our interpretation is that before this male arrived there was no population but just a strongly inbred family. The arrival of this newcomer allowed young wolves to find partners outside of their own family, and this sparked off a rapid initial increase, but has not prevented the succeeding inbreeding.

### (c) *Conservation implications*

This study has general implications for the 'small population paradigm' (Caughley 1994), and is especially relevant for the conservation of large carnivores. These are charismatic species with large public support, but as powerful predators also highly controversial, they are often forced into small fragmented populations. The wolf could be useful as a model species for this dilemma, in part because there are several studies of inbreeding in captive populations of this species. A captive Swedish wolf population, partly founded from the same source as our study population, also expressed severe inbreeding effects (Laikre 1999), while in two American captive populations of Red and Mexican wolf, no effects were noted on demographic parameters (Kalinowski *et al.* 1999), although effects on body size was noted in the Mexican wolves (Fredrickson & Hedrick 2002). The genetic load of our wild population (6.04(+3.44), 95% CI) was substantially heavier than that for the Red and Mexican wolves (0.63 and 0.71, respectively), and also clearly higher than the average estimate of 3.14 in a study of 40 captive mammal populations (Ralls *et al.* 1988). This indicates that impact of inbreeding can vary substantially, even within the same species, depending on the random subset of genes from the source population drawn by the founders, and succeeding random drift. The famous wild wolf population on Isle Royale in MN, USA, still fails to show any obvious demographic effects of inbreeding almost 50 years after its founding by only two individuals (Wayne 1991; Peterson 1995), but a detailed analysis of inbreeding, of the type demonstrated in this paper, has not been employed.

The conservation implication for our study population is that genetic exchange with the source population should be strongly promoted. In the meanwhile, the close demographic and genetic monitoring of the population should be continued. The potential for further exploration of inbreeding effects on more demographic parameters should be pursued.

**Acknowledgements**

Funding was supported to O.L., H.A. and H.S. by the Swedish Environmental Protection Agency, the World Wide Fund for Nature (Sweden), the Swedish Association for Hunting and Wildlife Management, the private foundation 'Olle och Signhild Engkvists Stiftelser', to H-C.P. and P.W. by the Norwegian Directorate for Nature Management, the Norwegian Institute for Nature Research, Norwegian Ministry of Environment, and to S.B. by the Swedish Research Council. We thank Philip Hedrick, John Vucetich and Josephine Pemberton and two anonymous referees for insightful comments and suggestions to an earlier version of the manuscript.

Caughley, G. 1994 Directions in conservation biology. *J. Anim. Ecol.* **63**, 215–244.

Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J.M 1999 Parasite-mediated selection against inbred Soay sheep in a free-living island population. *Evolution* **53**, 1259–1267.

Fredrickson, R. J. & Hedrick, P. W. 2002 Body size in endangered Mexican wolves: effects of captivity, inbreeding, and cross-lineage matings. *Anim. Cons.* **5**, 39–43.

Gilpin, M. E. & Soulé, M. E. 1986 Minimum viable populations: processes and extinction. *Conservation biology. The science of scarcity and diversity* (ed. M. E. Soulé). pp. 19–34, Sunderland, Massachusetts: Sinauer Associates.

Hedrick, P. W. & Kalinowski, S. T. 2000 Inbreeding depression in conservation biology. *Ann. Rev. Ecol. Syst.* **31**, 139–162.

Hörnberg, S. 2001 Changes in population density of moose (*Alces alces*) and damage to the forests in Sweden. *Forest Ecol. Manag.* **149**, 141–151.

Kalinowski, S. T., Hedrick, P. W. & Miller, P. S. 1999 No inbreeding depression observed in Mexican and red wolf captive breeding programs. *Conserv. Biol.* **13**, 1371–1377.

Keller, L. F. 1998 Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* **52**, 240–250.

Keller, L. F. & Waller, D. M. 2002 Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241.

Laikre, L. 1999 Conservation genetics of Nordic carnivores: lessons from zoos. *Hereditas* **130**, 203–216.

Loeske, E. B., Kruuk, L. E., Sheldon, B. C. & Merilä, J. 2002 Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proc. Roy. Soc. Lond. B* **269**, 1581–1589.

Mech, L. D. 1970 *The wolf: the ecology and behaviour of an endangered species*. New York: The Natural History Press.

Messier, F. 1994 Ungulate population models with predation: A case study with the North American moose. *Ecology* **75**, 478–488.

Miller, C. R., Joyce, P. & Waits, L. P. 2002 Assessing allelic dropout and genotype reliability using maximum likelihood. *Genetics* **160**, 357–366.

Peterson, R. O. 1995 *The wolves of Isle Royale. A broken balance*. Minocqua, Wisconsin: Willow Creek Press.

Ralls, K. J., Ballou, J. D. & Templeton, A. D. 1988 Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conserv. Biol.* **2**, 185–193.

Sand, H., Zimmerman, B., Wabakken, P., Andrén, H. & Q5 Pedersen, H. C. 2005 GPS-technology and GIS-cluster analyses as tools to estimate kill rates in wolf-ungulate ecosystems. *Wildlife Soc. Bull.*. In press.

Sarkar, G. & Sommer, S. 1990 Shedding light on PCR contamination. *Nature* **343**, 27.

Slate, J. L. E., Kruuk, B., Marshall, T. C., Pemberton, J. M. & Clutton-Brock, T. H. 2000 Inbreeding depression influences lifetime breeding success in a wild population of red deer. *Proc. R. Soc.* **267**, 1657–1662.

Vila, C. *et al.* 2003 Rescue of a severly bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proc. R. Soc. Lond. B* **270**, 91–97.

Wabakken, P., Sand, H., Liberg, O. & Bjärvall, A. 2001 The recovery, distribution, and population dynamics of wolves on the Scandinavian peninsula, 1978–1998. *Can. J. Zool.* **79**, 710–725.

Wayne, R. K. *et al.* 1991 Conservation genetics of the endangered Isle Royale wolf. *Conserv. Biol.* **5**, 41–51.

## Author Queries

*JOB NUMBER:* 0266
*JOURNAL:* RSBL

| | |
|---|---|
| Q1 | Please note that Reference Taberlet et al. (1999) is cited in the text but not given in the list. |
| Q2 | Kindly note that Reference Miller et al. (1999) cited in the text has been changed to Miller et al. (2002) as per reference list. |
| Q3 | Please note that Reference Kalinowski and Hedrick (1998) has been cited in the text but not given in the list. |
| Q4 | Kindly note that Reference Keller and Waller (2003) cited in the text has been changed to Keller and Waller (2002) as per reference list. |
| Q5 | Kindly update Ref. "Sand et al., 2005". |

*Animal Conservation* (1999) **2**, 59–68 © 1999 The Zoological Society of London Printed in the United Kingdom

# Biological and technical considerations of carnivore translocation: a review

Brian Miller[1], Katherine Ralls[2], Richard P. Reading[1], J. Michael Scott[3] and James Estes[4]

[1]Dept. of Conservation Biology, Denver Zoo, Denver, CO 80205, USA
[2]National Zoological Park, Smithsonian Institution, Washington, DC 20008, USA
[3]College of Forestry, Wildlife and Range Science, University of Idaho, Moscow, ID 83843, USA
[4]Earth and Marine Science Building, University of California, Santa Cruz, CA 96064, USA

*(Received 30 June 1998; accepted 21 September 1998)*

**Abstract**

Carnivore translocations are usually risky and expensive, and a number of biological and non-biological factors can influence success. Biological considerations include knowledge of genetics, demographics, behavior, disease, and habitat requirements. This information is critical for determining if the translocation should be attempted, if it could be successful, and how it could be implemented in an efficient and effective manner. We stress that individual species will vary in their responses, and ideas should be tested scientifically. The technical considerations of translocation are closely related to the biological questions. They include legal framework, fiscal and intellectual resources, monitoring capacity, goals of the translocation, logistic challenges, and organizational structure of decision-making. We do not discuss socio-economic aspects of translocation because those challenges require detailed discussion in a separate paper. We suggest that because large carnivores often play key roles in regulating ecological interactions between trophic levels, restoring them is more than a single-species activity. By restoring carnivores in viable numbers, we can take a large step toward recovering ecological integrity of geographically extensive landscapes.

## INTRODUCTION

While this paper draws specific attention to carnivores, most of the issues discussed apply to many, if not all, translocation efforts. We focus on carnivores in this paper for four basic reasons.

First, humans have drastically changed most of the Earth's ecosystems (Vitousek *et al*., 1997). As a result of these ecosystem changes, and direct persecution, carnivores have been eliminated from most areas in a manner disproportionate to species of other trophic levels. The consequences of habitat fragmentation, such as area effect, edge effect, distance effect, rarity effect, age effect, and disturbance dynamics, have been well documented (e.g. Frankel & Soulé, 1981; Wilcox & Murphy, 1985; Wilcove, McLellan, & Dobson, 1986; Noss, 1987; Noss & Cooperrider, 1994; Soulé, 1995). Basically, as habitat patch size decreases, more species disappear: larger, wide-ranging, and specialized species are disproportionately represented in those losses (Soulé, 1995).

Second, large carnivores often have disproportionate effects on ecosystem processes (Terborgh, 1988; Estes, 1996; Power *et al*., 1996; Terborgh, Lopez *et al*., 1997; Terborgh, Estes *et al*., 1999). Since the ground-breaking studies by Paine (1966), the effects of predators have been demonstrated in numerous systems, and many of these investigations have been reviewed by Terborgh, Estes *et al*. (1999).

Protecting top carnivore species, therefore, can have positive effects on the entire system. Where carnivores have been eliminated, events such as herbivore release (McShea, Underwood & Rappole, 1997) and mesopredator release (Soulé *et al*., 1988) have produced trophic cascades that have severely disrupted ecological communities and extirpated species (Estes, 1996; Terborgh, Lopez *et al*., 1997; Terborgh, Estes *et al*., 1999). Because many carnivores play umbrella, flagship, indicator, and keystone roles, reintroducing a suite of extirpated carnivores is a step toward restoring the natural integrity to large sections of land (Miller, Reading, Strittholt *et al*., 1999).

Third, in most cases, natural recolonization is no longer an option. Large carnivores have been widely extirpated, and severe habitat disruption poses a barrier to their natural dispersal. Finally, carnivores seem to be disproportionately difficult to re-establish via translocation.

---

All correspondence to: Brian Miller. Tel: 303-376-4944;
E-mail: zooconservation@denverzoo.org.

In this paper, we discuss some of the variables that influence the success of carnivore translocation, or moving organisms from one area to another. We emphasize returning species to areas where their populations have been extirpated (reintroduction) because releasing animals to augment an existing population (restocking) and releasing animals outside their historical range (introduction) are generally inadvisable (IUCN, 1987), although they can be useful under special circumstances (e.g. Gerrodette & Gilmartin, 1990).

Because the focus of this paper is biological, we will not discuss the socio-economic aspects of translocation in great detail. That does not diminish their importance. The social challenges of carnivore reintroduction are even more daunting than the biological ones (Reading & Clark, 1996), and a successful program will need a holistic and truly inter-disciplinary approach that integrates social and biological sciences toward the goal of conservation.

## BIOLOGICAL CONSIDERATIONS FOR TRANSLOCATIONS

An array of biological factors affect the success of translocations. Knowledge of genetics, demography, behavior, disease, and habitat requirements can lead to more effective reintroduction methods and provide baseline data against which the results of a translocation program can be evaluated (Kleiman, 1989; Stanley Price, 1989; Miller, Biggins *et al*., 1993; Reading & Clark, 1996). Unfortunately, such information is often lacking, as only 15% of terrestrial carnivore species have been the subject of at least one field investigation, and the status of most remains obscure (Schaller, 1996).

A translocation program should include a feasibility study, a preparation phase, a release phase, and a monitoring phase (IUCN, 1987). Several biological questions should be addressed during the feasibility study (Reading, Clark, & Kellert, 1991; Kleiman, Stanley Price & Beck, 1993). These include: is there a need to reintroduce or restock a wild population? Did the species occur as a viable population in the proposed release area? If restocking is a possibility, would it pose a threat to the existing wild population? Have the causes of the population decline or extirpation been eliminated? Is there sufficient protected habitat for the translocated animals to survive? Are there suitable animals available that are surplus to the genetic and demographic needs of the source population? Is there sufficient knowledge to formulate a plan of action and evaluate its success?

If a reintroduction is deemed feasible, a myriad of additional biological considerations should be addressed. We explore several of these considerations in more detail.

### Selecting animals for translocations

#### *Taxonomy*

Animals chosen for reintroduction should be as similar as possible to those that originally inhabited the release site. However, existing subspecific frameworks should be examined critically because they may not reflect the true distribution of genetic variation and phylogenetic discontinuities within species (Ryder, 1986; Avise, 1989; Avise & Nelson, 1989). Early mammalogists described and named large numbers of subspecies within most species of carnivores, and they often based their subspecific classifications on a small number of morphological characters from a small number of specimens. These subspecies are still listed in many reference works: for example, Hall & Kelson (1959) name 24 subspecies of gray wolves (*Canis lupus*). This probably overestimates the number of wolf subspecies because minor differences between clinal distributions of neighboring populations are unlikely to merit subspecific status (Carbyn, 1987). Wolves can disperse over long distances, and ‘the vast expanses of boreal areas on the North American continent resulted in a wide-spread unrestricted gene flow from one area to another during the period prior to European settlement’ (Carbyn, 1987).

#### *Molecular genetic data*

Descriptive genetic studies using modern molecular techniques can help to define appropriate genetic subdivisions. In large North American canids, patterns of mitochondrial DNA variation suggest ‘that gene flow may occur across the continent and suppress genetic differentiation among even widely separated populations’ (Mercure *et al*., 1993). For example, widely separated populations of coyotes (*Canis latrans*) show little mitochondrial DNA differentiation (Lehman *et al*., 1991). However, in kit foxes (*Vulpes macrotis*), a small canid with limited dispersal capabilities, there are significant genetic differences between populations that reflect geographical barriers (Mercure *et al*., 1993). Molecular genetic differentiation among populations must be interpreted critically because it does not always reflect historical barriers to gene flow. For example, Wayne *et al*. (1992) believed that apparent genetic differences among extant gray wolf populations reflect population declines and habitat fragmentation rather than a long history of genetic isolation.

In summary, spatial heterogeneity in population genetic structure is probably not a relevant concern for large carnivores that range widely, but it can be for smaller carnivores with limited dispersal capability. We emphasize, however, that each species be weighed individually. For example, grizzly bears (*Ursus arctos*) have more limited dispersal patterns than expected for an animal of their size.

Maximizing genetic diversity among release animals is probably the best strategy for most species. Haig, Ballou & Derrickson (1990) suggested selection of animals for release based on maximizing founder genome equivalents as a good compromise between maximizing founder contributions and allelic diversity. However, this strategy should not jeopardize the genetic integrity of the source population (Kleiman, 1989). Greater genetic diversity among release animals would reduce the chances for founder effects and inbreeding depression, which may be important in a small population struggling to become established. Greater diversity may also enable the population to better adapt to its habitat.

### *Wild versus captive animals*

Wild-born animals are preferable to captive-born animals for translocations (Griffith *et al*., 1989), and we recommend releasing captive carnivores only when there are no other alternatives. Captive breeding is a strategy in conservation, and captive breeding and reintroduction of black-footed ferrets (*Mustela nigripes*) has saved that species from extinction (Miller, Reading & Forrest, 1996). In addition, captive animals can be used for education and research whether or not they are ever translocated to the wild. For example, some questions important to conservation, such as understanding energetic needs or reproductive habits, can be difficult to answer from wild animals.

Yet, captive breeding for purposes of translocation is expensive in time, space, and money and can be risky (e.g. see Scott & Carpenter, 1987; Leader-Williams, 1990; Derrickson & Snyder, 1992; Hutchins, Willis & Weise, 1995; Miller, Reading & Forest, 1996; and Snyder *et al*., 1996). The captive environment may erode the genetic basis for important morphological, physiological, and behavioral traits via artificial selection. For example, while captive-born animals may still exhibit the correct behavior in a given situation, they may not perform at the level of efficiency needed for survival in the wild.

Indeed, during a captive-breeding program, learned behavioral traits can degenerate much more rapidly than genetic diversity (May, 1991). Some examples of behavioral traits that may be adversely affected by the captive environment include: searching for food, killing, predator avoidance, recognition of home sites, movement patterns (such as seasonal migrations), methods of raising young, ability of young to follow mothers to kill sites, and negative response to human presence (Derrickson & Snyder, 1992; Miller, Biggins *et al*., 1993; Beldon & McCown, 1996; Miller, Reading & Forest, 1996; Snyder *et al*., 1996). As a result, when captive-born animals are reintroduced, mortality rates are often high. Reducing the impact of these problems during reintroduction of captive-raised black-footed ferrets was time-consuming and expensive (see Miller, Reading & Forest, 1996; Biggins, Godbey, Hanebury *et al*., 1998).

Different species respond variably to captive conditions, but more generations in captivity will likely increase the degeneration of survival skills. Pre-release preparation and post-release training may not be able to restore survival traits to full efficiency. Effective development of adaptive behaviors requires the correct environment for learning (including a skilled parent) or, in the case of critical periods, the correct stimulus at the proper time during development (Gossow, 1970). Captive conditions can make it difficult to provide these requirements. Furthermore, selection for tameness and other genetic adaptations to the captive environment are likely to become increasingly serious as populations are maintained in captivity for many generations, reducing the probability of a successful reintroduction (Frankham, 1995; Snyder *et al*., 1996). Frankham (1995) provides suggestions for minimizing genetic adaptations to the captive environment.

When captive-raised and wild-born individuals of the same species have been released experimentally, captive-raised animals exhibited different behaviors and lower survival times than their wild-born counterparts (Schadweiler & Tester, 1972; Cade, Redig & Tordoff, 1989; Griffith *et al*., 1989; Beck, Kleiman *et al*., 1991; Biggins, Hanebury, *et al*., 1991; Wiley, Snyder & Gnam, 1992; Beldon & McCown, 1996; Miller, Reading & Forest, 1996). Important to the release of large predators, are their interactions with humans and livestock. Captive-raised pumas (*Puma concolor*) in Florida had less fear of humans and were more likely to engage in puma–human and puma–livestock encounters than wild-caught animals (Beldon & McCown, 1996). Similarly, orphan sea otter (*Enhydra lutris*) pups raised in captivity and released into the wild often approach people, and two such animals attacked humans (C. Benz, pers. comm.; J. Estes, pers. obs.).

### *Age-sex categories*

Individuals in different age-sex classes vary in reproductive value and often exhibit different behaviors. It is usually advisable to release animals in sex ratios similar to that exhibited by wild populations to ensure reproductive encounters (Erickson & Hamilton, 1988). This often entails releasing more females (Short *et al*., 1992). Differences between male and female behavior may influence release considerations, and age is also crucial. Young animals often display greater behavioral plasticity than adults and are less important to the source population (Gordon, 1991; Logan *et al*., 1996; Miller, Reading & Forest, 1996). Some translocations use releases of mixed sexes and ages that replicate natural social groups, such as wolf packs (Moore & Smith, 1991; Bangs & Fritts, 1996).

In many cases, both genetic and demographic considerations are constrained by the availability of animals from the source population (i.e. some translocations must take whatever animals they can get). This is especially true when the source population is a captive-breeding program. In many such situations managers are concerned with the genetic and demographic management of the source population rather than the translocated population (Gordon, 1991; Moore & Smith, 1991; Miller, Reading & Forest, 1996). This is a tactic we support, especially in the initial stages of release, when the translocated population is just getting established and experiencing high mortality.

Studies of puma translocations illustrate several of these points. In the Florida puma release, wild-caught females with kittens did not move far from their release site, and the kittens behaved normally; however, wild-caught and released males covered large areas until they located females (Beldon & McCown, 1996). Logan *et al.* (1996) translocated wild-caught pumas in New Mexico, and they found success was affected by sex, age, and social status. The best results came with translocated pumas between 12 and 27 months of age (Logan *et al.*, 1996). They moved the shortest distance from the release site and quickly established areas of use. Pumas of this age group may settle more quickly because, being at dispersal age, they may be predisposed to accept an unfamiliar area (Logan *et al.*, 1996). In addition, the females of this age group moved less and had higher survival rates than males. The removal of pumas less than 27 months of age from a self-sustaining population would probably not jeopardize the source population genetically or demographically (Logan *et al.*, 1996), an important consideration in selecting animals for translocation (Kleiman, 1989; Stanley Price, 1989).

Adult translocated pumas (28–96 months of age) taken from established territories traveled the farthest from their release site, often showing homing tendencies (Logan *et al.*, 1996). Indeed, two pumas in this age class returned to their original home territories, over 400 km away. Older pumas (over 96 months of age) showed high, immediate risk of death (Logan *et al.*, 1996). Similarly, adult male sea otters had a greater risk of death during capture and translocation than individuals in other age-sex classes (T. Williams, pers. comm.).

In general, puma translocation increased mortality over that observed in the source population (Logan *et al.*, 1996). The risks were long-term, and a number of deaths occurred in the second year after release. Chronic stress may have been a factor, particularly for adults. Combining suggestions for puma translocation from Logan *et al.* (1996) and Beldon & McCown (1996), it may be preferable to first release dispersal age females. After the female pumas establish areas of use, young males could be released in the presence of those females to keep them from wandering long distances. We caution, however, that it can sometimes be difficult to determine optimum ages for translocation. For example, in some species, juveniles may have higher survival rates after translocation, but their future reproductive potential must be balanced against the immediate reproductive capacity of any adults that establish in the release area. Even after years of data on sea otters, there is still some disagreement as to the optimum sex and age composition for translocation.

Homing behavior and excessive movement from the release site has been a major problem in translocation of ursids, canids, felids, and mustelids (Linnell *et al.*, 1997). For example, when 139 California sea otters were translocated to San Nicolas Island, the majority dispersed away from the island, and a minimum of 30 individuals, including both juvenile and adult females, returned to their capture location (G. Rathbun, pers. comm.). Excessive movement from the release site is a major reason for low survival and poor reproductive rates of translocated carnivores. There is often a correlation between movement distances after release and mortality (Biggins, Godbey & Vargas 1993a; Logan *et al.*, 1996). Linnell *et al.* (1997) suggest holding animals on a release site for a time prior to release to reduce post-release movements, and moving large carnivores far from their capture site to reduce homing.

## Genetics

Understanding genetic considerations is important to translocation, yet genetic screening was performed in only 37% of the reintroduction projects using captive-raised animals (Beck, Rapaport *et al.*, 1993). As discussed above, translocated animals should be as genetically diverse as possible because of the potential for founder effects and inbreeding depression within the small populations typical of translocation programs (Templeton, 1990). This is especially true in the early stages.

Inbreeding depression (reduced reproductive fitness due to matings between close relatives) has been documented in a large number of mammals (Ralls, Ballou & Templeton, 1988; Lacy, Petric & Warneke, 1993; Lacy, 1997), including wolves (Laikre & Ryman, 1991) and Florida panthers (Roelke, Martenson & O'Brien, 1993; O'Brien, 1994). Inbreeding depression is a potential problem in small, reintroduced populations of large mammals because these species probably had low inbreeding rates prior to European settlement (Ralls, Harvey & Lyles, 1986; Frankham, 1995).

In Wyoming, translocated big horn sheep (*Ovis canadensis*) have been living in small isolated populations, and genetic changes (including shifts in allele frequencies, decreases in number of alleles, and changes in heterozygosity) in those animals were detected within 10 to 20 years after release (Fitzsimmons, Buskirk & Smith, 1997). Genetic problems may be contributing to declining numbers in the translocated herds (Berger, 1990; Fitzsimmons *et al.*, 1997). Wildt *et al.* (1995) demonstrated that felid populations with reduced genetic diversity ejaculate lower total sperm counts and extraordinarily high numbers of malformed spermatozoa, than do populations of the same species with high levels of genetic diversity. They also showed homozygous populations are plagued with an array of physiological defects, including cardiac and immune-system problems.

Outbreeding depression (reduced reproductive fitness due to matings between individuals that are genetically dissimilar) is much less likely to be a problem than inbreeding depression (Ballou, 1995; Frankham, 1995). Evidence for outbreeding depression comes primarily from plants and animals with extremely limited dispersal (Ballou, 1995). Serious outbreeding depression in mammals appears to result mainly from crosses between individuals with significant genetic (e.g. chromosomal) differences resulting in sterility in the F1 generation (Ballou, 1995).

Furthermore, several studies of captive animals failed to find evidence of outbreeding depression in mammals. Smith *et al.* (1987) observed no adverse effects of crossing rhesus macaques (*Macaca mulatta*) from India and China. Jaquish (1994) found no outbreeding depression from crosses between subspecies of saddle-back tamarins (*Saguinus fuscicollis*). Ballou (1995) found no evidence for outbreeding depression in captive mammals, including orangutan (*Pongo pymaeus*) subspecies from Borneo and Sumatra. Finally, Lacy has conducted extensive crosses between several subspecies of *Peromycus polionotus* and found that all crosses display heterosis, with respect to percent of pairs breeding, litter size, juvenile survival, and growth rates, at the F1 and subsequent generations (R. C. Lacy, pers. comm.). Importantly, these studies were all conducted in captivity. Theoretically, outbreeding to genetically dissimilar reintroduced animals could have repercussions, such as birthing at inappropriate times and reduced fitness with a particular, more restricted, habitat (Leberg, 1990; May, 1991). However, such effects have not yet been documented in large mammals such as carnivores.

Many conservationists caution against simply trying to bolster numbers or to maximize genetic heterogeneity by translocating animals into an area with a remnant population. The result could be ‘contamination’, or even swamping, of unique, remnant genetic stocks by the translocated animals (Berg, 1982; Betram & Moltu, 1986; Sale, 1986; Stanley Price, 1989; R. R. Johnson, 1990). This effect has been documented when hatchery fish are released into wild waters and is one of the arguments against restocking (IUCN, 1987). For example, native breeding populations of coho salmon (*Oncorhynchus kisutch*) have been replaced in the lower Columbia River basin by feral hatchery fish (O. W. Johnson *et al*., 1991). For a mammalian example, red wolves interbreeding with congeneric species living at the release site could lead to genetic contamination or swamping (Phillips, 1990; Moore & Smith, 1991).

In addition, it is problematic to use translocation of animals between isolated patches of habitat as an alternative to restoring the historical connections between those isolated patches. While animals may be captured and moved between fragments, there may be no functional benefit from those efforts. Homing behavior and excessive movement from release site have been a major problem in carnivore translocations (Linnel *et al*., 1997). As mentioned above, several translocated pumas traveled over 400 km to return to their original territories (Local *et al*., 1996), and a young male tiger (*Panthera tigris*) translocated to a new area was quickly killed by a resident male (Seidensticker, 1976).

Most importantly, simply moving animals between fragments is not a viable attempt to restore wilderness or expanses of habitat similar to those that existed prior to extensive human development. Indeed, relying on such half-way technology can preserve existing patterns of habitat fragmentation. So, even if genetic material can be successfully exchanged, the small fragments would still be susceptible to demographic events, environmental events, and poaching. Even if large animals persist over the short-term in these fragments, important ecological processes such as fire, nutrient cycling, grazing, and flooding would remain altered by isolation and reduced scale. Following the same logic, translocating ‘problem animals’ as a cure for livestock depredation will probably have more cosmetic value than conservation substance, and it may only deflect attention from the deeper questions about existing ecological conditions that encourage predation on livestock.

## Demography

Colonies of reintroduced animals must become large enough, as quickly as possible, to withstand fluctuations in both the environment and population size, because vacillations in either can drastically increase the chance of extinction in small populations (Gilpin & Soulé, 1986). To understand these population dynamics, biologists must analyze demographic parameters such as fecundity, mortality, population growth rate, age structure, sex ratio, and life expectancy in natural populations (Stanley Price, 1989; Reading & Clark, 1996). Comparing demographic traits of reintroduced populations with wild populations will help managers determine when a reintroduced population has become an established, viable population.

Demographic characteristics are also important for defining habitat quality, which is the foundation of any management plan. Van Horne (1983) discussed misleading conclusions about habitat quality when simple density estimates (and presence/absence data) were used without knowledge of age structure or social structure. For example, density surveys can be taken in the warm months when winter habitat may be the critical factor for mortality (Van Horne, 1983). Additionally, social interactions can push juvenile, dispersing animals into poorer quality habitat, or even habitat sinks, because all good habitat is occupied by a stable population of territorial adults. Even though numbers of individuals can be temporarily high in the poor habitat, very few of those animals will survive to reproduce (Van Horne, 1983).

In polygynous carnivores, adult females with young will center their activities where critical resources are concentrated and easiest to obtain. When caring for offspring, females are restricted to optimal habitat as they need to satisfy elevated energetic requirements with minimum time away from the young (Lindstedt, Miller & Buskirk, 1986). Male carnivores, on the other hand, wander over extensive areas searching for females. Their movements are highly variable and often more related to reproductive needs and social status than habitat quality (Ewer, 1973; Powell, 1979). For that reason, adult females, which form the demographic base of a population, will often best represent the habitat needs of a species. Without attention to demographic factors (such as age structure, mortality, and reproduction) and behavioral information (such as social structure) one can not truly differentiate the quality of habitat types.

## Behavior

Behavioral traits must be performed efficiently in a variety of situations. The expression of a given trait is also influenced by a host of simultaneous behaviors that are also necessary for survival. Indeed, several authors have suggested using behavior as a measure of reintroduction success (Kleiman, Beck, Dietz *et al.*, 1986; Miller, Kleiman, Beck, Baker *et al.*, 1990; Miller, Biggins *et al.*, 1993; Miller, Reading & Forest, 1996). Box (1991) suggests using expression of behavioral traits in the selection of individuals for release. Knowledge of hunting, killing, caching, predator avoidance, reproduction, parenting, imprinting periods, social organization, communication, territoriality, locomotion, daily movements, seasonal movements, and habitat choices will affect the demographic selection of individuals for release, timing of reintroductions, method of release, and choice of sites. We have discussed many of these factors in previous sections.

As mentioned earlier, site fidelity and homing behavior, are important behavioral traits affecting large carnivore reintroduction success (Linnell *et al.*, 1997). Habituating animals to release sites appears to help reduce dispersal following reintroduction for many species (Berg, 1982; Jacuart *et al.*, 1986; Stanley Price, 1989; Linnell *et al.*, 1997). Permitting animals to become habituated to release sites also permits them to hone behavioral skills, such as locomotion, social skills, and foraging (Bangs & Fritts, 1996).

## Health and disease

The health and physical condition of animals selected for release should be carefully assessed. Despite the fact that Griffith *et al.* (1989) found no correlation between success and physical condition of animals at time of release, we believe only animals in good physical condition should be used in translocations. In addition, translocation should not introduce diseases to the release site, yet only 46% of the translocation programs using captive-born animals conducted any kind of medical screening before release (Beck, Rapaport *et al.*, 1993). In a survey including captive-raised and wild-born animals for translocation, 24% utilized medical screening (Griffith *et al.*, 1989) while about 25% of the programs had data that was inadequate for calculating the proportion of translocated animals lost as a result of disease (Griffith *et al.*, 1993). These figures are shockingly low. Many of these translocation programs used animals that were housed in multi-species facilities, and that increases the possibility of contacting an exotic disease. Risks can be minimized by veterinary intervention at the founder site, screening at the proposed release site, through vaccination if necessary, and by post-release monitoring (Woodford & Rossiter, 1993). A paper by Ballou & Wildt (1991) provides a vehicle to assess the risk of disease. The ultimate success of black-footed ferret reintroductions will probably depend on a better understanding of the dynamics of both canine distemper and plague (Williams, Thorne *et al.*, 1988; Williams, Mills *et al.*, 1994; Reading, Clark, Vargas *et al.*, 1996).

It should be remembered that acts of capture and holding until release will likely stress the animals, particularly wild-born animals, and that can increase susceptibility to new or latent infectious diseases (Woodford & Kock, 1991; Woodford & Rossiter, 1993). Logan *et al.* (1996) speculated that stress was an agent in the death of some translocated wild-born pumas, particularly adults older than 27 months of age.

## Habitat

Among the most important points in assessing a release site are determining the amount and type of habitat required and the cause of decline for the species to be translocated. If sufficient habitat is not available or the cause of decline has not been eliminated, it is nearly impossible to justify a translocation (Kleiman, 1989; Stanley Price, 1989; Short *et al.*, 1992; Reading & Clark, 1996). For many large carnivores (e.g. gray wolves), effectively halting harvest or control of the species may be enough, but other species (e.g. jaguars) may be much more sensitive to human presence and disturbance. A baseline study before translocation could determine the impact of the translocation on prey and competitors (Reading & Clark, 1996).

Translocation sites shold be evaluated in terms of habitat requirements, spatial characteristics, and management considerations (Reading & Clark, 1996). We caution, however, that *a priori* it is relatively easy to determine if habitat is inadequate (demonstrating that one or more critical elements are missing) but nearly impossible to demonstrate that habitat is adequate (determining that all critical elements are present).

Sites should be compared quantitatively during the selection process (e.g. Biggins, Miller *et al.*, 1993b). Some obvious examples are prey, cover, denning sites, water sources, competitors, predators, and the presence of exotics. More difficult to assess are ecosystem resilience and the effects of disturbance such as fires, droughts, catastrophic storms, etc (Kleiman, 1989; Stanley Price, 1989; Reading & Clark 1996). Such disturbances will have effects that are scale dependent, and issues of scale are some of the most difficult to understand (Soulé, 1996). But the presence of large carnivores, with their extensive movements, allows managers to evaluate conservation issues across a landscape. Because the landscape level is important to regional biodiversity, and habitat fragmentation has its most drastic effects at that level of scale, large carnivores can be a good indicator of wilderness quality (Miller, Reading, Strittholt *et al.*, 1999).

The degree of isolation, size, shape, and site location (in the context of historical range) are important spatial considerations (Kleiman, 1989; Reading & Clark, 1996). In North America, many of the native ecosystems are unrepresented or underrepresented in protected areas and only a small fraction of the reserves are large enough to maintain a full range of ecological processes or viable populations of middle-sized or large carnivores (Newmark, 1985; Caicco *et al.*, 1995; Davis *et al.*, 1995).

Habitat area is especially important for large carnivores. They exist at the top of the food chain and their densities are lower than species living at other trophic levels. So, when the average area of habitat patches declines through fragmentation and alteration, carnivore populations are among the first to disappear. Conflict with people on reserve borders is the major cause of mortality of large carnivores living in reserves, and it represents roughly 89% of the mortality for grizzly bears (Woodroffe & Ginsberg, 1998). Therefore, wide-ranging carnivores in small reserves are most vulnerable because they are more often exposed to the population sink that exists at the reserve boundary (Woodroffe & Ginsberg, 1998).

For that reason, sufficient prey is also a critical habitat trait (Sharps & Whitcher, 1982; Scott-Brown, Herrero & Mamo, 1986). With an adequate and constant prey base, carnivores will have smaller home ranges and wander over less territory. Fewer animals will therefore be exposed to the high mortality associated with reserve boundaries. Adequate prey densities also reduce the amount of livestock depredation and its consequent conflicts (Ravi Chellam & Saberwal, in press).

Even if large animals survive in fragmented habitats for long periods of time, their evolutionary potential is diminished. The forces of natural selection in small, isolated populations will be eventually overwhelmed by the randomized effects of genetic drift (Soulé, 1980, 1995, 1996). Evolutionary potential of large carnivores is necessary if they are to play a long-term role in ecosystem processes. Maintaining evolutionary potential in large animals will be impossible unless we can protect and restore large, and geographically extensive, populations (Soulé, 1995, 1996). By geographically extensive we mean for example, a system of core areas, linked by wildlife corridors, forming habitat connections throughout North America (Soulé, 1991, 1995; Noss & Cooperrider, 1994).

We recognize that while, in theory, corridors are a solution to habitat fragmentation, they are still a complex and controversial issue. Nevertheless, different types of connections could benefit carnivores. One involves connecting habitat patches within a protected area or the immediate region. Some large carnivores, like pumas, can negotiate through intra-reserve corridors even if there is an occasional bottleneck in the connection (Beier, 1993; B. Miller, pers. obs.). On the other hand, corridors to facilitate long-distance interchange between populations of a metapopulation may need to support residents of the focal species (Noss & Cooperrider, 1994). Even though there are records of dispersing large mammalian carnivores covering hundreds of kilometers, those individuals are usually juvenile males; conversely, the juvenile females establish territories relatively close to their area of birth (Greenwood, 1980). If we wish to maintain the capacity to naturally reestablish populations that have winked-out, we must create habitat connections that allow the movement of females.

## Non-biological considerations

Technical considerations are closely related to the biological factors, and difficult management issues should be considered during the feasibility study (Reading & Clark, 1996). Questions posed by Kleiman, Stanley-Price & Beck (1993) include: what legal framework exists, and does the program comply with laws? Is there an active research program to devise tactics? Are there sufficient fiscal and intellectual resources to maintain the program? Will the program be adequately monitored? To these questions we might add: what are the goals of the reintroduction? What logistic challenges must be overcome? Is there an appropriate organizational structure for making decisions?

The reintroduction should be carefully monitored to determine causes of mortality, movements and behaviors of released animals, life history attributes, and changes in habitat. The results of monitoring can guide future releases; therefore, records need to be detailed and should extend to offspring of the released animals (Miller, Biggins *et al*., 1993). Unfortunately, monitoring is one of the first things many organizations eliminate in an effort to reduce expenses (Noss & Cooperrider, 1994).

Goals should be defined carefully to provide accurate evaluation. Defining success solely by survival can be misleading because mortality is likely to be high during early releases; alternatively, analysis of behavioral traits during early releases many provide clues as to how animals respond to their new environment and that can result in improved techniques (Kleiman, Beck, Dietz *et al*., 1986; Kleiman, Beck, Baker *et al*., 1990; Miller, Biggins *et al*., 1993). Knowledge gained toward improved translocation methodology may be the most important goal of early releases. High mortality is not a failure unless biologists do not learn enough to increase survival in future reintroductions. For that reason, careful planning with a sound scientific approach, and effective monitoring, will offer the most efficient path toward recovery (Miller, Biggins *et al*., 1993).

Funding and physical resources are always a problem in biology, and reintroduction programs are expensive. As we have discussed, reintroduction can involve a variable amount of pre-release conditioning and training. Different techniques require different resources, and since resources are always limited, cost–benefit analyses can be important. We suggest comparing techniques on the basis of cost per successfully reproducing female released.

A well-trained and dedicated staff with the appropriate expertise is crucial to program success. We contend that reintroduction programs may be even more vulnerable to staff changes than other biological programs because reintroduction programs are long-lived, require many difficult decisions made in near-crisis situations, and mistakes with small populations can be hard to reverse (Snyder *et al*., 1996). For that reason, careful attention to the organizational structure of the decision-making body is crucial to maintaining an efficient and effective program (Miller, Reading & Forest, 1996; Clark, 1997).

In conclusion, we have discussed some general guidelines for reintroducing carnivores, and included issues of taxonomy, age and sex, genetics, demographics, behavior, health, habitat, and some general non-biological considerations. Many of these issues apply to all types of translocation efforts, but we have concentrated on carnivores for several reasons. Carnivores often play a strong role in top-down interactions among trophic levels, they have been disproportionately extirpated from most of the world's ecosystems, fragmentation has rendered natural colonization difficult, and carnivores are disproportionately harder to reestablish via translocation. For additional 'how to' information on reintroduction issues please refer to the IUCN guidelines for reintroduction (IUCN, 1987) supplemented by Beck, Rapaport *et al*. (1993) and Kleiman, Stanley Price & Beck (1993).

## Acknowledgements

We thank Michael Soulé, John Terborgh, David Wildt, and an anonymous reviewer for constructive comments on the paper.

## REFERENCES

Avise, J. C. (1989). A role for molecular genetics in the recognition and conservation of endangered species. *Trends Ecol. Evol.* **4**: 279–281.

Avise, J. C. & Nelson, W. S. (1989). Molecular genetic relationships of the extinct dusky seaside sparrow. *Science* **243**: 646–648.

Ballou, J. (1995). *Genetic management, inbreeding depression and outbreeding depression in captive populations*. PhD thesis: University of Maryland.

Ballou, J. & Wildt, A. M. (1991). Working group report on risk assessment and population dynamics. *J. Zoo Wildl. Med*. **24**: 398–405.

Bangs, E. E. & Fritts, S. H. (1996). Reintroducing the gray wolf to central Idaho and Yellowstone National Park. *Wildl. Soc. Bull*. **24**: 402–413.

Beck, B. B., Kleiman, D. G., Dietz, J. M., Castro, I., Carvalho, C., Martins, A. & Rhettberg-Beck, B. (1991). Losses and reintroduced golden-lion tamarins (*Leontopithicus rosalia*). *Dodo, J. Jersey Wildl. Pres. Trust* **27**: 50–61.

Beck, B. B., Rapaport, L. G., Stanley Price, M. R. & Wilson, A. C. (1993). Reintroduction of captive-raised animals. In *Creative conservation: interactive management of wild and captive animals:* 265–286. Olney, P. J. S., Mace, G. M. & Feistner, A. T. C. (Eds). London: Chapman and Hall.

Beier, P. (1993). Determining minimum habitat areas and habitat corridors for cougars. *Conserv. Biol*. **7**: 94–108.

Beldon, R. C. & McCown, J. W. (1996). *Florida panther reintroduction feasibility study*. Final Report, Study no. 7507. Tallahassee, FL: Florida Game and Fresh Water Fish Commission.

Berg, W. E. (1982). Reintroduction of fisher, pine marten, and river otter. In *Midwest furbearer management:* 159–173. Sanderson, G. C. (Ed.). Wichita, KS: Kansas Chapter of the Wildlife Society.

Berger, J. (1990). Persistence of different-sized populations: an empirical assessment of rapid extinctions in bighorn sheep. *Conserv. Biol*. **4**: 91–98.

Betram, B. C. R. & Moltu, D. P. (1986). Reintroducing red squirrels into Regent's Park. *Mamm. Rev.* **16**: 81–88.

Biggins, D. E., Godbey, J. L., Hanebury, L. H., Luce, B., Marinari, P., Matchett, M. R. & Vargas, A. (1998). The effect of rearing methods on survival of reintroduced black-footed ferrets. *J. Wildl. Mgmt*. **62**: 643–653.

Biggins, D. E., Godbey, J. L. & Vargas, A. (1993a). *Influence of pre-release experience on reintroduced black-footed ferrets (**Mustela nigripes**)*. Internal Report. Ft. Collins, CO: U.S. Fish and Wildlife Service.

Biggins, D. E., Hanebury, L. H., Miller, B., Powell, R. A. & Wemmer, C. (1991). *Release of Siberian ferrets (**Mustela eversmanni**) to facilitate reintroduction of black-footed ferrets.* Internal Report. Ft. Collins CO: U.S. Fish and Wildlife Service.

Biggins, D. E., Miller, B., Hanebury, L., Oakleaf, B., Farmer, A., Crete, R. & Dood, A. (1993b). A system for evaluating black-footed ferret habitat. In *Management of prairie dog complexes for black-footed ferret reintroduction sites:* 73–92. Oldemeyer, J., Biggins, D., Miller, B., & Crete, R. (Eds). Report no. 13. Denver, CO: U.S. Fish and Wildlife Service Publishing.

Box, H. O. (1991). Training for life after release: simian primates as examples. In *Beyond captive breeding: re-introducing endangered mammals to the wild:* 111–123. Gipps. J. H. W. (Ed.). Oxford: Clarendon Press.

Cade, T. J., Redig, P. T. & Tordoff, H. B. (1989). Peregrine falcon restoration: expectation versus reality. *The Loon* **61**: 160–162.

Caicco, S., Scott, J. M., Field, B. & Csuti, B. (1995). Gap analysis of the management status of the vegetation of Idaho (U.S.A.). *Conserv. Biol*. **9**: 498–511.

Carbyn, L. N. (1987). Gray wolf and red wolf. In *Wild furbearer management and conservation in North America:* 359–376. Novak, M., Baker, J. A., Obbard, M. E. & Malloch, B. (Eds). Ontario, Canda: Ministry of Natural Resources.

Clark, T. W. (1997). *Averting extinction: reconstructing endangered species recovery*. New Haven, CT: Yale University Press.

Davis, F. W., Stine, P. A., Stoms, D. M., Borchert, M. I. & Hollander, A. D. (1995). Gap analysis of the actual vegetation of California. The southwestern region. *Madrono* **42**: 40–78.

Derrickson, S. R. & Snyder, N. F. R. (1992). Potential and limits of captive breeding in parrot conservation. In *New World parrots in crisis: solutions from conservation biology:* 133–163 Beissinger, S. R. & Snyder, N. F. R. (Eds). Washington DC: Smithsonian Institution Press.

Erickson, D. W. & Hamilton, D. A. (1988). Approaches to river otter restoration in Missouri. *Trans. N. Am. Wildl. Nat. Res. Conf*. **53**: 404–413.

Estes, J. A. (1996). Predators and ecosystem management. *Wildl. Soc. Bull*. **24**: 390–396.

Ewer, R. F. (1973). *The carnivores*. Ithaca, NY: Cornell University Press.

Fitzsimmons, N. N., Buskirk, S. W. & Smith, M. W. (1997). Genetic changes in reintroduced rocky mountain bighorn sheep populations. *J. Wildl. Mgmt*. **61**: 863–872.

Frankel, O. H. & Soulé, M. E. (1981). *Conservation and evolution*. New York: Cambridge University Press.

Frankham, R. (1995). Conservation genetics. *Annu. Rev. Gen*. **29**: 305–337.

Gerrodette, T. & Gilmartin, W. G. (1990). Demographic consequences of changed pupping and hauling sites of the Hawaiian monk seal. *Conserv. Biol*. **4**: 423–430.

Gilpin, M. & Soulé, M. E. (1986). Minimum viable populations: processes of species extinctions. In *Conservation biology: science of scarcity and diversity:* 19–34. Soulé, M. E. (Ed.). Sunderland, MA: Sinauer Associates.

Gordon, I. L. (1991). Ungulate re-introductions: the case of the scimitar-horned oryx. In *Beyond captive breeding: re-introducing endangered mammals to the wild:* 217–240. Gipps, J. H. W. (Ed.). Oxford: Clarendon Press.

Gossow, H. (1970). Vergleichende verhaltensstudien an Marderartigen. I. Uber LautauBerungen und zum Beuteurhalten. *Zeit. Tierpsychol*. **27**: 405–480.

Greenwood, P. J. (1980). Mating systems, philopatry, and dispersal in birds and mammals. *Anim. Behav.* **28**: 1140–1162.

Griffith, B., Scott, J. M., Carpenter, J. W. & Reed, C. (1989). Translocation as a species conservation tool: status and strategy. *Science* **345**: 447–480.

Griffith, B., Scott, J. M., Carpenter, J. W. & Reed, C. (1993). Animal translocations and potential disease transmission. *J. Wildl. Med*. **24**: 231–236.

Haig, S. M., Ballou, J. D. & Derrickson, S. R. (1990). Management options for preserving genetic diversity: reintroduction of Guam rails to the wild. *Conserv. Biol*. **4**: 290–300.

Hall, E. R. & Kelson, K. R. (1959). *The mammals of North America*. vol. 2. New York: The Ronald Press Co.

Hutchins, M., Willis, K. & Wiese, R. J. (1995). Strategic collection planning: theory and practice. *Zoo Biol*. **14**: 5–25.

IUCN (1987). *Translocation of living organisms: introductions, reintroductions, and restocking*. IUCN Position Statement. Gland, Switzerland: IUCN.

Jacuart, H. C., Flinders, J. T., Coffeen, M. P. & Hassenyager, R. (1986). *Prescriptive transplanting and monitoring of Utah prairie dogs (**Cynomys parvidens**) populations*. Internal report. Salt Lake City, Utah: Division of Wildlife Resources.

Jaquish, C. E. (1994). Evidence of hybrid vigor in subspecific hybrids of the saddle-back tamarin (*Saguinus fuscicollis*). *Am. J. Primatol*. **33**: 263–276.

Johnson, O. W., Flagg, T. A., Maynard, D. J., Milner, G. B. & Waknitz, F. W. (1991). *Status review for the Lower Columbia River coho salmon*. Seattle, WA: National Oceanic and Atmospheric Administration Tech. Mem. National Marine Fisheries Service F/NWC-202.

Johnson, R. R. (1990). Release and translocation strategies for the Puerto Rican crested toad, *Peltophryne lemur. End. Spec. Update* **8**: 54–57.

Kleiman, D. G. (1989). Reintroductions of captive mammals for conservation: guidelines for reintroducing endangered species into the wild. *BioScience* **39**: 152–161.

Kleiman, D. G., Beck, B. B., Baker, A. J., Ballou, J. D., Dietz, L. A. & Dietz, J. M. (1990). The conservation program for the golden lion tamarin, *Leontopithecus rosalia. End. Spec. Update* **8**: 82–85.

Kleiman, D. G., Beck, B. B., Dietz, J. M., Dietz, L. A., Ballou, J. D. & Coimbra-Filho, A. F. (1986). Conservation program for the golden lion tamarin: captive research and management, ecological studies, educational strategies, and reintroduction. In *Primates; the road to self-sustaining populations:* 959–979. Benerschke, K. (Ed.). New York: Springer-Verlag, Inc.

Kleiman, D. G., Stanley Price, M. R. & Beck, B. B. (1993). Criteria for reintroductions. In *Creative conservation: interactive management of wild and captive animals:* 287–303. Olney, P. J. S., Mace, G. M. & Feistner, A. T. C. (Eds). London: Chapman and Hall.

Lacy, R. C. (1997). Importance of genetic variation to the viability of mammalian populations. *J. Mamm*. **78**: 320–335.

Lacy, R. C., Petric, A. & Warneke, M. (1993). Inbreeding and outbreeding in captive populations of wild animal species. In *The natural history of inbreeding and outbreeding:* 352–374. Thornhill, N. W. (Ed.) Chicago: The University of Chicago Press.

Laikre, L. & Ryman, N. (1991). Inbreeding depression in a captive wolf (*Canis lupus*) population. *Conserv. Biol*. **5**: 33–40.

Leader-Williams, N. (1990). Black rhinos and African elephants: lessons for conservation funding. *Oryx* **24**: 23–29.

Leberg, P. L. (1990). Genetic considerations in the design of introduction programs. *Trans. N. Am. Wildl. Nat. Res. Conf*. **55**: 609–619.

Lehman, N., Eisenhawer, A., Hansen, A., Mech, L. D., Peterson, R. O., Gogan, P. J. & Wayne, R. K. (1991). Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution* **45**: 104–119.

Lindstedt, S. L., Miller, B. J. & Buskirk, S. W. (1986). Home range, time, and body size in mammals. *Ecology* **67**: 413–418.

Linnell, J. D. C., Aanes, R., Swenson, J. E., Odden, J. & Smith, M. E. (1997). Translocation of carnivores as a method for managing problem animals: A review. *Biodiv. Conserv*. **6**: 1245–1257.

Logan, K. A., Sweanor, L. L., Ruth, T. K. & Hornocker, M. G. (1996). *Cougars of the San Andres Mountains, New Mexico*. Final Report to New Mexico Department of Game and Fish. Santa Fe, NM: New Mexico Department of Game and Fish.

McShea, W. J., Underwood, H. B. & Rappole, J. H. (Eds) (1997). *The science of overabundance*. Washington, DC: Smithsonian Institution Press.

May, R. M. (1991). The role of ecological theory in planning the reintroduction of endangered species. *Symp. Zool. Soc. Lond*. **62**: 145–163.

Mercure, A., Ralls, K., Koepfli, K. P. & Wayne, R. K. (1993). Genetic subdivisions among small canids: mitochondrial DNA differentiation of swift, kit, and arctic foxes. *Evolution* **47**: 1313–1328.

Miller, B., Biggins, D., Hanebury, L. & Vargas, A. (1993). Reintroduction of the black-footed ferret. In *Creative conservation: interactive management of wild and captive animals:* 455–463. Olney, P. J. S., Mace, G. M. & Feister, A. T. C. (Eds). London: Chapman and Hall.

Miller, B., Reading, R. P. & Forrest, S. (1996). *Prairie night: black-footed ferrets and the recovery of endangered species*. Washington, DC: Smithsonian Institution Press.

Miller, B., Reading, R., Strittholt, J., Carroll, C., Noss, R., Soulé, M., Sánchez, O., Terborgh, J., Brightsmith, D., Cheeseman, T. & Foreman, D. (1999). Focal species in design of reserve networks. *Wild Earth* (in press).

Moore, D. E. III & Smith, R. (1991). The red wolf as a model for carnivore re-introductions. In *Beyond captive breeding: re-introducing endangered mammals to the wild:* 263–278. Gipps, J. H. W. (Ed.). Oxford: Clarendon Press.

Newmark, W. D. (1985). Legal and biotic boundaries of western North American national parks: a problem of congruence. *Biol. Conserv*. **33**: 197–208.

Noss, R. F. (1987). Protecting natural areas in fragmented landscapes. *Nat. Areas J*. **7**: 2–13.

Noss, R. F. & Cooperrider, A. Y. (1994). *Saving nature's legacy: protecting and restoring biodiversity*. Covelo, CA: Island Press.

O'Brien, S. J. (1994). A role for molecular genetics in biological conservation. *Proc. Nat. Acad. Sci. USA* **91**: 5748–5755.

Paine, R. (1966). Food web complexity and species diversity. *Am. Nat*. **100**: 65–75.

Phillips, M. K. (1990). The red wolf: recovery an endangered species. *End. Spec. Update* **8**: 24–26.

Powell, R. A. (1979). Mustelid spacing patterns: variations on a theme by *Mustela*. *Zeit. Tierpsychol*. **50**: 153–165.

Power, M. E., Tilman, D., Estes, J. A., Menge, B. A., Bond, W. J., Mills, L. S., Daily, G., Castilla, J. C., Lubenchenco, J. & Paine, R. T. (1996). Challenges in the quest for keystones. *BioScience* **46**: 609–620.

Ralls, K., Ballou, J. D. & Templeton, A. R. (1988). Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conserv. Biol*. **2**: 185–193.

Ralls, K., Harvey, P. H. & Lyles, A. M. (1986). Inbreeding in natural populations of birds and mammals. In *Conservation biology: science of scarcity and diversity:* 35–56. Soulé, M. E. (Ed.) Sunderland, MA: Sinauer Associates, Inc.

Ravi Chellam & Saberwal, V. (in press). Living on the edge: lion conservation in the Gir Forest, India. In *Endangered species: conflict and context*. Reading, R. & Miller, B. (Eds). Westport: Greenwood Press.

Reading, R. P. & Clark, T. W. (1996). Carnivore reintroductions. In *Carnivore behavior, ecology, and evolution, Vol. 2:* 296–336. Gittleman, J. L. (Ed.). Ithaca, NY: Comstock Publishing Associates.

Reading, R. P., Clark, T. W. & Kellert, S. R. (1991). Toward an endangered species reintroduction paradigm. *End. Spec. Update* **8**: 1–4.

Reading, R. P., Clark, T. W., Vargas, A., Hanebury, L. H., Miller, B. J. & Biggins, D. (1996). Recent directions in black-footed ferret recovery. *End. Spec. Update* **13**: 1–6.

Roelke, M. E., Martenson, J. S. & O'Brien, S. J. (1993). The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biol.* **3**: 340–350.

Ryder, O. A. (1986). Species conservation and systematics: the dilemma of subspecies. *Trends Ecol. Evol.* **1**: 9–10.

Sale, J. B. (1986). Reintroduction in Indian wildlife management. *Ind. Forester* (**Oct**.): 867–873.

Schadweiler, J. D. & Tester, J. R. (1972). Survival and behaviour of hand-reared mallards released into the wild. *J. Wildl. Mgmt.* **36**: 1118–1127.

Schaller, G. B. (1996). Introduction: carnivores and conservation biology. In *Carnivore behavior, ecology, and evolution, Vol. 2:* 1–10. Gittleman, J. L. (Ed.). Ithaca, NY: Comstock Publishing Associates.

Scott, J. M. & Carpenter, J. W. (1987). Release of captive-reared or translocated birds: what do we need to know. *Auk* **104**: 540–545.

Scott-Brown, J. M., Herrero, S. & Mamo, C. (1986). *Monitoring of released swift foxes in Alberta and Saskatchewan*. Unpublished report to the Canadian Wildlife Service.

Seidensticker, J. (1976). Problem tiger in the Sundarbans. *Oryx* **13**: 267–273.

Sharps, J. C. & Whitcher, M. F. (1982). *Swift fox reintroduction techniques*. Internal report. Rapid City, South Dakota Game, Fish, and Parks Report.

Short, J., Bradshaw, S. D., Giles, J., Prince, T. I. T. & Wilson, G. R. (1992). Reintroduction of macropods (Marsupialia: Macropodoidea) in Australia – a review. *Biol. Conserv.* **61**: 189–204.

Smith, D. G., Lorey, F. W., Suzuki, J. & Abe, M. (1987). Effect of outbreeding on weight and growth rate of captive infant rhesus macaques. *Zoo Biol.* **6**: 201–212.

Snyder, N. R. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D. & Miller, B. (1996). Limitations of captive breeding in endangered species recovery. *Conserv. Biol.* **10**: 338–348.

Soulé, M. E. (1980). Thresholds for survival: criteria for maintenance of fitness and evolutionary potential. In *Conservation biology: an evolutionary-ecological perspective:* 151–170. Soulé, M. E. & Wilcox, B. M. (Eds). Sunderland, MA: Sinauer Associates.

Soulé, M. (1991). Theory and strategy. In *Landscape linkages and biodiversity:* 91–104. Hudson, W. E. (Ed.). Covelo, CA: Island Press.

Soulé, M. E. (1995). An unflinching vision: networks of people defending networks of lands. In *Nature conservation 4: The role of networks:* 1–8. Saunders, D. A., Craig, J. L. & Mattiske, E. M. (Eds). Sydney: Surrey Beatty and Sons Limited.

Soulé, M. (1996). The end of evolution. *Wld Conserv.* **1/96**: 24–25. (Originally IUCN Bulletin).

Soulé, M. E., Bolger, D. T., Alberts, A. C., Sauvajot, R., Wright, J., Sorice, M. & Hill, S. (1988). Restructured dynamics of rapid extinctions of chaparral-requiring birds in urban habitat islands. *Conserv. Biol.* **2**: 75–92.

Stanley Price, M. R. (1989). *Animal reintroductions: the Arabian oryx in Oman*. New York: Cambridge University Press.

Templeton, A. R. (1990). The role of genetics in captive breeding and reintroduction for species conservation. *End. Spec. Update* **8**: 14–17.

Terborgh, J. (1988). The big things that run the world – a sequel to E. O. Wilson. *Conserv. Biol.* **2**: 402–403.

Terborgh, J., Estes, J., Paquet, P., Ralls, K., Boyd, D., Miller, B. & Noss, R. (1999). Role of top carnivores in regulating terrestrial ecosystems. In *The science of continental scale reserve design*. Soulé, M. & Terborgh, J. (Eds). Covelo, CA: Island Press.

Terborgh, J., Lopez, L., Tello, J., Yu, D. & Bruni, A. R. (1997). Transitory states in relaxing land bridge islands. In *Tropical forest remnants: ecology, management, and conservation of fragmented communities:* 256–274. Laurance, W. F. & Bierregaard, R. O. Jr (Eds). Chicago: University of Chicago Press.

Van Horne, B. (1983). Density as a misleading indicator of habitat quality. *J. Wildl. Mgmt.* **47**: 893–901.

Vitousek, P. M., Mooney, H. A., Lubchenko, J. & Melillo, J. M. (1997). Human domination of earth's ecosystems. *Science* **277**: 494–499.

Wayne, R. K., Lehman, N., Allard, M. W. & Honeycutt, R. L. (1992). Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. *Conserv. Biol.* **6**: 559–569.

Wilcove, D. S., McLellan, C. H. & Dobson, A. P. (1986). Habitat fragmentation in the temperate zone. In *Conservation biology: science of scarcity and diversity:* 237–256. Soulé, M. E. (Ed.). Sunderland, MA: Sinauer Associates.

Wilcox, B. A. & Murphy, D. D. (1985). Conservation strategy: the effects of fragmentation on extinction. *Am. Nat.* **125**: 879–887.

Wildt, D., Pukazhenthi, B., Brown, J., Monfort, S., Howard, J. & Roth, T. (1995). Spermatology for understanding and managing rare species. *Reprod. Fert. Develop.* **7**: 811–824.

Wiley, J. W., Snyder, N. F. W. & Gnam, R. S. (1992). Reintroduction as a strategy for parrots. In *New World parrots in crisis:* 165–200. Beissinger, S. R. & Snyder, N. F. R. (Eds). Washington, DC: Smithsonian Institution Press.

Williams, E. S., Mills, K., Kwiatkowski, D. R., Thorne, E. T. & Boelger-Field, A. (1994). Plague in a black-footed ferret (*Mustela nigripes*). *J. Wildl. Dis.* **30**: 581–585.

Williams, E. S., Thorne, E. T., Appel, M. J. G. & Belitch, D. W. (1988). Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. *J. Wildl. Dis.* **24**: 387–398.

Woodford, M. H. & Kock, R. A. (1991). Veterinary considerations in re-introduction and translocation projects. In *Beyond captive breeding: re-introducing endangered mammals to the wild:* 101–110. Gipps, J. H. K. (Ed.). Oxford: Clarendon Press.

Woodford, M. H. & Rossiter, P. B. (1993). Disease risks associated with wildlife translocation projects. In *Creative conservation: interactive management of wild and captive animals:* 178–200. Olney, P. J. S., Mace, G. M. & Feistner, A. T. C. (Eds). London: Chapman and Hall.

Woodroffe, R. & Ginsberg, J. R. (1998). Edge effects and the extinction of populations inside protected areas. *Science* **280**: 2126–2128.

Population Dynamics of a Recolonizing Wolf Population
Author(s): Daniel H. Pletscher, Robert R. Ream, Diane K. Boyd, Michael W. Fairchild, Kyran E. Kunkel
Source: *The Journal of Wildlife Management*, Vol. 61, No. 2 (Apr., 1997), pp. 459-465
Published by: Allen Press
Stable URL: http://www.jstor.org/stable/3802604
Accessed: 01/08/2011 17:38

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at http://www.jstor.org/page/info/about/policies/terms.jsp. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/action/showPublisher?publisherCode=acg.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

*Allen Press* is collaborating with JSTOR to digitize, preserve and extend access to *The Journal of Wildlife Management.*

http://www.jstor.org

dynamic equilibria of animals with environment. Ecol. Monogr. 39:227–244.

SAS INSTITUE INC. 1989. SAS/STAT user's guide. Version 6. SAS Inst. Inc., Cary, N.C. 1686pp.

SMITH, R. J. 1973. Weather in relation to behavior of confined cottontail rabbits. M. S. Thesis, Purdue Univ., Lafayette, Ind. 106pp.

TABER, R. D., AND I. MCT. COWAN. 1969. Capturing and marking wild animals. Pages 277–317 *in* R. H. Giles, Jr., ed. Wildlife management techniques. The Wildl. Soc., Washington, D.C.

TRENT, T. T., AND O. J. RONGSTAD. 1974. Home range and survival of cottontail rabbits in southwestern Wisconsin. J. Wildl. Manage. 38:459–472.

TRIPPENSEE, R. E. 1934. The biology and management of the cottontail rabbit (*Sylvilagus floridanus mearnsii* Allen). Ph.D. Thesis, Univ. Michigan, Ann Arbor. 217pp.

WALLER, R. A., AND D. B. DUNCAN. 1969. A Bayes Rule for the symmetric multiple comparisons problem. J. Am. Stat. Assoc. 64:1484–1503.

*Received 15 March 1995.*
*Accepted 25 November 1996.*
*Associate Editor: McDonald.*

# POPULATION DYNAMICS OF A RECOLONIZING WOLF POPULATION

DANIEL H. PLETSCHER, Wildlife Biology Program, School of Forestry, University of Montana, Missoula, MT 59812, USA.
ROBERT R. REAM, Wildlife Biology Program, School of Forestry, University of Montana, Missoula, MT 59812, USA.
DIANE K. BOYD, Wildlife Biology Program, School of Forestry, University of Montana, Missoula, MT 59812, USA.
MICHAEL W. FAIRCHILD,[1] Wildlife Biology Program, School of Forestry, University of Montana, Missoula, MT 59812, USA.
KYRAN E. KUNKEL, Wildlife Biology Program, School of Forestry, University of Montana, Missoula, MT 59812, USA.

*Abstract:* Breeding populations of wolves (*Canis lupus*) were absent from the western United States for about 50 years following their extirpation by humans in the 1930s. Here we describe the recolonization by wolves of northwestern Montana and southeastern British Columbia, from the initial production of a litter by a pair of wolves in 1982 through the mid-1990s when 3–4 packs produced litters. Sex ratio of captured wolves favored females (38/54 = 70%; $\chi^2 = 8.96$, 1 df, $P < 0.005$). Litter size in early summer ($\bar{x} = 5.3$, SE = 0.4, $n = 26$) and in December ($\bar{x} = 4.5$, SE = 0.5, $n = 26$) were relatively high compared to similar counts in established populations elsewhere. Pack size in May was unrelated to litter size in June ($r_s = -0.13$, 23 df, $P = 0.25$) or the following December ($r_s = -0.12$, 23 df, $P = 0.28$). Annual adult survival rate (0.80) was relatively high in this semi-protected population and was higher among residents (0.84) than among wolves that dispersed (0.66) from the study area ($Z = 2.24$, $P = 0.025$). Although dispersal was common among radiocollared wolves (19/43 = 44%), population growth within the study area averaged 20% per year from 1982 to 1995. Low human-caused mortality rates and maintenance of connectivity for wolves between this small population in the United States and larger populations in Canada will enhance the probability of persistence and expansion of this population.

***J. WILDL. MANAGE. 61(2):459–465***

**Key words:** British Columbia, *Canis lupus*, dispersal, endangered species, Glacier National Park, Montana, mortality, natality, population dynamics, sex ratio, wolf

Wolves were extirpated from much of their historic range in North America through intensive human efforts during the 19th and early 20th centuries (Mech 1970). Eradication from Montana was complete by the 1930s, although a few individual animals were killed in later years (Singer 1979, Day 1981, Ream and Mattson 1982). Recolonization by wolves in the western United States began in the late 1970s and was focused in Glacier National Park, Montana (Ream et al. 1985, 1989, 1991). The nearest breeding population at the time was at least 100 km, and may have been 250 km, north in Canada (Ream and Harris 1986).

Several factors facilitated the increase of this wolf population. Wolves were listed as endangered in Montana in 1973 under the Endangered Species Act and were fully protected in southeastern British Columbia (BC) from 1967 until limited hunting was allowed starting in the late 1980s (Pletscher et al. 1991). Glacier National Park (GNP) provided additional security for wolves in the United States. Prey populations were high due to a series of relatively mild

---

[1] Present address: 120 Derns Road, Kalispell, MT 59901, USA.

winters (H. E. Nyberg, Mont. Dep. Fish, Wildl. and Parks, Kalispell, pers. commun.).

Our objectives were to document wolf reproduction, survival, immigration, and dispersal in this population within and near GNP that is apparently a source for wolves repopulating the western United States. We believe results from our study may provide insights into what may occur elsewhere in the region.

We thank the U.S. Fish and Wildlife Service, the National Park Service, the U.S. Forest Service, British Columbia Wildlife Branch, the Montana Department of Fish, Wildlife and Parks, the McIntire-Stennis Program at the School of Forestry, University of Montana, and private donors for financial contributions. E. E. Bangs, J. J. Fontaine, S. H. Fritts, L. D. Mech, W. G. Brewster, J. T. Tilmant, B. N. Hird, R. A. Demarchi, A. J. Fontana, and M. D. Jimenez were particularly helpful. I. J. Ball commented on the manuscript. We thank the many volunteers who assisted during the project.

## STUDY AREA

Our study was initiated in the Flathead River drainage in the southeast corner of BC and encompassed the adjacent Wigwam River drainage to the west and GNP to the south. In the United States, the Flathead River separates GNP on the east from U.S. Forest Service, state, and private lands on the west. The river valley is 4–10 km wide and about 1,200 m in elevation, rising to forested slopes and steeper, subalpine peaks. The Wigwam River valley is narrower and steeper. Both valleys were dominated by dense coniferous forests interspersed with meadows, marshes, and riparian habitats (Habeck 1970, Krahmer 1989). Lodgepole pine (*Pinus contorta*) was the dominant tree species, associated with spruce (*Picea engelmannii*), larch (*Larix occidentalis*), sub-alpine fir (*Abies lasiocarpa*), and Douglas-fir (*Pseudotsuga menziesii*).

As the wolf population increased, wolves dispersed and the study area grew to include northeastern Idaho, westcentral Montana near Missoula, the Sun River Game Range near Augusta, Montana, and the areas surrounding Banff National Park, Alberta (Ream et al. 1991, Boyd et al. 1995).

The density of humans in the study area was less than 0.005 people/km$^2$ in BC and less than 0.1 people/km$^2$ in Montana. Logging, petroleum exploration, and hunting occurred on forest service, state and provincial, and private lands. Three ranchers grazed cattle in the Flathead west of GNP; cattle were not present during winter. No cattle were permitted in the BC portion of the study area. None of these consumptive uses was permitted within GNP where wolves spent most of their time when in the United States.

Ungulate prey of wolves within the study area included white-tailed deer (*Odocoileus virginianus*), elk (*Cervus elaphus*), moose (*Alces alces*), and mule deer (*O. hemionus*; Boyd et al. 1994). In addition, bighorn sheep (*Ovis canadensis*) occurred in the Wigwam area and snowshoe hare (*Lepus americanus*) and beaver (*Castor canadensis*) inhabited the entire area. Other predators competing for some of the same prey in this ecosystem included grizzly bears (*Ursus arctos*), black bears (*U. americanus*), cougars (*Puma concolor*), coyotes (*Canis latrans*), and humans.

## METHODS

We captured wolves and sedated them following techniques described by Mech (1974) and Ream et al. (1991). Wolves >20 kg were radiocollared; smaller wolves were eartagged only. We assumed equal catchability of males and females (Mech 1975).

Radiocollared wolves were located from the ground about 3 times per week and from an airplane about once per week. We determined pack sizes from aerial observations and from track counts along winter travel routes. Pelage color varied from black to light gray, and this factor aided in determining pack sizes during aerial counts by color combination as well as total number observed. We noted possible immigrants during aerial counts using known pack sizes and color combinations.

The first observations of pups generally were made in late June or July while aerially locating radiocollared adults. Some pup mortality may have occurred before initial observation. Pups were distinguished from adults based on size differences and behavioral observations through September. By October, pups and adults were of similar size and differentiation became increasingly difficult; color combinations (black or gray) to identify individuals, and known mortalities and dispersals were then used to track pup survival until December.

We investigated radiocollared wolves that died to determine the cause of mortality. Mortalities of nonradioed wolves were sometimes discovered at these same locations. Other mortalities were found while back-tracking wolves during winter and at den sites following abandonment. Wolves legally harvested from the study area in Canada were reported to us by Canadian officials.

We estimated age- and sex-specific survival rates and survival rates of dispersing and resident wolves for radiocollared and eartagged wolves (Trent and Rongstad 1974) using program MICROMORT (Heisey and Fuller 1985). One of 4 eartagged wolves was excluded because its fate was unknown. Radiocollared wolves that were dispersal-aged or showed typical pre-dispersal movements (from and back to the natal pack territory, generally over a period of weeks) and subsequently disappeared were assumed to have dispersed. Three wolves that did not show pre-dispersal movements were assumed to have dispersed in the first survival and dispersal analysis, and to have been killed illegally in the second analysis. We assigned mortality dates as halfway between the last known live location and the first indication that the wolf had died, unless carcass evidence indicated otherwise.

Age was divided into 2 classes ($<3$ yr vs. $\geq 3$ yr) because most dispersal and resettling is done by wolves $<3$ years of age (Gese and Mech 1991, Boyd et al. 1995). We compared survival between sexes, between ages, and between residents and dispersers with $z$ tests. For MICROMORT analysis, the biological year began on 1 April because denning occurred during this month. Initially, each month was considered an interval with a constant daily survival rate. Daily survival rates for each interval were compared, and data from intervals pooled if rates were not significantly different (Heisey and Fuller 1985).

Dispersal in wolves usually is defined as occurring when an animal leaves its natal territory (Gese and Mech 1991). Because we were interested primarily in the population within and adjacent to GNP, we classified wolves as dispersers only if they permanently left the study area.

## RESULTS

Sex ratios of all captured wolves favored females (38 F:16 M, 70% F; $\chi^2 = 8.96$, 1 df, $P < 0.005$). We also evaluated sex ratio only among those wolves younger than the minimum known age of dispersal (16 months) to explore the possibility that the skewed sex ratio existed because dispersal differed between the sexes. A weaker bias toward females remained evident in this sample (25 F:14 M, 64% F; $\chi^2 = 3.10$, 1 df, $P = 0.08$). There was no significant difference from parity in our small sample of pups (8 F:5 M, 62% F; $\chi^2 = 0.69$, 1 df, $P > 0.25$).

Observed immigration during the study period was minimal. We documented 2 females (1 in 1986 and 1 in 1987) and 1 male (in 1992) joining known packs. While all may have been immigrants (based on color combinations of missing wolves from the study area), the female in 1987 is the only known immigrant from outside the study area. While we know of no other immigrants, we cannot dismiss the possibility that other wolves immigrated at about the same time resident wolves dispersed or disappeared.

Twenty-six known denning efforts were documented (Table 1). Maximum pup counts ranged from 1 to 9 ($\bar{x} = 5.3$, SE = 0.4, $n = 26$). December pup counts (through 1994) ranged from 0 to 9 ($\bar{x} = 4.5$, SE = 0.5, $n = 26$). Four additional packs had $\geq 3$ adults in them during spring but apparently did not den. No significant relations were found between number of adults in May and maximum pup counts ($r_s = -0.13$, 23 df, $P = 0.25$) or December pup counts ($r_s = -0.12$, 23 df, $P = 0.28$).

Of the 137 pups known to have been born in the study area through 1994, 117 (85.4%) survived until at least December (Table 1). Eight of the 20 (40%) pups that died were known to be human-caused; 3 pups (15%) died of unknown causes at their natal dens, and 9 (45%) disappeared during summer and their fates are unknown.

We examined carcasses from 46 wolf mortalities. Mortalities occurred in all months; of the 43 non-neonatal mortalities, 36 (83.7%) were human-caused (Table 2) and we suspect 2 others were also human-caused. Twenty-two wolves were killed legally in BC and Alberta, and 11 wolves were killed illegally (BC and the U.S.). Only 4 of the non-neonatal mortalities occurred within GNP.

Twenty-nine radiocollared wolves died or were assumed to have died during the study. Survival data were pooled for the study period because of small sample sizes, especially before 1987. Daily survival rates for each monthly interval from April through August and September through March were similar ($G^2$ test, $P > 0.05$), thus these periods were defined as intervals during which daily survival rates were assumed constant.

Table 1. Production and survival of wolf pups in the northwestern Montana and southeastern BC study area, 1982–94.

| Year | Packs | Adults in May | Max. pup count | Pups in Dec |
|---|---|---|---|---|
| 1982 | 1 | 2 | 7 | 7 |
| 1985 | 1 | 6 | 7 | 7 |
| 1986 | 1 | 8 | 5 | 3 |
| 1987 | 3 | 5, 3, 2 | 5, 6, 6 | 1, 6, 5 |
| 1988 | 3 | 5–8, 5–7, 3 | 6, 6, 1 | 6, 4, 1 |
| 1989 | 2 | 9–10, 3 | $2^1$, 9 | 0, 9 |
| 1990 | 3 | 2, 2–5, 11 | 6, 6, 2 | 6, 6, 2 |
| 1991 | 4 | 7, 5–7, 1, 5 | —, 7, 2, 4 | —, 7, 2, 1 |
| 1992 | 4 | 5, 11, 3, 4 | 5, 2, 6, 7 | 5, 2, 4, 7 |
| 1993 | 4 | 10, 5–10, 5, 6 | 8, 8, 7, — | 7, 7, 7, — |
| 1994 | 3 | 11, 3, 7 | —, $2^2$, 5 | —, 0, 5 |
| TOTAL | | | 137 | 117 |
| | | | $\bar{x} = 5.3$ | $\bar{x} = 4.5$ |
| | | | SE = 0.4 | SE = 0.5 |

[1] These pups were found dead at the den (Johnson et al. 1994).

[2] Two pups were heard howling but never seen.

Interval and annual survival rates were not different ($z$ tests, $P > 0.54$) between males and females (Table 3). Annual survival of wolves ≥3.0 years of age was not different than for wolves <3.0 years old (0.80 vs. 0.74, $P = 0.45$; Table 3).

Annual survival of radiocollared wolves within the study area was significantly greater than survival following dispersal (0.84 vs. 0.66, $P = 0.025$; Table 3). The overall annual survival rate of all radiocollared wolves when dispersal was assumed for the 3 wolves showing no pre-dispersal movements was 0.80 (0.77 when the 3 wolves were assumed to have been illegally killed). The annual rate of known, human-caused mortality (assuming dispersal for the 3 wolves showing no pre-dispersal behavior was 0.07 for illegal and 0.10 for legal mortalities (Table 4).

Table 2. Non-neonatal wolf mortalities in Montana, southeastern British Columbia, and southwestern Alberta 1982–95.

| Cause of mortality | No. of mortalities |
|---|---|
| Human-caused | |
| Legal | |
| Shot | 22 |
| Research/control action | 2 |
| Illegal | |
| Shot | |
| Verified | 5 |
| Probable | $2^1$ |
| Poison | 5 |
| Unknown | $2^2$ |
| Other causes | |
| Avalanche[3] | 1 |
| Ungulate | 1 |
| Wolves | 1 |
| Unknown | 2 |
| TOTAL | 43 |

[1] One wolf starved following a probable bullet wound; the radiosignal from the second wolf came from a garage.

[2] One was killed during fall hunting season in BC; the other's radiocollar was found in a river near Missoula, Mont.

[3] See Boyd et al. 1992.

From 26 August 1984 through 31 May 1995, 19 of 43 (44.2%) radiocollared wolves dispersed out of the study area. Fourteen (73.7%) of these were females, comparable to the sex ratio of captured wolves (70%).

The number of contiguous packs grew from 1 in 1982 to 4 in 1987. The 1987 hunting season resulted in the demise of 1 pack, and the number of packs in the study area did not reach 4 again until another pack split in 1990. The number of packs remained at 4 through 1993. During 1993, the northernmost pack disappeared. We are currently unsure of the status of this pack, though a pair of tracks were seen in its former territory during May 1995. The pre-denning population grew from 2 wolves in 1982 to a minimum of 23 in 1995, an average finite rate of increase of 1.20 ($r = 0.18$) for the 13 year period. The annual rate of increase ranged from 0.74 to 1.44 with the highest rate occurring in the first years and the lowest rates occurring in the last years. The population density within the study area in 1994 (Singleton 1995) was 35 wolves/1,000 km$^2$.

Of the 140 wolves known to have been in the study area (the 2 original wolves, 137 pups born, and assuming 1 immigrant), we can account for the fates of 80 (26 in the study area as of Dec 1994, 46 known mortalities, 6 probable pup mortalities, and 2 known dispersers still alive).

Table 3. Survival rates of 52 radiocollared wolves in northwestern Montana for August 1984–May 1995.

| | Apr–Aug | | | Sep–Mar | | | Annual | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | Rate[a] | 95% CI | *n*[b] | Rate[a] | 95% CI | *n*[b] | Rate[a] | 95% CI | *n*[b] |
| Male | 0.97* | 0.92–1.00 | 5765 | 0.82* | 0.72–0.94 | 8688 | 0.80* | 0.69–0.93 | 14453 |
| Female | 0.95* | 0.91–1.00 | 12745 | 0.82* | 0.74–0.90 | 17893 | 0.78* | 0.70–0.87 | 30638 |
| <3.0 yr | $0.92^{+}$ | 0.84–1.00 | 7095 | $0.81^{+}$ | 0.72–0.91 | 12107 | $0.74^{+}$ | 0.64–0.86 | 19202 |
| ≥3.0 yr | $0.97^{+}$ | 0.93–1.00 | 10260 | $0.82^{+}$ | 0.73–0.92 | 13018 | $0.80^{+}$ | 0.71–0.90 | 23278 |
| Resident | $0.98^{x}$ | 0.94–1.00 | 12173 | $0.86^{x}$ | 0.79–0.93 | 18282 | $0.84^{x}$ | 0.77–0.92 | 30455 |
| Disperser | $0.92^{x}$ | 0.83–1.00 | 5333 | $0.72^{x}$ | 0.60–0.88 | 7157 | $0.66^{xx}$ | 0.53–0.82 | 12490 |
| Overall[c] | 0.96 | 0.93–1.00 | 18951 | 0.83 | 0.77–0.89 | 27255 | 0.80 | 0.73–0.87 | 46206 |
| Overall[d] | 0.94 | 0.90–0.99 | 18510 | 0.82 | 0.76–0.89 | 27573 | 0.77 | 0.71–0.85 | 46083 |

[a] Rates followed by 2 superscript symbols are significantly different ($P < 0.05$) than rates above them with only 1 of the same superscript.

[b] No. of transmitter-days.

[c] Assuming dispersal of 3 wolves showing no pre-dispersal behavior.

[d] Assuming mortality of 3 wolves showing no pre-dispersal behavior.

## DISCUSSION

The sex ratio of offspring in wolves favors males in saturated, high density populations on a relatively low nutritional plane (Mech 1975). Conversely, females would be favored in low density populations where the nutritional plane was higher. The preponderance of females in our expanding population appears to support Mech's hypothesis, though we have few data for pups.

Immigration into a population is difficult to monitor with certainty, even in a population as intensively monitored as ours. We recorded 1 known and 2 possible immigrants following the initial recolonization. Recent genetic findings (Forbes and Boyd 1996) indicate immigration was greater than that suggested by our direct observations.

Pup production in our study area was relatively high compared to studies reviewed by Fuller (1989). The only study with a comparable number of pups produced was a heavily exploited wolf population in southcentral Alaska (Ballard et al. 1987).

Pack size and surviving litter size in canids generally are correlated positively, presumably because pack members help feed pups (Harrington et al. 1983). The one negative correlation was in a study area where the wolf population was declining. We found no significant correlation between pack size and litter size; our results concurred with results from several other studies (Peterson et al. 1984, Ballard et al. 1987, Fuller 1989). Our pack sizes (Table 1) were greater than most of the sizes reported by Harrington et al. (1983), probably because survival rates of both pups and adults were high. These traits may characterize reintroduced and recolonizing populations in the western United States.

Survival for wolves is generally higher within their territories (Messier 1985). Lower survival of dispersing wolves than resident wolves was also reported in Alaska by Peterson et al. (1984) where survival of wolves outside their natal territory was half that of wolves within their territories. The lower rate of survival in dispersing wolves was probably due to travel in unfamiliar areas and a reduced tendency among dispersers to avoid settled areas (Peterson et al. 1984). These factors likely also played a role in our study. Many dispersers in our study left the relative security of GNP and travelled to Canada where wolf hunting and trapping were legal. A result of the recovery of the wolf population in southern Alberta and BC (coinciding with

Table 4. Cause-specific mortality rates of 52 radiocollared wolves in northwestern Montana for August 1984–May 1995.

| | Apr–Aug | | | Sep–Mar | | | Annual | | |
|---|---|---|---|---|---|---|---|---|---|
| Cause | Rate | 95% CI | *n*[a] | Rate | 95% CI | *n*[a] | Rate | 95% CI | *n*[a] |
| Unknown | 0.01 | 0.00–0.02 | 18798 | 0.01 | 0.00–0.02 | 27043 | 0.02 | 0.00–0.04 | 45841 |
| Non-human | 0.00 | 0.00–0.00 | 18798 | 0.02 | 0.00–0.05 | 27043 | 0.02 | 0.00–0.05 | 45841 |
| Illegal | 0.02 | 0.00–0.04 | 18798 | 0.06 | 0.02–0.10 | 27043 | 0.07 | 0.03–0.11 | 45841 |
| Legal | 0.02 | 0.00–0.04 | 18798 | 0.09 | 0.04–0.13 | 27043 | 0.10 | 0.05–0.15 | 45841 |

[a] No. of transmitter-days.

population growth in northwestern Mont.) was a liberalization of wolf hunting and control practices that resulted in the death of 8 of our radiocollared wolves in 1994–95. The survival rate for August 1993–May 1995 was lower than the rate for August 1984–July 1993 (0.61 vs. 0.85, $P = 0.009$). Had the 1994–95 mortalities been excluded from our analysis, survival rates of resident and dispersing wolves would not have differed. No significant differences in survival related to dispersal, however, were found in Minnesota (Fuller 1989).

Our adult and pup survival rates were similar to the highest rates reported in the literature (Fuller 1989). We used Fuller's (1989) linear model to predict an exponential rate of increase based on annual mortality rate and predicted a value of $r$ substantially higher than we found (0.32 vs. 0.18). We believe the difference may be due in part to many of our dispersers leaving the study area, and therefore they and their offspring were not counted. In addition, the relation between mortality rate and $r$ would not remain linear at low annual mortality if pup production declines as pack size increases.

The density of wolves at the end of our study was comparable to the highest densities reported by Fuller (1989:40). We expect more dispersal, to adjacent and distant areas, rather than a further increase in density within currently occupied areas.

Our annual human-caused mortality rate of 0.17 was low compared to the studies reviewed by Fuller (1989; range = 0.15–0.68). The proportion of total mortalities attributed to humans in our study area, however, was high but not unusual (Fuller 1989). In Banff and Kootenay national parks from 1986 to 1993, 28 of 29 wolf mortalities (96.6%) were caused by humans (hit by cars and trains; P. Paquet, Banff Natl. Park, pers. commun.). Thus, despite protection, humans are the most common cause of mortality in many wolf populations.

Cause-specific mortality rates can be biased when a transmitter fails but is returned later when the animal is killed by humans because the transmitter probably would not have been recovered if the death was not human-caused (White and Garrott 1990:225). Five wolves fell into this category. As a result, we may have underestimated nonhuman caused mortality rates.

The dispersal rate we documented (44%) was higher than for any other study we found. Dispersal rates from other studies ranged from 21% in northwestern Minnesota (Fritts and Mech 1981) to 35% in northcentral Minnesota (Fuller 1989). Yearling and pup dispersal rates increased during population increases and declined during stable population phases in Minnesota (Gese and Mech 1991). Increased dispersal and success at pairing also occurred in an expanding wolf population with an ample prey base in Minnesota (Fritts and Mech 1981). The opportunity for dispersing wolves to successfully establish their own pack in unoccupied territory was high during our study and may have been a factor in the high dispersal rate (Boyd et al. 1995).

Dispersal occurred in all directions. Dispersers went north to Canadian national parks and beyond, to the Missoula area, to Idaho (Boyd et al. 1995), to the Rocky Mountain front near Augusta, Montana (J. Fontaine, U.S. Fish and Wildl. Serv., Helena, pers. commun.), and possibly south of Yellowstone National Park (S. Fain, Wildl. Forensic Lab, Ashland, Oreg., pers commun.). By estimating the growth rate of the population of adjacent packs within our study area, we have underestimated the effect of the GNP population on population growth at a larger scale.

Glacier National Park was without a breeding population of wolves from the 1930s until 1986 (Ream et al. 1989), although occasional dispersers were reported throughout that period (Singer 1979). Human actions surrounding the park certainly played a role in this absence (Curnow 1969), but the role of genetic or other biological factors are unknown.

Glacier National Park is likely to remain an important core area for wolves dispersing into the western United States. Wolves have a high reproductive rate, and wolf recovery should proceed relatively rapidly in the Northwestern Montana Recovery area if connectivity with wolf populations further north is maintained and human-caused mortality rates remain relatively low.

## LITERATURE CITED

BALLARD, W. B., J. S. WHITMAN, AND C. L. GARDNER. 1987. Ecology of an exploited wolf population in south-central Alaska. Wildl. Monogr. 98. 54pp.

BOYD, D. K., P. C. PAQUET, S. DONELON, R. R. REAM, D. H. PLETSCHER, AND C. C. WHITE. 1995. Dispersal characteristics of a colonizing wolf population in the Rocky Mountains. Pages 135–140 *in* L. N. Carbyn, S. H. Fritts, and

D. R. Seip, eds. Ecology and conservation of wolves in a changing world. Can. Circumpolar Inst., Univ. Alberta, Edmonton.

———, R. R. Ream, D. H. Pletscher, and M. W. Fairchild. 1994. Prey taken by colonizing wolves and hunters in the Glacier National Park area. J. Wildl. Manage. 58:289–295.

———, L. Secrest, and D. H. Pletscher. 1992. A wolf (*Canis lupus*) killed in an avalanche in southwestern Alberta. Can. Field-Nat. 106:524–525.

Curnow, E. 1969. The history of the eradication of the wolf in Montana. M.S. Thesis. Univ. Montana, Missoula. 99pp.

Day, G. L. 1981. The status and distribution of wolves in the northern Rocky Mountains of the United States. M.S. Thesis, Univ. Montana, Missoula. 130pp.

Forbes, S. H., and D. K. Boyd. 1996. Genetic variation of naturally colonizing wolves in the Central Rocky Mountains. Conserv. Biol. 10:1082–1090.

Fritts, S. H., and L. D. Mech. 1981. Dynamics, movements, and feeding ecology of a newly-protected wolf population in northwestern Minnesota. Wildl. Monogr. 80. 79pp.

Fuller, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildl. Monogr. 105. 41pp.

Gese, E. M., and L. D. Mech. 1991. Dispersal of wolves in northeastern Minnesota, 1969–1989. Can. J. Zool. 69:2946–2955.

Habeck, J. R. 1970. The vegetation of Glacier National Park. Natl. Park Serv., and Univ. Montana, Missoula. 123pp.

Harrington, F. H., L. D. Mech, and S. H. Fritts. 1983. Pack size and wolf pup survival: their relationship under varying ecological conditions. Behav. Ecol. Sociobiol. 13:19–26.

Heisey, D. M., and T. K. Fuller. 1985. Evaluation of survival and cause-specific mortality rates using telemetry data. J. Wildl. Manage. 49:668–674.

Johnson, M. R., D. K. Boyd, and D. H. Pletscher. 1994. Serologic investigations of canine parvovirus and canine distemper in relation to wolf (*Canis lupus*) pup mortalities. J. Wildl. Dis. 30:270–273.

Krahmer, R. W. 1989. Seasonal habitat relationships of white-tailed deer in northwestern Montana. M.S. Thesis. Univ. Montana, Missoula. 104pp.

Mech, L. D. 1970. The wolf: the ecology and behavior of an endangered species. Nat. Hist. Press, Garden City, N.Y. 384pp.

———. 1974. Current techniques in the study of elusive wilderness carnivores. Proc. Int. Congr. Game Biol. 11:315–322.

———. 1975. Disproportionate sex ratios of wolf pups. J. Wildl. Manage. 39:737–740.

Messier, F. 1985. Social organization, spatial distribution, and population density of wolves in relation to social status and prey abundance. Can. J. Zool. 63:1068–1077.

Peterson, R. O., J. D. Woolington, and T. N. Bailey. 1984. Wolves of the Kenai Peninsula, Alaska. Wildl. Monogr. 88. 52pp.

Pletscher, D. H., R. R. Ream, R. Demarchi, W. G. Brewster, and E. E. Bangs. 1991. Managing wolf and ungulate populations in an international ecosystem. Trans. North Am. Wildl. Nat. Resour. Conf. 56:539–549.

Ream, R. R., M. W. Fairchild, D. K. Boyd, and A. Blakesley. 1989. First wolf den in western U.S. in recent history. Northwest. Nat. 70:39–40.

———, ———, ———, and D. H. Pletscher. 1991. Population dynamics and home range changes in a colonizing wolf population. Pages 349–366 *in* R. B. Keiter and M. S. Boyce, eds. The Greater Yellowstone Ecosystem: redefining America's wilderness heritage. Yale Univ. Press, New Haven, Conn.

———, and R. Harris. 1986. Wolf movements in southern Alberta. Natl. Geogr. Res. 21:405–409.

———, ———, J. Smith, and D. K. Boyd. 1985. Movement patterns of a lone wolf, *Canis lupus*, in unoccupied wolf range, southeastern British Columbia. Can. Field-Nat. 99:234–239.

———, and U. Mattson. 1982. Wolf status in the northern Rockies. Pages 362–381 *in* F. H. Harrington, and P. C. Paquet, eds. Wolves of the world. Noyes Publ., Park Ridge, N.J.

Singer, F. J. 1979. Status and history of timber wolves in Glacier National Park, Montana. Pages 19–42 *in* E. Klinghammer, ed. The behavior and ecology of wolves. Garland STPM Press, New York, N.Y.

Singleton, P. H. 1995. Winter habitat selection by wolves in the North Fork of the Flathead River Basin, Montana and British Columbia. M.S. Thesis, Univ. Montana, Missoula. 116pp.

Trent, T. T., and O. J. Rongstad. 1974. Home range and survival of cottontail rabbits in southwestern Wisconsin. J. Wildl. Manage. 38:459–472.

White, G. C., and R. A. Garrott. 1990. Analysis of wildlife radio- tracking data. Acad. Press, San Diego, Calif. 383 pp.

*Received: 7 November 1995.*
*Accepted: 18 October 1996.*
*Associate Editor: McDonald*

Journal of Wildlife Management 74(4):620–634; 2010; DOI: 10.2193/2008-584

*Management and Conservation Article*

# Survival of Colonizing Wolves in the Northern Rocky Mountains of the United States, 1982–2004

**DOUGLAS W. SMITH,**[1] *National Park Service, Yellowstone Center for Resources, Wolf Project, P.O. Box 168, Yellowstone National Park, WY 82190, USA*

**EDWARD E. BANGS,** *United States Fish and Wildlife Service, 585 Shepard Way, Helena, MT 59601, USA*

**JOHN K. OAKLEAF,** *United States Fish and Wildlife Service, Mexican Wolf Project, P.O. Box 856, Alpine, AZ 85920, USA*

**CURTIS MACK,** *Nez Perce Tribe, P.O. Box 1922, McCall, ID 83638, USA*

**JOSEPH FONTAINE,** *United States Fish and Wildlife Service, 585 Shepard Way, Helena, MT 59601, USA*

**DIANE BOYD,** *College of Forestry and Conservation, University of Montana, 32 Campus Drive, Missoula, MT 59812, USA*

**MICHAEL JIMENEZ,** *United States Fish and Wildlife Service, P.O. Box 2645, Jackson, WY 83001, USA*

**DANIEL H. PLETSCHER,** *College of Forestry and Conservation, University of Montana, 32 Campus Drive, Missoula, MT 59812, USA*

**CARTER C. NIEMEYER,** *United States Fish and Wildlife Service, 1387 S Vinnell Way, Boise, ID 83709, USA*

**THOMAS J. MEIER,** *National Park Service, Denali National Park and Preserve, P.O. Box 9, Denali Park, AK 99755, USA*

**DANIEL R. STAHLER,** *National Park Service, Yellowstone Center for Resources, Wolf Project, P.O. Box 168, Yellowstone National Park, WY 82190, USA*

**JAMES HOLYAN,** *Nez Perce Tribe, P.O. Box 1922, McCall, ID 83638, USA*

**VALPHA J. ASHER,** *Turner Endangered Species Fund, 1123 Research Drive, Bozeman, MT 59718, USA*

**DENNIS L. MURRAY,** *Department of Biology, Trent University, Peterborough, ONT K9J 7B8, Canada*

**ABSTRACT** After roughly a 60-year absence, wolves (*Canis lupus*) immigrated (1979) and were reintroduced (1995–1996) into the northern Rocky Mountains (NRM), USA, where wolves are protected under the Endangered Species Act. The wolf recovery goal is to restore an equitably distributed metapopulation of $\geq 30$ breeding pairs and 300 wolves in Montana, Idaho, and Wyoming, while minimizing damage to livestock; ultimately, the objective is to establish state-managed conservation programs for wolf populations in NRM. Previously, wolves were eradicated from the NRM because of excessive human killing. We used Andersen–Gill hazard models to assess biological, habitat, and anthropogenic factors contributing to current wolf mortality risk and whether federal protection was adequate to provide acceptably low hazards. We radiocollared 711 wolves in Idaho, Montana, and Wyoming (e.g., NRM region of the United States) from 1982 to 2004 and recorded 363 mortalities. Overall, annual survival rate of wolves in the recovery areas was 0.750 (95% CI = 0.728–0.772), which is generally considered adequate for wolf population sustainability and thereby allowed the NRM wolf population to increase. Contrary to our prediction, wolf mortality risk was higher in the northwest Montana (NWMT) recovery area, likely due to less abundant public land being secure wolf habitat compared to other recovery areas. In contrast, lower hazards in the Greater Yellowstone Area (GYA) and central Idaho (CID) likely were due to larger core areas that offered stronger wolf protection. We also found that wolves collared for damage management purposes (targeted sample) had substantially lower survival than those collared for monitoring purposes (representative sample) because most mortality was due to human factors (e.g., illegal take, control). This difference in survival underscores the importance of human-caused mortality in this recovering NRM population. Other factors contributing to increased mortality risk were pup and yearling age class, or dispersing status, which was related to younger age cohorts. When we included habitat variables in our analysis, we found that wolves having abundant agricultural and private land as well as livestock in their territory had higher mortality risk. Wolf survival was higher in areas with increased wolf density, implying that secure core habitat, particularly in GYA and CID, is important for wolf protection. We failed to detect changes in wolf hazards according to either gender or season. Maintaining wolves in NWMT will require greater attention to human harvest, conflict resolution, and illegal mortality than in either CID or GYA; however, if human access increases in the future in either of the latter 2 areas hazards to wolves also may increase. Indeed, because overall suitable habitat is more fragmented and the NRM has higher human access than many places where wolves roam freely and are subject to harvest (e.g., Canada and AK), monitoring of wolf vital rates, along with concomitant conservation and management strategies directed at wolves, their habitat, and humans, will be important for ensuring long-term viability of wolves in the region.

**KEY WORDS** *Canis lupus*, gray wolf, mortality, Northern Rocky Mountains, protected areas, survival.

Gray wolves (*Canis lupus*) were eradicated from the northern Rocky Mountains (NRM) of the United States by the 1930s (Young and Goldman 1944, McIntyre 1995). For the next 50 years, wolves were only occasionally reported and there was no functional wolf population in the area (U.S. Fish and Wildlife Service [USFWS] 1994). Reestablishment of wolves to northwest Montana (NWMT) began in 1979 through dispersal from Canada, and reproduction was first documented in 1986 (Ream et al. 1991, Pletscher et al. 1997). Wolves from Canada were reintroduced to central Idaho (CID) and Yellowstone National Park (YNP) in 1995 and 1996 to establish wolves in Idaho and the Greater Yellowstone Area (GYA; USFWS 1994, Bangs and Fritts 1996).

The Endangered Species Act (ESA) was passed in 1973 and wolves were listed in the contiguous United States in 1974. Wolf recovery plans were formulated for the NRM (ID, MT, and WY) in 1980 and 1987 and reintroductions

---

[1] *E-mail: doug_smith@nps.gov*

to CID and YNP using wild wolves from Canada were recommended in an Environmental Impact Statement in 1994. Configured as a 3-segment metapopulation and one recovery area, the objective of the program was to restore wolves as a viable population to the NRM and return management to the affected States. Recovery plans included genetic exchange, either natural or artificial, between the 3 populations (USFWS 1994). Genetic exchange was assumed to be primarily natural because of the distance between recovery areas and dispersal capability of wolves (>500 km; Fritts 1983, Boyd and Pletscher 1999).

The minimum goal for restoration was to establish a metapopulation of $\geq 30$ breeding pairs, with a breeding pair defined as an adult male and female wolf that raise 2 young to 31 December, and $\geq 300$ wolves equitably distributed among the 3 core recovery areas for a $\geq 3$ successive years (USFWS 1994). In addition to a minimum population requirement, each state needed a USFWS-approved management plan. Once this was achieved, wolves would be removed from the Endangered Species list and managed solely by the States of Idaho, Montana, and Wyoming, USA. These minimum population requirements were reached in 2002, but approved state plans were not completed until 2007. Wolf delisting occurred in 2008 but was remanded back to the USFWS after litigation for further consideration. Wolves were again delisted, except in Wyoming, in 2009.

The strategy for recovery was to nurture natural wolf immigration and to protect as endangered any population of wolves that became established in NWMT (USFWS 1987, 1994). Unlike NWMT, wolf restoration in CID and GYA called for reintroduction of wolves from Canada and management not as endangered but as experimental–nonessential, which allowed for more management flexibility in conflict situations (e.g., allowing ranchers to legally shoot wolves depredating on livestock) and less administration (e.g., no ESA Section 7 consultation).

Beginning in 1982, radiocollars were placed on wolves in NWMT to aid management and research (Ream et al. 1991, Pletscher et al. 1997). From 1995 to 1996, 35 and 31 wolves from Canada were reintroduced to CID and YNP, respectively (Bangs and Fritts 1996, Phillips and Smith 1996). All reintroduced wolves were fitted with radiocollars and survival was monitored. In all 3 areas annual radiocollaring efforts directed at wild-born animals continues. The annual proportion of the population collared ranged from 20% to 50% (Mitchell et al. 2008). We used radiocollar data from 1982 to 2004, a period of USFWS oversight for wolf management prior to proposed delisting and state management, to examine factors associated with wolf hazard.

The wolf population expanded rapidly in the 2 areas where wolves were reintroduced but more slowly where they had recolonized naturally (Bangs et al. 1998, Fritts et al. 2001). In 2004, 324 wolves were present in GYA, 452 in CID, and 59 in NWMT (USFWS et al. 2005).

The recovery plan for wolves in the NRM emphasized establishing successfully reproducing packs in core areas of secure habitat where wolf mortality would be minimal (USFWS 1987, 1994). The CID wilderness complex, YNP, Glacier National Park (GNP), and the Bob Marshall wilderness area (BMWA) of NWMT, and the extensive areas of multiple-use public land surrounding those areas, were selected to function as core areas–refugia for wolf recovery (USFWS 1987). All of these areas encompass large areas of public land where livestock grazing and motorized vehicle use, 2 factors contributing to higher rates of wolf mortality, are limited (USFWS 1994, Mitchell et al. 2008). Outside these core areas, habitat for wolves is less suitable and dominated by agriculture, and wolf protection accordingly is more tenuous. However, wolves were expected to be able to disperse between these 3 core recovery areas and survive in less secure habitat, facilitating connection between the 3 areas and thereby creating a large metapopulation (USFWS 1987, Pletscher et al. 1997, Boyd and Pletscher 1999). Critical to this plan was understanding if the 3 core areas functioned as presumed, or in other words that mortality outside core areas would not overwhelm source populations of wolves leading to population declines.

Human-caused mortality in the NRM strongly affects wolf population viability (Mitchell et al. 2008) as it does for other wolf populations (Fuller 1989, Adams et al. 2008, Person and Russell 2008). In addition, each NRM area differed in land status or ownership (e.g., park, wilderness, state, private, national forest) and management policy, so it was open to question how wolves would fare in each area. In general human access across the NRM is much greater than other areas where wolves have been studied (e.g., AK and Canada) even in wilderness areas (e.g., outfitter horse access for big-game hunting). Lastly, wolves in NWMT were managed as endangered, giving them greater legal protection than reintroduced wolves in CID and GYA.

Despite these differences, and based on research from NWMT during their colonization phase that indicated high survival among wolves there (Pletscher et al. 1997, Boyd and Pletscher 1999), we predicted that wolf survival would not differ between recovery areas nor threaten the NRM population because wolves were increasing most years (except for some yr in NWMT; USFWS et al. 2005). Because excessive levels of human-caused mortality were the primary reason wolves were extirpated, evaluation was important because delisting requires that the causes of endangerment be reduced to a level that no longer threatens the population (ESA of 1973). Survival data were also important beyond population counts because we could assess factors associated with high risk for wolves, which would inform management action. Further, sustainable survival rates for wolves are already known and indicative of population status (Keith 1983, Fuller et al. 2003, Adams et al. 2008), and because radiocollars were being used extensively as part of the recovery effort, we could easily use them for survival estimation comparing them to this larger data set, which would inform us about the status of our population. Therefore, our objectives were to determine demographic, behavioral, and anthropogenic determinants of wolf survival across the 3 recovery areas of the NRM.

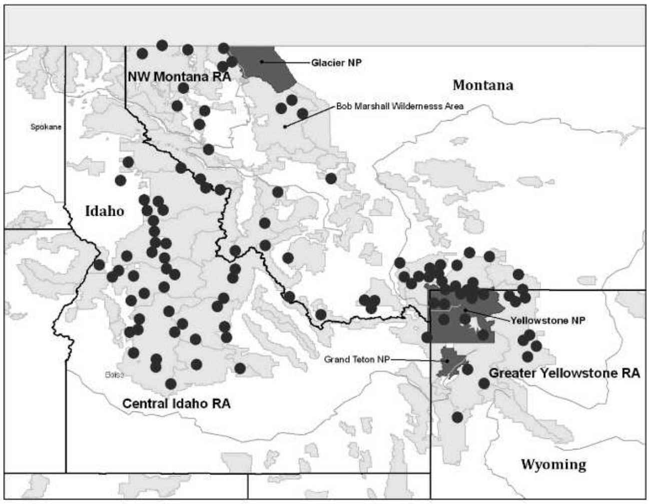


**Figure 1.** Location of wolf packs (black dots) in the northern Rocky Mountain study area (USA) in 2004. Note few packs in the Glacier National Park (NP)–Bob Marshall Wilderness Area portion of the northwestern Montana recovery area (RA) relative to Central Idaho and Greater Yellowstone Area.

## STUDY AREA

Our large study area (>266,400 km$^2$) is difficult to characterize specifically because of wide local and regional variation (Fig. 1). Each of the 3 recovery areas is mountainous; a mountain–valley dichotomy prevails and this habitat is critical to supporting wildlife in the region. Many species of wildlife in this study area typically spend winter in the mountain valleys, where human population density is high, and summer in more remote mountains (Hansen et al. 2002). Ungulate seasonal movements follow this pattern, as do wolves, within their territorial constraints (wolves in the NRM are not migratory). Elevations ranged from about 200 m to 4,200 m; annual precipitation ranged from 25 cm to 150 cm. Temperatures ranged from −40° C to 35° C due to variation in elevation. Vegetation was dominated by coniferous forests of lodgepole pine (*Pinus contorta*), Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*), subalpine fir (*Abies lasiocarpa*), Englemann spruce (*Picea engelmannii*), and whitebark pine (*Pinus albicaulis*), with quaking aspen (*Populus tremuloides*) and cottonwood (*Populus* spp.) at lower elevations. Willow (*Salix* spp.) was also common throughout the area. Grasslands, high mountain meadows, and shrub-steppe habitats were interspersed throughout the region. Each area had several major river systems and lesser watercourses creating the mountain–valley dynamic important to the vegetation and summer–winter movement of ungulates and wolves. In general, winters were from October to April with most precipitation coming as snow, but increasing variability in snowfall and duration has been recorded (Wilmers and Post 2006; YNP, unpublished data).

Each wolf recovery area was centered on a large area of public land including National Parks, National Forest, and designated wilderness (Table 1). The NWMT recovery area encompassed GNP and the BMWA, comprising 11,770 km$^2$, and was surrounded by national forest lands, Blackfeet tribal, or private lands. The NWMT was the most fragmented recovery area and was interspersed with private lands mainly used for timber production (Table 1). The CID recovery area was about 53,000 km$^2$ of primarily national forest including 15,800 km$^2$ of wilderness, but there was also permitted grazing on public land. In the GYA recovery area, YNP occupied 8,991 km$^2$ in a 68,000-km$^2$ recovery area comprised mostly (62%) of public land (national forest, national wildlife refuges, and Bureau of Land Management areas).

Like physiographic features and climate, the potential prey of wolves varied across areas but was generally similar. Each area had a mix of elk (*Cervus elaphus*), deer (mule [*Odocoileus hemionus*] and white-tailed [*O. virginianus*]), moose (*Alces alces*), bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), and pronghorn antelope (*Antilocarpa americana*). Bison (*Bison bison*) were unique to YNP and Grand Teton National Park. Primary prey for wolves varied across the region but was generally either elk or deer, although some wolves in YNP used bison as well (Smith et al. 2000). Most areas had the full complement of large carnivores, except that grizzly bears (*Ursus arctos*) were absent in the CID recovery area. Besides wolves, black (*U. americanus*) and grizzly bears, cougars (*Felis concolor*), coyotes (*Canis latrans*), and humans preyed on most of these ungulates. Livestock, mostly cattle and sheep, were also accessible to wolves throughout the year but were more vulnerable in summer. A more detailed description specific to each study area is presented in Mitchell et al. (2008).

## METHODS

We captured and radiocollared wolves as adults, yearlings, and pups (>20 kg; usually >5 months of age) either by foot-hold trapping or helicopter darting and netting. Capture efforts in NWMT were almost exclusively foot-hold traps during May–October; capture in the Yellowstone area was almost exclusively darting in YNP November–February and a combination of trapping and darting outside (yr-round); Idaho was predominately trapping with some

**Table 1.** Land characteristics of the 3 wolf recovery areas (Central ID [CID], Greater Yellowstone Area [GYA], and northwestern MT [NWMT]) in the Northern Rocky Mountains of the United States. We based land characteristics and area for densities in 2004 on the intensive study area identified in Oakleaf et al. (2006) and on the average of 9-km$^2$ cells in each recovery area.

| Recovery area | Wolf density (no./1,000 km$^2$) | % private | % federal | % forest | Human density (no./km$^2$) | Road density (km/km$^2$) |
|---|---|---|---|---|---|---|
| GYA | 1.77 | 31.7 | 62.2 | 31.1 | 2.34 | 0.66 |
| CID | 3.03 | 23.2 | 72.4 | 47.3 | 2.64 | 0.60 |
| NWMT | 0.72 | 36.3 | 56.3 | 59.8 | 3.825 | 0.77 |

**Table 2.** Description of variables we used in analyses of wolf survival in northwestern United States (1982–2004).

| Variable | Description and coding system |
|---|---|
| Demographic | |
| GENDER | Gender (M = 1) |
| AGECLASS[a] | Dummy variable representing age class (pup; yearling; ad; old ad [>9 yr]) |
| AGEYEARS[b,c] | Age (yr) |
| Behavioral | |
| DISPERSER | Dummy variable representing dispersal status (disperser = 1) |
| BREEDING[b] | Dummy variable representing breeding status (current breeder = 1) |
| PACKMEMBER[b] | Dummy variable representing pack affiliation status (with pack = 1) |
| SMALLPACK[b] | Dummy variable representing small pack size ([pack size <5] = 1) |
| HOMERANGESIZE[b,c] | 95% fixed kernel home range size ($km^2$) |
| Temporal | |
| YEAR[a] | Dummy variables representing each yr of the study (1982–2004) |
| SEASON[a] | Dummy variable representing each season of the study (Jan–Mar; Apr–Jun; Jul–Sep; Oct–Dec) |
| Anthropogenic | |
| ROADS[b,c] | Road density in home range ($km^2$) |
| HUMANS[b,c] | Human density in home range ($km^2$) |
| FEDERAL[b] | % of home range managed by Federal government |
| PRIVATE[b] | % of home range under private ownership |
| STATE | % of home range managed by the State government |
| CATTLE[b,c] | Cattle density in home range ($km^2$) |
| SHEEP[b,c] | Sheep density in home range ($km^2$) |
| PROTECTION[b] | Average protection status in home range, determined by Gap Analysis Program (GAP) |
| Habitat | |
| ELEVATION[b] | Average elevation in home range (m)/1,000 |
| SLOPE[b] | Average slope in home range (°) |
| ELK[b] | Dummy variable representing elk as primary prey in home range (elk = 1) |
| MULEDEER[b] | Dummy variable representing mule deer as primary prey in home range (mule deer = 1) |
| FOREST[b] | % of home range covered by forest habitat |
| AGRICULTURAL[b] | % of home range covered by modified agricultural land |
| Other | |
| RECOVERYAREA[a] | Recovery area where the subject was resident |
| RECRUITMENT | Whether subject was recruited as part of representative vs. targeted capture efforts (representative = 1) |
| PACKSADJACENT[b] | No. of wolf packs adjacent to home range |

[a] We developed variable by coding each category into a separate dummy variable.
[b] Available only for a subsample of subjects.
[c] Also includes separate variable representing quadratic relationship ($x + x^2$).

darting and netting (yr-round). Once captured, standard measurements and biological samples such as blood were obtained from each wolf and a very high frequency and occasionally a Global Positioning System radiocollar (Telonics Inc., Mesa, AZ and Televilt Inc., Lindesberg, Sweden) was attached. All radiocollars contained mortality switches that increased radio-signal pulse from approximately 55 beats/minute to approximately 110 beats/minute if a collar was stationary for 4 hours. Radiocollars in mortality mode were retrieved as soon as feasible, usually within 1 week, but some circumstances prevented quick recovery. Cause of death was determined through on-site exams or lab necropsies either because field exams were inconclusive or a law-enforcement investigation was involved. We focused specifically on wolf mortality-rate determinants; wolf cause of death in the context of risk is addressed in other research (D. Murray, Trent University, unpublished data).

Once collared, wolves were typically tracked from aircraft every 7–14 days, but often more frequently, as in YNP where wolves were sometimes tracked daily. When radio contact was lost with a particular wolf, search efforts ensued in the local area for several months. Coordinated high-elevation flights among recovery areas were also flown each year to look for missing wolves.

We related wolf mortality risk to a variety of independent variables (Table 2; Oakleaf et al. 2006). We assessed variables not in Oakleaf et al. (2006) as follows: we categorized PACKSIZE (we considered <5 wolves a small pack and ≥5 wolves a large pack) by observing pack size during winter observations, which typically occurred multiple times per season. We based DISPERSER status of study animals on knowledge of the territory of the radioed wolf after collaring versus its pack affiliation prior to dispersal. We considered a subject animal as resident if it was traveling with pack-mates within its territory and a disperser if it left its established territory not to return. We did not know breeder status for all collared wolves, so we only used related variables when we positively knew breeding status for animals that we either frequently observed or determined to be a breeder through observation of lactation or later pedigree analysis.

## Statistical Analysis and Modeling

*Hazard modeling.*—We right-censored wolves that either died of capture-related causes or whose radio signal was lost (e.g., transmitter failure, collar loss, emigration from the study area) at their final monitoring date; we censored those that survived until the end of the study on 31 December 2004. We assessed determinants of wolf mortality using Andersen–Gill (AG) hazard models (Fleming and Harrington 1991, Andersen et al. 1993). Briefly, AG methods are analogous to better known Cox proportional hazard models except that AG methods are based on counting process methodology and have greater flexibility including allowing discontinuous risk intervals, which makes AG models particularly well suited for telemetry-based survival analysis using subjects having punctuated survival timelines (Johnson et al. 2004, Murray 2006). The AG method records subject survival time as a function of a binomial censoring variable (1 = failure, 0 = censored) relative to counting, risk, and intensity processes; the counting process is an indicator function equal to 1 when mortality occurs, the risk process is 1 when monitoring is ongoing, and the intensity process is a product of the risk process and hazard function $h(t)$ (Fleming and Harrington 1991, Hosmer and Lemeshow 1999). Integrating the intensity process over time yields the expected number of deaths at $t$, or the cumulative intensity process. In the resulting models, hazards associated with variables $i$ and $j$ are proportional through time and differ only multiplicatively by the exponential term involving the covariates $[h_i(t)/h_j(t) = \exp(\beta_1)]$. Thus, we can easily evaluate determinants of mortality risk in a subject population using the AG framework, and it follows that such hazard models are considered as semi-parametric because distribution of lifetimes and the baseline hazard function are unspecified and the hazard ratio does not depend specifically on time.

Fitting hazard models to a large set of candidate variables presents a variety of challenges, including dealing with inconsistent functional roles of some variables among groups of subjects or across space and time. We also contended with variables that were incomplete, served as proxies for other variables or survival determinants, or whose role on hazard was interactive. Accordingly, we conducted hazard modeling through a cautious approach that emphasized phenomenological relationships between variables and wolf hazard rather than a rigid approach focused on quantitative cause-and-effect relationships. We fit several families of AG models, with the first series (demographic models) involving demographic, behavioral, and temporal variables (Table 2) for all 711 wolves monitored during the study. Variables under consideration as potential mortality-risk determinants included both continuous and categorical (dummy) variables of which several were time-dependent and could be considered time-varying (i.e., interactive with time, see Table 2); we updated most time-dependent covariates annually (e.g., age, habitat variables) but we updated seasonally those related to wolf behavior (e.g., dispersal, pack size, breeding status). Our limited a priori knowledge of the functional relationship between time-dependent variables and wolf hazard justified using a variety of modeling frameworks to assess variable significance; we conducted analyses using the same variables as either time-dependent or time-varying, and noted that results were usually qualitatively similar irrespective of variable classification scheme. Therefore, we inferred that the general relationships presented herein are robust and do not depend on variable relationships with time. In most cases we report results from the more conservative time-varying classification scheme.

The main demographic models under evaluation involved complete sets of independent variables (i.e., no missing data) such that we included all subjects in analyses; later models also included partially complete variables and, therefore, used a restricted set of subjects (see below). The first series of analyses pooled recovery areas into an all-inclusive model set, but because we detected area-specific differences in hazard, subsequent analyses considered recovery areas separately, which allowed us to examine determinants of wolf hazard both overall as well as in separate recovery areas with differing baseline habitat and prey availability, level of wolf protection, etc. The method by which we recruited subjects to the study had a profound influence on mortality risk, and this influence failed to conform to the proportional hazards assumption (see below). Therefore, we stratified most models according to subject recruitment method, later segregating demographic models by the RECRUITMENT variable specifically to evaluate hazard differences between groups. Stratification is an important process in hazard modeling and allows for calculation of a stratum-specific baseline hazard function where the assumption of hazard proportionality fails to be upheld (Hosmer and Lemeshow 1999).

The second series of models (habitat models) included spatially explicit behavioral, anthropogenic, and habitat variables associated with the 95% fixed-kernel home range of each subject (Table 2), along with other demographic and behavioral variables considered in the first series of models. We excluded temporal variables from this latter series because their evidence ratios generally were low and precision was poor. Habitat models were restricted to wolves with estimable home ranges and, therefore, excluded subjects that were either nonresident or dispersing or radiolocated too infrequently (<20 locations/yr; see Oakleaf et al. 2006) to estimate their home range. Approaches for dealing with wolf recovery areas and study recruitment method followed those described previously for demographic models. Because we re-estimated home ranges each year, we updated spatially explicit time-dependent covariates according to an annual schedule.

*Testing hazard model assumptions.*—We can easily test basic distributional and associated assumptions underlying AG models using martingale theory (Fleming and Harrington 1991, Andersen et al. 1993). The functional form of several continuous variables under consideration (e.g., AGEYEARS, ROADS, SHEEP) was not necessarily linear but could be quadratic (Johnson et al. 2004). We evaluated the most appropriate functional form of such variables by examining martingale residuals of fitted AG models against untransformed ($x$) and transformed ($x + x^2$) forms of each variable. We used the LOWESS regression yielding approximately linear fit to select the most appropriate functional form (Cleves et al. 2003). The assumption of proportional hazards is critical to AG model fit, and we determined it primarily by assessing proportionality in plots of ln-transformed analysis time versus −ln[−ln(survival probability)] (Hougaard 2000, Therneau and Grambsch 2000). We assessed model goodness-of-fit by checking Cox–Snell residuals for a standard exponential distribution where the hazard function equals 1 for all $t$ and, thus, the cumulative hazard for the residuals is linear at approximately 45° (Cleves et al. 2003). We conducted influence and leverage analysis by refitting best-fit candidate models with $n - 1$ observations and evaluating differences between the efficient score residual matrix and the variance–covariance matrix, relative to time (Cleves et al. 2003). We do not report the above diagnostics because test results were consistently favorable. Other assumption checking and diagnostic tests are outlined in the Discussion. We constructed hazard models using STATA (Stata Corporation, College Station, TX).

Given the many independent variables under consideration and the phenomenological approach we advocated when developing hazard models, we were unable to model all combinations and our analyses should be considered as exploratory. We examined 2-way interaction terms between all variables in model sets for significance (Hosmer and Lemeshow 1999). Multicollinearity is an important concern in any multivariate regression, but acceptance criteria are poorly identified especially for hazard models having many time-dependent covariates. We assessed variable multicollinearity by sequentially adding variables to our selected models and evaluating stability of the parameter estimates (Mitra and Golder 2002, Van den Poel and Larivière 2004); we considered our use of multimodel inferencing procedures (see below) to help mitigate against the influence of collinearity. We also further assessed inter-relationships between variables via standard collinearity diagnostics and appropriate thresholds (mean variance inflation factor [VIF] $> 6.0$; individual VIF $> 10.0$; tolerance $< 0.10$; condition no. $> 30.0$; Belsley et al. 1980). Where appropriate, we eliminated models including redundant variables from candidate sets to achieve independence.

We compared hazard models within each set using standard model-selection methods (Burnham and Anderson 2002), and we calculated Akaike's Information Criterion corrected for sample size ($\mathrm{AIC}_c$), $\mathrm{AIC}_c$ differences ($\Delta_i$), and $\mathrm{AIC}_c$ weights ($w_i$) to guide model selection. We used $\Delta_i < 10$ for model evaluation, and $P < 0.10$ for all individual variables, to restrict our set of candidate models to a smaller number with high ecological plausibility. We considered models with $\Delta_i < 2.0$ to be indistinguishable from the best-fit model (Burnham and Anderson 2002). Variables that were not complete for all individuals (e.g., AGEYEARS, BREEDING, PACKMEMBER, SMALLPACK) were subject to restricted analysis where we selected the best-fit model for the complete data set and used a backward stepwise procedure to remove any nonsignificant ($P > 0.10$) variables. Using $\Delta_i$, we then compared the best-fit model with versus without the restricted variable to assess its significance. Throughout, we report model-averaged hazard ratios, unconditional variances, and weight of evidence for individual variables (Burnham and Anderson 2002). For time-dependent covariates the unit was hazard ratio per day, and we used 90-day and 365-day intervals to describe their influence on subject mortality risk. Where appropriate, we report annual survival rates as determined from a piecewise exponential model (Heisey and Fuller 1985, Hougaard 2000), after having first ascertained that the assumption of constant hazards within the time interval was upheld.

# RESULTS

During 1982–2004, we monitored survival of 711 radio-collared wolves across the 3 recovery areas. Animals monitored during 1982–1994 were exclusively from NWMT, whereas those tracked during 1995–2004 also included individuals resulting from reintroductions in GYA and CID (Table 3). Number of individual animals monitored by year initially was low in NWMT and even after 1995 generally remained below numbers for GYA and CID. Numbers of monitored wolves in the GYA and CID increased steadily post-1995 and peaked at the end of the study period in 2004, whereas in NWMT monitored wolves and the wolf population did not increase (Table 3). Notably, number of wolves monitored in all 3 areas increased after 2004 (E. E. Bangs, United States Fish and Wildlife Service, unpublished data). One animal marked in YNP emigrated to Utah, whereas another emigrated from YNP and ultimately died in Colorado; we right-censored both wolves after they left GYA. Most wolves (51.1%) died during the study whereas 26.0% survived until study completion (31 Dec 2004). We censored at the last known live signal wolves either succumbing to unknown fate due to radio-signal loss (21.4%) or dying of capture-related causes (1.5%). Overall, during our study wolves died from legal control (30.0%; $n = 363$ deaths), illegal mortality (24.0%), natural causes (11.8%), other causes (e.g., vehicle accidents, strife; 21.4%), and unknown causes (11.8%). Overall, annual survival rate of wolves across all recovery areas was 0.750 (0.728, 0.772; $n = 363$ deaths).

Computed as a hazard rate, the method by which we recruited wolves into the study influenced risk of death, with those obtained through targeted sampling having consistently higher risk than the representative sample (log-rank test: $\chi^2_1 = 42.89$, $P < 0.001$; Fig. 2). Overall, 47.3% ($n = 579$) of wolves recruited through representative sampling and 64.9% ($n = 134$) of those recruited via targeted sampling died during the study. The RECRUITMENT variable failed to conform to the assumption of proportional hazards ($\chi^2_1 = 5.24$, $P = 0.022$) and we therefore stratified it in subsequent analyses. The proportion of animals that we recruited via representative sampling differed among recovery areas (GYA: 88.0% [$n = 299$], CID: 79.0% [$n = 219$], NWMT: 73.1% [$n = 193$]; $\chi^2_2 = 17.995$, $P < 0.001$), and in each recovery area wolves recruited via targeted sampling had higher mortality risk (GYA: $\chi^2_1 = 31.954$, $P < 0.001$; CID: $\chi^2_1 = 5.444$, $P = 0.020$; NWMT: $\chi^2_1 =$

**Table 3.** Numbers of radiocollared wolves monitored for survival in western United States (1982–2004). We provide total number (total no. of subjects monitored during the calendar yr), number alive (no. alive on 31 Dec), number dead (no. dying during the calendar yr), and number censored (no. whose fate was unknown during the calendar yr). Censored animals also include 11 subjects that died from capture-related causes and one that died in Colorado.

| | Greater Yellowstone Area | | | | Central ID | | | | Northwestern MT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yr | No. | Alive | Dead | Censored | No. | Alive | Dead | Censored | No. | Alive | Dead | Censored |
| 1982 | | | | | | | | | 1 | 1 | 0 | 0 |
| 1983 | | | | | | | | | 0 | 0 | 0 | 0 |
| 1984 | | | | | | | | | 1 | 1 | 0 | 0 |
| 1985 | | | | | | | | | 4 | 4 | 0 | 0 |
| 1986 | | | | | | | | | 6 | 5 | 1 | 0 |
| 1987 | | | | | | | | | 11 | 7 | 4 | 0 |
| 1988 | | | | | | | | | 13 | 11 | 1 | 1 |
| 1989 | | | | | | | | | 20 | 14 | 6 | 0 |
| 1990 | | | | | | | | | 21 | 19 | 1 | 1 |
| 1991 | | | | | | | | | 23 | 12 | 7 | 4 |
| 1992 | | | | | | | | | 23 | 22 | 1 | 0 |
| 1993 | | | | | | | | | 38 | 25 | 13 | 0 |
| 1994 | | | | | | | | | 34 | 16 | 12 | 6 |
| 1995 | 20 | 18 | 2 | 0 | 15 | 12 | 2 | 1 | 27 | 17 | 8 | 2 |
| 1996 | 39 | 31 | 8 | 0 | 32 | 30 | 1 | 1 | 20 | 11 | 7 | 2 |
| 1997 | 45 | 29 | 15 | 1 | 36 | 33 | 1 | 2 | 22 | 13 | 8 | 1 |
| 1998 | 46 | 34 | 8 | 4 | 51 | 43 | 6 | 2 | 29 | 17 | 10 | 2 |
| 1999 | 54 | 45 | 6 | 3 | 59 | 45 | 11 | 3 | 38 | 25 | 12 | 1 |
| 2000 | 59 | 44 | 11 | 4 | 61 | 41 | 15 | 4 | 33 | 15 | 16 | 2 |
| 2001 | 92 | 71 | 15 | 6 | 61 | 41 | 11 | 9 | 36 | 31 | 5 | 6 |
| 2002 | 105 | 76 | 12 | 17 | 62 | 38 | 16 | 8 | 43 | 22 | 11 | 10 |
| 2003 | 133 | 100 | 27 | 6 | 70 | 46 | 6 | 18 | 27 | 16 | 5 | 6 |
| 2004 | 156 | 94 | 38 | 24 | 100 | 71 | 20 | 9 | 27 | 23 | 3 | 1 |

16.290, $P < 0.001$; Fig. 3). Overall, mortality rates tended to be higher in NWMT than in the remaining recovery areas (using GYA as reference; CID: $z_1 = 1.55$, $P = 0.12$; NWMT: $z_1 = 2.87$, $P = 0.004$). The recovery area variable conformed to the proportional hazards assumption (global $\chi^2_2 = 2.62$, $P = 0.27$), so we conducted subsequent analyses either with recovery areas pooled or by separate recovery area. Because we monitored only 6 (0.84%) subjects in multiple recovery areas (0.34% of total radio-days), we considered recovery area as a fixed variable.

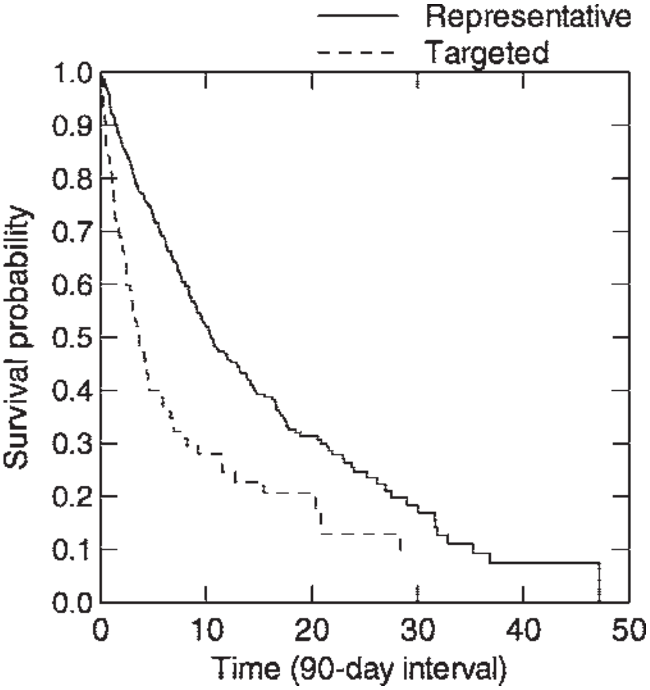


**Figure 2.** Kaplan–Meyer survivorship probability for wolves in northwestern United States (1982–2004) relative to whether subjects were radiomonitored as part of standard sampling (representative sample) or following focused capture efforts in response to livestock depredation or other problems (targeted sample). The origin (time = 0) corresponds to time of recruitment to the study.

### Pooled Recovery Areas

Using RECRUITMENT as stratum and a dummy variable (MONTANA) to isolate subjects from NWMT, we

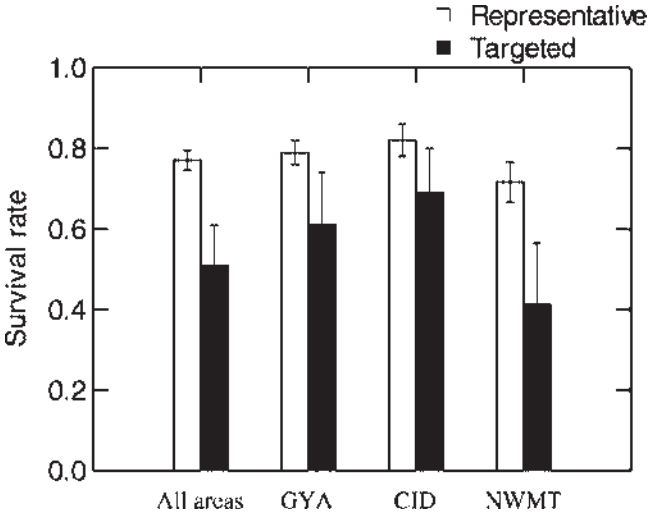


**Figure 3.** Annual survival rate (±95% CI) for wolves in western United States (1982–2004) by recovery area (GYA: Greater Yellowstone Area; CID: central Idaho; NWMT: northwestern Montana; $n = 711$ wolves, 363 deaths). Wolves were radiomonitored either as part of standard sampling (representative sample) or following focused capture efforts in response to livestock depredation or other problems (targeted sample).

**Table 4.** Candidate Andersen–Gill hazard models for wolves in northwestern United States (1982–2004), generated from models using demographic, behavioral, and temporal variables (see Table 2 for coding scheme). Sample sizes vary depending on whether models include all recovery areas (subjects = 711, deaths = 361), or are restricted to Greater Yellowstone Area (subjects = 269, deaths = 142), central Idaho (subjects = 175, deaths = 89), or northwestern Montana (subjects = 192, deaths = 130). We provide model parameter number ($K$), Akaike's Information Criterion corrected for sample size ($AIC_c$), $AIC_c$ difference ($\Delta_i$), and $AIC_c$ weight ($w_i$). Likelihood ratio chi-square and $P$ indicate goodness-of-fit for each model relative to the best-fit model. Individual parameter estimates for each model were significant ($P < 0.10$), and we provide only models with $<10\ \Delta_i$. All models were stratified according to whether subjects were recruited to the study for standard monitoring purposes (representative sample) versus following livestock depredations or other perceived problems (targeted sample).

| Model | $K$ | $AIC_c$ | $\Delta_i$ | $w_i$ | $\chi^2$ | $P$ |
|---|---|---|---|---|---|---|
| All recovery areas | | | | | | |
| MONTANA + PUP + YEARLING + DISPERSER + (YR 2004) | 5 | 3,543.1 | 0 | 0.889 | 59.10 | <0.001 |
| MONTANA + PUP + DISPERSER + (YR 2004) | 4 | 3,548.6 | 5.5 | 0.057 | 51.54 | <0.001 |
| MONTANA + PUP + YEARLING + DISPERSER | 4 | 3,548.8 | 5.7 | 0.051 | 51.33 | <0.001 |
| MONTANA + YEARLING + DISPERSER + (YR 2004) | 4 | 3,552.3 | 9.3 | 0.009 | 47.77 | <0.001 |
| Greater Yellowstone Area | | | | | | |
| DISPERSER + (YR 2002) | 2 | 1,165.8 | 0 | 0.917 | 25.91 | <0.001 |
| DISPERSER + (YR 2004) | 2 | 1,171.6 | 5.6 | 0.055 | 20.27 | <0.001 |
| DISPERSER | 1 | 1,172.9 | 7.0 | 0.029 | 16.93 | <0.001 |
| Central ID | | | | | | |
| YEARLING + (JUL–SEP) + (YR 2004) | 3 | 656.8 | 0 | 0.582 | 19.92 | <0.001 |
| YEARLING + (JUL–SEP) | 2 | 658.1 | 1.4 | 0.295 | 16.41 | <0.001 |
| YEARLING + (YR 2004) | 2 | 662.1 | 1.4 | 0.039 | 12.37 | 0.002 |
| YEARLING + (JAN–MAR) | 2 | 662.2 | 5.5 | 0.037 | 12.25 | 0.002 |
| (JUL–SEP) + (YR 2004) | 2 | 663.6 | 6.9 | 0.018 | 10.87 | 0.004 |
| YEARLING | 1 | 663.8 | 7.0 | 0.017 | 8.62 | 0.003 |
| (JUL–SEP) | 1 | 664.6 | 7.9 | 0.11 | 7.76 | 0.005 |
| Northwestern MT | | | | | | |
| PUP | 1 | 932.4 | 0 | 0.711 | 22.65 | <0.001 |
| PUP + (APR–JUN) | 2 | 934.0 | 1.9 | 0.289 | 22.91 | <0.001 |

determined that a range of variables influenced wolf mortality risk. For analyses including all recovery areas, the best model had a high degree of plausibility ($w_i = 0.889$; Table 4) and model variables had high weight of evidence (Table 5). Subjects from NWMT were 1.63 times more likely to die than their counterparts in other recovery areas. Wolf hazards also were influenced by demographic and behavioral variables, with the PUP, YEARLING, and DISPERSER variables each associated with increased mortality risk (Table 4). Daily hazard ratios for time-varying covariates tended to be high. For example, the DISPERSER variable was associated with 8.4% higher mortality risk/90 days ($1.0009^{90} = 1.084$) and 38.9% higher risk/365 days (Table 4). Wolves also experienced higher mortality in 2004. Inclusion of a single 2-way interaction term (MONTANA × PUPS) in the best-fit model improved fit ($\Delta_i = -11.437$; all other interaction terms: $\Delta_i > 2.061$), but small sample sizes precluded robust variance estimation for the PUP variable in this particular model.

Annual survival rate for pups (estimated from autumn to spring monitoring) was 0.398 (0.273, 0.579; 95% CI; $n$ = 23 deaths) for NWMT compared to 0.756 (0.635, 0.899; 95% CI; $n$ = 3 deaths) and 0.889 (0.777, 1.000; 95% CI; $n$ = 10 deaths) for GYA and CID, respectively. Annual survival rates for nonpups (yearlings and ad) were 0.680 (0.643, 0.740; 95% CI; $n$ = 107 deaths), 0.771 (0.737, 0.806; 95% CI; $n$ = 131 deaths), and 0.789 (0.750, 0.829; 95% CI; $n$ = 86 deaths) for NWMT, GYA, and CID, respectively. The proportional hazards assumption was upheld by the best-fit model pooling

**Table 5.** Model-averaged hazard ratios, unconditional variances, and weight of evidence [$w$(E)] for individual variables in Andersen–Gill models of wolf mortality risk in northwestern United States (1982–2004). We generated model sets from best-fit models using demographic, behavioral, and temporal variables (see Table 2 for coding scheme). Sample sizes vary depending on whether models include all recovery areas (subjects = 711, deaths = 361) or are restricted to Greater Yellowstone Area (subjects = 269, deaths = 142), central Idaho (subjects = 175, deaths = 89) or northwestern Montana (subjects = 192, deaths = 130). All models were stratified according to whether subjects were recruited to the study for standard monitoring purposes (representative sample) versus following livestock depredations or other perceived problems (targeted sample). Hazard ratios >1.0 indicate increased mortality risk.

| Variable | Hazard ratio | SE | Lower 95% CI | Upper 95% CI | $w$(E) |
|---|---|---|---|---|---|
| All recovery areas | | | | | |
| MONTANA | 1.6274 | 0.1840 | 1.2669 | 1.9880 | 1.000 |
| DISPERSER | 1.0009 | 0.0002 | 1.0006 | 1.0013 | 1.000 |
| PUP | 1.0050 | 0.0013 | 1.0025 | 1.0076 | 0.991 |
| (YR 2004) | 1.0004 | 0.0002 | 1.0001 | 1.0007 | 0.949 |
| YEARLING | 1.0014 | 0.0005 | 1.0004 | 1.0023 | 0.944 |
| Greater Yellowstone Area | | | | | |
| DISPERSER | 1.0015 | 0.0003 | 1.0009 | 1.0022 | 1.000 |
| (YR 2002) | 0.9990 | 0.0004 | 0.9982 | 0.9997 | 0.917 |
| (YR 2004) | 1.0004 | 0.0002 | 1.0000 | 1.0009 | 0.055 |
| Central ID | | | | | |
| YEARLING | 1.0026 | 0.0009 | 1.0011 | 1.0045 | 0.970 |
| (JUL–SEP) | 1.0009 | 0.0003 | 1.0003 | 1.0015 | 0.907 |
| (YR 2004) | 1.0006 | 0.0003 | 1.0000 | 1.0012 | 0.640 |
| (JAN–MAR) | 0.9993 | 0.0004 | 0.9986 | 1.0001 | 0.037 |
| Northwestern MT | | | | | |
| PUP | 1.0081 | 0.0016 | 1.0050 | 1.0112 | 1.000 |
| (APR–JUN) | 0.9994 | 0.0003 | 0.9998 | 1.0000 | 0.289 |

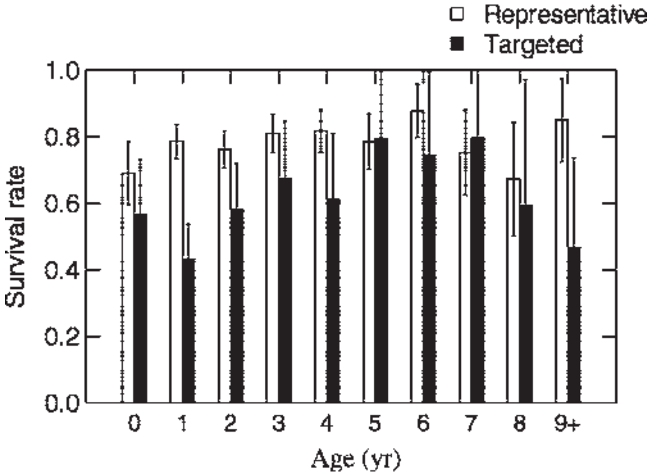


**Figure 4.** Annual survival rate (±95% CI) for wolves in western United States (1982–2004) by age ($n$ = 618 wolves, 307 deaths). Wolves were radiomonitored either as part of standard sampling (representative sample) or following focused capture efforts in response to livestock depredation or other problems (targeted sample). Survival rates of 0-aged animals (pups) were largely restricted to autumn and winter of their first year (northwestern MT) or winter-only (Greater Yellowstone Area, central ID).

recovery areas (without interaction term; global $\chi^2_5 = 3.96$, $P = 0.56$, all variables $P > 0.20$), independent variables were not strongly correlated (mean VIF = 1.03; all individual VIF < 1.03; all tolerance > 0.960; condition no. = 2.67), and goodness-of-fit tests indicated high concordance between the set of models and the data.

Next, we restricted our analysis to wolves whose precise age was known (86.9% of subjects, $n$ = 711; 85.0% of deaths, $n$ = 361), and replaced PUP and YEARLING from the above best-fit model with the continuous linear variable representing age (AGEYEARS). This variable failed to improve model fit ($\Delta_i$ = 13.126), indicating that discrete differences in mortality risk were restricted primarily to pups (representative sample) or pups and yearlings (targeted sample) and that adults did not have marked fine-scale variability in hazards (Fig. 4). A similar analysis restricted to wolves whose current breeding status was known (77.9% of subjects, 66.5% of deaths) did not provide additional explanatory power when we added BREEDING to the best-fit model ($\Delta_i$ = 2.175). However, when we restricted the analysis to wolves whose status with respect to pack membership (PACKMEMBER) was known (76.1% of subjects, 63.2% of deaths), the model replacing the dispersal status dummy variable with the variable isolating wolves that were solitary did improve model fit ($\Delta_i$ = −7.080; hazard ratio: 1.0008 [1.0004, 1.0013; 95% CI]). Dispersers tended to be solitary, but DISPERSER and PACKMEMBER variables had acceptably low collinearity (mean VIF = 6.65; all individual VIF < 6.65; all tolerance > 0.150; condition no. = 5.474). Thus, we surmised that pack membership likely was a more important determinant of wolf mortality risk than was dispersal status. Yet, even when wolves belonged to a pack the actual size of the group apparently influenced mortality risk; an analysis restricted to animals known to be in a pack and whose pack size could be approximated by a dummy variable (SMALLPACK; 50.9% of subjects, 39.1% of deaths) revealed that mortality risk was elevated among animals belonging to smaller packs ($\Delta_i$ = −4.257; hazard ratio: 1.0007 [1.0003, 1.0011; 95% CI]).

### Separate Recovery Areas

We refined our hazard models by isolating each recovery area through separate analysis. In GYA, the best-fit model had high certainty compared to other candidates (Table 4). Model-averaged hazards indicated that the time-varying DISPERSER variable increased hazards by 14.4% ($1.0015^{90} = 1.14444$)/90 days and 72.8%/365 days, compared to residents (Table 5). Mortality rates in GYA appeared to be lower in 2002 and higher in 2004 than other years, although precision and weight of evidence for the latter variable were particularly low (Table 5). When we restricted the analysis only to animals whose pack membership status was known (19.7% of subjects, $n$ = 30 4; 23.2% of deaths, $n$ = 142), we found that models including PACKMEMBER versus DISPERSER were indistinguishable ($\Delta_i$ = −0.555; hazard ratio: 1.0015 [1.0007, 1.0023; 95% CI]), implying that the ultimate factor contributing to mortality risk in GYA was unclear. However, in analyses restricted only to wolves that were members of packs, we determined that the SMALLPACK variable was associated with higher mortality risk ($\Delta_i$ = −3.355; hazard ratio: 1.0007 [1.0002, 1.0013; 95% CI).

For CID, the best-fit candidate model had weak certainty, with several other candidate models having comparable $\Delta_i$ (Table 4). The YEARLING variable was present in each of the better models and had a high weight of evidence (Tables 4, 5); hazards for yearling wolves was 26.3% higher/90 days, and 2.580 times higher/365 days, than for nonyearling animals. Annual survival rate was 0.580 (0.395, 0.708; 95% CI) for yearlings ($n$ = 29 deaths) and 0.812 (0.758, 0.854; 95% CI) for nonyearlings ($n$ = 60 deaths). Mortality risk in CID was lower during 2002 and possibly higher in 2004 (Table 4). Restricted analysis for CID indicated that PACKMEMBER (86.5% of subjects, $n$ = 221; 81.9% of deaths, $n$ = 89) provided comparable explanatory power to the best-fit model excluding this variable ($\Delta_i$ = 1.136; hazard ratio: 1.0005 [0.9995, 1.0015; 95% CI]). Similarly, SMALLPACK failed to provide additional explanatory power ($\Delta_i$ = −0.487; hazard ratio: 0.9984 [0.9963, 1.0001; 95% CI]). Thus, we infer that neither pack membership nor pack size influenced mortality risk in CID.

For NWMT, the univariate model including PUP was the best fit, and both candidate models contained the PUP variable (Table 4). Based on their September–March survival rate, pups had 2.1 and 19.0 times higher mortality risk/90 days and 365 days, respectively, compared to nonpups (Table 4). Annual survival rate was 0.398 (0.273, 0.579; 95% CI) for pups ($n$ = 23 deaths) and 0.690 (0.643, 0.740; 95% CI) for nonpups (yearlings and ad pooled; $n$ = 107 deaths). Restricted analysis did not indicate that either PACKMEMBER ($\Delta_i$ = 8.102; 30.3% of subjects, $n$ = 192; 23.6% of deaths, $n$ = 130) or SMALLPACK ($\Delta_i$ = 2.441;

**Table 6.** Model-averaged hazard ratios, unconditional variances, and weight of evidence [*w*(E)] for Andersen–Gill models of wolf mortality risk in northwestern United States (1982–2004). We generated model sets for recovery areas pooled from best-fit models using demographic and behavioral variables (see Table 2 for coding scheme). We ran separate models depending on whether subjects were recruited to the study for standard monitoring purposes (representative sample) versus following livestock depredations or other perceived problems (targeted sample). Hazard ratios >1.0 indicate increased mortality risk.

| | Representative | | | | | Targeted | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Hazard | SE | Lower 95% CI | Upper 95% CI | *w*(E) | Hazard | SE | Lower 95% CI | Upper 95% CI | *w*(E) |
| PUP | 1.0060 | 0.0013 | 1.0035 | 1.0084 | 1.00 | | | | | |
| DISPERSER | 1.0009 | 0.0002 | 1.0005 | 1.0013 | 1.00 | 1.0011 | 0.0006 | 1.0000 | 1.0023 | 0.566 |
| MONTANA | 1.5020 | 0.1948 | 1.1202 | 1.8839 | 0.982 | 1.7281 | 0.3887 | 0.9663 | 2.4899 | 0.910 |
| (DISPERSER × YEARLING) | 1.0027 | 0.0009 | 1.0010 | 1.0045 | 0.638 | 0.9975 | 0.0015 | 0.9945 | 1.0004 | 0.397 |
| YEARLING | 1.0012 | 0.0006 | 1.0001 | 1.0023 | 0.318 | 1.0021 | 0.0011 | 1.0000 | 1.0042 | 0.571 |
| GENDER | 1.2342 | 0.1546 | 0.9655 | 1.5778 | 0.188 | 0.6491 | 0.1429 | 0.3689 | 0.9292 | 0.492 |

30.3% of subjects, 23.6% of deaths) influenced wolf hazards in northwestern Montana.

### Subject Recruitment Method

Hazards models developed for subjects recruited via representative versus targeted sampling (all recovery areas pooled) revealed several similarities but also differences between groups of subjects. Hazard was consistently higher in Montana and among dispersers, and effect sizes also were comparable between groups (Table 6). However, pups only had higher mortality risk in the representative sample. Dispersing yearlings (DISPERSER × YEARLING interaction term) had higher and lower than average hazard in the representative and targeted samples, respectively, whereas risk was higher among males versus females in the representative versus targeted sample (Table 6). Restricted analysis indicated that for the representative sample BREEDING did not influence mortality risk ($\Delta_i > 1.199$). Although addition of the PACKMEMBER variable to the best-fit model for the representative sample provided equivocal results compared to the best-fit model ($\Delta_i = -1.669$; hazard ratio: 0.9989 [0.9978, 0.9999; 95% CI]), inclusion of SMALLPACK revealed negative effects of membership in small packs ($\Delta_i = -5.957$; hazard ratio: 1.0006 [1.0002, 1.0010; 95% CI]). For the targeted sample of wolves, none of the restricted variables were associated with hazards (all $\Delta_i > 1.710$) although statistical power was notably lower due to the smaller sample size. Thus, we infer that mortality risk patterns likely differed between the representative and targeted sample of wolves, but the particular association between covariates and mortality risk was largely equivocal in the latter group.

### Habitat and Anthropogenic Variables

The next series of hazard models was restricted to 297 individuals (41.8% of total sample) that had a fixed home range with estimable habitat and anthropogenic variables. When we pooled recovery areas, 15 candidate models met our criteria for consideration ($\Delta_i < 10.0$, all $P < 0.10$) although 10 models had markedly low explanatory power ($w_i < 0.05$ and all $\Delta_i > 3.480$). Overall, 9 variables were associated with wolf hazard, with the MONTANA variable being present in all models and the PUP variable common to most (Table 7). Wolf mortality risk also was negatively associated with the index of wolf density (PACKSADJACENT); parameter estimates for this variable indicated that on average, wolf mortality risk decreased by 2.7% ($0.9997^{90} = 0.973$)/90-day (10.4%/365-day) interval for each additional wolf pack with a home range that was adjacent to that of the subject in question (Table 7). Hazards tended to be higher among wolves at high elevations, in areas where agricultural cover was more abundant, and where forest cover was scarce. Wolf hazard was higher in areas where mule deer were the most common wild ungulate prey, as well as where cattle and sheep were more abundant (Table 7). Overall, the weight of evidence for most variables supported their strong association with wolf mortality risk (Table 7). All 2-way interaction terms were nonsignificant (all $P > 0.19$), and the best-fit hazard model satisfied the assumption of proportional hazards (global $\chi^{2}{}_{4} = 1.00$, $P = 0.91$ all variables $P > 0.17$). Variables in the best-fit model set had acceptably low correlation (mean VIF = 1.77; all individual VIF < 2.83; all tolerance > 0.354; condition no. = 26.74), and all models had good fit. Analyses restricted to subjects with known breeding status (CURRENTBREEDING: 91.2% of subjects, $n = 297$; 84.4% of deaths, $n = 109$; $\Delta_i = 2.199$), known pack status (PACKMEMBER: 87.9% of subjects, 82.6% of deaths; $\Delta_i = 2.001$), and known pack size (SMALLPACK: 60.3% of subjects, 42.2% of deaths; $\Delta_i = 2.144$) failed to improve model fit.

For GYA, 15 models and 10 main variables were included in our candidate set, with 7 models having markedly low explanatory power ($w_i < 0.05$, all $\Delta_i > 3.210$). Notably, the best-fit model also had low explanatory power ($w_i = 0.214$), implying that several candidates were in contention. Model-averaged hazards indicated that the index of wolf density (PACKSADJACENT) was importantly associated with wolf hazards, with higher local wolf density correlating with reduced mortality risk (Table 7). Wolves with a higher percentage of their home range under State management, and also having more agricultural cover in their territory, also had higher risk in GYA. Several additional variables also were associated with higher hazards, but these tended to have low effect size and poor weight of evidence (Table 7). No demographic or behavioral variables were included in habitat-based hazard models for GYA, and current breeding

**Table 7.** Model-averaged hazard ratios and unconditional variances, and weight of evidence [$w$(E)] for Andersen–Gill hazard models, for wolves in northwestern United States (1982–2004) generated from models using demographic, behavioral, and habitat variables (see Table 2 for coding scheme). Habitat variables were available for a subsample of animals used in previous analyses, and sample sizes vary depending on whether models include all recovery areas (subjects = 297, deaths = 109), Greater Yellowstone Area (subjects = 139, deaths = 39), central Idaho (subjects = 89, deaths = 25), or northwestern Montana (subjects = 69, deaths = 45). All models were stratified according to whether subjects were recruited to the study for standard monitoring purposes (representative sample) versus following livestock depredations or other perceived problems (targeted sample). Hazard ratios >1.0 indicate increased mortality risk.

| Variable | Hazard ratio | SE | Lower 95% CI | Upper 95% CI | $w$(E) |
|---|---|---|---|---|---|
| All recovery areas | | | | | |
| MONTANA | 6.3129 | 2.1108 | 2.1758 | 10.4500 | 1.00 |
| PACKSADJACENT | 0.9997 | 0.0001 | 0.9996 | 1.0000 | 0.974 |
| PUP | 1.0064 | 0.0023 | 1.0019 | 1.0109 | 0.893 |
| ELEVATION | 1.0012 | 0.0005 | 1.0001 | 1.0022 | 0.849 |
| AGRICULTURAL | 1.0004 | 0.0002 | 1.0001 | 1.0008 | 0.374 |
| MULEDEER | 1.0007 | 0.0003 | 1.0001 | 1.0014 | 0.360 |
| CATTLE | 1.0010 | 0.0005 | 0.9999 | 1.0020 | 0.293 |
| SHEEP | 1.0016 | 0.0006 | 1.0005 | 1.0028 | 0.072 |
| FOREST | 1.0000 | 0.0000 | 1.0000 | 1.0000 | 0.025 |
| Greater Yellowstone Area | | | | | |
| PACKSADJACENT | 0.9997 | 0.0001 | 0.9995 | 1.0000 | 0.875 |
| STATE | 1.0002 | 0.0000 | 1.0001 | 1.0002 | 0.380 |
| AGRICULTURAL | 1.0004 | 0.0002 | 1.0000 | 1.0008 | 0.287 |
| FEDERAL | 1.0000 | 0.0000 | 1.0000 | 1.0000 | 0.129 |
| PROTECTION | 1.0005 | 0.0002 | 1.0001 | 1.0010 | 0.158 |
| ROADS[a] | 1.0012 | 0.0008 | 0.9996 | 1.0028 | 0.099 |
| ELEVATION | 1.0024 | 0.0011 | 1.0003 | 1.0046 | 0.076 |
| PRIVATE | 1.0000 | 0.0000 | 1.0000 | 1.0000 | 0.075 |
| MULEDEER | 1.0007 | 0.0004 | 1.0000 | 1.0014 | 0.073 |
| CATTLE | 1.0001 | 0.0000 | 1.0000 | 1.0002 | 0.064 |
| Central ID | | | | | |
| MULEDEER | 0.9997 | 0.0035 | 0.9900 | 1.0039 | 0.994 |
| CATTLE | 0.9941 | 0.0029 | 0.9884 | 0.9998 | 0.908 |
| (MULEDEER × CATTLE) | 1.0036 | 0.0011 | 1.0013 | 1.0058 | 0.811 |
| SHEEP | 1.0030 | 0.0014 | 1.0002 | 1.0057 | 0.046 |
| ELEVATION | 1.0007 | 0.0012 | 0.9984 | 1.0031 | 0.018 |
| Northwestern MT | | | | | |
| PUP | 1.0089 | 0.0049 | 0.9994 | 1.0185 | 0.774 |
| PRIVATE | 1.0000 | 0.0000 | 1.0000 | 1.0001 | 0.379 |
| FEDERAL | 1.0000 | 0.0000 | 0.9999 | 1.0000 | 0.216 |
| FOREST | 0.9999 | 0.0000 | 0.9999 | 1.0000 | 0.188 |
| (PUP × PRIVATE) | 0.9998 | 0.0001 | 0.9996 | 1.0000 | 0.142 |
| CATTLE | 1.0005 | 0.0002 | 1.0001 | 1.0009 | 0.113 |
| SHEEP | 1.0081 | 0.0045 | 0.9993 | 1.0168 | 0.099 |

[a] Quadratic relationship.

status, pack membership, and membership in a small pack were not related to hazards (all $\Delta_i > 2.085$).

For CID, 6 models and 6 variables were in the model set, with 4 models having low power ($w_i < 0.05$, all $\Delta_i > 5.741$). The best-fit model ($w_i = 0.811$) included variables MULEDEER and CATTLE, with the model-averaged estimates indicating an overall lower mortality risk within areas with higher mule deer and cattle numbers. However, the MULEDEER × CATTLE interaction term was significant, indicating that wolves had higher mortality risk in areas where both were abundant (Table 7). Annual survival rates, segregated according to the 50th percentile for the MULEDEER × CATTLE term, were 0.920 (0.847, 0.958; 95% CI) versus 0.704 (0.640, 0.756; 95% CI) for the lower versus upper group, respectively. Additional spatially explicit variables related to mortality risk included SHEEP and ELEVATION; no demographic or behavioral variables were included in the candidate set of models (Table 7).

For NWMT, 12 models were considered as candidates with 4 having $w_i < 0.05$ (all $\Delta_i > 2.597$). As noted in demographic models, pups had higher mortality risk compared to nonpups (Table 7). Model-averaged hazards also were weakly associated with land management status (PRIVATE, FEDERAL), agricultural activities (CATTLE, SHEEP), and presence of forest cover in the home range. However, several variables had markedly low parameter estimates and large uncertainty. The significant PUP × PRIVATE interaction term indicated that pups had lower hazards where there was a high degree of private land ownership (Table 7).

## DISCUSSION

Overall, we contend that annual wolf survival rates were likely adequate to sustain all 3 populations (Keith 1983, Fuller et al. 2003, Adams et al. 2008). When considered with even modest connectivity, whether natural or artificial, population viability is likely enhanced (USFWS 1994). Wolves in NWMT, however, were especially susceptible to human-caused mortality (E. E. Bangs, unpublished data) and in some cases mortality risk could have limited population growth rate. Indeed, population growth in NWMT was stationary or negative some years from 1995 to 2004 compared to the 2 other recovery areas where numbers increased concurrently (USFWS et al. 2005). Lower survival in NWMT was a finding contrary to our prediction of equal survival across recovery areas, and surprising given that wolves naturally recolonized this area, had a highest level of legal protection, and had been present for a longer period (Ream et al. 1991). This finding of lower survival in NWMT underscores the importance of metapopulation structure and refugia where populations function in a quasi-independent fashion, yet are sufficiently connected to allow for possible rescue effects (Levins 1969).

Our finding that wolf hazards were higher in NWMT was probably partly because GNP and BMWA did not function as high-quality wolf habitat (most of both areas are high in elevation and have low densities of potential prey during winter) and few wolves lived there (USFWS et al. 2005; Fig. 1). In contrast, CID and GYA were either inaccessible wilderness areas or, in the case of YNP, supported many wolves with high survival. For example, because most wolf mortality in our study area was of anthropogenic origin (E. E. Bangs, unpublished data) we considered that human access contributed negatively to wolf survival and that national parks (YNP) and remote wilderness areas (CID) where such access was limited or actively controlled are favorable to wolf survival. In NWMT, most wolves lived outside protected areas (Fig. 1), probably because year-round prey were scarce within those protected areas and more private land was present than in either CID or GYA

(Table 1). Thus, it is not surprising that wolves in NWMT had lower survival rates than their counterparts in other recovery areas.

In NWMT, poor survival was associated with cattle and sheep, low forest cover, and mule deer as primary prey; each of these factors is related to low-elevation habitat, private land, or agricultural activities. A habitat model by Oakleaf et al. (2006) also found less natural habitat available to wolves in NWMT. Agriculture per se was not unique to NWMT because all 3 recovery areas had substantial portions of land in agricultural use (Oakleaf et al. 2006), but NWMT was the most fragmented landscape (Table 1) with fewest wolves living in truly protected areas (Fig. 1). In contrast, both YNP and CID wilderness provided large areas of forested cover with abundant prey and protection from humans or infrequent human use.

In GYA, hazards were notably high among dispersers, suggesting that as wolves emigrated from YNP they encountered humans outside a park setting and, thereby, were subject to higher risk compared to YNP residents (Smith et al. 2007). In CID, yearlings had higher mortality risk, which, because most dispersing wolves tend to be yearling animals, likely identifies the same cohort of animals as those observed in GYA as being particularly susceptible to mortality when leaving protected areas (Adams et al. 2008, Person and Russell 2008). Pack membership increased survival, which probably acted to limit movements and retain wolves on a territory where conflicts were at least less than wandering wolves. Lower survival of dispersing and young wolves may only occur in exploited wolf populations because disperser survival is high in protected areas (Pletscher et al. 1997, Smith et al. 2007, Adams et al. 2008). Further support for this idea comes from differing survival rates for wolves collared due to livestock damage (targeted sampling) versus monitoring (representative) purposes. More wolves were collared in NWMT because of conflicts with livestock and these wolves had lower survival compared to wolves collared for monitoring purposes in the other recovery areas.

Pack density was highest in the northern reaches of YNP, which decreased mortality risk and is suggestive of positive density dependence, the opposite of NWMT in the early recovery period where there was low density and higher survival compared to our results (Ream et al. 1991, Pletscher et al. 1997). Further, our study found pack membership increased survival (but not breeding status), probably by reducing movement through high-risk areas both because of intraspecific mortality (territorial attacks) and livestock conflicts. But pack membership alone did not connote higher survival because in packs of ≤5 wolves survival was lower. These small packs were likely newly formed (and therefore small) and in marginal areas, or they were in chronically high risk areas and small because of control actions or illegal killing, and either cause would contribute to lower survival. For example, in GYA wolf packs outside YNP were smaller probably because of mortality associated with control actions, again suggestive of the source–sink dynamic that may be functioning in all of the recovery areas (NWMT, and especially GNP and BMWA, may be a sink; USFWS et al. 2005). In YNP, anecdotal evidence also suggests that small packs were at a competitive disadvantage to larger packs and suffered more intraspecific death (the leading cause of mortality in YNP; Smith et al. 2007).

Our results contrast with early studies of wolves in NWMT (Ream et al. 1991, Pletscher et al. 1997, Boyd and Pletcsher 1999). Especially poor survival for NWMT pups is notable and may be partially due to time of collaring. In NWMT pups were typically collared in autumn when they were just large enough to support a collar. Calculations of survival then included autumn (a time potentially difficult for pups, especially if food is limiting) and overwinter mortality, whereas in CID and GYA pups were mostly collared in winter, making comparisons problematic. Regardless, these high mortality rates are suggestive of poor recruitment in NWMT compared to the other recovery areas and may be reflective of habitat quality (e.g., ungulate density and vulnerability to humans) compared to when wolves were colonizing the area (Ream et al. 1991). Adult survival from NWMT was higher during the colonization phase, which was probably due to low wolf density and more prey, but also because most wolves then lived within protected areas (GNP; Pletscher et al. 1997).

Our findings are not surprising in light of wolf studies elsewhere and are strongly supportive of the influence of human-caused mortality on wolf populations (Adams et al. 2008, Person and Russell 2008). Humans were important to survival of wolves in other regions and outside of protected areas dispersers had lower survival (Pletscher et al. 1997, Fuller 1989, Adams et al. 2008, Person and Russell 2008). This same dynamic appears to be operating across the NRM with no legal harvest but where human access and settlement is high compared to other wolf populations. Both CID and GYA have an area of overlay on secure habitat with adequate ungulate density contributing to high survival (Oakleaf et al. 2006). It is possible that once human harvest is allowed within the NRM this will substitute for mortality due to control and illegal take. This, however, is speculation because total mortality will still be important as harvest may be additive or partially additive.

Eventual harvest mortality will probably lower wolf survival across all 3 recovery areas, but without harvest there does not appear to be region-wide synchrony in survival. Lack of a region-wide trend enhances metapopulation structure for wolf recovery in the NRM allowing for population rescue if necessary. Wolf survival was low in 1999 in YNP but not in GYA due to disease, yet this was not the case anywhere else (Smith and Almberg 2007). Year and season were also not significant, except weakly in 2002 and 2004, suggesting lack of mortality synchrony region-wide, bolstering population stability in the event any one population should decline. Most wolves died of natural mortality in the core areas of CID and GYA (E. E. Bangs, unpublished data) and each population has different causes and rates, further strengthening wolf population viability in the NRM (USFWS et al. 2005, Smith et al. 2007).

## Analytical Issues Potentially Influencing Results

We relied extensively on continuous-time survival analysis to elucidate patterns of wolf mortality risk. Such methods are bound by several important restrictions that may be particularly relevant in wildlife research (Murray 2006). Wolf recruitment to our study occurred either via standard capture efforts involving opportunistic radiotransmitter deployment (representative sampling) or specifically in response to livestock depredations or other perceived problems (targeted sampling). Deployment of transmitters through representative sampling usually was stratified across wolf packs within a recovery area, with a representative sample of packs monitored continuously in each area and $<3$ animals/pack typically monitored at any given time. Targeted sampling usually involved transmitter deployment near livestock grazing areas and included either solitary individuals or 1–2 members of a pack. Animals obtained through targeted sampling were recruited specifically to facilitate relocation in the event of further problems so that appropriate management actions (e.g., euthanasia, transplant) could be implemented. Thus, the latter sample was biased toward animals having higher mortality risk. Because recruitment method influenced wolf hazard and the RECRUITMENT variable did not conform to the proportional hazards assumption, in our main analyses we stratified AG models according to recruitment method. We also ran separate analyses for representative versus targeted subjects specifically to evaluate potential hazard differences between groups.

Wolf mortality risk often is correlated among pack members, implying that multiple transmitter deployments per pack could violate the assumption of independence. Robust variance estimation (clustering) adjusts hazards to reflect lack of independence (Hosmer and Lemeshow 1999), but we were limited in our ability to cluster because of the many wolf packs monitored (no. of packs: GYA: 57; CID: 65; NWMT: 53), lack of pack affiliation among many subjects (e.g., dispersers), and unknown pack affiliation status of many animals. However, because a subset of our analyses involved models with spatially explicit variables that were restricted to animals with known home ranges, we adjusted variances via clustering in a subset of analyses. Note that clustering improved model precision but did not alter our results qualitatively.

Subjects that we censored from analysis should represent a random sample of the population but could be biased toward those prone to dispersing or having defective transmitters. Censoring bias also could be incurred if wolves were killed illegally and their transmitters were destroyed intentionally at the time of death (Murray 2006). We minimized the influence of these potential confounds by searching for missing transmitters via wide-ranging telemetry flights conducted several times per year and intensifying local monitoring when specific radio frequencies went missing. The proportion of radiocollared wolves disappearing during the study was comparable among recovery areas (GYA: 20.4%, $n = 299$; CID: 23.3%, $n = 219$; NWMT: 19.7%, $P = 0.62$), implying that we likely detected emigrating animals at similar rates in all areas. Because we always deployed transmitters with new batteries (normal transmitter lifespan was approx. 3.5 yr with low known premature failure rate), the comparable duration of transmitter lifespan between censored versus uncensored animals (mean censored timeline: 661.9 [355.5; median] days, $n = 150$; mean uncensored timeline: 635.1 [370.0] days, $n = 561$) implies an absence of bias. In addition, differences in survival timelines were not related to gender or age class (all $P > 0.12$) despite the likely disparity in dispersal rates among these cohorts.

The proportion of dispersers whose signal was lost (31.4%, $n = 108$) differed from that for residents succumbing to a similar fate (18.1%, $n = 557$; $P = 0.002$). Also, we detected a relationship between anthropogenic factors and signal loss, where CATTLE, ROADS, and PRIVATE (all $P < 0.003$) each differed between fate unknown versus known dead or alive animals. However, in each case the odds ratio for the logistic regression model indicated reduced risk of signal loss in areas of high human activity, contrary to our a priori prediction (i.e., humans were responsible for lost signals due to tampering with the collar after a wolf was killed illegally), and likely implied reduced monitoring intensity (and higher signal loss and censoring) in remote areas of each recovery area. Thus, we consider that modest informative censoring was present in our sample and was associated principally with dispersal status rather than human-caused mortality.

Survival research requires that the time origin be clearly identified, which may be problematic in wildlife research where recruitment is staggered (left truncation) and early mortalities can be common (Pollock et al. 1989, Winterstein et al. 2002). To be comprehensive, our analysis included a sample of subjects monitored in Montana in the 1980s, but most animals were recruited to the study after releases in GYA and CID in 1995 (Table 2). We controlled for variable start times by isolating recovery areas in specific analyses, but analyses restricted to the 1995–2004 period did not differ qualitatively from those reported herein (D. Murray, unpublished data). Furthermore, inclusion of temporal variables (i.e., season, yr) in our models generally did not alter our findings qualitatively and parameter estimates associated with temporal variables tended to have low precision and poor weight of evidence. Thus we infer that the extensive left truncation in our study did not markedly alter our results.

If subjects are not monitored continuously in time, timing of death events may be imprecise and survival times may be artificially tied (Bunk et al. 1995, Murray 2006). In our study frequency of survival assessment varied both temporally and among the 3 recovery areas but generally occurred at $<14$-day intervals (E. E. Bangs, unpublished data). This level of discontinuity is characteristic of many survival studies involving large mammals and should impose limited loss of precision in death time assessment if mortalities were assumed to have occurred at the interval midpoint (Murray 2006). Yet, we used the Breslow approximation (Hosmer and Lemeshow 1999) to address tied failure times and consider that any lack of death-time precision should be negligible relative to the overall duration of wolf timelines. Accordingly, the high relocation certainty and continuous nature of wolf survival timelines confirmed that continuous-time analytical methods were most appropriate (see Murray and Patterson 2006).

## MANAGEMENT IMPLICATIONS

Unlike Canada and Alaska, where wolves have persisted and been harvested for decades, the NRM does not have large reservoirs of wolves away from areas of high human population density (Boitani 2003, Adams et al. 2008). Consequently management will need to be more intensive, both to resolve conflicts and to maintain wolf populations. As such, we offer these 3 management recommendations. First, we found that GNP and BMWA do not function as a large refugium from which wolves could emigrate into the surrounding area (USFWS 1987; Fig. 1). Therefore, increasing the survival of wolves around this area or in nearby Canada or CID would improve population status in NWMT by retention of resident animals or by emigration. Increasing survival here would involve reducing conflicts with livestock and reducing illegal killing. Second, we recommend that survival rates continue to be monitored if each segment of the NRM wolf population is not managed at high levels that are well above minimum recovery requirements. If the NRM wolf subpopulations are managed at lower levels, intensive monitoring of wolf survival rates is likely necessary. Further, our study found greater survival in wolves collared for monitoring purposes compared to those collared because of livestock conflict (representative vs. targeted), thereby emphasizing the need to monitor survival with the onset of legal human harvest to learn if harvest may be compensatory or additive. The source–sink dynamic we found depends on high survival somewhere in the region, so any change in management action must monitor survival in both sink and source areas. Third, higher wolf survival in some areas outside core recovery areas is necessary to maintain connectivity and natural dispersal. Because young or dispersing wolves had lower survival in both CID and GYA, and because managing age-specific harvest is not possible, we recommend harvest regulations that enhance opportunity for natural dispersal between recovery areas, especially linkages with GYA.

## ACKNOWLEDGMENTS

We would like to highlight the collaborative nature of both data gathering and funding for this project. Federal, state, private, and university cooperation were critical to its success. Primary collaborators were the USFWS, YNP, Trent University, The Nez Perce Tribe, the University of Montana, the University of Idaho, and the Turner Endangered Species Fund. Funding came from all of these agencies and organizations as well as an anonymous donor, the Tapeats Foundation, Frank and Kay Yeager, Masterfoods Corporation, Patagonia, the California Wolf Center, and National Science Foundation grant DEB-0613730. Many individuals also donated to the effort through contributions to the Yellowstone Park Foundation. We would also like to highlight help from various pilots, both fixed-wing and helicopter, who were critical to the safe capture and monitoring of wolves in the northern Rocky Mountain region of the United States. We appreciate reviews of the manuscript by D. Ausband, O. Liberg, M. Mitchell, S. Nadeau, C. Sime, and P. J. White. We especially thank D. Guernsey for help with data management and E. Albers with graphics both of the Yellowstone Wolf Project. Finally, numerous technicians and landowners assisted with data gathering and we recognize their important contribution.

## LITERATURE CITED

Adams, L. G., R. O. Stephenson, B. W. Dale, R. T. Ahgook, and D. J. Demma. 2008. Population dynamics and harvest characteristics of wolves in the central Brooks Range, Alaska. Wildlife Monographs 170.

Andersen, P. K., Ø. Borgan, R. D. Gill, and N. Keiding. 1993. Statistical models based on counting processes. Springer-Verlag, New York, New York, USA.

Bangs, E. E., and S. H. Fritts. 1996. Reintroducing the gray wolf to central Idaho and Yellowstone National Park. Wildlife Society Bulletin 24:785–798.

Bangs, E. E., S. H. Fritts, J. A. Fontaine, D. W. Smith, K. M. Murphy, C. M. Mack, and C. C. Niemeyer. 1998. Status of gray wolf restoration in Montana, Idaho, and Wyoming. Wildlife Society Bulletin 26:785–798.

Belsley, D. A., E. Kuh, and R. E. Welsch. 1980. Regression diagnostics: identifying influential data and sources of collinearity. John Wiley & Sons, New York, New York, USA.

Boitani, L. 2003. Wolf conservation and recovery. Pages 317–340 *in* L. D. Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, Illinois, USA.

Boyd, D. K., and D. H. Pletscher. 1999. Characteristics of dispersal in a colonizing wolf population in the central Rocky Mountains. Journal of Wildlife Management 63:1094–1108.

Bunk, C. M., C. L. Chen, and K. H. Pollock. 1995. Robustness of survival estimates from radio-telemetry studies with uncertain relocation of individuals. Journal of Wildlife Management 59:790–794.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York, New York, USA.

Cleves, M. A., W. W. Gould, and R. G. Gutierez. 2003. An introduction to survival analysis using Stata. Stata Press, College Station, Texas, USA.

Fleming, T., and D. Harrington. 1991. Counting processes and survival analysis. John Wiley & Sons, New York, New York, USA.

Fritts, S. H. 1983. Record dispersal by a wolf from Minnesota. Journal of Mammalogy 64:166–167.

Fritts, S. H., C. M. Mack, D. W. Smith, K. M. Murphy, M. K. Phillips, M. D. Jimenez, E. E. Bangs, J. A. Fontaine, C. C. Niemeyer, W. G. Brewster, and T. J. Kaminski. 2001. Outcomes of hard and soft releases of reintroduced wolves to central Idaho and the greater Yellowstone area. Pages 125–147 *in* D. S. Maehr, R. F. Noss, and J. L. Larkin, editors. Large mammal restoration: ecological and sociological challenges in the 21st century. Island Press, Washington, D.C., USA.

Fuller, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs 105.

Fuller, T. K., L. D. Mech, and J. F. Cochrane. 2003. Wolf population dynamics. Pages 161–191 *in* L. D. Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, Illinois, USA.

Hansen, A. J., R. Rasker, B. Maxwell, J. J. Rotella, J. D. Johnson, A. W. Parmenter, U. Langen, W. B. Cohen, R. L. Lawrence, and M. P. V. Kraska. 2002. Ecological causes and consequences of demographic change in the new west. BioScience 52:151–162.

Heisey, D. M., and T. K. Fuller. 1985. Evaluation of survival and cause-specific mortality rates using telemetry data. Journal of Wildlife Management 49:668–674.

Hosmer, D. W., and S. Lemeshow. 1999. Applied survival analysis. John Wiley and Sons, New York, New York, USA.

Hougaard, P. 2000. Analysis of multivariate survival data. Springer-Verlag, New York, New York, USA.

Johnson, C. J., M. S. Boyce, C. C. Schwartz, and M. A. Haroldson. 2004. Modeling survival: application of the Andersen–Gill model to Yellowstone Grizzly bears. Journal of Wildlife Management 68:966–978.

Keith, L. B. 1983. Population dynamics of wolves. Pages 66–77 *in* L. N. Carbyn, editor. Wolves in Canada and Alaska: their status, biology, and management. Canadian Wildlife Service Report Series 45, Edmonton, Alberta, Canada.

Levins, R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bulletin of Entomological Society of America 15:237–240.

McIntyre, R. 1995. War against the wolf: America's campaign to exterminate the wolf. Voyageur Press, Stillwater, Minnesota, USA.

Mitchell, M. S., D. E. Ausband, C. A. Sime, E. E. Bangs, J. A. Gude, M. D. Jimenez, C. M. Mack, T. J. Meier, M. S. Nadeau, and D. W. Smith. 2008. Estimation of successful breeding pairs for wolves in the northern Rocky Mountains, USA. Journal of Wildlife Management 72:881–891.

Mitra, D., and P. N. Golder. 2002. Whose culture matters? Near-market knowledge and its impact on foreign market entry timing. Journal of Marketing Research 39:350–365.

Murray, D. L. 2006. On improving telemetry-based survival estimation. Journal of Wildlife Management 70:1530–1543.

Murray, D. L., and B. R. Patterson. 2006. Wildlife survival estimation: recent advances and future directions. Journal of Wildlife Management 70:1499–1503.

Oakleaf, J. K., D. L. Murray, J. R. Oakleaf, E. E. Bangs, C. M. Mack, D. W. Smith, J. A. Fontaine, M. D. Jimenez, T. J. Meier, and C. C. Niemeyer. 2006. Habitat selection by recolonizing wolves in the northern Rocky Mountains of the United States. Journal of Wildlife Management 70:554–563.

Person, D. K., and A. L. Russell. 2008. Correlates of mortality in an exploited wolf population. Journal of Wildlife Management 72:1540–1549.

Phillips, M. K., and D. W. Smith. 1996. The wolves of Yellowstone. Voyageur Press, Stillwater, Minnesota, USA.

Pletscher, D. H, R. R. Ream, D. K. Boyd, M. W. Fairchild, and K. E. Kunkel. 1997. Population dynamics of a recolonizing wolf population. Journal of Wildlife Management 61:459–465.

Pollock, K. H., S. R. Winterstein, and M. J. Conroy. 1989. Estimation and analysis of survival distributions for radio-tagged animals. Biometrics 45:99–109.

Ream, R. R., M. W. Fairchild, D. K. Boyd, and D. H. Pletscher. 1991. Population dynamics and home range changes in a colonizing wolf population. Pages 349–366 *in* R. B. Keiter and M. S. Boyce, editors. The greater Yellowstone ecosystem. Yale University Press, New Haven, Connecticut, USA.

Smith, D. W., and E. Almberg. 2007. Wolf diseases in Yellowstone National Park. Yellowstone Science 15:17–19.

Smith, D. W., L. D. Mech, M. Meagher, W. E. Clark, R. Jaffe, M. K. Phillips, and J. A. Mack. 2000. Wolf–bison interactions in Yellowstone National Park. Journal of Mammalogy 81:1128–1135.

Smith, D. W., D. R. Stahler, D. S. Guernsey, M. Metz, A. Nelson, E. Albers, and R. McIntyre. 2007. Yellowstone Wolf Project: annual report, 2006. National Park Service, Yellowstone Center for Resources, YCR-2007-01, Yellowstone National Park, Wyoming, USA.

Therneau, T. M., and P. M. Grambsch. 2000. Modeling survival data: extending the Cox model. Springer-Verlag, New York, New York, USA.

U.S. Fish and Wildlife Service [USFWS]. 1987. Northern Rocky Mountain wolf recovery plan. U.S. Fish and Wildlife Service, Denver, Colorado, USA.

U.S. Fish and Wildlife Service [USFWS]. 1994. The reintroduction of gray wolves to Yellowstone National Park and central Idaho: final environmental impact statement. U.S Fish and Wildlife Service, Helena, Montana, USA.

U.S. Fish and Wildlife Service [USFWS], Nez Perce Tribe, National Park Service, Montana Fish, Wildlife and Parks, Idaho Fish and Game, and USDA Wildlife Services. 2005. Rocky Mountain wolf recovery 2004 interagency annual report. D. Boyd, editor. U.S. Fish and Wildlife Service, Ecological Services, Helena, Montana, USA.

Van den Poel, D., and B. Larivière. 2004. Attrition analysis for financial services using proportional hazard models. European Journal of Operational Research 157:196–217.

Wilmers, C. C., and E. Post. 2006. Predicting the influence of wolf-provided carrion on scavenger community dynamics under climate change scenarios. Global Change Biology 12:403–409.

Young, S. P., and E. A. Goldman. 1944. The wolves of North America. American Wildlife Institute, Washington, D.C., USA.

*Associate Editor: Gese.*

PLOS | ONE

RESEARCH ARTICLE

# Demographic and Component Allee Effects in Southern Lake Superior Gray Wolves

**Jennifer L. Stenglein**¤*, **Timothy R. Van Deelen**

Department of Forest and Wildlife Ecology, University of Wisconsin–Madison, Madison, Wisconsin, United States of America

¤ Current address: Bureau of Science Services, Wisconsin Department of Natural Resources, Madison, Wisconsin, United States of America
* jennifer.stenglein@wisconsin.gov

**Citation:** Stenglein JL, Van Deelen TR (2016) Demographic and Component Allee Effects in Southern Lake Superior Gray Wolves. PLoS ONE 11 (3): e0150535. doi:10.1371/journal.pone.0150535

**Editor:** Joseph K. Bump, Michigan Technological University, UNITED STATES

**Received:** September 18, 2015

**Accepted:** February 15, 2016

**Published:** March 1, 2016

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** The authors thank NSF-IGERT award DGE-1144752: Novel ecosystems, rapid change, and no-analog conditions: the future of biodiversity conservation in human-dominated landscapes and a USDA Hatch grant for research funding. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

## Abstract

Recovering populations of carnivores suffering Allee effects risk extinction because positive population growth requires a minimum number of cooperating individuals. Conservationists seldom consider these issues in planning for carnivore recovery because of data limitations, but ignoring Allee effects could lead to overly optimistic predictions for growth and underestimates of extinction risk. We used Bayesian splines to document a demographic Allee effect in the time series of gray wolf (*Canis lupus*) population counts (1980–2011) in the southern Lake Superior region (SLS, Wisconsin and the upper peninsula of Michigan, USA) in each of four measures of population growth. We estimated that the population crossed the Allee threshold at roughly 20 wolves in four to five packs. Maximum per-capita population growth occurred in the mid-1990s when there were approximately 135 wolves in the SLS population. To infer mechanisms behind the demographic Allee effect, we evaluated a potential component Allee effect using an individual-based spatially explicit model for gray wolves in the SLS region. Our simulations varied the perception neighborhoods for mate-finding and the mean dispersal distances of wolves. Simulation of wolves with long-distance dispersals and reduced perception neighborhoods were most likely to go extinct or experience Allee effects. These phenomena likely restricted population growth in early years of SLS wolf population recovery.

## Introduction

Allee effects threaten small populations with extinction when growth rate (demographic Allee effect) or a component of individual fitness (component Allee effect) is related positively to population size or density [1, 2]. Demonstrating an Allee effect contradicts expectations that resource abundance is the primary determinant of population growth across all population sizes or densities. A demographic Allee effect is a hump-shaped form of density dependence wherein growth at low relative density shows positive density dependence before transitioning to negative density dependence at a higher relative density [3]. Strong demographic Allee effects exhibit negative population growth at the lowest population sizes whereas weak demographic Allee effects have a pattern of reduced population growth rates (but still positive) at low population sizes. The consequences of strong demographic Allee effects are more severe than weak Allee effects because negative population growth can lead to extinction directly rather than contributing to small-population stochastic risks through slower than expected population growth (a weak Allee effect). A component Allee effect occurs when a component of growth (e.g., survival, reproduction) shows similar positive density dependence at low relative density [3]. Observing a demographic Allee effect indicates the presence of at least one component Allee effect although the reverse may not be true because of compensatory interactions between components of growth [2, 4].

Allee effects are a small population phenomenon and therefore may be particularly influential in reintroduced, newly established, or struggling carnivore populations because carnivores typically exist at low densities, have elaborate social structures, and are sensitive to human activities [5–10]. In addition, small populations may be especially vulnerable to stochastic variation in intrinsic (e.g., age structure) and extrinsic (e.g., habitat) variation. Small populations of carnivores that exhibit long periods of negative or slow growth followed by a sudden increase in growth may indicate the presence of an Allee effect, although frequently it is unidentified or confounded by other sources of variation. Examples of Allee effects identified in small populations of carnivores include: African wild dogs (*Lycaon pictus*) [6], island foxes (*Urocyon littoralis*) [5] and gray wolves in Scandinavia [9] and Yellowstone National Park, USA [7].

Given difficulty in detecting demographic Allee effects in wildlife populations, research has focused on mechanisms influencing component Allee effects. The best evidence for an Allee effect is identification of both demographic effects and component mechanisms, but these cases are rare [11]. In a meta-analysis of 20 studies of Allee effects in mammal populations, five studies could not confirm Allee effects, six examined both demographic and component Allee effects, one study examined only demographic Allee effects, and eight studies examined only component Allee effects [11]. Consequences of Allee effects are reduced population growth, elevated extinction risk, and potential bias in estimation of population parameters; consequently identifying populations prone to Allee effects can improve wildlife conservation efforts [10, 12]. Knowledge of demographic Allee effects helps predict critical numeric population thresholds and elevated extinction risk at low relative density, and knowledge of component Allee effects assists in understanding and potentially mitigating Allee effects.

Reduced breeding interactions at low density is the most commonly cited component Allee effect and usually manifests as a shortage of receptive mate encounters at low-density [13, 14]. Finding a mate is an outcome of individual-based behaviors and decisions on the landscape, and an individual's perception neighborhood (the range over which an individual can find a mate) is one component of mate-finding [13]. Consequently, individual-based modeling is useful for studying mate-finding and other mechanisms driving Allee effects [13, 15–17].

## Southern Lake Superior (SLS) wolf population

We studied the southern Lake Superior (SLS) wolf population (Northern Wisconsin and the upper peninsula of Michigan, USA), which is part of the larger western Great Lakes population of wolves. The SLS region is dominated by mixed forest and has moderate to high quality wolf habitat [18, 19]. SLS wolves are mostly isolated from wolf populations in Minnesota and Ontario because narrow corridors that connect wolf habitat are surrounded by agriculture or water (Lakes Superior and Michigan) and are interrupted by human development (Superior and Duluth in Wisconsin and Minnesota, Sault St. Marie in Michigan and Ontario; Fig 1) [18, 19]. Even so, immigrants are periodically exchanged into the larger population (especially Minnesota) and the SLS population established through natural recolonization from Minnesota into Wisconsin and then to Michigan [20–22]. Recolonization began in the mid-1970s and by

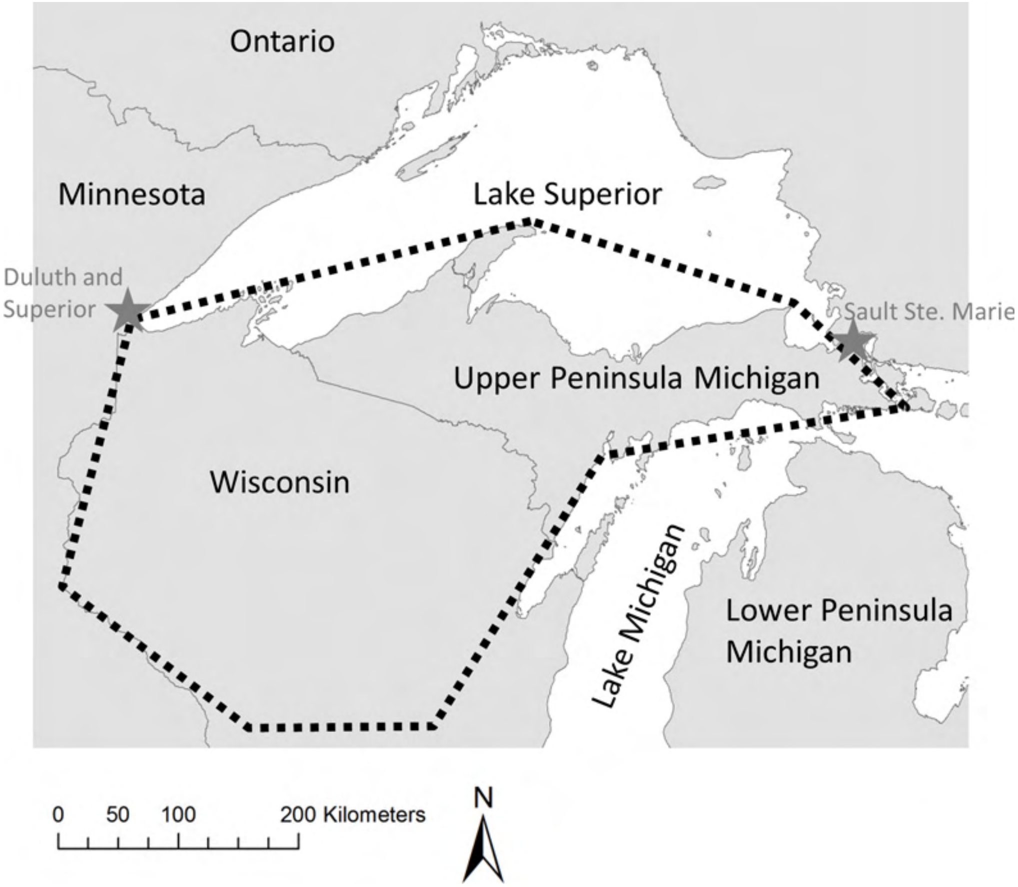


**Fig 1. Map of the location of the southern Lake Superior wolf population.** Black dotted polygon outlines the primary southern Lake Superior wolf range made up of Wisconsin and the upper peninsula of Michigan (each with currently around 600–800 wolves) and stars show the major cities limiting connectivity to Minnesota and Ontario [26, 27].

doi:10.1371/journal.pone.0150535.g001

1979 five wolf packs were detected in the northwestern portion of Wisconsin [22]. By the mid-1980s wolves had recolonized the upper peninsula of Michigan, and by the mid-1990s wolves recolonized the central forest region of Wisconsin [23]. Prior to this, the wolf population grew very little and even decreased in some years [24]. Since the mid-1990s, the SLS population has grown at a median rate of 14% per year to >1500 wolves in 2011. An extended period of little or no growth during early recovery is inconsistent with simple negative density dependence across all densities and may suggest an Allee effect. We hypothesized that the interplay between dispersal and mate-finding abilities of wolves varied with density and may have produced an Allee effect similar to that observed in recolonizing wolves in Yellowstone National Park, USA [7]. At low population sizes, high dispersal rates and distances may impede a recovering population if mate-finding is restricted because of a limited ability to detect sparsely distributed mates at a distance, and this combination may lead to an Allee effect [17, 25]. Conversely, a high rate of dispersal matched with increased ability to detect mates rescues a population from an Allee effect and promotes recovery [17].

We were interested in a particular biological hypothesis about wolves' perception neighborhood in a human-dominated landscape because this is a poorly understood component of wolf behavior. Our objectives were to test for a demographic Allee effect in the early recovery of SLS wolves and, if found, to test whether the high dispersal of colonizing wolves exacerbated a mate-finding Allee effect when the population size was low and dispersers were sparsely distributed. We hypothesized that dispersing wolves had difficulty locating mates during recolonization because potential mates were located mostly outside of the disperser's perception neighborhood at low density.

## Materials and Methods

### Demographic Allee effect

We tested for a demographic Allee effect in four measures of population growth in SLS wolves (1980–2011) using published data [22, 28, 29]: 1) SLS wolf population size (Michigan and Wisconsin together), 2) Wisconsin wolf population size, 3) number of wolf packs in Wisconsin, and 4) amount of occupied territory in Wisconsin (S1 Appendix). These measures are all highly correlated (i.e., population size, number of packs, and occupied territory all increased over time), although growth rates calculated from these time series are not necessarily highly correlated (S1 Appendix). Therefore, these four measures may reveal different patterns of density dependence that could influence our ability to detect a demographic Allee effect.

For each measure of population growth $i$, for $i = 1,2,3,4$, we fit the relationship between per capita population growth rate, $pgr_{i,t} = \ln(N_{i,t}/N_{i,t-1})$, and log population size, $ln(N_{i,t})$, in year $t$ for $t = 1981,1982,\ldots,2011$, with a penalized spline using Bayesian methods (S2 Appendix) [30] where:

$$pgr_{i,t} \sim Normal(\mu_{i,t}, \sigma_i^2)$$

$$\mu_{i,t} = \beta_i \times ln(N_{i,t}) + \alpha_{i,t,k} \times Z_{i,t,k}$$

The spline portion is $\alpha_{i,t,k} \times Z_{i,t,k}$, where $k$ is the number of knots for $k = 1,2,\ldots,20$, and we assigned vague priors $\alpha_{i,t,k} \sim Normal(0, v_i^2)$ and $v_i \sim Uniform(0,100)$. Also, we assigned vague priors $\beta_i \sim Normal(0,100^2)$ and $\sigma_i \sim Uniform(0,100)$. Heuristically, using a spline enables the data to determine the shape of the relationship between $pgr_{i,t}$ and $ln(N_{i,t})$ instead of assuming a functional form for this relationship *a-priori* through a parametric (e.g., linear, quadratic) model. Evidence for an Allee effect would be a hump-shaped spline [25]. A spline crossing the x-axis at two non-negative values would identify the Allee threshold (low-density unstable equilibrium also called the extinction threshold) and the carrying capacity (high-density stable equilibrium), respectively [3]. We chose to use penalized splines, specifically low-rank thin-plate splines in our analysis because of their good mixing properties in the Markov Chain Monte Carlo (MCMC) chains of a Bayesian analysis [30, 31].

We ran the models in program R (version 2.14) [32], library 'rjags' [33] with program JAGS (version 3.3.0) [34]. We ran three MCMC chains for each model for 150,000 iterations and discarded the first 100,000 iterations as burn-in (the testing period that is thrown out prior to stabilization of chains). For each model, we assessed convergence using visual inspection of chain mixing and univariate ($\hat{R}$) and multiple potential scale reduction factors ($\hat{R}^p$, where $p$ is the number of parameters) [35, 36]. Generally, convergence is adequate when upper 97.5% confidence limits of the $\hat{R}$s and $\hat{R}^p$ statistics are close to 1 and here we declared convergence attained if the upper 97.5% confidence limits of all $\hat{R}$s and $\hat{R}^p$ were $<1.1$ [36].

### Component Allee effect

We evaluated hypothetical mechanisms [7] leading to an Allee effect by simulating population growth under various mate-finding distances and dispersal distance functions for wolves in an individual-based spatially explicit (IBSE) model of the SLS wolf population [37]. We derived parameters for the model from empirical research specific to the Great Lakes wolf population [22] using NetLogo (version 4.1) [38]. Our model is described in detail in Stenglein, Gilbert (37) following the Overview, Design, and Details protocol (ODD) [39, 40]. Here, we give a brief overview (Table 1; Fig 2).

In our IBSE model, unmated individual male and female adult wolves experienced an annual cycle of life history events culminating in a goal of finding a mate and establishing a territory to become a reproducing pack (Table 1). Simulated unmated wolves could move around the model landscape during mate-finding, winter dispersal, and fall dispersal events. Wolves could die from targeted lethal control efforts, fall dispersal mortality, spatially varying mortality risk reflecting human activity, and aging (Table 1). Wolves could enter the simulation annually through reproduction and through a winter dispersal event by replacing a number of immigrants equal to the number of emigrants that dispersed beyond the bounds of the simulated

**Table 1. Life history events and sequence of events for simulated southern Lake Superior wolves in an individual-based spatially explicit model [37].**

| Life history event | Sequence | Description |
|---|---|---|
| Mate-finding | 1, 3 | A breeding wolf in a territory[a], if not mated (mate died in previous year), searches for an unrelated wolf of the opposite sex, first in their own territory and then within their perception neighborhood of up to 1, 2, 3, 4 or 5 territories away, depending on the simulation. Next, any non-breeding wolves that are unrelated to other wolves within its territory or that are located outside of breeding range[b] will look for each other within their perception neighborhood, pair up if unrelated and of the opposite sex, move to the nearest territory, and establish themselves as the breeding pair if there are no other breeders in that territory. |
| Winter dispersal | 2 | All wolves that are not breeders and without other wolves nearby disperse to increase their chances of finding a mate by choosing a random direction and moving a distance drawn from lognormal distribution with the log mean equal to 3.23, 3.92, or 4.61 depending on the simulation and log standard deviation equal to 1.01. |
| Reproduce | 4 | All breeding females reproduce a number of pups drawn from a normal distribution with mean equal to 5.41 and standard deviation equal to 0.79 and rounded to the nearest whole number. The sex of each pup is chosen randomly. |
| Targeted lethal control | 5 | To simulate the lethal control of wolves to alleviate livestock depredation in the summer months, wolves are killed from within 5 km of areas where there have been reported livestock depredations in Wisconsin in the late 2000s. A total of 10% of the last winter count of wolves in Wisconsin are killed from these high depredation areas once the simulated population reaches 350 wolves in Wisconsin. |
| Fall dispersal | 6 | To simulate resource limitation, the number of non-breeding wolves within a pack in excess of 10 wolves will disperse out of their natal pack by choosing a random direction and moving a distance drawn from a lognormal distribution with log mean equal to 3.23, 3.92, or 4.61 depending on the simulation and log standard deviation equal to 1.01. If these wolves do not disperse far enough to leave the pack, they die. |
| Spatial mortality risk | 7 | Wolves survive with a probability prescribed by the spatial mortality risk determined by local road density and amount of agriculture [37]. |
| Age | 8 | Wolves age each year and die if they reach 12 years of age. |

[a] 225 km$^2$ that support up to 1 pack and exist in areas with low background risk. There are 363 potential territories with 151 of them in Wisconsin.

[b] Areas of Minnesota, Wisconsin, and the upper peninsula of Michigan where the spatial mortality risk is <0.75.

doi:10.1371/journal.pone.0150535.t001

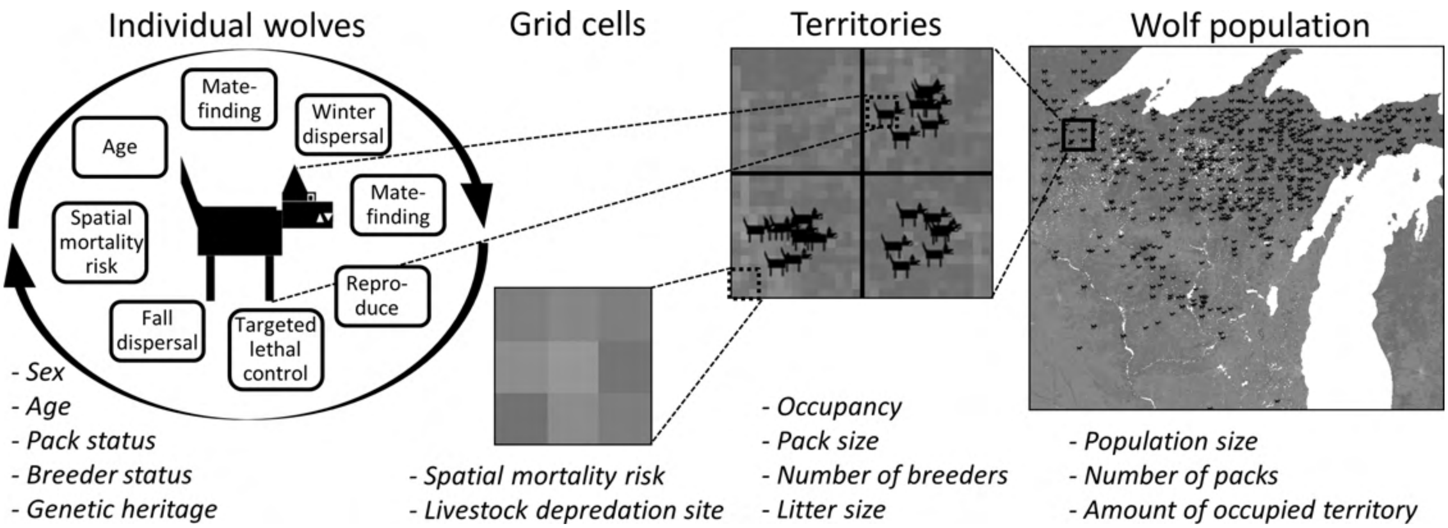


**Fig 2. Depiction of an individual-based spatially explicit model for growth of the southern Lake Superior wolf population [37].** The hierarchical levels of organization are the individual wolves, grid cells that make up the landscape, territories, and wolf population and the lists (e.g., sex, age, pack status) are the variables that characterize each level.

doi:10.1371/journal.pone.0150535.g002

landscape (Table 1; Fig 2). Each repetition began with 20 breeding pairs in territories in Minnesota and ran for 40 years or until all wolves died. We varied the individual-level perception neighborhoods (5 categories) and dispersal distances (drawn from a lognormal distribution with varying log mean parameters (3 categories; Table 1) and ran 100 repetitions for each of the 3 × 5 = 15 simulations for a total of 1500 repetitions.

A wolf's perception neighborhood for detecting mates is largely unknown. We varied this parameter in the IBSE model in territory-based increments from a perception neighborhood of one territory away (15 km) to five territories away (75 km; Fig 3). Dispersal distance is better understood for Great Lakes wolves, but there are still many uncertainties with defining a dispersal event and determining how to deal with bias associated with radio-collared wolves that may have dispersed but are lost from radio-contact. We fit a lognormal distribution to 110 observations of Great Lakes wolf dispersal distances [20] and used the maximum likelihood estimates for the mean, $d_{ave}$, and standard deviation, $s$: $\ln(d_{ave}) = 3.92$ and $\ln(s) = 1.01$. We took $d_{ave}$ to be our best estimate of true mean dispersal distance, and then considered alternate dispersal distance functions where mean dispersal distance was half of $d_{ave}$, $d_{low}$, and where dispersal distance was double $d_{ave}$, $d_{high}$ (Fig 3).

To test whether mate-finding limitations would lead to a demographic Allee effect, we looked for evidence of Allee effects in the relationship between simulated per capita population growth and the SLS population size. For each repetition, we calculated $pgr_t$ and plotted $pgr_t$ versus $\ln(N_t)$ for $t = 2,3,\ldots,T$ where $T$ was the number of years in the time series and $N_2$ was the population size in the first year the simulated SLS population was $\geq$15 wolves because this was the minimum number of wolves detected in the SLS population since wolf recovery in the SLS region [22]. For each plot, we fit a cubic smoothing spline with six knots using the function "smooth.spline" with its default values in program R. We categorized each simulation outcome as: 1) 'extinct' when we could not assess because simulations never reached 15 wolves or $\geq$10 data points, 2) 'strong Allee effect' when the spline started with a positive slope and negative values for per capita growth, 3) 'weak Allee effect' when the spline started with a positive slope and positive values for per capita growth, or 4) 'no evidence for an Allee effect' when the spline started with a negative slope.

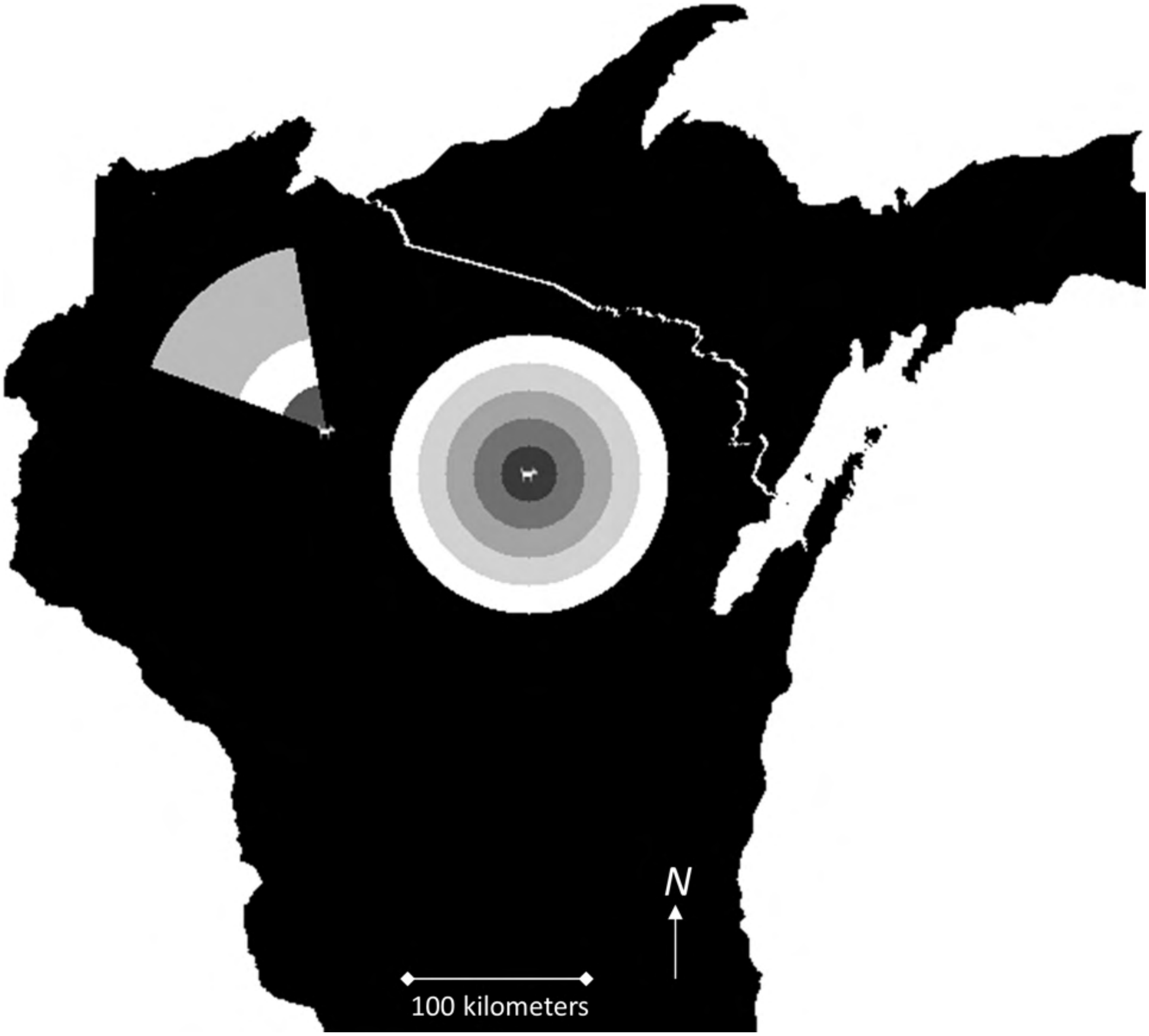


**Fig 3. Simulations for an individual-based spatially explicit model for southern Lake Superior wolves.** We varied perception neighborhoods where simulated wolves could search for mates 1, 2, 3, 4 and 5 territories away (concentric circles) and the log mean parameter in the lognormal distribution used to calculate individual dispersal distance with average dispersal distances of 25, 50, and 100 kilometers (sectors) on a simulated landscape.

doi:10.1371/journal.pone.0150535.g003

We explored how the probability of evidence for an Allee effect, $p_i$ where $i = 1,2,\ldots1500$ indexed the repetition, was affected by the choice of perception neighborhood and mean dispersal distance with a logistic regression model:

$$Y_i \sim Bernoulli(p_i)$$

$$logit(p_i) = \beta_0 + x_1 \times \beta_1 + x_2 \times \beta_2 + \cdots + x_K \times \beta_K$$

The response $Y_i = 1$ if there was evidence for a strong or weak Allee effect. The predictors $x_k$ for $k = 1,2,\ldots,K$ for $K$ total predictors were dummy variables for the different perception neighborhood and dispersal distance combinations. We considered aggregating some categories depending on whether there appeared to be an interaction between perception neighborhood and dispersal distance. We assigned vague priors to the parameters, $\beta_k \sim normal(0,100^2)$. We ran this model in a Bayesian framework following the methods outlined above.

# Results

## Demographic Allee effect

The MCMC algorithms converged adequately for all models (upper 97.5% estimates of $\hat{R}$ were $< 1.04$ for all parameters, and the overall $\hat{R}^p$ statistics were $< 1.02$ for all models). Strong demographic Allee effects were evident from all models because the splines fit to the data were all hump-shaped and the spline changed from negative to positive growth rates at a low population size (i.e., positive Allee thresholds; Fig 4). No models had fitted splines that passed into negative growth rates at high population size which would have provided an estimate of carrying capacity (Fig 4). Mean posterior fitted values from the SLS population dataset and the Wisconsin population dataset both had an Allee threshold around 1987–1988 when there were approximately 20 wolves in these populations. Mean posterior fitted values reached maximum growth in the SLS population in 1994–1995 with approximately 135 wolves and in 1996–1997 in the Wisconsin population with approximately 111 wolves. For the pack dataset, the Allee threshold was estimated to have occurred slightly earlier in 1985–1986 when there were four to five packs of wolves, and maximum growth was estimated to have occurred in 1995–1996 when there were 26–27 packs of wolves in Wisconsin. The territory dataset had the latest estimated Allee threshold in 1990–1991 when there was approximately 1100 $km^2$ of occupied wolf territory in Wisconsin and the maximum growth was reached in 1993–1994 when there was approximately 2705 $km^2$ of occupied wolf territory.

## Component Allee effect

Of the 1500 repetitions from the IBSE model, 33 (2.2%) of them went functionally extinct in the sense that they could not be assessed because the population did not grow to $\geq$15 wolves or did not persist for $\geq$10 years with $\geq$15 wolves. All extinctions occurred when the perception neighborhood was simulated to be one territory away, and extinction was >4 times as frequent in the simulations with high dispersal distance compared to average or low dispersal distances (Table 2). There were 545 (36.3%) repetitions with a probable Allee effect and there were approximately twice as many weak Allee effects compared to strong Allee effects. A third of the strong Allee effects occurred under high dispersal when the perception neighborhood was one territory away. Of the strong Allee effects, 72.1% occurred when the perception neighborhood was one territory away and 48.1% of them occurred under high dispersal distance (Table 2). There was little difference in the number of probable Allee effects for simulations that had perception neighborhoods for $\geq$3 territories away (Table 2).

Consequently in the logistic regression, we grouped the simulations with perception neighborhoods $\geq$3 territories away. The MCMC algorithm converged adequately, and the upper 97.5% estimates of $\hat{R}$ and $\hat{R}^p$ were 1. Simulations with perception neighborhoods of $\geq$3 territories away for low, average or high dispersal distances and simulations with a perception neighborhood of two territories away for low and average dispersal distances were least likely to show evidence of an Allee effect (Fig 5). High dispersal distance simulations with a perception neighborhood of two territories away were just as likely to have an Allee effect as to have no evidence for an Allee effect. Simulations with perception neighborhoods of one territory away and low and average dispersal distances were more likely to have an Allee effect than not (Fig 5). Finally, the simulation most likely to go extinct with a perception neighborhood of one territory away and high dispersal was also the simulation with the highest probability of Allee effects (Fig 5).

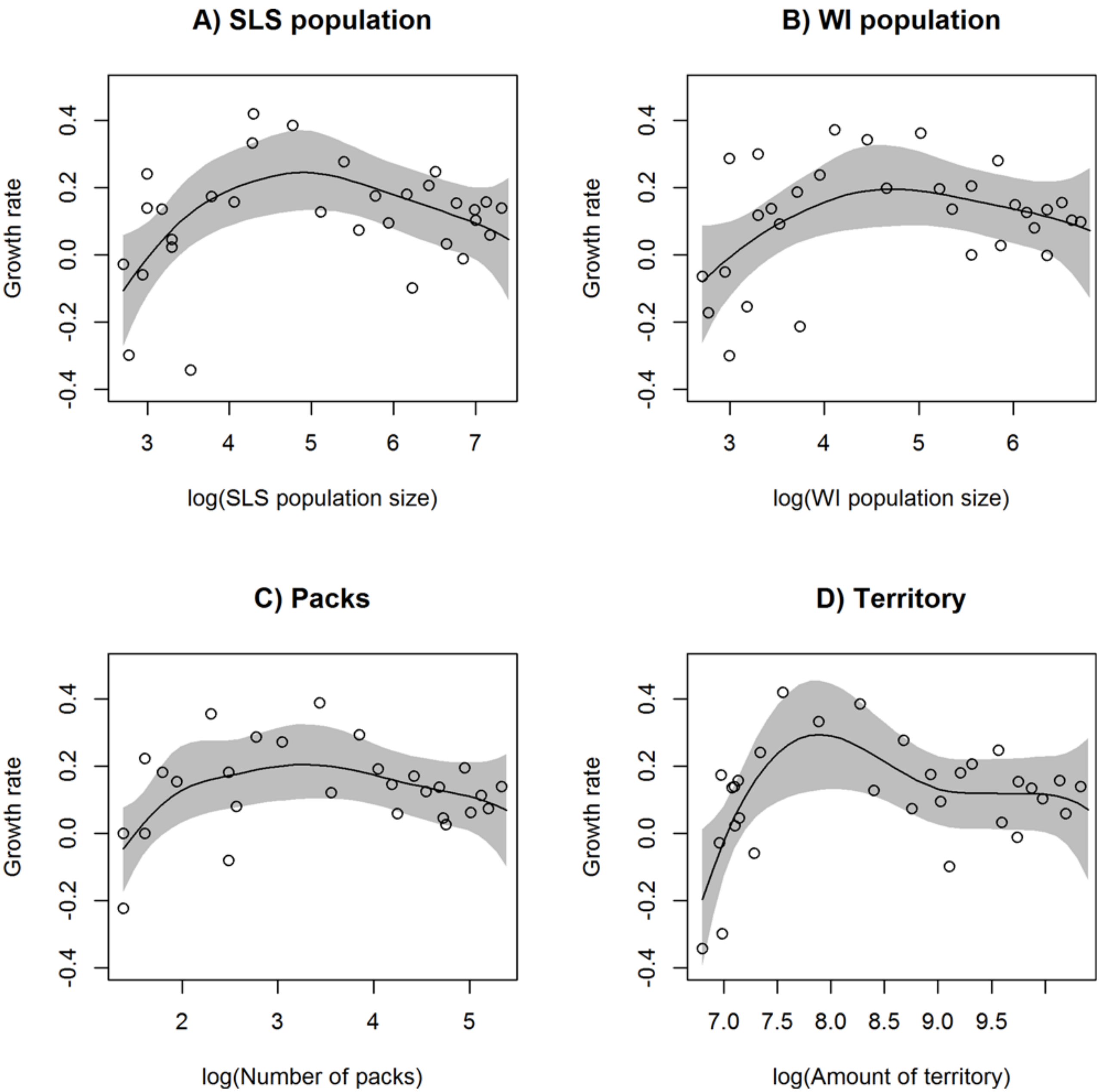


**Fig 4. Splines fit to growth versus population size of the southern Lake Superior wolf population in 1980–2011.** Fitted curves with 95% credible intervals from splines fit to the relationship between per capita growth and four measure of population size for gray wolves in the southern Lake Superior wolf (SLS) population (A) and Wisconsin (B), including the number of packs (C) and the proportion of occupied territory in Wisconsin (D).

doi:10.1371/journal.pone.0150535.g004

## Discussion

We detected a strong demographic Allee effect in the SLS wolf population. Simulations from an IBSE model suggested that the Allee effect could have resulted from wolves dispersing far from population centers and into vacant territories leading to an inability to find mates [7]. In addition, simulation scenarios that produced Allee effects associated with increased simulation failures (extinctions of simulated populations). The combination of high dispersal potential and a restricted perception neighborhood for mate-finding may have restricted population

**Table 2. Number of repetitions with evidence for Allee effects from simulations of an individual-based spatially explicit model for gray wolves [37] in the southern Lake Superior region.**

| | | | Allee effect | | |
|---|---|---|---|---|---|
| **Perception neighborhood (territories)** | **Mean dispersal distance** | **Extinct** | **Strong** | **Weak** | **No evidence** |
| 1 | Low | 1 | 31 | 24 | 44 |
| 1 | Ave | 6 | 38 | 17 | 39 |
| 1 | High | 26 | 63 | 3 | 8 |
| 2 | Low | 0 | 8 | 26 | 66 |
| 2 | Ave | 0 | 4 | 21 | 75 |
| 2 | High | 0 | 15 | 35 | 50 |
| 3 | Low | 0 | 3 | 32 | 65 |
| 3 | Ave | 0 | 7 | 25 | 68 |
| 3 | High | 0 | 7 | 27 | 66 |
| 4 | Low | 0 | 2 | 19 | 79 |
| 4 | Ave | 0 | 1 | 28 | 71 |
| 4 | High | 0 | 2 | 24 | 74 |
| 5 | Low | 0 | 1 | 28 | 71 |
| 5 | Ave | 0 | 0 | 30 | 70 |
| 5 | High | 0 | 1 | 23 | 76 |

The perception neighborhood for mates was varied from 1–5 territories away and the mean dispersal distance from the dispersal function was either low (25 km), average (50 km), or high (100 km). Please see text for category descriptions.

doi:10.1371/journal.pone.0150535.t002

growth in early years of population recovery in SLS wolves. Social carnivores can be particularly vulnerable to Allee effects because of their need for conspecifics in hunting and rearing of young and because they often exist at low densities [5, 8]. Hence, carnivore recovery likely requires careful consideration of Allee effects because of the numerous, intertwined factors that influence dynamics of the population related to population size and density [8, 12, 25].

Demographic Allee effects are notoriously difficult to detect because of the need for a long-term dataset of population counts spanning a range of densities and the potential complications of observer error and demographic stochasticity which can be prevalent at low population sizes [14, 25, 41, 42]. Therefore, it is especially notable that we detected a demographic Allee effect in the SLS wolf population. Further, we detected a strong demographic Allee effect which allowed us to estimate the Allee threshold in this population. From the relationship between per capita growth and population size in the wolf population, we estimated an Allee threshold was passed in the mid- to late-1980s, nearly a decade into population recovery. Hence, the SLS wolf population was probably at or below the Allee threshold for the first decade of reestablishment and could have just as likely become extinct as successfully recolonized during this time. It may be that immigration from Minnesota or Ontario prevented extinction by supplementing population growth sufficiently to exceed the Allee threshold [20]. The population achieved maximum growth and switched from positive to negative density dependence in the mid-1990s coincident with colonization of the central forest region of Wisconsin and the upper peninsula of Michigan–the last remaining patches of high quality habitat [18, 23] and may support an interpretation that growth at high relative density was limited by the availability of high quality habitat or vacant territories.

Examples of mate-finding Allee effects leading to a demographic Allee effect for a species that has evolved life-history strategies to improve mate-finding probability are rare [14]. We found compelling evidence of wolves, a territorial and vagile species with long distance

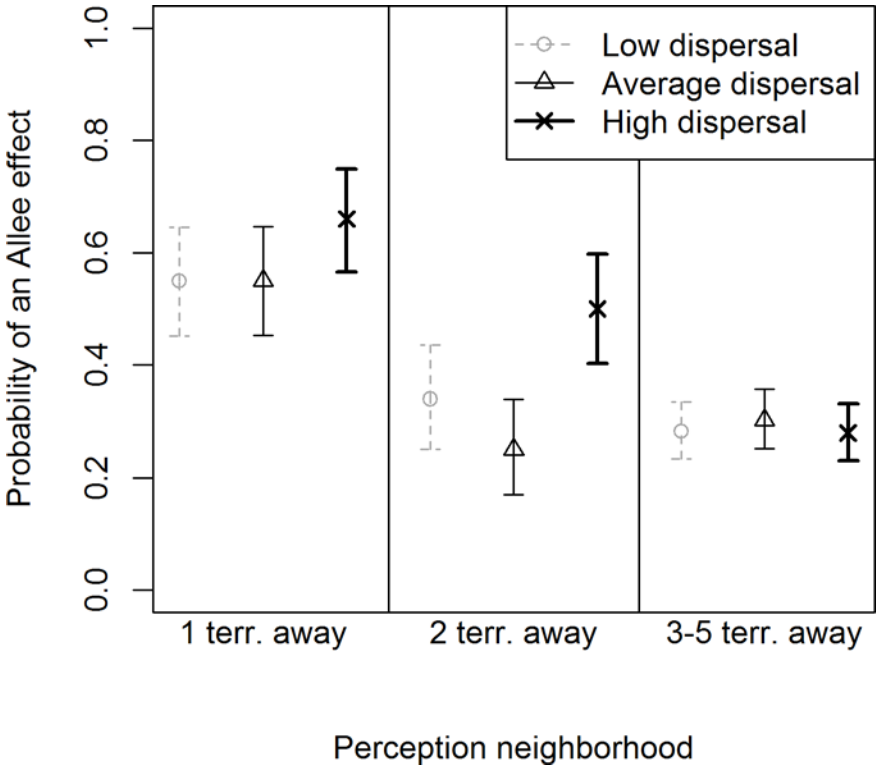


**Fig 5. The probability of an Allee effect from simulations of an individual-based spatially explicit model.** Posterior mean and 95% credible intervals of the probability of an Allee effect from simulations varying the perception neighborhood for mate-finding as 1, 2 or >3 territories (terr) away and the mean dispersal distances as low (25 km), average (50 km) and high (100 km) in an individual-based spatially explicit model for gray wolves in the southern Lake Superior region [37].

doi:10.1371/journal.pone.0150535.g005

communication capabilities that plausibly facilitate mate-finding (howling, scent-marking), experiencing mate-finding and demographic Allee effects. Wolves' ability to detect mates through communication and searching was probably evolved because until recently wolves were the most widely-distributed terrestrial species without many limitations on their movement or establishment [43]. Presently, wolf populations are reduced to fragments of their historic range. In the SLS region, the probability of mortality from human activity has restricted population expansion into a corridor of the northern forested portion of Wisconsin and the upper peninsula of Michigan where human influence is relatively reduced (small portion of historic range) [22]. Hence anthropogenic factors have excluded wolves from a region where population growth would not otherwise be so limited. In a human-dominated landscape, wolf use of space interacts with spatially varying risk of human-caused mortality [44] such that unnatural sparseness or low density likely inhibits mate-finding capacity.

Early reestablishment of the SLS wolf population probably was not slowed because of lack of territory (1547 wolves in the SLS region in 2011) or lack of food [24]. We evaluated potential for a mate-finding Allee effect in the recolonizing SLS wolf population because it is the most-cited Allee effect mechanism [13] and other wolf populations have documented or suspected mate-finding Allee effects [7, 9]. Additionally, we assessed changes in fecundity and the proportion of lone wolves over time (Stenglein unpublished) in Wisconsin's wolf population data [22] and found no evidence of other Allee effect mechanisms. We did not find reduced fecundity in pups per pack or in the proportion of breeding females in the population pre-1995 compared to 1995–2007 (Stenglein unpublished). However, the proportion of lone wolves prior to 1995 (roughly 10% of the population) was higher compared to 1995–2007 when only 4% were lone wolves [22]. The difference in proportion of lone wolves could be due to sampling and detection issues; however a real difference provides support for a mate-finding component Allee effect in early recovery because it suggests that wolves had difficulty finding mates at low densities, resulting in more lone wolves.

Pathogens with long infection cycles or stable reservoirs can persist in small populations and impede population growth [45]. A population affected by pathogens and Allee effects may be more prone to extinction than a population suffering from Allee effects alone and these effects can be more pronounced in social species, like wolves [8, 46–48]. We did not model pathogens explicitly as a source of mortality for simulated wolves in our individual-based model, although we suggest this as an extension if empirical data on density dependent population effects in social carnivore become available. Wolf pathogens identified in the SLS region include canine parvovirus, canine distemper virus, mange, blastomycosis, Lyme disease, anaplasmosis, canine ehrlichiosis and heartworm [49–51]. During early population recovery in the mid-1980s, canine parvovirus was detected in the Wisconsin wolf population and may have reduced survival of wolf pups [52]. In Minnesota, a negative correlation between number of pups captured and canine parvovirus seroprevalence was found during this same time period, also suggesting a reduction in pup survival [53, 54]. However, no population-level effect was detected during the time of the canine parvovirus outbreak suggesting compensatory interactions with other mortalities [54]. In wild wolf populations, canine parvovirus has trivial impacts on adult survival and population size despite elevated pup mortality [51, 54, 55].

Factors other than Allee effect mechanisms could cause observations of negative population growth followed by a sudden increase in growth. Observer error estimating the four measures of population growth we used could have contributed to the appearance of an Allee effect. When the wolf population was small, it may have been more difficult to count wolves, packs and occupied territory. At small population sizes, failing to count just one pack and then finding and counting it in the next year could lead to the appearance of substantial population growth which would be due to observer error rather real growth. Demographic stochasticity in small populations can result in perceived Allee effects [56]. However, demographic stochasticity itself is sometimes considered an Allee effect mechanism when a skewed sex ratio occurring by chance results in mate limitation and subsequent decreased fitness [57, 58]. Our individual-based model incorporated demographic stochasticities by drawing litter sizes, sex assignments of pups, dispersal distances and survival from characteristic probability distributions which resulted in some repetitions within a simulation showing evidence for an Allee effect and others not. Even so, an overall pattern emerged from the simulations that supported a mate-finding Allee effect.

A potential improvement to our model would be to incorporate a more sophisticated mate-finding process for wolves. We treated mate-finding simply in our individual-based model; individual wolves were able to search for mates up to two times each year but only within a maximum distance of their current location and not during dispersal events. This resulted in a circular search area for mates and was not based on landscape information. Wolves may travel most often in long, linear routes [59]. Similarly wolves and other mammals may move across paths of least resistance or choose paths through preferred habitat [20, 60]. However, to our knowledge, nobody has measured the shape of a wolf's perception neighborhood. If perception is based on auditory cues (howling) it could be relatively independent of habitat and therefore circular, and especially if howling can be detected by wolves at great distances. As understanding of the mate-finding process for wolves improves, modelers can design and parameterize a more sophisticated mate-finding process that could incorporate measures of landscape resistance to allow simulated wolves to locate mates in a more informed way [60].

The confluence of long-term datasets and computational power that can support individual-based models expanded opportunities for studying and understanding population dynamics. Splines are an improvement over parametric models when looking for evidence for a demographic Allee effect because they provide useful flexibility in letting the data determine functional relationships [25, 30]. Once a demographic Allee effect is detected, hypothesized mechanisms leading to the Allee effect should be evaluated, and individual-based models provide a useful framework for testing these hypotheses. A well-parameterized individual-based model can be used to study specific mechanisms as well as the emergent population properties to which they contribute [44] and can inform important conservation concerns such as long-term population viability, or how novel mortalities that vary in space and time (e.g., hunting, illegal killing, infectious disease) will affect the population [37, 61, 62].

The SLS wolf population size is >60 times higher than the Allee threshold that we detected; therefore it is very unlikely that the SLS wolf population size would be reduced to a level where it would be prone to Allee effects in the near future [26, 29]. However, as wolves become more established in the SLS region, they are moving to other areas, including the lower peninsula of Michigan, southern Wisconsin and surrounding states [22, 26, 63]. If conservation and expansion for wolves is a goal in these areas, conservationist may need to monitor population growth data in newly established populations to infer whether Allee effects are occurring. Understanding whether an Allee threshold exists (and at what population size) will help predict population growth and expansion probabilities in new areas. Further, an IBSE model has potential to test hypotheses about dynamics other than Allee effects in small populations, such as the effect of demographic stochasticity in newly established populations and the effect of inbreeding in small populations [64, 65].

Difficulty in detecting Allee effects does not diminish the importance that they may play in the dynamics of small and recovering populations, and particularly in the case of social carnivores where social facilitation is a key feature of reproduction [6–8, 14]. Rigorous simulation techniques (e.g., Bayesian MCMC approaches, individual-based models) may offer an optimal strategy for integrating field and published data on population dynamics. Our analysis of complexities in the density dependent growth of the SLS wolf population suggests that similar approaches might provide new insights on the dynamics of small and sparse populations.

## Supporting Information

**S1 Appendix. Datasets of southern Lake Superior wolf population growth.**
(DOCX)

**S2 Appendix. Bayesian spline model with R code.**
(DOCX)

**S1 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 1 territory away and an average dispersal distance of 50 km (average dispersal).
(TIFF)

**S2 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 1 territory away and an average dispersal distance of 100 km (high dispersal).
(TIFF)

**S3 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 1 territory away and an average dispersal distance of 25 km (low dispersal).
(TIFF)

**S4 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 2 territories away and an average dispersal distance of 50 km (average dispersal). (TIFF)

**S5 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 2 territories away and an average dispersal distance of 100 km (high dispersal). (TIFF)

**S6 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 2 territories away and an average dispersal distance of 25 km (low dispersal). (TIFF)

**S7 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 3 territories away and an average dispersal distance of 50 km (average dispersal). (TIFF)

**S8 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 3 territories away and an average dispersal distance of 100 km (high dispersal). (TIFF)

**S9 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 3 territories away and an average dispersal distance of 25 km (low dispersal). (TIFF)

**S10 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 4 territories away and an average dispersal distance of 50 km (average dispersal). (TIFF)

**S11 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 4 territories away and an average dispersal distance of 100 km (high dispersal). (TIFF)

**S12 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 4 territories away and an average dispersal distance of 25 km (low dispersal). (TIFF)

**S13 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 5 territories away and an average dispersal distance of 50 km (average dispersal). (TIFF)

**S14 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 5 territories away and an average dispersal distance of 100 km (high dispersal). (TIFF)

**S15 Fig. Individual-based model simulation results.** Simulation with a perception neighborhood of 5 territories away and an average dispersal distance of 25 km (low dispersal). (TIFF)

**S1 Table. Summary individual-based model simulation results.**
(XLSX)

## Acknowledgments

We thank R. Jurewitz, D. MacFarland, R. Schultz, D. Thiel, J. Weidenhoeft, and A. Wydeven for their development of the wolf monitoring program in Wisconsin, on-going data collection, and feedback on this research. We thank R. Chappell, D. Mladenoff, and M. Samuel for their statistical ideas and friendly review. Thank you to J. Bump and three anonymous reviewers whose comments helped us improve this manuscript. Thank you to the Department of Forest and Wildlife Ecology at University of Wisconsin–Madison and the Wisconsin Department of Natural Resources for data and office support.

## Author Contributions

Conceived and designed the experiments: JLS TRV. Performed the experiments: JLS TRV. Analyzed the data: JLS. Contributed reagents/materials/analysis tools: JLS TRV. Wrote the paper: JLS TRV.

## References

1. Allee WC, Park O, Emerson AE, Park T, Schmidt KP. Principles of animal ecology. 1st ed. St. Louis, Missouri: WB Saundere Company; 1949.
2. Stephens PA, Sutherland WJ, Freckleton RP. What is the Allee effect? Oikos. 1999; 87(1):185–90.
3. Stephens PA, Sutherland WJ. Consequences of the Allee effect for behaviour, ecology and conservation. Trends in Ecology & Evolution. 1999; 14(10):401–5.
4. Berec L, Angulo E, Courchamp F. Multiple Allee effects and population management. Trends in Ecology & Evolution. 2007; 22(4):185–91.
5. Angulo E, Roemer GW, Berec L, Gascoigne J, Courchamp F. Double Allee effects and extinction in the island fox. Conservation Biology. 2007; 21(4):1082–91. PMID: 17650257
6. Courchamp F, Clutton-Brock T, Grenfell B. Multipack dynamics and the Allee effect in the African wild dog, Lycaon pictus. Animal Conservation. 2000; 3(4):277–85.
7. Hurford A, Hebblewhite M, Lewis M. A spatially explicit model for an Allee effect: Why wolves recolonize so slowly in Greater Yellowstone. Theoretical Population Biology. 2006; 70(3):244–54. PMID: 16916526
8. Sanderson CE, Jobbins SE, Alexander KA. With Allee effects, life for the social carnivore is complicated. Population ecology. 2014; 56(2):417–25.
9. Wabakken P, Sand H, Liberg O, Bjärvall A. The recovery, distribution, and population dynamics of wolves on the Scandinavian peninsula, 1978–1998. Canadian Journal of Zoology. 2001; 79(4):710–25.
10. Molnár PK, Lewis MA, Derocher AE. Estimating Allee dynamics before they can be observed: Polar bears as a case study. PloS One. 2014. doi: 10.1371/journal.pone.0085410
11. Kramer AM, Dennis B, Liebhold AM, Drake JM. The evidence for Allee effects. Population Ecology. 2009; 51(3):341–54.
12. Hart EM, Avilés L. Reconstructing local population dynamics in noisy metapopulations—the role of random catastrophes and Allee effects. Plos One. 2014. doi: 10.1371/journal.pone.0110049
13. Berec L, Boukal D, Berec M. Linking the Allee effect, sexual reproduction, and temperature-dependent sex determination via spatial dynamics. American Naturalist. 2001; 157(2):217–30. doi: 10.1086/318626 PMID: 18707273
14. Gascoigne J, Berec L, Gregory S, Courchamp F. Dangerously few liaisons: a review of mate-finding Allee effects. Population Ecology. 2009; 51(3):355–72.
15. Grimm V. Ten years of individual-based modelling in ecology: What have we learned and what could we learn in the future? Ecological Modelling. 1999; 115(2–3):129–48.
16. Huston M, DeAngelis D, Post W. New computer models unify ecological theory. BioScience. 1988; 38 (10):682–91.
17. South AB, Kenward RE. Mate finding, dispersal distances and population growth in invading species: a spatially explicit model. Oikos. 2001; 95(1):53–8.
18. Mladenoff DJ, Clayton MK, Pratt SD, Sickley TA, Wydeven AP. Change in occupied wolf habitat in the northern Great Lakes region. In: Wydeven AP, Van Deelen TR, Heske EJ, editors. Recovery of Gray wolves in the Great Lakes region of the United States: An Endangered Species Success Story. New York, NY, USA: Springer; 2009. p. 119–38.
19. Mladenoff D, Sickley T, Haight R, Wydeven A. A regional landscape analysis and prediction of favorable gray wolf habitat in the norther Great Lakes region. Conservation Biology. 1995; 9(2):279–94.

20. Treves A, Martin KA, Wiedenhoeft JE, Wydeven AP. Dispersal of gray wolves in the Great Lakes region. In: Wydeven AP, Van Deelen TR, Heske EJ, editors. Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. New York, NY, USA: Springer; 2009. p. 191–204.
21. Wydeven AP, Van Deelen TR, Heske EJ. Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. New York, NY, USA: Springer; 2009.
22. Wydeven AP, Wiedenhoeft JE, Schultz RN, Thiel RP, Jurewicz RL, Kohn BE, et al. History, population growth, and management of wolves in Wisconsin. In: Wydeven AP, Van Deelen TR, Heske EJ, editors. Recovery of Gray Wolves in the Great Lakes Region of the United States: an endangered species success story. New York, NY, USA: Springer; 2009. p. 87–105.
23. Thiel RP, Hall W Jr, Heillhecker E, Wydeven AP. A disjunct gray wolf population in Central Wisconsin. In: Wydeven AP, Van Deelen TR, Heske EJ, editors. Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. New York, NY, USA: Springer; 2009. p. 107–17.
24. Van Deelen TR. Growth characteristics of a recovering wolf population in the Great Lakes region In: Wydeven AP, Van Deelen TR, Heske EJ, editors. Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. New York, NY, USA: Springer; 2009. p. 139–53.
25. Courchamp F, Berec L, Gascoigne J. Allee effects in ecology and conservation. Environ Conserv. 2008; 36(1):80–5.
26. Michigan Department of Natural Resources. Michigan Wolf Management Plan. Lansing, Michigan: 2015.
27. Wisconsin Department of Natural Resources. Preliminary Pack and Lone Wolf Summaries for Wisconsin Winter 2014–2015. Madison, WI: Wisconsin Department of Natural Resources, 2015.
28. Beyer DE Jr, Peterson RO, Vucetich JA, Hammill JH. Wolf population changes in Michigan. In: Wydeven AP, Van Deelen TR, Heske EJ, editors. Recovery of Gray Wolves in the Great Lakes Region of the United States: An Endangered Species Success Story. New York, NY, USA: Springer; 2009. p. 65–85.
29. MacFarland D, Wiedenhoeft JE. Wisconsin gray wolf post-delisting monitoring: 27 January 2012 through 14 April 2013. Madison, Wisconsin: Bureau of Wildlife Management, Wisconsin Department of Natural Resources; 2013.
30. Ruppert D, Wand MP, Carroll RJ. Semiparametric regression. New York, NY, USA: Cambridge University Press; 2003.
31. Crainiceanu CM, Ruppert D, Wand MP. Bayesian Analysis for Penalized Spline Regression Using WinBUGS. Journal of Statistical Software. 2005; 14(14):1–24.
32. R developement Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
33. Plummer M. rjags: Bayesian graphical models using MCMC. R package version 3–5. 2011.
34. Plummer M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. In: Hornik K, Leisch F, Zeileis A, editors. Proceedings of the 3rd International Workshop on Distributed Statistical Computing; Vienna, Austria: Technische Universit at Wien; 2003. p. 1–10.
35. Brooks S, Gelman A. General methods for monitoring convergence of iterative simulations. Journal of Computational and Graphical Statistics. 1998; 7(4):434–55.
36. Gelman A, Rubin DB. Inference from iterative simulation using multiple sequences. Statistical science. 1992; 7(4):457–72.
37. Stenglein JL, Gilbert JH, Wydeven AP, Van Deelen TR. An individual-based model for southern Lake Superior wolves: A tool to explore the effect of human-caused mortality on a landscape of risk. Ecological Modelling. 2015; 302:13–24.
38. Wilensky U. NetLogo. Evanston, IL: Center for Connected Learning and Computer-Based Modeling; 1999.
39. Grimm V, Berger U, Bastiansen F, Eliassen S, Ginot V, Giske J, et al. A standard protocol for describing individual-based and agent-based models. Ecological Modelling. 2006; 198(1–2):115–26.
40. Grimm V, Berger U, DeAngelis D, Polhill J, Giske J, Railsback S. The ODD protocol: A review and first update. Ecological Modelling. 2010; 221(23):2760–8.
41. Gregory SD, Bradshaw CJA, Brook BW, Courchamp F. Limited evidence for the demographic Allee effect from numerous species across taxa. Ecology. 2010; 91(7):2151–61. PMID: 20715637
42. Sibly RM, Barker D, Denham MC, Hone J, Pagel M. On the regulation of populations of mammals, birds, fish, and insects. Science. 2005; 309(5734):607–10. PMID: 16040705

**43.** Young SP, Goldman EA. The wolves of North America: Part I. Their history, life habits, economic status, and control. Washington D.C., USA: The American Wildlife Institute; 1944.

**44.** Stenglein JL. Survival of Wisconsin's gray wolves from endangered to harvested, 1980–2013 [Dissertation]. Madison, WI: University of Wisconsin—Madison; 2014.

**45.** Cleaveland S, Mlengeya T, Kaare M, Haydon DAN, Lembo T, Laurenson MK, et al. The conservation relevance of epidemiological research into carnivore viral diseases in the Serengeti. Conservation Biology. 2007; 21(3):612–22. PMID: 17531040

**46.** Deredec A, Courchamp F. Combined impacts of Allee effects and parasitism. Oikos. 2006; 112(3):667–79.

**47.** Hilker FM. Population collapse to extinction: the catastrophic combination of parasitism and Allee effect. Journal of Biological Dynamics. 2010; 4(1):86–101. doi: 10.1080/17513750903026429 PMID: 22881072

**48.** Hilker FM, Langlais M, Malchow H. The Allee Effect and Infectious Diseases: Extinction, Multistability, and the (Dis-) Appearance of Oscillations. The American Naturalist. 2009; 173(1):72–88. doi: 10.1086/593357 PMID: 19072071

**49.** Jara RF. Spatial and temporal patterns of gray wolf exposure to vector-borne diseases in Wisconsin. Madison, WI: University of Wisconsin—Madison; 2013.

**50.** Thiel RP, Mech LD, Ruth GR, Archer JR, Kaufman L. Blastomycosis in wild wolves. Journal of wildlife diseases. 1987; 23(2):321–3. PMID: 3586211

**51.** Kreeger TJ. The internal wolf: physiology, pathology, and pharmacology. In: Mech LD, Boitani L, editors. Wolves: Behavior, Ecology and Conservation. Chicago and London: The University of Chicago Press; 2003. p. 192–217.

**52.** Wydeven AP, Schultz RN, Thiel RP. Monitoring of a recovering gray wolf population in Wisconsin, 1979–1991. In: Carbyn LN, Fritts SH, Seip DR, editors. Ecology and Conservation of Wolves in a Changing World. Edmonton, Alberta: Canadian Circumpolar Institute; 1995. p. 147–56.

**53.** Goyal SM, Mech LD, Rademacher RA, Khan MA, Seal US. Antibodies against canine parvovirus in wolves of Minnesota: a serologic study from 1975 through 1985. Journal of the American Veterinary Medical Association. 1986; 189(9):1092–4. PMID: 2851572

**54.** Mech LD, Goyal SM. Effects of canine parvovirus on gray wolves in Minnesota. The Journal of wildlife management. 1995; 59(3):565–70.

**55.** Johnson MR, Boyd DK, Pletscher DH. Serologic investigations of canine parvovirus and canine distemper in relation to wolf (Canis lupus) pup mortalities. Journal of Wildlife Diseases. 1994; 30(2):270–3. PMID: 8028116

**56.** Lande R. Demographic stochasticity and Allee effect on a scale with isotropic noise. Oikos. 1998; 83 (2):353–8.

**57.** Engen S, Lande R, SÆther B-E. Demographic stochasticity and Allee effects in populations with two sexes. Ecology. 2003; 84(9):2378–86.

**58.** Bessa-Gomes C, Legendre S, Clobert J. Allee effects, mating systems and the extinction risk in populations with two sexes. Ecology Letters. 2004; 7(9):802–12.

**59.** Mech LD, Boitani L. Wolf Social Ecology. In: Mech LD, Boitani L, editors. Wolves: Behavior, Ecology, and Conservation. Chicago, Illinois: The University of Chicago Press; 2003. p. 1–34.

**60.** Zeller KA, McGarigal K, Whiteley AR. Estimating landscape resistance to movement: a review. Landscape Ecology. 2012; 27(6):777–97.

**61.** Olson ER, Stenglein JL, Shelley V, Rissman AR, Browne-Nuñez C, Voyles Z, et al. Pendulum swings in wolf management led to conflict, illegal kills, and a legislated wolf hunt. Conservation Letters. 2015; 8 (5):351–60.

**62.** Stenglein JL, Zhu J, Clayton MK, Van Deelen TR. Are the numbers adding up? Exploiting discrepancies among complementary population models. Ecology and Evolution. 2015; 5(2):368–76. doi: 10.1002/ece3.1365 PMID: 25691964

**63.** Gehring TM, Potter BA. Wolf habitat analysis in Michigan: an example of the need for proactive land management for carnivore species. Wildlife Society Bulletin. 2005; 33(4):1237–44.

**64.** Liberg O, Andrén H, Pedersen H-C, Sand H, Sejberg D, Wabakken P, et al. Severe inbreeding depression in a wild wolf Canis lupus population. Biology letters. 2005; 1(1):17–20. PMID: 17148117

**65.** Räikkönen J, Vucetich JA, Peterson RO, Nelson MP. Congenital bone deformities and the inbred wolves (Canis lupus) of Isle Royale. Biological Conservation. 2009; 142(5):1025–31.

Molecular Ecology (2007) doi: 10.1111/j.1365-294X.2007.03468.x

# The genealogy and genetic viability of reintroduced Yellowstone grey wolves

BRIDGETT M. VONHOLDT,* DANIEL R. STAHLER,*† DOUGLAS W. SMITH,† DENT A. EARL,*
JOHN P. POLLINGER* and ROBERT K. WAYNE*
**University of California, Los Angeles, Ecology and Evolutionary Biology, 621 Charles E. Young Dr South, Los Angeles, CA 90095, USA, †Yellowstone Center for Resources, National Park Service, PO Box 168; Yellowstone National Park, Wyoming 82190, USA*

## Abstract

**The recovery of the grey wolf in Yellowstone National Park is an outstanding example of a successful reintroduction. A general question concerning reintroduction is the degree to which genetic variation has been preserved and the specific behavioural mechanisms that enhance the preservation of genetic diversity and reduce inbreeding. We have analysed 200 Yellowstone wolves, including all 31 founders, for variation in 26 microsatellite loci over the 10-year reintroduction period (1995–2004). The population maintained high levels of variation (1995 $H_0 = 0.69$; 2004 $H_0 = 0.73$) with low levels of inbreeding (1995 $F_{IS} = -0.063$; 2004 $F_{IS} = -0.051$) and throughout, the population expanded rapidly ($N_{1995} = 21$; $N_{2004} = 169$). Pedigree-based effective population size ratios did not vary appreciably over the duration of population expansion (1995 $N_e/N_g = 0.29$; 2000 $N_e/N_g = 0.26$; 2004 $N_e/N_g = 0.33$). We estimated kinship and found only two of 30 natural breeding pairs showed evidence of being related (average $r = -0.026$, SE = 0.03). We reconstructed the genealogy of 200 wolves based on genetic and field data and discovered that they avoid inbreeding through a wide variety of behavioural mechanisms including absolute avoidance of breeding with related pack members, male-biased dispersal to packs where they breed with nonrelatives, and female-biased subordinate breeding. We documented a greater diversity of such population assembly patterns in Yellowstone than previously observed in any other natural wolf population. Inbreeding avoidance is nearly absolute despite the high probability of within-pack inbreeding opportunities and extensive interpack kinship ties between adjacent packs. Simulations showed that the Yellowstone population has levels of genetic variation similar to that of a population managed for high variation and low inbreeding, and greater than that expected for random breeding within packs or across the entire breeding pool. Although short-term losses in variation seem minimal, future projections of the population at carrying capacity suggest significant inbreeding depression will occur without connectivity and migratory exchange with other populations.**

*Keywords*: conservation, genealogy, heterozygosity, inbreeding, viability, wolves

*Received 19 March 2007; revision 19 June 2007*

## Introduction

The reintroduction of extirpated species to their native habitats is an important step in the restoration of ecosystem function in human-altered landscapes. However, many reintroductions are actual or functional failures due to poor quality of the recovery habitat, the inexperience of reintroduced captive-reared individuals, too few founding individuals, or failure to release individuals into a region of their historic distribution (Griffith *et al*. 1989; Wolf *et al*. 1998; Breitenmoser *et al*. 2001; Frankham *et al*. 2002). Few model reintroductions are available for study to understand how long-term viability can be retained. A key element of successful reintroduction programmes is the integration of genetic management into the scientific design, in addition to an understanding of ecology and demography of the reintroduced species (Wolf *et al*. 1998; Miller *et al*. 1999;

Correspondence: Robert K. Wayne, Fax: 310-825-3987; E-mail: rwayne@ucla.edu

Breitenmoser *et al*. 2001; Frankham *et al*. 2002). Because reintroduced populations typically have small effective population sizes and are isolated, the effect of inbreeding and loss of genetic variation on population viability can be substantial (Wright 1931; Nei *et al*. 1975; Frankham *et al*. 2002). Thus, founding populations should be large and composed of genetically diverse individuals to reduce fitness costs associated with inbreeding depression and to allow for an adaptive response to changing conditions (Lande 1995; Keller & Waller 2002). Factors that influence the genetic structure of the reintroduced population also must be considered so as to maximize the genetic variation retained during the recovery process. For example, mating system and the degree of sociality can influence fine-scale genetic structure through patterns of breeding and population assembly rules (Chesser 1991a, b; Sugg *et al*. 1996; Randall *et al*. 2007). In this regard, population structure is predicted to be largely influenced by sex and kinship bias in dispersal patterns, inbreeding avoidance, and social barriers to gene flow (Sugg *et al*. 1996; Girman *et al*. 1997; Smith *et al*. 1997; Peakall *et al*. 2003). Finally, management should aim to establish and maintain population connectivity, which can greatly influence population growth, gene flow, and genetic variation (Keller & Waller 2002; Riley *et al*. 2006).

With respect to the presence and relative abundance of large carnivores, all natural habitats in the contiguous United States are highly altered from pre-Columbian conditions. Large carnivores often exert a top-down impact on ecosystems with effects on prey and vegetation (Terborgh *et al*. 1999, 2006; Schmitz *et al*. 2000; Smith *et al*. 2003; Soule *et al*. 2003; Hebblewhite *et al*. 2005). The grey wolf (*Canis lupus*) is the only top carnivore to be effectively extirpated from the American West and historic populations had higher genetic variability than elsewhere in North America, suggesting a population of more than 350 000 wolves (Leonard *et al*. 2005). After being absent for approximately 70 years, the grey wolf was reintroduced to Yellowstone National Park (YNP), Wyoming, in 1995 and 1996 as part of wolf restoration to the northern Rocky Mountains (Bangs & Fritts 1996; Phillips & Smith 1996). A founding stock of 31 wild-born individuals from Canada was used to establish a breeding population, with 10 additional wolves translocated from northwestern Montana augmenting the population in 1997 (Bangs *et al*. 1998). Strategic guidelines regarding genetic aspects of recovery included selecting an adequate number of founders from two distinct source populations and reintroducing extant family groups to promote early reproduction and social stability (USFWS 1994; Fritts *et al*. 1997). In the 10 years following their initial release, wolves have recolonized the 8991 km$^2$ park and several adjacent portions of the 72 800 km$^2$ Greater Yellowstone Area (GYA). The population expanded rapidly due to an abundance of prey and reduced human exploitation, which are both considered key habitat criteria for wolves (Mech 1970; Fritts *et al*. 2001). This growth represents the products of founder reproduction only, as the isolation of the GYA from other wolf populations prevented any influential immigration (Oakleaf *et al*. 2006; von Holdt unpublished data). Currently, there are over 300 wolves in the GYA (USFWS 2007) which affect many aspects of ecosystem function (Smith *et al*. 2003). The remarkable success of the reintroduction of the wolf to Yellowstone provides a unique opportunity to understand the role of individual breeding patterns and social structure in the preservation of genetic diversity.

Wolves live in territorial social groups whose members cooperate to capture prey, raise young, and defend resources from competitors (Mech 1970; Mech & Boitani 2003b). Wolf packs most commonly represent families consisting of a single breeding pair and their offspring of one or more litters (Murie 1944; Mech 1970). However, packs may also include siblings or previous offspring of one of the breeding pair, and may include nonreproductive individuals unrelated to pack members (Mech & Boitani 2003a). Due to such kinship structuring in wolf populations, levels of genetic heterogeneity will be influenced by aspects of their mating system, such as reproductive skew, inbreeding avoidance, and access to unrelated mates. For example, although it has been shown that inbreeding avoidance is an important constraint on wolf behavioural ecology (Smith *et al*. 1997), some wolf populations have experienced bottlenecks or founding events resulting in genetic deterioration and inbreeding depression (Wayne *et al*. 1991; Peterson *et al*. 1998; Liberg *et al*. 2005). Additionally, the effective population size ($N_e$) is much smaller than census population size ($N_c$) in wolf populations, reflecting the limited number of breeders (Nunney 1995; Frankham 1996; Aspi *et al*. 2006). Because $N_e$ determines the rate of loss of genetic variation, inbreeding, and the fixation of deleterious alleles (Wright 1969), understanding changes in $N_e$ are important for long-term genetic viability of wolf populations (Peterson *et al*. 1998; Randi *et al*. 2000; Aspi *et al*. 2006).

In this study, we constructed a pedigree of the Yellowstone population involving all founder individuals and 169 of their descendants over the past decade. Few studies to date have been able to resolve extensive relationships in a wild endangered species (Taylor *et al*. 1997; Kalinowski *et al*. 1999; Ralls & Ballou 2004; Liberg *et al*. 2005). This pedigree, based on field and genetic data, was used to explore trends in genetic diversity, population structure, and effective population size. We describe how reproduction, pack formation and kinship influenced the observed genetic variation, and identify population assembly rules governing the preservation of variation in this rapidly expanding wolf population. Additionally, we evaluated the success of observed breeding behaviour in maintaining genetic variation as compared to simulated breeding

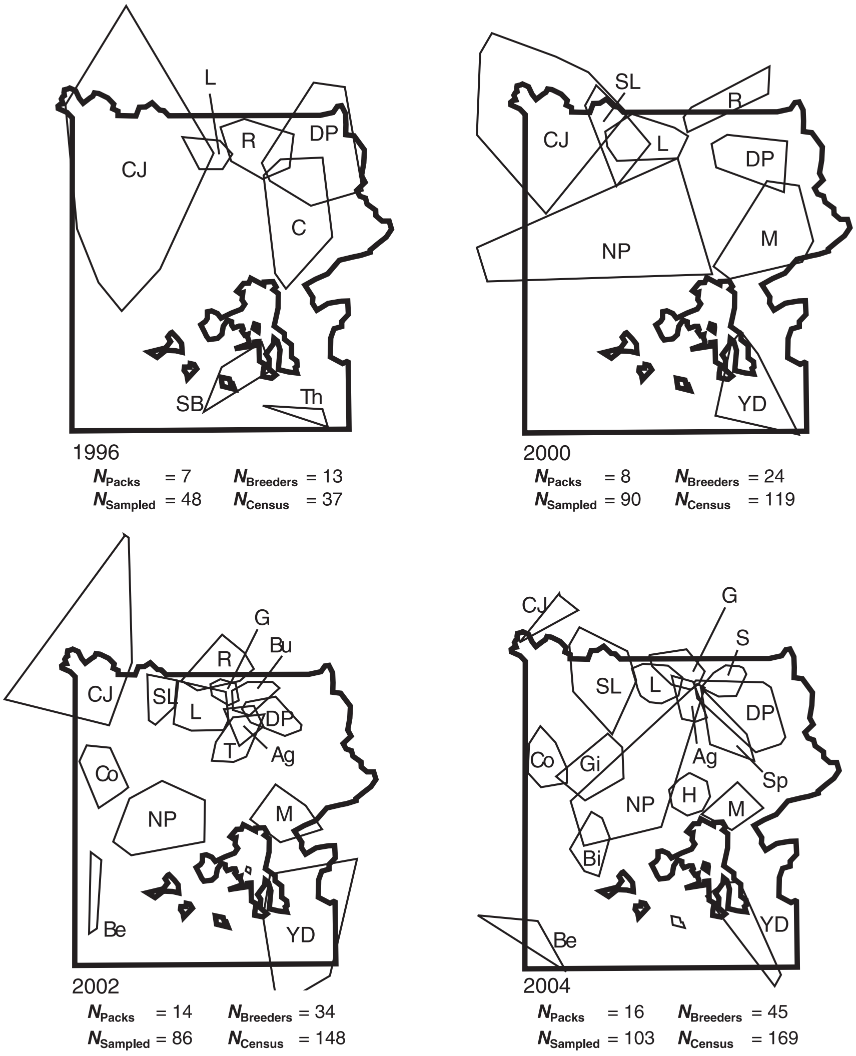


**Fig. 1** Pack distributions, number of packs, number of individuals sampled, number of breeding individuals, and census size for Yellowstone National Park wolves (1995–2004). Polygons represent the pack territories. Number of breeders was based on field observations of attempted or actual copulations as well as documented pup production in packs. (Ag, Agate Creek; Be, Bechler; Bi, Biscuit Basin; Bu, Buffalo Fork; CJ, Chief Joseph; Co, Cougar Creek; C, Crystal Creek; D, Druid Peak; G, Geode Creek; Gi, Gibbon Meadows; H, Hayden; L, Leopold; M, Mollie's; NP, Nez Perce; R, Rose Creek; Sh, Sheep Mountain; S, Slough Creek; SB, Soda Butte; Sp, Specimen Ridge; SL, Swan Lake; Th, Thorofare; T, Tower; and YD, Yellowstone Delta).

scenarios. Finally, we projected changes in genetic variability into the future and discuss management strategies for maintaining variation. This study provides a unique assessment of endangered species recovery, and facilitates a greater understanding of the importance of conservation actions on genetic viability and population persistence.

## Materials and methods

### *Sample collection design*

Blood and tissue samples were collected between 1995 and 2004 from 200 wolves from YNP by means of helicopter darting and post-mortality sampling (Fig. 1). Our sampling represents 23 packs: Agate Creek, Bechler, Biscuit Basin, Buffalo Fork, Chief Joseph, Cougar Creek, Crystal Creek, Druid Peak, Geode Creek, Gibbon Meadows, Hayden, Leopold, Lone Star, Mollie's, Nez Perce, Rose Creek, Sheep Mountain, Slough Creek, Soda Butte, Swan Lake, Thorofare, Tower, and Yellowstone Delta. All 31 founding Canadian wolves were sampled prior to their release in YNP. Additionally, 10 pups (Sawtooth pack) were translocated from northwestern Montana in 1996 after their parents were killed as part of a livestock depredation control action in 1996 and represented additional individuals unrelated to Yellowstone founders. Only two of these, however, were observed to reproduce in the wild. The proportion of individuals radio-collared during the study period ranged from 25 to 86% of the total Yellowstone census size ($N_C$; range 21–174 wolves), defined as all living wolves at the end of the calendar year. All radio-collared individuals were aged and sexed and pack membership, social status, and location of the pack within the park were recorded at least once per week. Most (81%) of the radio-collared wolves were genetically sampled. Changes in pack membership, new pack formation and an individuals' social status were determined via aerial and ground monitoring of collared and uncollared individuals. Field-based parentage was used to corroborate genetic analysis, and was based on observed dominant status of males and females, copulatory ties, morphological evidence of pregnancy prior to denning period, and denning behaviour. At dens, the presence of a lactating female indicated maternity; however, multiple breeders at den sites made it difficult to resolve field-estimated parentage in these circumstances without confirming genetic data.

### *Microsatellite genotyping*

DNA was extracted from whole blood, tissue, hair, and serum using the QIAGEN QIAamp DNA Mini kit and the manufacturer's protocol. We genotyped individuals for 30 domestic dog microsatellite loci that were screened for amplification and polymorphic content on a test panel of 24 grey wolf samples: PEZ5, PEZ6, PEZ8, PEZ11, PEZ12, PEZ15, PEZ19 (J. Halverson in Neff *et al*. 1999), FHC2001, FHC2004, FHC2010, FHC2054, FHC2088, FHC2137, FHC2324, FHC2611, FHC2658, FHC2670, FHC2766, FHC2785, FHC2790, FHC2869, FHC2914, FHC3047, FHC3313, FHC3398, FHC3399, FHC3725, FHC3853, FHC3965, and FHC4027 (Neff *et al*. 1999; Breen *et al*. 2001; Guyon *et al*. 2003).

Genotypes were obtained by polymerase chain reaction (PCR) amplification using QIAGEN Multiplex PCR kits with a hybrid forward primer consisting of the published forward primer with an M13F (–20) sequence (16 bp) added to the 5′ end and a fluorescent dye-labelled M13F (–20) primer (Boutin-Ganache *et al*. 2001). The reverse primer was unlabelled. Reactions were performed in 10 μL volumes containing 1.5 μL DNA, 1.0 μL primer mix, 0.4 μL 10 mg/mL bovine serum albumin, 5.0 μL QIAGEN master-mix and double-distilled water. Loci were multiplexed in sets of two to five, using primer mix prepared according to the manufacturer's guidelines. Amplifications were performed on a Peltier Thermal Cycler (MJ Research PTC-200) using the multiplex cycling profile: 95 °C for 15 min; 25 cycles at 94 °C for 30 s, 59 °C for 90 s, and 72 °C for 60 s; then 20 cycles at 94 °C for 30 s, 53 °C for 90 s, and 72 °C for 60 s with a final extension at 60 °C for 30 min. PCR products were analysed on an ABI 3730XL capillary sequencer and alleles were analysed using ABI GENEMAPPER version 3.0 software (Applied Biosystems). Allele repeats were checked with Excel Microsatellite Toolkit (Parker 2001).

### *Genetic diversity*

The total pedigree data set included 200 genotyped individuals with > 70% of the loci typed. We utilized population- and individual-based approaches for calculating heterozygosity. We used CERVUS (Marshall *et al*. 1998) for calculating population-based variation indices. The observed heterozygosity was obtained by dividing the total number of heterozygotes by the total number of individuals typed and the multilocus expected heterozygosity was calculated and averaged across all loci using the unbiased formula of Nei (1987) from allele frequencies assuming Hardy–Weinberg equilibrium (Marshall *et al*. 1998). Uncorrected individual-based heterozygosity was the proportion of heterozygous loci typed for each individual and as in Bensch *et al*. (2006), was used to compare individuals, such as parent–offspring or breeding pairs and to investigate mate choice based on individual heterozygosities. Annual heterozygosities were calculated based on the calendar year for all living animals. We also estimated relatedness and inbreeding coefficients ($F_{IS}$) to assess trends in genetic diversity over the study period. We tested for significant deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for individuals in the pedigree data set using the web version of GENEPOP version 3.4 (Raymond & Rousset 1995) with an adjusted *P* value corresponding to alpha = 0.05 after Bonferroni correction (Rice 1989). We tested for the presence of null alleles using MICROCHECKER (Van Oosterhout *et al*. 2004). Inbreeding coefficients were calculated as population-based estimates with FSTAT version 2.9.3.2 (Goudet *et al*. 2002). Data were assessed for normality using quantile–quantile plots. Breeding pair relatedness was assessed with KINSHIP (Goodnight & Queller 1999).

To compare genetic diversity in the reintroduced population to that from alternative breeding strategies, we created breeding pools consisting of individuals with pedigreed ancestry for three scenarios: (i) open gene pool; (ii) restricted gene pool; and (iii) managed gene pool. The open gene pool scenario placed all reproductively mature individuals (> 2 years) in an annual breeding population without regard to kinship or pack membership. Under the restricted gene pool scenario, females were restricted to breeding with males from the same pack. This scenario presumed no inbreeding avoidance and reflected only a preference for mates in close proximity. Such a scenario may approximate conditions of low mate availability due to high pack isolation (such as after a long distance colonization event) or low pack density (Wayne *et al*. 1991; Schröder & Promberger 1993; Ellegren *et al*. 1996; Liberg *et al*. 2005). The managed gene pool was created following rules commonly used in captive breeding programmes: minimizing mean kinship, maximizing gene diversity, increasing population size and eliminating unknown pedigree lineages (Ballou *et al*. 2001; Frankham *et al*. 2002). Using PM2000 (Pollak *et al*. 2002) and MATERX (Ballou *et al*. 2001; Ralls & Ballou 2004), we identified ideal breeding pairs that would maximize these breeding goals according to the joint measure of the Mate suitability index (MSI). The MSI provides a value for each male–female pair based on how well the pairing maximizes genetic diversity while minimizing inbreeding, unknown ancestry and mean kinship. Pairs were ranked on a scale of 1 (beneficial mate pair) to 6 (detrimental mate pair). Using default settings in $\text{MATER}_x$, we identified the same number of ideal mate pairs as were actually observed annually.

We used these breeding pools in a simulation to estimate heterozygosity of adults and offspring in 2004 with the simulation model WOLFY version 0.1 developed for this study and available online (http://taylor0.biology.ucla.edu/~daearl/software/wolf/). We focused on the year 2004 after the population reached carrying capacity and 24 male–female breeding pairs were observed. We chose 24 breeding pairs from each of the three breeding pools discussed above (restricted, open and managed) and allowed them to produce the average number of offspring observed in that year ($N = 6$) based on Mendelian inheritance of 26 loci (see Results). We then calculated the average individual heterozygosity of the parents and offspring (the new population). For the restricted scenario, each pack had at least one breeding pair. For each scenario, we simulated 24 breeding pairs for 1 million iterations by resampling males with replacement and females without replacement (assuming no multiple paternity of litters but allowing for males to fertilize multiple females). The resulting heterozygosity histogram is displayed as the frequency of individuals within each of 800 heterozygosity bins. The simulation was not intended to incorporate all possible complexities of wolf pack breeding structure; rather, this simulation was used to assess the effect on heterozygosity of random and restricted breeding scenarios without regard to kinship and managed breeding with regard to kinship across the entire breeding pool.

We determined the opportunities for inbreeding within a pack for the restricted breeding strategy data set. For each pack, we divided the number of possible male–female adult pairs (= 2 years old) between close relatives ($r > 0.25$) by the number of all possible adult pairings. We averaged these proportional values across packs for each year as a measure of possible breeding opportunities within packs that would constitute inbreeding.

### *Effective population size estimates and generation time estimate*

We estimated annual effective population sizes ($N_e$) based only on those individuals genotyped and included in the pedigree data set ($N = 200$) with the population management software PM2000 (Pollak *et al*. 2002). This method excludes individuals whose parentage assignments have not been resolved. We estimated the mean generation time using the population viability analysis (PVA) simulation program VORTEX (Lacy *et al*. 2005; Miller & Lacy 2005) and the observational life history and breeding demographic data for the 2004 population (Table S1, Supplementary material).

### *Parentage and pedigree reconstruction*

We calculated the probability that two siblings would have identical genotypes by chance (PIDsib, as in Evett & Weir 1998; Waits *et al*. 2001) using the program GIMLET version 1.3.1 (Valière 2002). PIDsib is a conservative estimate of the power to resolve individuals given population substructure or when comparisons are made between related individuals (Waits *et al*. 2001). The population genealogy was determined by sibship and parentage analysis of 200 grey wolves. Sibship was inferred using COLONY version 1.3 to identify groups of full and half-sib offspring utilizing a maximum- likelihood approach for relationship estimation (Wang 2004). The groups that are produced include all individuals that share approximately 50% of their genes. From these groups, we identified putative full- and half-sib dyads as those sharing two and one parent, respectively. Dyads were then grouped to construct putative litters to reduce analytical complexity. This narrowed the pool of candidate parents for additional pups in parentage testing that had no parentage information. Parentage analyses and assignments for parent–offspring dyads were completed under a likelihood approach employed in CERVUS version 3.0 (Marshall *et al*. 1998). Parentage assignments were determined initially through exclusion with field and genetic data where possible followed by use of log likelihood (LOD) scores for candidate parents given the offspring genotypes and allele frequencies in the population. Simulations were performed to determine the likelihood of random individuals as parents and the ratio between this value and that for candidate individuals is expressed as the delta value. We considered delta values that were significant at 95% and 80% levels. To generate delta values, we simulated 10 000 offspring and 50 candidate males allowing for 20% of the population to be unsampled and 20% incomplete multilocus genotypes. CERVUS was also used to calculate the polymorphic information content (PIC) and the probability that a single-locus genotype is identical between two randomly chosen individuals.

We used two general approaches for resolving parentage: open and restricted. Open paternity/maternity analyses were utilized for individuals having unresolved sibship groups or had no a priori assessments of parentage based on field observations and involved testing for parentage using all reproductively mature males/females (> 2 years). Candidate parent pools were not based on geographical proximity to potential offspring as extra-pack copulations have been observed (Yellowstone Wolf Project, NPS, unpublished data). Restricted analyses used pools of candidate parents identified by sibship analysis or field observations to reduce the pool of potential parents and increase the certainty of parentage assignments that might be obscured by the presence of close relatives. Individuals were placed into pack pedigrees based on assigned parentage and year of birth as determined by the age estimate of the individual at the time of sampling. The genealogies reported here include sampled individuals only and therefore do not completely reflect actual annual pack compositions and pup production. The genealogy was prepared with PEDIGREE VIEWER (Kinghorn 1994).

### *Breeding pair and interpack relatedness*

Relatedness was assessed by likelihood simulations and significance testing with KINSHIP (Goodnight & Queller 1999). To corroborate inbreeding events identified by field and pedigree-based relationships, we evaluated relatedness among breeding pairs with likelihood simulations executed in KINSHIP, testing the hypothesis that breeding pairs are related at $r = 0.25$ or greater. We assessed interpack kinship ties for 2002 by use of the inferred genealogy at two relatedness levels: (i) $r = 0.5$, indicating first order relationships of parent–offspring or full-sibling; and (ii) $r = 0.25$, indicating second-order relationships such as half-sibling, aunt/uncle–nephew/niece, or grandparent–grand offspring. We choose 2002 because this year is well sampled and is representative of years when the population reached carrying capacity.

### *Long-term genetic trajectory predictions*

The future genetic trajectory of the YNP wolves was investigated using the population viability analysis (PVA) simulation program VORTEX (Lacy *et al.* 2005; Miller & Lacy 2005). The demographic characteristics during 2004 and a carrying capacity of 170 individuals (see Results) were used as input parameters (Table S1). With respect to breeding system parameters, we found no significant difference between running short- and long-term monogamy in the model, and polygamy was not appropriate. We used long-term monogamy in the model because as a first order approximation, this adequately characterizes wolf-mating structure (Mech & Boitani 2003a). We assumed no immigration and evaluated 0, 5 and 10% emigration each year to represent observed one-way emigration of individuals from the park into the Greater Yellowstone Area. We simulated 100 years of population dynamics using 1000 iterations to estimate change in observed and expected genetic heterozygosity and in the inbreeding coefficient. We also investigated the population size required (assuming no immigration) to maintain heterozygosity at 95% of its current level over the next 100 years for a population with the observed demographic characteristics by increasing the VORTEX model population carrying capacity. Finally, we investigated the amount of immigration needed to prevent decreases in heterozygosity by adding wolves to the population each year using the supplementation scenario option in VORTEX, which assumes added individuals are unrelated to the current population.

## Results

### *Observed demographic history of reintroduction*

The Yellowstone population expanded rapidly after the reintroductions of 1995 and 1996. In total, 41 wild-born wolves were reintroduced from 1995 through 1997; 31 founders were from Canada and 10 from northwest Montana. The Montana individuals were derived from an independent Canadian stock and only three of these pups lived past 1 year with just two reproducing in the wild. Population growth was initially very high through the reintroduction phase (40–50% per year, 1995–1997) and continued to increase through 2003 (10–15% per year, 1999–2003) reaching a maximum of 174 wolves before declining to 169 wolves in 2004 (Smith 2005). Pack formation and territory establishment followed a similar pattern, increasing from three packs in 1995 and stabilizing at 16 packs in 2003 and 2004 (Fig. 1). Life table analysis calculated from age-specific birth and death rates of the Yellowstone population as of 2004 (Table S1) were used in VORTEX to estimate a mean generation time of 4.16 years (Miller & Lacy 2005). Since 2004, population size and the number of packs have stabilized, indicating that carrying capacity in Yellowstone has been reached (Yellowstone Wolf Project, NPS, unpublished data). Increasing interpack conflict and intraspecific mortality have been associated with higher wolf densities, suggesting that carrying capacity has been socially mediated (Yellowstone Wolf Project, NPS, unpublished data). Emigration of wolves from YNP was common throughout the study period, and was responsible for the establishment of packs in the GYA outside of the park (USFWS *et al.* 2005). Immigration of wolves to YNP from outside the recovery area was not observed (Yellowstone Wolf Project, NPS, unpublished data) or revealed from genetic studies of wolves within or outside the park (vonHoldt unpublished data; see below).

### *General trends in genetic diversity*

Deviations from Hardy–Weinberg expectations occurred in less than 10% of the original 30 loci on average after a Bonferroni correction for multiple testing (see Table S2, Supplementary material). However, when inheritance patterns were examined with known field genealogies, four loci (Pez6, Pez11, 3313 and 4027) consistently exhibited non-Mendelian patterns of inheritance and were dropped from all subsequent analyses. The remaining 26 loci were polymorphic in each year, ranging from three to 18 alleles per locus with an allelic richness ($A_R$) of 6.3–9.1 alleles per locus for the pedigree population ($N = 200$; Table 1 and Table S3, Supplementary material). Tests were insignificant for the presence of null alleles for all 26 loci (using Bonferroni correction for multiple tests). Eighteen of 325

**Table 1** Population census size ($N_C$), total number of Yellowstone packs ($N_{Packs}$), number of individual genotyped ($N_g$), observed/expected heterozygosity ($H_O$ and $H_E$, respectively), allelic diversity ($A_R$), inbreeding coefficient ($F_{IS}$), pedigree-based effective population size estimates ($N_e$), and within-pack inbreeding opportunities for individuals in the reconstructed pedigree of Yellowstone National Park (1995–2004; $N$ = 200; 26 microsatellite loci)

| | 1995 | 1996‡ | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 |
|---|---|---|---|---|---|---|---|---|---|---|
| Population $N_C$* | 21 | 37 | 80 | 83 | 72 | 119 | 132 | 148 | 174 | 169 |
| $N_{Packs}$ | 3 | 8 | 8 | 8 | 7 | 9 | 10 | 14 | 14 | 16 |
| $N_g$† | 21 | 45 | 69 | 62 | 52 | 67 | 65 | 65 | 61 | 66 |
| Mean $H_O$ | 0.694 | 0.698 | 0.702 | 0.697 | 0.698 | 0.714 | 0.723 | 0.727 | 0.721 | 0.725 |
| Mean $H_E$ | 0.717 | 0.754 | 0.760 | 0.750 | 0.740 | 0.744 | 0.740 | 0.735 | 0.733 | 0.737 |
| $A_R$ | 6.3 | 8.8 | 9.1 | 9.0 | 8.6 | 9.0 | 8.7 | 8.7 | 8.5 | 8.6 |
| $F_{IS}$ | –0.063 | –0.016 | –0.012 | –0.026 | –0.044 | –0.043 | –0.052 | –0.054 | –0.050 | –0.051 |
| $N_e$ | 6 | 13.7 | 22.6 | 16.9 | 16.3 | 17.3 | 16.3 | 14.4 | 17.1 | 22.1 |
| $N_e/N_g$ | 0.29 | 0.30 | 0.33 | 0.27 | 0.31 | 0.26 | 0.25 | 0.22 | 0.28 | 0.33 |
| No. of total within pack possible male–female pairs | 8 | 14 | 20 | 20 | 37 | 38 | 39 | 60 | 56 | 100 |
| Within-pack inbreeding opportunities | 0.00 | 0.14 | 0.10 | 0.10 | 0.24 | 0.24 | 0.13 | 0.35 | 0.55 | 0.59 |

*Annual census size as of 31 December.

†Individuals in the pedigree data set having at least 70% of the 26 loci genotyped.

‡Includes individuals that died before annual census count.

pairwise comparisons showed significant linkage disequilibrium (LD) following Bonferroni correction ($P < 0.05$) but none of the 18 pairings were loci located on the same chromosome, suggesting that LD is probably due to population structure rather than physical linkage. Thus, we included all remaining 26 loci in our analyses.

With an increase in allelic diversity when the second founder population was introduced in 1996, genetic diversity remained high and inbreeding was near zero for the entire study period. Observed heterozygosity was maintained in the pedigree population at high levels ($H_{1995} = 0.694$; $H_{2004} = 0.725$) whereas inbreeding ($F_{IS}$) has only increased slightly over time (1995 $F_{IS} = –0.063$; 2004 $F_{IS} = –0.051$), results that are consistent with the rapid population expansion ($N_{1995} = 21$; $N_{2004} = 169$; Table 1 and Fig. 1). The marked increase in the inbreeding coefficient observed in 1996 and 1997 was associated with the addition of a litter from a full-sib inbreeding event of wolves housed in the same pen (1996 $F_{IS} = –0.016$; 1997 $F_{IS} = –0.012$, see below).

Mean levels of heterozygosity for the observed breeding pool over the 10-year study were not significantly different from those chosen in the managed breeding scenario (see Methods; $H_{Observed} = 0.750$, SE = 0.013; $H_{Managed} = 0.761$, SE = 0.017; Fig. 2A). Relatedness of the observed breeders is higher for the first 4 years (1995–1998) than the managed scenario, and significantly higher for two of these years (Fig. 2B). Thereafter, values are similar except for the last 2 years when relatedness is significantly higher for the observed population (2003; $r_{Observed} = 0.017$, SE = 0.002; $r_{Managed} = –0.009$, SE = 0.007; 2004; $r_{Observed} = 0.010$, SE = 0.002; $r_{Managed} = –0.009$, SE = 0.006; Fig. 2B). Mean inbreeding coefficients ($F_{IS}$) of breeders decreased over time for both scenarios but were more often lower in the managed population ($F_{Observed} = 0.012$, SE = 0.023; $F_{Managed} = –0.011$, SE = 0.022; Fig. 2C).

Finally, we simulated breeding in 2004 to compare heterozygosities of open, restricted and managed breeding scenarios (Fig. 3). The mean heterozygosity of the observed population ($H_{Observed} = 0.750$, SD = 0.013) was higher than the restricted ($H_{Restricted} = 0.690$, SD = 0.019) and open breeding strategies ($H_{Open} = 0.728$ SD = 0.026) and not significantly different from the managed scenario ($H_{Managed} = 0.764$, SD = 0.008). High heterozygosity in the observed population can be attributed to the active choice of wolves to breed with unrelated individuals within or outside of their natal pack (see below).

### *Sibship groups and parentage assignments*

From sibship analysis, we identified 52 sibship groups consisting of multiple individuals from 14 packs. From these groupings we resolved 31 full-sib dyad relationships and 19 half-sib dyads based on sharing of one parent, with only two dyads being unresolved. All genetically deduced sib groupings were consistent with field data. The 26 microsatellites in our data set had a very low overall probability of identity among siblings (PIDsib ranged from $9.33 \times 10^{-13}$ in 1996 to $2.91 \times 10^{-12}$ in 2004). This result implies that full-siblings sharing the same genotype by chance were highly unlikely in our population.

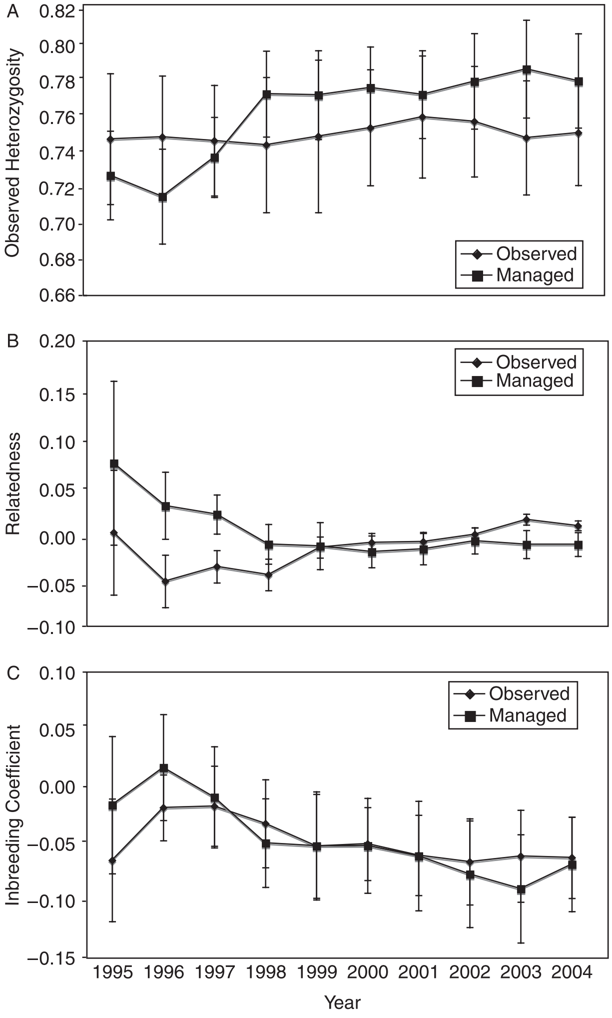


**Fig. 2** Annual heterozygosity (A), relatedness (B) and inbreeding coefficient (C) for the observed breeding population as compared to breeders selected by MATER$_x$ under a managed breeding strategy. Error bars represent 1.96 standard deviations from the mean and significance is defined by mean values being separated by more than two standard errors.

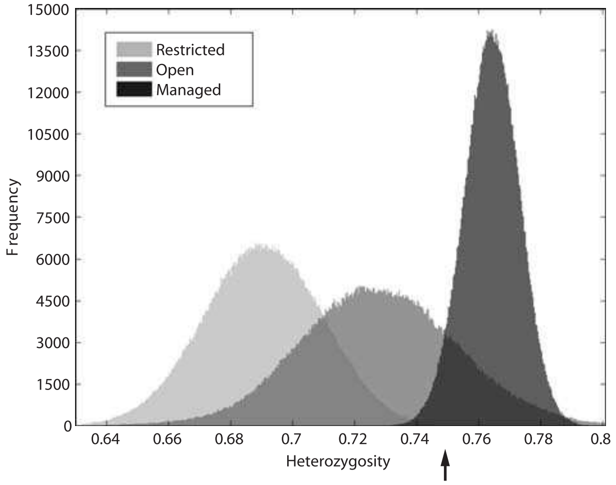


**Fig. 3** Histograms of the average heterozygosity for simulated populations of parent and offspring (for 1 million iterations) for open, restricted, and managed breeding scenarios compared with observed breeding strategy (arrow) in 2004.

Initial a priori field-based (nongenetic) parentage data resolved 12 two-parent, eight paternity and 22 maternity assignments. Genetic analyses resolved parentage for 200 individuals, 183 assignments (91.5%) at the 95% confidence level and 17 (8.5%) at the 80% confidence level. We found no multiple-paternity within a litter. The polymorphic information content was high (PIC = 0.733) and probabilities of nonexclusion were on the order of $10^{-12}$ for the set of 26 loci. Of the 200 individuals, genetic parentage analyses resolved 126 (77.3%) offspring with two-parent assignments and 37 (22.7%) offspring having single-parent assignments (28 paternity and nine maternity assignments). Five two-parent assignments were resolved by sibship reconstruction. There were unresolved parentage assignments for seven paternities with known maternity (two due to unsampled males) and 15 maternities with known paternity (seven due to unsampled females). The 10 Sawtooth pups were assumed to be full-sibs and were included in all parentage analyses.

## *Relatedness of breeding pairs*

We determined the relatedness of 31 breeding pairs using field and genetic confirmation of parentage, and found 28 (90%) were unrelated. However, only two of the three related mating events represent a natural event (see below); hence, the rate of naturally occurring unrelated matings is 93%. The mean (± SE) pairwise relatedness values between breeding pairs was –0.026 ± 0.03 (range –0.313–0.515, $N = 31$) and their mean (± SE) inbreeding coefficient was $F = -0.005 \pm 0.007$ (range –0.087–0.084, $N = 31$; Table 2). Breeding pairs had high mean levels of heterozygosity ($H_{\text{parents}} = 0.787 \pm 0.015$) that differed from their offspring ($H_{\text{pups}} = 0.741 \pm 0.014$, $N = 151$; pairwise $t$-test: $t = 2.12$, d.f. = 30, $P = 0.043$; Table 2). Further, we partitioned the data set into known breeding ($N = 65$) and nonbreeding ($N = 135$) individuals and found no significant differences between groups in heterozygosity ($H_{\text{breeding}} = 0.749$, SE = 0.010; $H_{\text{nonbreeding}} = 0.727$, SE = 0.010, $t = 1.543$, d.f. = 164, $P = 0.125$). Consequently, this latter result does not support a bias toward matings of individuals with higher heterozygosity as found in inbred wolf populations (Bensch *et al*. 2006).

The mechanism of formation for 34 breeding pairs was documented (Table 3). Five breeding pairs were established prior to their release, with 29 other pairs forming in

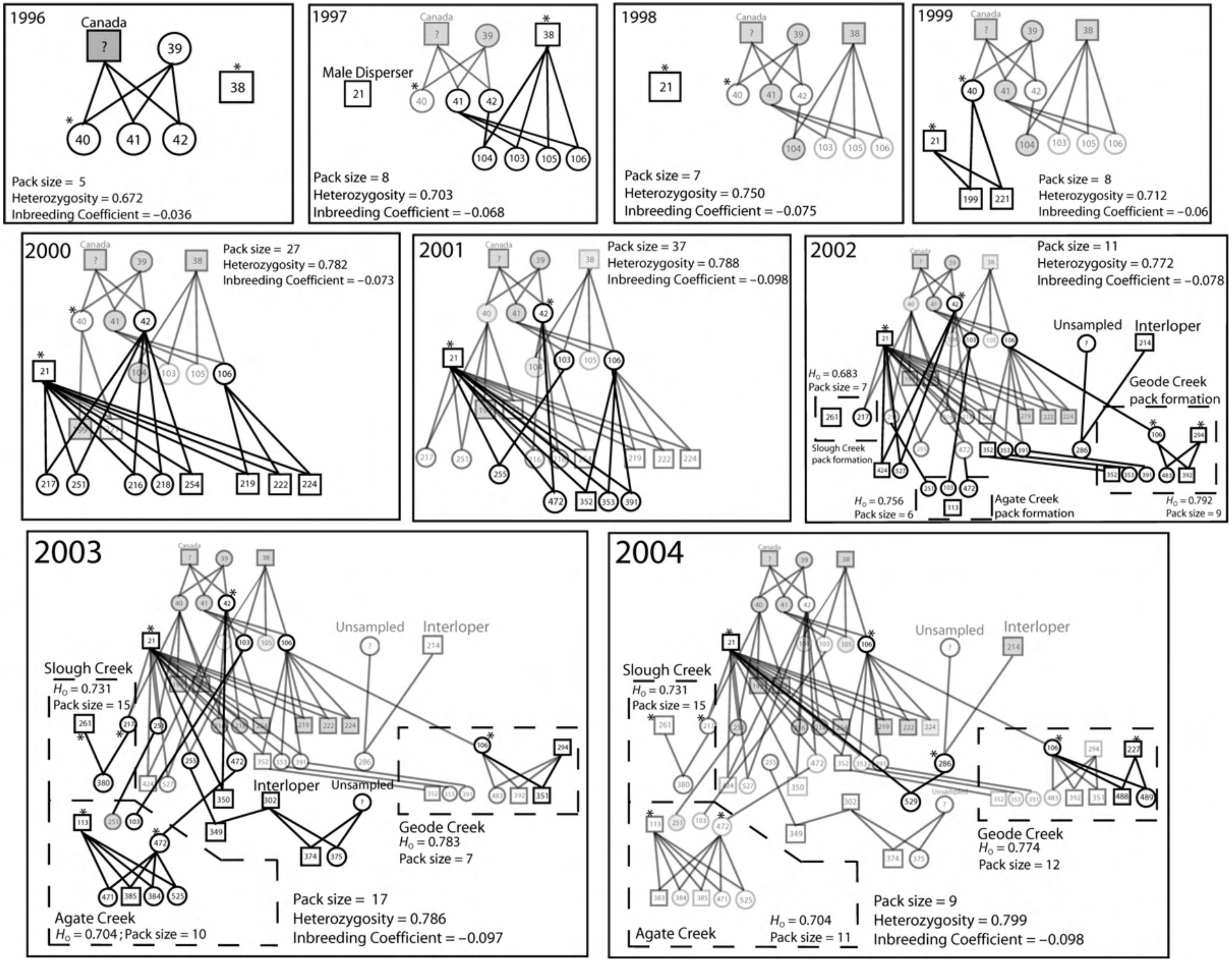


**Fig. 4** Druid Peak pack genealogy of genotyped individuals. Circles represent females and squares represent males. Pack size reflects end-of-year count. Asterisk indicates dominant individual; shaded symbols represent death, dispersal or presence unknown. ($H_O$, observed heterozygosity).

**Table 2** Parental and offspring observed heterozygosity ($H_O$), number of genetically verified offspring ($N_{offspring}$) and the pedigree-based inbreeding coefficient (F) of the breeding pair ($N = 31$)

| Sire | Dam | Pack | Mean parental $H_O$ | Relatedness | $N_{offspring}$ | Mean offspring $H_O$ | $F_{BreedPair}$ |
|---|---|---|---|---|---|---|---|
| 2M | 7F | Leopold | 0.720 | –0.167 | 25 | 0.727 | 0.010 |
| 4M | 5F | Mollie's | 0.654 | –0.033 | 3 | 0.780 | –0.028 |
| 6M | 5F | Mollie's | 0.654 | 0.227* | 4 | 0.660 | 0.022 |
| 8M | 19F | Rose Creek | 0.965 | –0.145 | 1 | 0.769 | 0.019 |
| 10M | 9F | Rose Creek | 0.850 | –0.065 | 6 | 0.766 | –0.027 |
| 13M | 14F | Yellowstone Delta | 0.876 | –0.110 | 6 | 0.798 | –0.044 |
| 21M | 286F | Druid Peak | 0.825 | 0.172† | 2 | 0.563 | 0.044 |
| 21M | 40F | Druid Peak | 0.825 | –0.213 | 4 | 0.682 | –0.014 |
| 21M | 42F | Druid Peak | 0.820 | –0.037 | 7 | 0.720 | –0.010 |
| 2M | 106F | Druid Peak | 0.902 | –0.188 | 6 | 0.776 | 0.043 |
| 28M | 27F | Nez Perce | 0.750 | –0.135 | 6 | 0.813 | –0.057 |
| 29M | 37F | Nez Perce | 0.704 | 0.515‡ | 1 | 0.550 | 0.084 |
| 34M | 16F | Chief Joseph | 0.908 | 0.008 | 2 | 0.635 | –0.016 |
| 34M | 33F | Chief Joseph | 0.734 | –0.154 | 6 | 0.852 | –0.087 |
| 34M | 17F | Chief Joseph | 0.784 | –0.046 | 2 | 0.708 | –0.005 |
| 35M | 30F | Thorofare | 0.844 | –0.186 | 2 | 0.827 | –0.043 |
| 38M | 41F | Druid Peak | 0.692 | 0.073 | 3 | 0.694 | –0.049 |
| 38M | 42F | Druid Peak | 0.706 | 0.039 | 1 | 0.885 | –0.063 |
| 70M | 48F | Nez Perce | 0.673 | –0.313 | 4 | 0.828 | –0.033 |
| 72M | 48F | Nez Perce | 0.685 | 0.082 | 11 | 0.784 | –0.005 |
| 120M | 14F | Yellowstone Delta | 0.759 | –0.049 | 4 | 0.672 | –0.020 |
| 165M | 16F | Sheep Mountain | 0.965 | –0.008 | 4 | 0.691 | 0.015 |
| 205M | 152F | Swan Lake | 0.778 | –0.034 | 1 | 0.750 | –0.005 |
| 206M | 152F | Swan Lake | 0.686 | 0.130 | 4 | 0.748 | –0.025 |
| 227M | 106F | Geode Creek | 0.782 | –0.005 | 2 | 0.839 | 0.029 |
| 294M | 106F | Geode Creek | 0.817 | –0.097 | 2 | 0.763 | 0.015 |
| 301M | 251F | Agate Creek | 0.813 | –0.005 | 1 | 0.808 | 0.004 |
| 302M | 255F | Druid Peak | 0.824 | –0.039 | 1 | 0.708 | 0.035 |
| 303M | 151F | Cougar Creek | 0.761 | 0.093 | 7 | 0.680 | 0.029 |
| 487M | 126F | Yellowstone Delta | 0.812 | –0.099 | 2 | 0.776 | 0.000 |
| 534M | 209F | Leopold | 0.827 | –0.005 | 1 | 0.731 | 0.039 |
| Average | | | 0.787 | –0.026 | 4.2 | 0.741 | –0.005 |

*Unknown ancestry and probably an aunt–nephew mating ($r = 0.25$; $P < 0.05$).
†Unknown ancestry ($r = 0.25$; $P < 0.05$).
‡Full-sib mating in acclimation pen prior to release ($r = 0.25$; $P < 0.001$).

the wild in YNP. Two of these naturally forming pairs (7%) were lone individuals that joined in 1996 (2M and 7M of Leopold, Fig 4; 35M and 30F of Thorofare pack), whereas the remaining 27 pairs (93%) formed in the context of a group. On six occasions, the vacancy created by the death of a dominant male breeder was filled by migration into the pack of an unrelated male, while no such events were documented for females. These events explained six pairs that formed as the result of the dominant female breeding the new male, as well as five subordinate females breeding with the new male. In total, we documented nine cases of subordinate individuals breeding, all of which were females related to the dominant female breeder as siblings, daughters or nieces. The Druid Peak pack exemplified a highly complex, multiple-breeding pack structure in which heterozygosity was maintained by these mechanisms (Fig. 4). For example, a male immigrant (21M) filled a vacant breeding position in 1997 after the death of the dominant male (38M), subsequently breeding unrelated females for multiple years until the pack split in 2001. There was an increase in heterozygosity through time associated with these years of multiple breeding ($H_{1997} = 0.743$, $H_{2001} = 0.778$; Fig. 4).

We genetically confirmed three extra-pack copulations when subordinate females formed temporary liaisons with interloping males during the breeding season, all in the Druid Peak pack. The first case was in 2002 when we confirmed parentage of at least one offspring by a dispersing Nez Perce male (214M) who paired temporarily with an unmarked subordinate female but did not join the

**Table 3** Observed mechanisms for first-time breeding pair formation in Yellowstone National Park for 32 pairings (1995–2004)*

| Category | Male | Female | Total |
|---|---|---|---|
| Reintroduced pair | | | 4 |
| Two dispersing individuals join | | | 2 |
| Multiple individuals join and at least two breed | | | 5 |
| Within-pack inheritance/succession of dominant breeder position | 2 | 2 | 4 |
| Immigrant usurps an active breeder | 1 | | 1 |
| Immigrant assumes vacant dominant breeder position | 6 | | 6 |
| Dominant breeds new immigrant | | 6 | 6 |
| Dominant breeds subordinate | 7 | | 7 |
| Subordinate breeder in natal pack | | 9 | 9 |
| Interloper breeds subordinate but does not join pack | 3 | | 3 |

*Includes only genotyped pairs where category was certain (not including inbreeding events).

pack (Fig. 4). A female offspring (286F) from this pairing then bred in the Druid Peak pack in 2004 with the alpha male, resulting in one of the two naturally occurring inbreeding events (see below; Table 2; Fig. 4). In 2003, we confirmed paternity by a dispersing male (302M) from the Leopold pack who fathered at least three pups with two different subordinate females in the Druid Peak pack before joining the pack in 2004 (Fig. 4).

Five breeding pairs formed in the context of a group outside of an established pack and involved an individual disperser joined by an opposite-sex group of wolves. This was the primary mechanism for forming new packs in Yellowstone after 1996 (see below). Four pairs formed as an individual inherited the dominant breeding position in their natal pack, and in each of these cases, the dominant, opposite-sex breeder was not their relative. We documented one event of a male disperser usurping the breeding position from a long-term dominant male. This immigrant male (534M) from the Nez Perce pack forced the long-time dominant male breeder (2M) to leave the pack (Fig. 5). The new male's subsequent breeding was associated with an increase in pack heterozygosity ($H_{2001} = 0.729$; $H_{2004} = 0.743$). These results demonstrate remarkable flexibility in the means by which pairs form and reveal a greater diversity of mechanisms within a single population than previously documented in other studies (e.g. Rothman & Mech 1979; Hayes *et al*. 1991; Mech & Boitani 2003a). However, despite this variability, all naturally observed mechanisms avoided breeding between highly related individuals (see below) and were often associated with increased heterozygosity in packs.

### *Inbreeding*

Over the 10-year study, we documented only three breeding pairs that were significantly related ($r = 0.515$, $P < 0.001$; $r = 0.227$, $P < 0.05$; $r = 0.172$, $P < 0.05$). One was a probable aunt–nephew mating in the Crystal Creek pack, a second was between a probable grandfather–granddaughter mating in the Druid Peak pack, and the third was a full-sib mating in Nez Perce pack (Table 2). The probable aunt–nephew inbred pair was significantly related but the exact relationships were not resolved due to unknown ancestry. However, this pairing occurred under extenuating circumstances as the female's unrelated mate died prior to the breeding season, leaving her nephew as the only remaining wolf in the pack during the breeding season in 1997, a year of low mate availability. The probable grandfather–granddaughter pair was significantly related, and field observations indicate that the shared relative was the breeding female's unsampled mother who was presumably a daughter of the breeding male (Table 2; Fig. 4). This pairing occurred immediately following the death of the breeding male's former long-term mate at the peak of the breeding season, leaving only related females as possible mates. The third mating reflected human interference as two full-sibs from the Nez Perce pack were penned in 1997 to act as surrogate parents for 10 orphaned pups from the Sawtooth pack of northwest Montana (Fig. 6). This resulted in the only full-sib breeding event in Yellowstone National Park in the 10-year period. The heterozygosity of the Nez Perce pack was initially high on reintroduction ($H_{1996} = 0.802$), then reduced as a consequence of this full-sib mating event the next year ($H_{1997} = 0.753$), but remained stable with the inclusion of the unrelated Sawtooth wolves ($H_{1997} = 0.790$; Fig. 6). Only two of these Sawtooth individuals (70M and 72M) acquired breeding status in the wild, both with the tenured Nez Perce dominant female (48F) that maintained heterozygosity over subsequent years ($H_{2000} = 0.786$ to $H_{2004} = 0.813$).

In 1997, the proportion of possible breeding opportunities within packs that would constitute inbreeding was 10%, increasing over subsequent years as the population expanded and peaking in 2004 at 59% as relatives accumulated within packs (Table 1). Consequently, Yellowstone wolves actively avoided breeding with close relatives, as no naturally occurring inbreeding events were documented between individuals with $r > 0.25$ despite an increasing opportunity to do so.

### *New pack formation*

The formation of new packs always involved the establishment of breeding pairs, as they are the fundamental unit of wolf social structure (Murie 1944; Mech 1970). Seven packs were established during the reintroduction (Chief

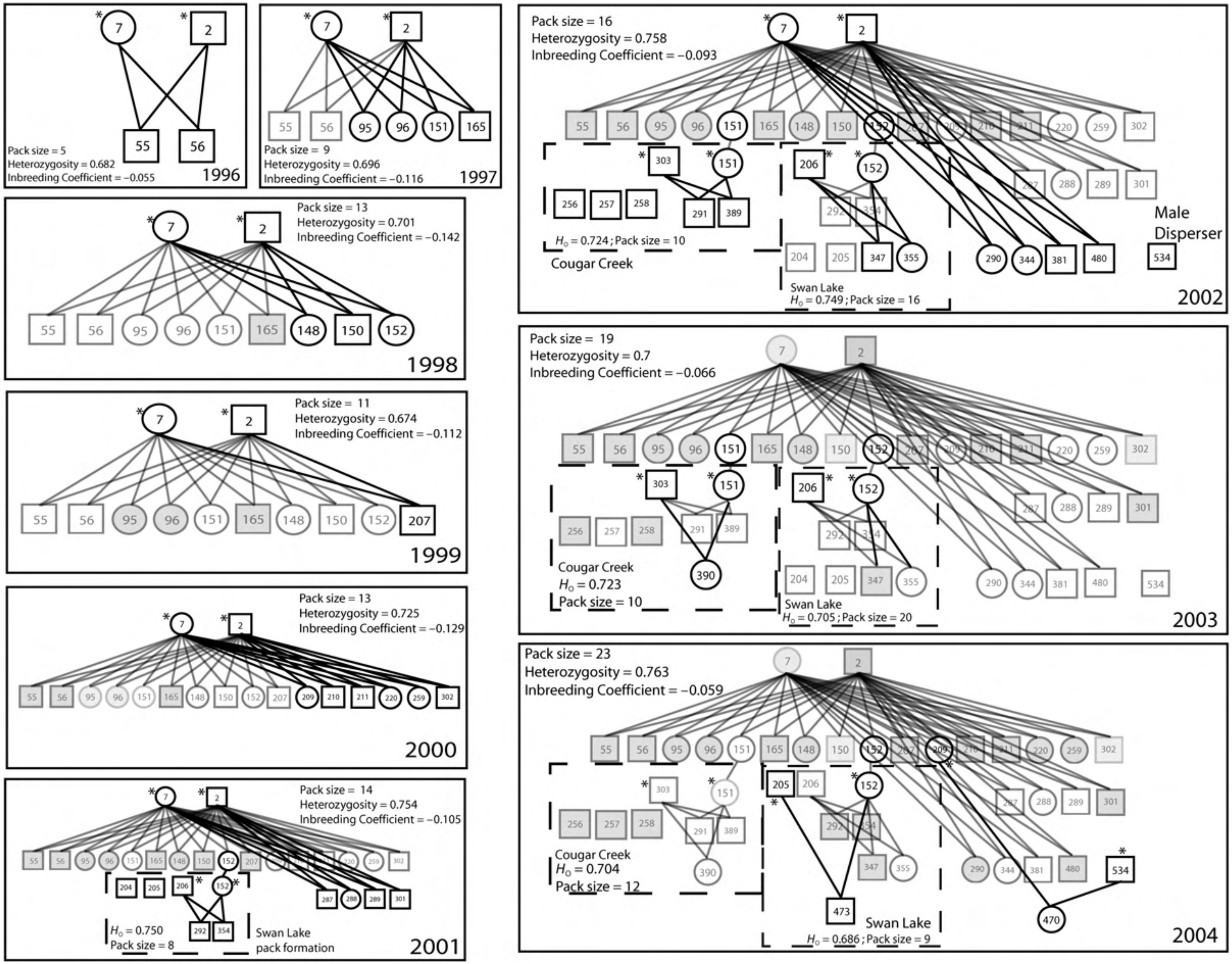


**Fig. 5** Leopold pack genealogy of genotyped individuals. Circles represent females and squares represent males. Pack size reflects end-of-year count. Asterisk indicates dominant individual; shaded symbols represent death, dispersal or presence unknown. ($H_O$, observed heterozygosity).

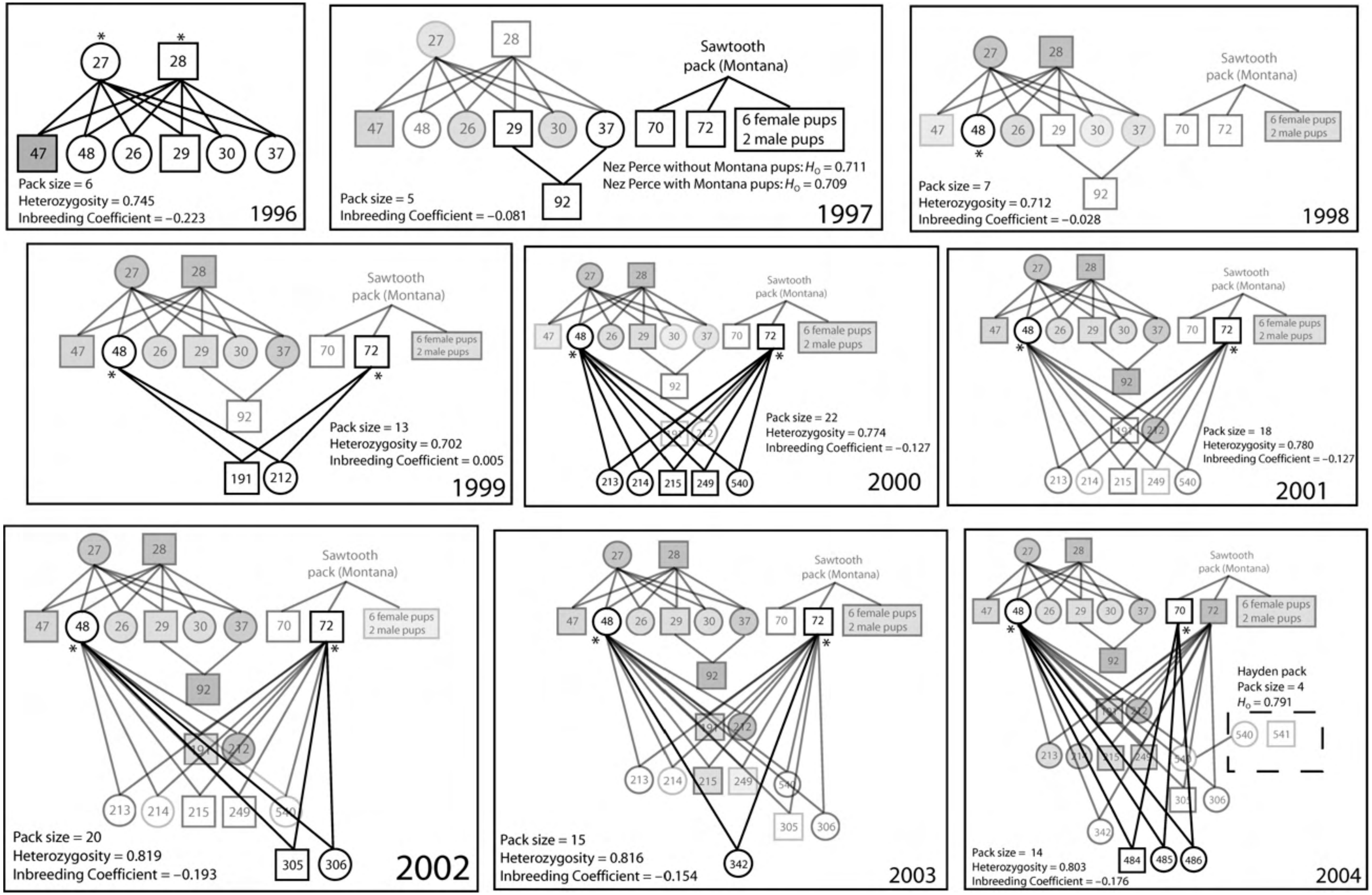


**Fig. 6** Nez Perce pack genealogy of genotyped individuals. Circles represent females and squares represent males. Pack size reflects end-of-year count. Asterisk indicates dominant individual; shaded symbols represent death, dispersal or presence unknown. ($H_O$, observed heterozygosity).

Joseph, Crystal Creek, Druid Peak, Lone Star, Nez Perce, Rose Creek, and Soda Butte) with four of them maintaining core group membership throughout all 10 years of the study (Fig. 1). Of the 15 naturally formed packs in subsequent years, only four (27%) of them resulted from the pairing of singletons (Bechler, Hayden, Leopold, and Thorofare), with two of these pairs genetically confirmed as being unrelated (Leopold and Thorofare; Fig. 1). Eleven (73%) new packs formed due to pack splitting (Agate Creek, Biscuit Basin, Buffalo Fork, Cougar Creek, Geode Creek, Gibbon Meadows, Sheep Mountain, Slough Creek, Specimen Creek, Swan Lake, and Tower). Pack splitting is defined here as a group of wolves leaving the pack together and joining with other dispersing individuals who then establish a new territory (Mech & Boitani 2003a). In five of these packs formed by splitting (Agate Creek, Cougar Creek, Geode Creek, Sheep Mountain, and Swan Lake), we genetically confirmed same-sex siblings or parent–offspring groups joining with unrelated individuals. Despite the fact that close relatives were involved in the formation of new packs, the breeders always mated with unrelated individuals preventing inbreeding.

The history of the Druid Peak pack exemplifies these trends. This pack rapidly expanded between 1997 and 2001, largely due to several years of multiple litters and high pup survivorship (Fig. 4). As a result, four new packs (Agate Creek, Buffalo Fork, Geode Creek, and Slough Creek) formed as groups of Druid wolves (comprised largely of female relatives) left and joined with unrelated individual males or groups of male relatives. Documented heterozygosities for three of these packs were high upon formation, as breeding pairs were comprised of unrelated individuals ($H_{\text{Agate}} = 0.734$, $H_{\text{Slough}} = 0.673$, $H_{\text{Geode}} = 0.787$). In contrast to Druid Peak pack's complexity, the Leopold pack conformed to the traditional structure of a monogamous breeding pair and their offspring (Fig. 5). Solitary female dispersers from Leopold, however, led to two new pack formations (Swan Lake and Cougar Creek) as groups of non-Leopold brothers splitting from their natal packs joined the females. These newly formed packs had high genetic variation ($H_{\text{Swan}} = 0.757$, $H_{\text{Cougar}} = 0.691$) and maintained relatively stable levels of heterozygosity throughout their tenure associated with the breeding of unrelated individuals as documented in 2004 ($H_{\text{Swan}} = 0.759$, $H_{\text{Cougar}} = 0.667$). These results are in contrast to past observations that most wolf packs form by two unrelated individuals joining (e.g. Rothman & Mech 1979; Mech & Boitani 2003a).

### *Interpack relatedness and breeder dispersal*

Based on the genealogical relationships in 2002, we identified 162 (55%) kinships ties of 296 possible interpack relatedness comparisons ($r = 0.5$ and $r = 0.25$). We documented a total of 90 (56%) kinship ties having $r = 0.5$ between individuals of different packs and 72 (44%) kinship ties with $r = 0.25$ in YNP in 2002 (Fig. 7). In that year, we found no ties between any of the packs with the Yellowstone Delta pack. The majority (94%) of the interpack kinship ties were between adjacent packs and only four ties with $r = 0.5$ and five ties with $r = 0.25$ joined two individuals of nonadjacent packs. For example, the Bechler pack was founded by a male that was a sibling and offspring of individuals born in the geographically nonproximate Rose Creek pack (Fig. 7). Kinship ties that spanned beyond adjacent territories were primarily the result of dispersing males becoming breeders in new or already established packs. In contrast, kinship ties between adjacent packs largely reflected female dispersal or female kin groups splitting from natal packs and establishing adjacent territories. Of the 90 kinship ties with $r = 0.5$, 18 (20%) were parent–offspring relationships and 72 ties (80%) were full-sibling relationships, both reflecting prior dispersal events from natal packs. One $r = 0.5$ kinship tie (1%) was due to an extra-pack copulation.

Overall, we documented a strong sex bias of dispersal into a pack as a breeder, with all successful immigrant breeders being males. For example, male 21M dispersed into Druid Peak pack in 1997 and male 227M dispersed into Geode Creek pack in 2004 (Fig. 4). In contrast, we documented no females dispersing into an already established pack and breeding. Alternatively, females became subordinate breeders in their natal pack or dominant breeders through new pack formation. Females did, however, disperse as singletons and joined with groups of males. For example, in 2000, dispersing Leopold female 152F joined with at least three male siblings (204M, 205M, 206M) from Chief Joseph pack to form the Swan Lake pack. The following year, Leopold disperser 151F joined with at least three male siblings (256M, 257M, 258M) also from the Chief Joseph pack, forming the Cougar Creek pack. Finally, we found no evidence for gene flow into YNP from outside as all individuals in the pedigree had ancestry derived from the population founders.

### *Effective population size estimates*

We calculated effective population size ($N_e$) from the pedigree data set (Table 1). $N_e$ increased with increasing population size after the founding events (1995 $N_e = 6$; 1996 $N_e = 13.7$; 2000 $N_e = 17.3$; 2004 $N_e = 22.1$). Using the ratio of $N_e$ to the genotyped population size ($N_g$; see Table 1), however, $N_e/N_g$ ratio estimates did not change appreciably after the founding events (1996 $N_e/N_g = 0.30$; 1997 $N_e/N_g = 0.33$; 2000 $N_e/N_g = 0.26$; 2004 $N_e/N_g = 0.33$).

### *Long-term genetic trajectory prediction*

As expected for an isolated small population of constant size ($N = 170$), our simulations predict a decrease in genetic

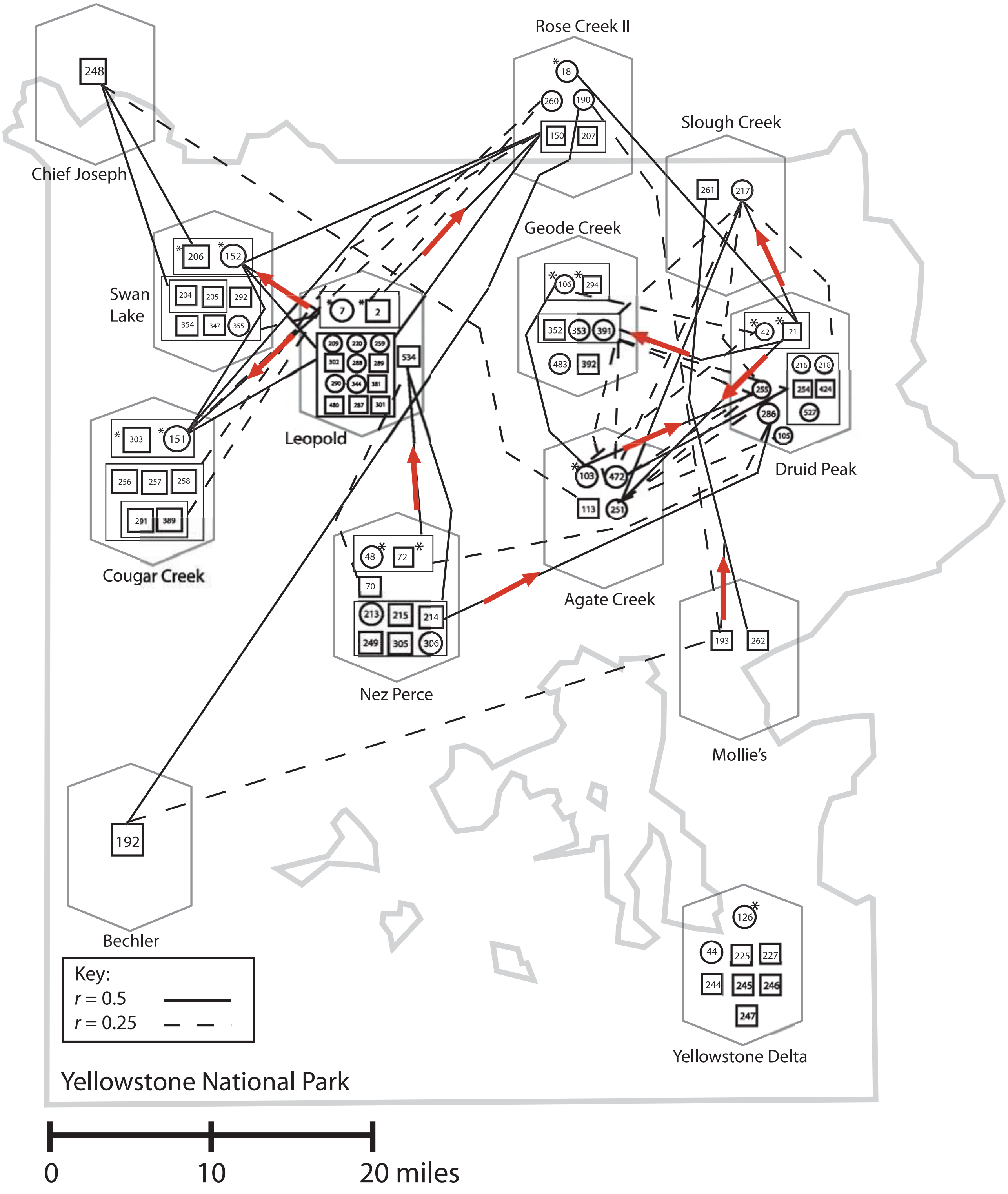


**Fig. 7** Interpack relatedness for 2002 based on pedigree data. Arrows represent parent–offspring relationships, and point towards the offspring. Boxes contain either full-siblings or dominant pairs (asterisk) for interpack comparisons. This figure does not represent the census population, as only individuals and packs with known lineages are shown.

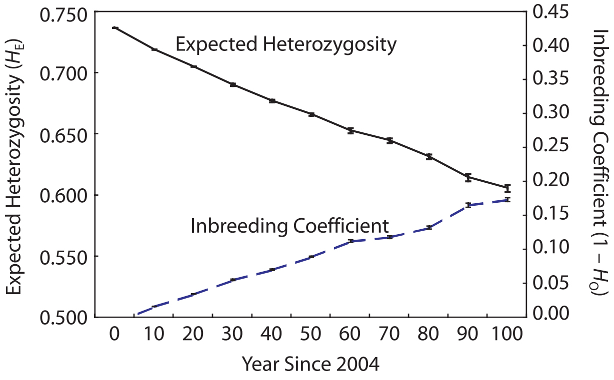


**Fig. 8** Predicted changes in heterozygosity ($H_E$) and inbreeding coefficient ($F_{IS}$) of Yellowstone wolves assuming a constant population size ($N = 170$) and no gene flow using VORTEX (1000 iterations). Input parameters were based on Yellowstone's 2004 demographic and pedigree data. Error bars denote standard error.

heterozygosity and increase in inbreeding coefficient over the next 100 years (Fig. 8). Compared to the population genetic heterozygosity in 2004 ($H_E = 0.74$), the VORTEX simulation analysis predicts a decrease of 18.1% over 100 years to 0.60. The estimated inbreeding coefficient increases from –0.007-0.174. For a constant-size isolated population of similar demography and life history, it is predicted that a population size of approximately 600 individuals would be needed to prevent a decrease in heterozygosity and increase in the inbreeding coefficients by less than 5% over 100 years (Table S3). Simulating the observed one-way migration of individuals out of YNP into the GYA indicates that the effect of 10% emigration per year decreases heterozygosity and increases the inbreeding coefficient by less than 0.5%, thus not having a significant impact on genetic variability. Simulation results also predict that immigration on the order of 12 individuals per year would be required to prevent significant decreases (< 1%) in heterozygosity and increase in inbreeding coefficient (Table S4, Supplementary material). Further, based on observed $N_e/N_c$ ratios of approximately 0.3 for the constant YNP population, these results predict that approximately four immigrating individuals per year would need to become breeders to maintain the genetic diversity of the Yellowstone population.

## Discussion

### *Temporal genetic dynamics*

Genetic diversity is reduced in small, isolated populations through increased drift and inbreeding and lack of migration from elsewhere (Taylor *et al*. 1994; Eldridge *et al*. 1999). Previous empirical studies of an isolated Swedish grey wolf population found that heterozygosity was lost at a rate of 2% per generation (Bensch *et al*. 2006). In Yellowstone, theory predicts a loss of heterozygosity of about $1/2N_e$ per generation and a corresponding increase in the inbreeding coefficient (Hartl & Clark 1997). Consequently, given a harmonic mean of effective population size close to 52 and generation time of 4 years (Table 1), we would expect heterozygosity to have decreased by about 2.4% and inbreeding coefficients to have increased by about the same fraction. However, despite an absence of gene flow into Yellowstone, we found no temporal decrease in genetic variability or increase in inbreeding coefficients over 10 years or 2.5 generations (Table 1). The observed heterozygosity in Yellowstone is similar and in some cases higher than previous estimates for grey wolf populations (e.g. Forbes & Boyd 1996, 1997; Jedrzejewski *et al*. 2005). The inbreeding coefficients are far lower than the values of 0.41 found in the inbred wolf population of Scandinavia (Liberg *et al*. 2005; Bensch *et al*. 2006) or as Hedrick *et al*. (1997) found in three Mexican wolf lineages ranging from 0.184 to 0.608. We suggest the maintenance of genetic variation in Yellowstone reflects the large founding size and rapid population expansion (Sugg *et al*. 1996; Toro *et al*. 2003; Alvarez *et al*. 2005) as well as specific mechanisms to avoid inbreeding with close relatives. Inbreeding avoidance may enhance genetic variability beyond that predicted by simple genetic models (Hartl & Clark 1997; Keller & Waller 2002; Saccheri & Brakefield 2002; Vilà *et al*. 2003; Hogg *et al*. 2006).

### *Population assembly rules*

We identified several factors governing the preservation of genetic variation that are important to a successful reintroduction. First, the founding population must be large and genetically diverse, a method preferred over repeated translocations over time (Wolf *et al*. 1996, 1998; Miller *et al*. 1999). A founding population in Yellowstone was established by 31 individuals from different packs belonging to two source populations in Canada. Additionally, 10 genetically distinct individuals were translocated from a northwestern Montana population early in the recovery process providing new genetic variation. The role that founding population size played in preserving high diversity in YNP is unique, as adequate number of founders are not common for reestablished wolf populations (Wayne *et al*. 1991; Hedrick *et al*. 1997; Liberg *et al*. 2005). Our results confirm the insight of the decision to select a large and diverse founding population for reintroduction (Forbes & Boyd 1997). Therefore, recovery programmes need to emphasize plans for an adequate number of founders to allow for the preservation of genetic diversity.

Second, there is clear evidence that given the choice, wolves avoid breeding with close relatives within their natal pack or elsewhere. We found that of 30 natural matings, none involved pairings between pack members at the sibship or parent–offspring level ($r = 0.5$) and only two pairs were more distantly related ($r > 0.25$). Avoidance of close incestuous matings occurred despite increasing opportunities that such matings would occur without active behavioural avoidance of inbreeding. Evidence from previous genetic studies on natural wolf populations showed that when given a choice, pairs consist of unrelated individuals (Smith *et al*. 1997), thereby preventing loss of heterozygosity through inbreeding (Wright 1922; 1931; Chesser 1991a). However, our study is unique for showing that inbreeding avoidance occurs across a wide variety of mating strategies and contributed to the maintenance high levels of variation. Thus, as was part of this original reintroduction design, future reintroductions and population management should focus on providing opportunities for wolves to avoid inbreeding through actions such as reintroduction of a genetically diverse founding stock to areas of high quality habitat where several wolf packs can coexist in adjacent territories. Promoting the formation of several packs by introduction of unrelated mated pairs might also assist in providing future generations with opportunities to avoid inbreeding. Further, efforts to facilitate immigration from other populations will increase the pool of unrelated individuals who can occupy breeding positions or territories.

Third, breeding pairs can form under a wide variety of different mechanisms when sufficient opportunities are available. Previous studies have shown that the primary method of breeding pair formation in grey wolves involves single wolves meeting and breeding in both recolonizing and established populations (Rothman & Mech 1979; Fritts & Mech 1981; Peterson *et al*. 1984; Hayes *et al*. 1991; Bergerud & Elliott 1998; Hayes & Harestad 2000). We find that only 7% of confirmed pairings have formed in this way (Table 3). The mechanisms that describe how Yellowstone wolves obtained mates included: (i) utilizing a breeding vacancy within a natal or neighbouring pack; (ii) becoming a subordinate breeder; (iii) joining with a group of wolves from either their natal or different pack; and (iv) usurping an established breeder. All of these methods have been previously documented in other wolf systems (Mech & Boitani 2003a), but not to the extent we observe in YNP. This diversity may be an artefact of the reintroduction and rapid population expansion in a prey-abundant ecosystem devoid of wolves, or reflect the unprecedented detail to which wolves were monitored in YNP. Regardless, we show that diversity in pair formation mechanisms in Yellowstone contributes to the maintenance of high heterozygosity. For example, we found that vacant male breeding positions were filled primarily by unrelated immigrants, resulting in subordinate female breeders producing litters unrelated to the previous dominant male breeder. The generality of this result is supported by a study of wolves from the Białowieża Primeval Forest where successors of breeding males were typically immigrant males in contrast to females who commonly obtained breeding positions within their natal pack (Jedrzejewski *et al*. 2005). Our study confirmed parentage and inbreeding avoidance in one of the most extreme cases of multiple breeding documented in a wild wolf population (Fig. 4), where the immigration of an unrelated male (21M) to the Druid Peak pack in 1997 led to breeding with multiple females in the pack for a series of years (Stahler *et al*. 2002).

This diversity of mating mechanisms may reflect conditions related to interpack competition or ecological constraints associated with dispersal (Brown 1974; Stacey 1979; Emlen 1982; Goldizen *et al*. 2002). For example, becoming a subordinate breeder or inheriting a dominant breeding position, in addition to the benefits received through group hunting and having a territory, may reflect the benefits of philopatry in a saturated landscape. Additionally, intraspecific strife has been the main cause of natural mortality for Yellowstone wolves (Smith 2005), and singletons are presumably at much greater risk during intraspecific interactions than individuals in a group (Yellowstone Wolf Project, NPS, unpublished data). Pack splitting may therefore reflect a less risky strategy for establishing territories as a larger group is more likely than singletons to establish a territory in a saturated landscape (Yellowstone Wolf Project, NPS, unpublished data). As with many species, variation in mating behaviour is presumably facilitated through mechanisms of asymmetric mate choice, dispersal and extra-group/pair copulations (Pusey & Wolf 1996; Smith *et al*. 1997; Ross 2001; Keller & Waller 2002; Packard 2003).

Both a diversity of mating mechanisms and inbreeding avoidance are facilitated by reintroduction to large protected areas where introduced populations can expand and new packs can readily be established. In contrast, populations that are more geographically constrained may allow fewer opportunities to avoid inbreeding. In captivity, inbreeding is common among wolves, suggesting that the desire to reproduce is stronger than inbreeding avoidance (Laikre & Ryman 1991; Kalinowski *et al*. 1999; Packard 2003). Similarly, the small wolf population in Isle Royale National Park, Michigan, has half the variation of mainland conspecifics, and heterozygosity has declined with every generation (Wayne *et al*. 1991; Peterson *et al*. 1998). Finnish and Swedish populations of grey wolves are small and restricted to limited areas and have lower levels of heterozygosity (Ellegren 1999; Vilà *et al*. 2003; Bensch *et al*. 2006). The Swedish population in particular suffers from inbreeding depression (Liberg *et al*. 2005; Bensch *et al*. 2006). Nonetheless, individuals in that population appeared to mate with wolves having higher levels of heterozygosity in the absence of unrelated mates (Bensch *et al*. 2006). In comparison, we found no evidence that breeders had higher levels of heterozygosity than nonbreeders. This finding may be more typical of wolf populations such as those in Yellowstone that have low levels of inbreeding and uniformly high levels of individual heterozygosity. These high levels of genetic diversity suggest that large-scale reintroductions can better preserve variation in the short term through a variety of mechanisms, given ample high quality habitat and the opportunity for the population to expand quickly. If such conditions are not possible, artificial migration may be the best option for preserving variation. For example, a single immigrant appears to have rescued the Swedish wolf population from high rates of inbreeding and loss of heterozygosity (Mills & Allendorf 1996; Ellegren 1999; Vila *et al*. 2003; Bensch *et al*. 2006). Future research is needed to determine how factors such as wolf density, prey density, dispersal, and territoriality influence pair and pack formation, and its subsequent influence on genetic diversity.

### *Interpack dispersal and relatedness*

Interpack dispersal was common during the study period and helped maintain the observed genetic trends of high heterozygosity and low inbreeding coefficients (Fig. 7). All genetically verified immigrants were males and all bred. Other studies have recorded the presence of adopted nonbreeding pack members (Mech & Boitani 2003a), although only one confirmed genetic relatedness of the adoptees (Lehman *et al*. 1992). We found that no females were immigrants into a pre-established pack; they were involved in pack splitting events with territories often established next to their natal pack. For example, Druid Peak female groups split to form adjacent packs of Agate Creek and Geode Creek (Fig. 4). However, single females also joined groups of males, as found in the formation of Swan Lake pack and Cougar Creek pack. In general, kinship ties were biased by proximity with the majority kinship ties existing between neighbouring packs. Conceivably, such kinship ties may promote social stability and pack persistence (Wayne 1996). However, such ties do not mitigate interpack strife in Yellowstone, as both are common between packs sharing territorial boundaries (Yellowstone Wolf Project, NPS, unpublished data), and overall rates of interpack interaction are as high in Yellowstone as elsewhere, even in areas were interpack relatedness values are low (Lehman *et al*. 1992).

### *Breeding scenarios and preservation of genetic variation*

In general, the breeding behaviours of Yellowstone wolves resulted in preservation of genetic variation in the breeding pool that did not differ substantially from that of a managed breeding strategy (Fig. 2). To understand the implications of different breeding strategies across one generation, we simulated a single generation of offspring for four mating schemes. These results showed that restricting potential mates to individuals within packs had a dramatic effect on genetic variability, with mean heterozygosity about 10% lower than observed. Even an open breeding strategy, which utilized the entire breeding pool, retained less variation than observed. These findings support pedigree evidence that active choice of unrelated mates within or outside of the pack structure is occurring. As expected, the managed breeding strategy preserved genetic variation most effectively overall. Strikingly, the observed population's level of heterozygosity was included in the distribution of the managed breeding strategy, indicating that the natural social behaviour of wolves is sufficient to preserve high levels of variation given access to unrelated mates and a large diverse breeding pool. Captive breeding strategies that mimic such natural breeding behaviours will only minimally reduce levels of genetic variability over a closely managed strategy and at the same time maintain natural patterns of social interactions. When feasible, captive breeding strategies should promote the natural formation of packs and diverse opportunities for pair formation, such as timely replacement of lost breeders with unrelated individuals and allowing for subordinate breeding.

### *Long-term concerns and conservation implications*

The Endangered Species Act in the United States (USFWS 1973) requires a recovery plan for species listed as endangered. The Western grey wolf is a listed species and has a modest recovery plan that has been enacted requiring only 30 breeding pairs for three consecutive years evenly distributed among the central Idaho, GYA and northwestern Montana recovery area. This recovery goal was met in 2002 (USFWS *et al*. 2005) but is far below historical values based on genetic analysis of over 350 000 individuals (Leonard *et al*. 2005). Currently, the states of Idaho, Wyoming and Montana have prepared plans for grey wolf management after proposed federal delisting with goals of maintaining at least 10 breeding pairs and at least 100 wolves per state (USFWS 2007). Our results show that populations of this size that remain isolated will lose genetic variation and become inbred over the long term (Fig. 8). Consequently, we suggest that future management of Western wolf populations incorporate genetic data regarding population structure, minimal viable population sizes, and the degree of isolation following population reestablishment. Such information will help assess recovery success and identify areas of concern for both short- and long-term genetic viability. For Yellowstone wolves, our viability predictions suggest that a minimum population size of 170 individuals is adequate for short-term retention of genetic variability. Over the long term, however, genetic variation will decrease and inbreeding will increase without additional migration from other populations or substantial increases in population size over this minimum value. In general, for each of the three recovery areas, an absence of gene flow will lead to decreased genetic variation and loss of the potential for recolonization in the event of population extinction (Eldridge *et al*. 1999; Frankham *et al*. 2002; Aspi *et al*. 2006; Hazlitt *et al*. 2006). However, northwestern Montana wolf populations genetically communicate with those in southern Canada (Forbes & Boyd 1997), and central Idaho currently has a large enough population size ($N > 700$) and connectivity to northwestern Montana populations to delay any immediate concerns about the loss of genetic variation.

In contrast, the YNP population appears to be genetically isolated and has reached carrying capacity at about 170 individuals. The rate of decrease in heterozygosity and increase in inbreeding over the near term (the next 20–30 years) are low enough that phenotypic signs of inbreeding depression such as skeletal defects or a significant decrease in offspring survivorship are not predicted (Hedrick *et al*. 2001; Raikkonen *et al*. 2006). However, a recent study examining the effect of inbreeding depression on offspring survivorship in an inbred Swedish population (Liberg *et al*. 2005) indicates a decrease in juvenile survivorship by approximately 15% with an increase in the inbreeding coefficient of 0.1. In the Yellowstone population, we predict that the inbreeding coefficient will rise to 0.1 in approximately 60 years without gene flow from outside the park. Given these results, we would expect to observe an increase in juvenile mortality from an average of 23 to 40%, an effect equivalent to losing an additional pup in each litter. To deter such inbreeding effects, migration will be needed, involving translocation of wolves from elsewhere or the development of specific habitat corridors. The latter may be feasible if populations in the northern Rocky Mountains are genetically connected and interpopulation dispersal occurs (Sunquist & Sunquist 2001; Waser *et al*. 2001). However, only low-quality corridors currently connect the GYA to the Idaho and northwestern wolf populations, exposing dispersers to high human-associated mortality risks (Oakleaf *et al*. 2006). The genetic impact of this isolation may take decades to accumulate but can be delayed if gene flow with other populations is established and maintained.

In conclusion, we show that in addition to a genetically diverse founding stock, the maintenance of genetic variation is dependent on a wide variety of behavioural mechanisms for avoiding inbreeding with close relatives. We found no natural breeding pairs that were closely related which confirmed previous results on wolves from Minnesota and Alaska (Smith *et al*. 1997). Such inbreeding avoidance was facilitated by specific population assembly patterns including avoidance of breeding with related pack members, dispersal of males to packs where they are unrelated to the breeding females, and the fission of packs with a high proportion of close relatives to include adult offspring that are joined by dispersing and unrelated adults of the opposite sex. In general, a wide diversity of mechanisms for breeding pair formation promoted retention of genetic variability in the Yellowstone population. Simulation results showed that the observed levels of genetic variation were higher than that expected by random breeding within packs or across the entire breeding pool. This observed bias in breeding occurs despite the high probability of mating with close relatives in the Yellowstone population and results in levels of variation similar to that of a population managed for high levels of variation and reduced inbreeding. Consequently, population management should include efforts to ensure that the social dynamics function remain unhindered, thus promoting the diversity of behaviours that allow for inbreeding avoidance and pack formation as found in the Yellowstone population. These actions might include the maintenance of a high quality core habitat that will allow a rapid increase and establishment of a founder population, and genetic communication between networks of adjoining packs. In Yellowstone, kinship ties predominate between packs sharing a common territorial boundary implying that interpack dispersal is a key feature of natural populations (Lehman *et al*. 1992). Over the short term, core areas the size of YNP containing 10–12 packs appear sufficient to maintain genetic variation and may act as source populations for nearby sink regions such as the GYA where control actions occur. However, intense control actions in the region may severely affect the continuity of pack systems and hinder genetic exchange. Moreover, if such actions result in the removal of breeding pairs, this may alter the stability of pack dynamics, leading to higher breeder turnover and more frequent occurrence of inbreeding as mating choices become limited to close relatives.

Overall, our findings demonstrate the effectiveness of the reintroduction in preserving genetic diversity over the first decade of wolf recovery in Yellowstone. Our analyses suggest that little more could have been done to improve the maintenance of variation, which is a testament to both the original reintroduction design as well as the importance of having large-scale and high quality ecosystems where natural behavioural processes can be maintained. Detailed population genealogies can provide valuable insight into the dynamics influencing both genetic and social structure of reintroduced populations, and in some cases, may identify some of the causes and consequences of limitations in breeding opportunities (e.g. Liberg *et al*. 2005; Bensch *et al*. 2006). These limitations can potentially be addressed by management actions such as increased protection, habitat restoration and population augmentation.

## Acknowledgements

We thank Steve Fain (USFWS Forensics Laboratory, Oregon), David Coltman (University of Alberta), and Ed Bangs (USFWS Montana) and Yellowstone National Park (National Park Service) for samples. Funding was provided by the USFWS's Idaho and Montana offices, Tapeats Foundation, Yellowstone Park Foundation, California Wolf Center, and by NSF grant DEB-0613730. We thank Mabel Chin for laboratory assistance. We are grateful to John Varley who supported and facilitated this project initially. We especially thank Roger Stradley (Gallatin Flying Services, Montana), Hawkins and Powers, and Central Copters for assisting in wolf monitoring and capture. Finally, Yellowstone Wolf Project's Deb Guernsey, Kerry Murphy, Rick McIntyre, and numerous field technicians were instrumental for collecting extensive field observations and genetic samples. We would also like to thank Phil Hedrick, Steve Forbes, and one anonymous reviewer for comments, which significantly improved the manuscript. Additional thanks to Laurie Bingaman-Lackey for technical support for SPARKS.

## References

Alvarez I, Gutierrez JP, Royo LJ *et al*. (2005) Testing the usefulness of the molecular coancestry information to assess genetic relationships in livestock using a set of Spanish sheep breeds. *Journal of Animal Science*, **83**, 737–744.

Aspi J, Roininen E, Ruokonen M, Kojola I, Vila C (2006) Genetic diversity, population structure, effective population size and demographic history of the Finnish wolf population. *Molecular Ecology*, **15**, 1564–1576.

Ballou JD, Earnhardt J, Thompson S (2001) *MATERX: Genetic Management Software*. Smithsonian Institution, National Zoological Park, Washington, D.C.

Bangs EE, Fritts SH (1996) Reintroducing the gray wolf to central Idaho and Yellowstone National Park. *Wildlife Society Bulletin*, **24**, 402–413.

Bangs EE, Fritts SH, Fontaine JA *et al*. (1998) Status of gray wolf restoration in Montana, Idaho, and Wyoming. *Wildlife Society Bulletin*, **26**, 785–798.

Bensch S, Andren H, Hansson B *et al*. (2006) Selection for heterozygosity gives hope to a wild population of inbred wolves. *Public Library of Science*, **1**, e72.

Bergerud AT, Elliott JP (1998) Wolf predation in a multiple-ungulate system in northern British Columbia. *Canadian Journal of Zoology*, **76**, 1551–1569.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques*, **31**, 24–28.

Breen M, Jouquand S, Renier C *et al*. (2001) Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome Research*, **11**, 1784–1795.

Breitenmoser R, Breitenmoser-Wursten C, Carbyn LN, Funk SM (2001) Assessment of carnivore reintroductions. In: *Carnivore Conservation* (eds Gittleman JL, Funk SM, Macdonald D, Wayne RK), pp. 241–281. Cambridge University Press, Cambridge, UK.

Brown JL (1974) Alternate routes to sociality in jays — with a theory for evolution of altruism and communal breeding. *American Zoologist*, **14**, 63–80.

Chesser RK (1991a) Gene diversity and female philopatry. *Genetics*, **127**, 437–447.

Chesser RK (1991b) Influence of gene flow and breeding tactics on gene diversity within populations. *Genetics*, **129**, 573–583.

Eldridge MDB, King JM, Loupis AK *et al*. (1999) Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black-footed rock-wallaby. *Conservation Biology*, **13**, 531–541.

Ellegren H (1999) Inbreeding and relatedness in Scandinavian grey wolves *Canis lupus*. *Hereditas*, **130**, 239–244.

Ellegren H, Savolainen P, Rosen B (1996) The genetical history of an isolated population of the endangered grey wolf *Canis lupus*: a study of nuclear and mitochondrial polymorphisms. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **351**, 1661–1669.

Emlen ST (1982) The evolution of helping.1. an ecological constraints model. *American Naturalist*, **119**, 29–39.

Evett IW, Weir BS (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sinauer Associates, Sunderland, Massachusetts.

Forbes SH, Boyd DK (1996) Genetic variation of naturally colonizing wolves in the Central Rocky Mountains. *Conservation Biology*, **10**, 1082–1090.

Forbes SH, Boyd DK (1997) Genetic structure and migration in native and reintroduced Rocky Mountain wolf populations. *Conservation Biology*, **11**, 1226–1234.

Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conservation Biology*, **10**, 1500–1508.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Fritts SH, Bangs EE, Fontaine JA *et al*. (1997) Planning and implementing a reintroduction of wolves to Yellowstone National Park and central Idaho. *Restoration Ecology*, **5**, 7–27.

Fritts SH, Mack CM, Smith DW *et al*. (2001) Outcomes of hard and soft releases of reintroduced wolves in central Idaho and the Greater Yellowstone Area. In: *Large Mammal Restoration* (eds Maehr DS, Noss RF, Larkin JL), pp. 125–147. Island Press, Washington, D.C.

Fritts SH, Mech LD (1981) Dynamics, movements, and feeding ecology of a newly protected wolf population in northwestern Minnesota. *Wildlife Monographs*, **80**, 1–79.

Girman D, Mills MGL, Geffen E, Wayne RK (1997) A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*). *Behavioral Ecology and Sociobiology*, **40**, 187–198.

Goldizen AW, Putland DA, Robertson KA (2002) Dispersal strategies in Tasmanian native hens (*Gallinula mortierii*). *Behavioral Ecology*, **13**, 328–336.

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, **8**, 1231–1234.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology*, **11**, 1103–1114.

Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool — status and strategy. *Science*, **245**, 477–480.

Guyon R, Lorentzen TD, Hitte C *et al*. (2003) A 1-Mb resolution radiation hybrid map of the canine genome. *Proceedings of the National Academy of Sciences, USA*, **100**, 5296–5301.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*, 3rd edn. Sinauer Associates, Sunderland, Massachusetts.

Hayes RD, Harestad AS (2000) Demography of a recovering wolf population in the Yukon. *Canadian Journal of Zoology*, **78**, 36–48.

Hayes RD, Baer AM, Larsen DG (1991) Population dynamics and prey relationships of an exploited and recovering wolf population in the southern Yukon. Yukon Territory, Fish and Wildlife Branch, Department of Renewable Resources. Final Report TR-91–1.

Hazlitt SL, Sigg DP, Eldridge MDB, Goldizen AW (2006) Restricted mating dispersal and strong breeding group structure in a mid-sized marsupial mammal (*Petrogale penicillata*). *Molecular Ecology*, **15**, 2997–3007.

Hebblewhite M, White CA, Nietvelt CG *et al*. (2005) Human activity mediates a trophic cascade caused by wolves. *Ecology*, **86**, 2135–2144.

Hedrick P, Fredrickson R, Ellegren H (2001) Evaluation of d$^2$, a microsatellite measure of inbreeding and outbreeding, in wolves with a known pedigree. *Evolution*, **55**, 1256–1260.

Hedrick P, Miller PS, Geffen E, Wayne RK (1997) Genetic evaluation of the three captive Mexican wolf lineages. *Zoo Biology*, **16**, 47–69.

Hogg JT, Forbes SH, Steele BM, Luikart G (2006) Genetic rescue of an insular population of large mammals. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **273**, 1491–1499.

Jedrzejewski W, Branicki W, Veit C *et al*. (2005) Genetic diversity and relatedness within packs in an intensely hunted population of wolves *Canis lupus*. *Acta Theriologica*, **50**, 3–22.

Kalinowski ST, Hedrick PW, Miller PS (1999) No inbreeding depression observed in Mexican and red wolf captive breeding programs. *Conservation Biology*, **13**, 1371–1377.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230–241.

Kinghorn BP (1994) PEDIGREE VIEWER — a graphical utility for browsing pedigreed data sets. In: *5th World Congress on Genetics Applied to Livestock Production* (eds Smith C, Gavora JS, Benkel B *et al*.), pp. 85–86. University of Guelph, Ontario, Canada.

Lacy RC, Borbat M, Pollak JP (2005) *VORTEX: A Stochastic Simulation of the Extinction Process*, Version 9.50. Chicago Zoological Society, Brookfield, Illinois.

Laikre L, Ryman N (1991) Inbreeding depression in a captive wolf (*Canis lupus*) population. *Conservation Biology*, **5**, 33–40.

Lande R (1995) Mutation and conservation. *Conservation Biology*, **9**, 782–791.

Lehman N, Clarkson P, Mech LD, Meier TJ, Wayne RK (1992) A Study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial-DNA. *Behavioral Ecology and Sociobiology*, **30**, 83–94.

Leonard JA, Vila C, Wayne RK (2005) Legacy lost: genetic variability and population size of extirpated US grey wolves (*Canis lupus*). *Molecular Ecology*, **14**, 9–17.

Liberg O, Andren H, Pedersen HC *et al*. (2005) Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biology Letters*, **1**, 17–20.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Mech LD (1970) *The Wolf: Ecology and Behavior of an Endangered Species*. Doubleday, New York.

Mech LD, Boitani L (2003a) Wolf social ecology. In: *Wolves: Behavior, Ecology, and Conservation* (eds Mech LD, Boitani L), pp. 1–34. University of Chicago Press, Chicago, Illinois.

Mech LD, Boitani L (2003b) *Wolf: Behavior, Ecology, and Conservation*. University of Chicago Press, Chicago, Illinois.

Miller PS, Lacy RC (2005) VORTEX: a stochastic simulation of the extinction process. Version 9.50 User's Manual. In: *Conservation Breeding Specialist Group (SSC/IUCN)*. Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, Minnesota.

Miller B, Ralls K, Reading RP, Scott JM, Estes J (1999) Biological and technical considerations of carnivore translocation: a review. *Animal Conservation*, **2**, 59–68.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509–1518.

Murie A (1944) *The Wolves of Mount McKinley*. US National Park Service Fauna Ser. no. 5, US Government Printing Office, Washington, D.C.

Neff MW, Broman KW, Mellersh CS *et al*. (1999) A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. *Genetics*, **151**, 803–820.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nei M, Maruyama T, Chakraborty R (1975) Bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Nunney L (1995) Measuring the ratio of effective population size to adult numbers using genetic and ecological data. *Evolution*, **49**, 389–392.

Oakleaf JK, Murray DL, Bangs EE *et al*. (2006) Habitat selection by recolonizing wolves in the northern Rocky Mountains of the United States. *Journal of Wildlife Management*, **70**, 554–563.

Packard JM (2003) Wolf behavior: reproductive, social, and intelligent. In: *Wolves: Behavior, Ecology, and Conservation* (eds Mech LD, Boitani L), pp. 35–65. University of Chicago Press, Chicago, Illinois.

Parker SDE (2001) *Trypanotolerance in West African cattle and the population genetic effects of selection*. PhD Thesis, University of Dublin.

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian Bush Rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.

Peel D, Ovenden JR, Peel SL (2004) *NEESTIMATOR: Software for Estimating Effective Population Size*. Queensland Government, Department of Primary Industries and Fisheries, St Lucia, Queensland.

Peterson RO, Page RE, Dodge KM (1984) Wolves, moose, and the allometry of population cycles. *Science*, **224**, 1350–1352.

Peterson RO, Thomas NJ, Thurber JM, Vucetich JA, Waite TA (1998) Population limitation and the wolves of Isle Royale. *Journal of Mammalogy*, **79**, 828–841.

Phillips MK, Smith DW (1996) *The Wolves of Yellowstone*. Voyageur Press, Stillwater, Minnesota.

Pollak JP, Lacy RC, Ballou JD (2002) *PM2000: Population Management Software*. Cornell University, Ithaca, New York.

Pusey A, Wolf M (1996) Inbreeding avoidance in animals. *Trends in Ecology & Evolution*, **11**, 201–206.

Raikkonen J, Bignert A, Mortensen P, Fernholm B (2006) Congenital defects in a highly inbred wild wolf population (*Canis lupus*). *Mammalian Biology*, **71**, 65–73.

Ralls K, Ballou JD (2004) Genetic status and management of California condors. *Condor*, **106**, 215–228.

Randall DA, Pollinger JP, Wayne RK *et al*. (2007) Inbreeding is reduced by female-biased dispersal and mating behavior in Ethiopian wolves. *Behavioral Ecology*, **18**, 579–589.

Randi E, Lucchini V, Christensen MF *et al*. (2000) Mitochondrial DNA variability in Italian and East European wolves: detecting the consequences of small population size and hybridization. *Conservation Biology*, **14**, 464–473.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Riley SPD, Pollinger J, Sauvajot RM *et al*. (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, **15**, 1733–1741.

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265–284.

Rothman RJ, Mech LD (1979) Scent marking in lone wolves and newly formed pairs. *Animal Behaviour*, **27**, 750–760.

Saccheri IJ, Brakefield PM (2002) Rapid spread of immigrant genomes into inbred populations. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 1073–1078.

Schmitz OJ, Hamback PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. *American Naturalist*, **155**, 141–153.

Schröder W, Promberger C (1993) Wolf conservation strategy for Europe. In: *Wolves in Europe. Status and Perspectives* (eds Promberger C, Schröder W), pp. 2–7. Munich Wildlife Society, Munich.

Smith DW (2005) Ten years of Yellowstone wolves: 1995–2005. Yellowstone Center for Resources, National Park Service. *Yellowstone Science*, **13**, 2–48.

Smith D, Meier T, Geffen E *et al*. (1997) Is incest common in gray wolf packs? *Behavioral Ecology*, **8**, 384–391.

Smith DW, Peterson RO, Houston DB (2003) Yellowstone after wolves. *Bioscience*, **53**, 330–340.

Soule ME, Estes JA, Berger J, Del Rio CM (2003) Ecological effectiveness: conservation goals for interactive species. *Conservation Biology*, **17**, 1238–1250.

Stacey PB (1979) Habitat saturation and communal breeding in the acorn woodpecker. *Animal Behaviour*, **27**, 1153–1166.

Stahler DR, Smith DW, Landis R (2002) The acceptance of a new breeding male into a wild wolf pack. *Canadian Journal of Zoology*, **80**, 360–365.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL (1996) Population genetics meets behavioral ecology. *Trends in Ecology & Evolution*, **11**, 338–342.

Sunquist ME, Sunquist F (2001) Changing landscapes: consequences for carnivores. In: *Carnivore Conservation* (eds Gittleman JL, Funk SM, Macdonald D, Wayne RK), pp. 399–418. Cambridge Unversity Press, Cambridge, UK.

Taylor AC, Horsup A, Johnson CN, Sunnucks P, Sherwin B (1997) Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **6**, 9–19.

Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation of microsatellite loci in a bottlenecked species — the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **3**, 277–290.

Terborgh J, Estes JA, Paquet P *et al*. (1999) The role of top carnivores in regulating terrestrial ecosystems. In: *Continental Conservation: Scientific Foundations of Regional Reserve Networks* (eds Terborgh J, Soule M), pp. 39–64. Island Press, Washington, D.C.

Terborgh J, Feeley K, Silman M, Nunez P, Balukjian B (2006) Vegetation dynamics of predator-free land-bridge islands. *Journal of Ecology*, **94**, 253–263.

Toro MA, Barragan C, Ovilo C (2003) Estimation of genetic variability of the founder population in a conservation scheme using microsatellites. *Animal Genetics*, **34**, 226–228.

US Fish & Wildlife Service (1973) *Endangered Species Act*. Department of the Interior, 108th Congress, Washington D.C.

US Fish & Wildlife Service (1994) Memorandum regarding a viable wolf population in the Northern Rocky Mountains. *Final Environmental Impact Statement: The Reintroduction of Gray Wolves to Yellowstone National Park and Central Idaho*, pp. 6–68 to 6–78. US Fish and Wildlife Service, Denver, Colorado.

US Fish & Wildlife Service (2007) Endangered and threatened wildlife and plants; designating the northern Rocky Mountain population of gray wolf as a distinct population segment and removing this distinct population segment from the federal list of endangered and threatened wildlife; Proposed Rule. US Fish and Wildlife Service, Department of the Interior. *Federal Register*, **72**, 6106–6139.

US Fish & Wildlife Service, Nez Perce Tribe, National. Park Service *et al*. (2005) Rocky mountain wolf recovery: 2004 annual report (ed. Boyd D. ), p. 72. US Fish and Wildlife Service, Helena, Montana.

Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Vilà C, Sundqvist AK, Flagstad O *et al*. (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 91–97.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*, **10**, 249–256.

Wang JL (2004) Sibship reconstruction from genetic data with typing errors. *Genetics*, **166**, 1963–1979.

Waser P, Strobeck C, Paetkau D (2001) Estimating interpopulation dispersal rates. In: *Carnivore Conservation* (eds Gittleman JL, Funk SM, Macdonald D, Wayne RK), pp. 484–497. Cambridge University Press, Cambridge, UK.

Wayne RK (1996) Conservation genetics in the Canidae. In: *Conservation Genetics: Case Histories from Nature* (eds Avise JC, Hamrick JL), pp. 75–118. Chapman & Hall, New York.

Wayne RK, Lehman N, Girman D *et al*. (1991) Conservation genetics of the endangered Isle Royale gray wolf. *Conservation Biology*, **5**, 41–51.

Wolf CM, Garland T, Griffith B (1998) Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts. *Biological Conservation*, **86**, 243–255.

Wolf CM, Griffith B, Reed C, Temple SA (1996) Avian and mammalian translocations: update and reanalysis of 1987 survey data. *Conservation Biology*, **10**, 1142–1154.

Wright S (1922) Coefficients of inbreeding and relationship. *American Naturalist*, **56**, 330–338.

Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

Wright S (1969) *Evolution and the Genetics of Populations*, *Vol. 2, The Theory of Gene Frequencies*. University of Chicago Press, Chicago, Illinois.

---

Bridgett vonHoldt studies evolutionary genomics, genetic mapping and domestication of wild canid populations and modern dogs. Dan Stahler is the Project Biologist of the Yellowstone Wolf Project interested in the ecology, behavior and ecosystem influence of carnivores. He is currently a doctoral student studying the behavioral and molecular ecology of Yellowstone's wolves. Doug Smith is the leader of the Yellowstone Wolf Project whose research interests include conservation biology, predator-prey relationships, population dynamics, behavior, and the role of wolves in ecosystems. Dent Earl applies computational and genetic techniques to study questions in conservation biology and population genetics theory. John Pollinger studies conservation genetics of carnivores and birds and directs UCLA's Conservation Genetics Resource Center. Robert Wayne applies molecular genetic techniques to study questions in ecology, behavior and evolution of animal.

---

## Supplementary material

The following supplementary material is available for this article:

**Table S1** Demographic input values for vortex analyses for Yellowstone National Park for 2004.

**Table S2** Descriptive statistics for the total population genotyped of Yellowstone National Park.

**Table S3** Descriptive statistics for the pedigree population genotyped of Yellowstone National Park.

**Table S4** Predicted population size needed to limit observed heterozygosity ($H_O$) drop to 5% over 100 years using demographic values for the population during 2004.

**Table S5** Predicted effect of annual immigration for YNP on maintenance of heterozygosity in a static population ($N = 170$) over the duration of 100 years.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/
10.1111/j.1365-294X.2007.03468.x
(This link will take you to the article abstract).

Received: 5 June 2023 | Revised: 19 November 2023 | Accepted: 21 November 2023

DOI: 10.1111/mec.17231

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

# Demographic history shapes North American gray wolf genomic diversity and informs species' conservation

**Bridgett M. vonHoldt**[1] | **Daniel R. Stahler**[2] | **Kristin E. Brzeski**[3] | **Marco Musiani**[4] | **Rolf Peterson**[3] | **Michael Phillips**[5] | **John Stephenson**[6] | **Kent Laudon**[7] | **Erin Meredith**[8] | **John A. Vucetich**[3] | **Jennifer A. Leonard**[9] | **Robert K. Wayne**[10,†]

[1]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, USA

[2]Yellowstone Center for Resources, Yellowstone National Park, Wyoming, USA

[3]College of Forest Resources and Environmental Science, Michigan Technological University, Houghton, Michigan, USA

[4]Dipartimento di Scienze Biologiche, Geologiche e Ambientali (BiGeA), Università di Bologna, Bologna, Italy

[5]Turner Endangered Species Fund, Bozeman, Montana, USA

[6]Grand Teton National Park, Moose, Wyoming, USA

[7]California Department of Fish and Wildlife, Northern Region, Redding, California, USA

[8]California Department of Fish and Wildlife, Wildlife Forensic Laboratory, Sacramento, California, USA

[9]Conservation and Evolutionary Genetics Group, Estación Biológica de Doñana (EBD-CSIC), Seville, Spain

[10]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, Los Angeles, California, USA

**Correspondence**
Bridgett M. vonHoldt, Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA.
Email: vonholdt@princeton.edu

**Funding information**
Turner Endangered Species Fund

**Handling Editor:** David Coltman

## Abstract

Effective population size estimates are critical information needed for evolutionary predictions and conservation decisions. This is particularly true for species with social factors that restrict access to breeding or experience repeated fluctuations in population size across generations. We investigated the genomic estimates of effective population size along with diversity, subdivision, and inbreeding from 162,109 minimally filtered and 81,595 statistically neutral and unlinked SNPs genotyped in 437 grey wolf samples from North America collected between 1986 and 2021. We found genetic structure across North America, represented by three distinct demographic histories of western, central, and eastern regions of the continent. Further, grey wolves in the northern Rocky Mountains have lower genomic diversity than wolves of the western Great Lakes and have declined over time. Effective population size estimates revealed the historical signatures of continental efforts of predator extermination, despite a quarter century of recovery efforts. We are the first to provide molecular estimates of effective population size across distinct grey wolf populations in North America, which ranged between $N_e \sim 275$ and 3050 since early 1980s. We provide data that inform managers regarding the status and importance of effective population size estimates for grey wolf conservation, which are on average 5.2–9.3% of census estimates for this species. We show that while grey wolves fall above minimum effective population sizes needed to avoid extinction due to inbreeding depression in the short term, they are below sizes predicted to be necessary to avoid long-term risk of extinction.

KEYWORDS
conservation, effective population size, genomics, grey wolf, RADseq

---

†Posthumous authorship.

## 1 | INTRODUCTION

The theory of the effective population size ($N_e$) was originally developed by Sewall Wright (1943, 1965) to provide a means for comparing structure across seemingly disparate populations to result in an estimate that represents an idealized population of randomly mating individuals (Crow & Kimura, 1970). Thus, social organization and non-random breeding will impact the distribution of genotypes over geographic space and concomitantly $N_e$ estimates. Any factor that results in deviations from random breeding (e.g. social factors, breeding strategies, site availability) or changes in population size across generations will result in an effective population size estimate that is a fraction of the census size ($N$) (Charlesworth & Willis, 2009; Clutton-Brock, 2016; Hedrick & Kalinowski, 2000; Keller & Reeve, 1994). For species with high reproductive skew and social structures that repress reproduction in subdominant ranks, the effective population size estimate inferred from sex ratios, dispersal or migration rates, number of reproductive individuals, or genetic assessments is critical information needed for evolutionary predictions (Lanfear et al., 2014; Wang et al., 2016).

Population sizes fluctuate over time, either through natural process or due to anthropogenic activity such as wildlife management (Rowe & Beebee, 2004). Any reduction in size, compounded with isolation, will erode genetic variation via random genetic drift to a degree that depends on the severity and duration of these bottlenecks (Fisher, 1958). Without inter-population connectivity, the only process that naturally introduces new variation into the gene pool is de novo mutations. New mutations are more likely to quickly drift to fixation in isolated small populations, resulting in continuing low levels of genetic diversity (Coyne et al., 1997; Fisher, 1930; Wade & Goodnight, 1998; Wright, 1931). The potential for a population to respond to evolutionary challenges deteriorates as genomic variation dwindles, thereby limiting adaptive outcomes (Allendorf, 2016; Frankham, 2005; Hoffmann et al., 2017; López-Cortegano et al., 2019). Anthropogenic effects that reduce population size and impact life history events central to individual-level fitness (e.g. reproduction, dispersal) are well known to degrade genomic variation and adaptive potential (Allendorf et al., 2008; Coltman, 2008; Frankel & Soulé, 1981; Frankham, 2005; Reed & Frankham, 2003).

In their recent evolutionary history, grey wolves (*Canis lupus*) in North America have been eradicated from much of their southern continental range through federal and state programmes first implemented during the mid-19th century. These programmes were highly effective and by the late 1950s had exterminated the wolf from the conterminous United States except for a few individuals on Isle Royale National Park in Lake Superior (Minnesota) and a few hundred individuals in northeastern mainland Minnesota (Boitani, 2003; Franzmann & Schwartz, 1997; Kolenosky & Standfield, 1975; Parker, 1995; Peterson, 1955; U.S. Fish and Wildlife Service, 1992; Young & Goldman, 1944). In the face of a near total elimination, coupled with social structure of the species and removal of dispersers, there was a growing concern regarding the future survival of the grey wolf species which led to the translocation of grey wolves to Yellowstone National Park (YNP) and central Idaho (Adams et al., 2008; Brainerd et al., 2008; Rick et al., 2017; Treves et al., 2016). A targeted study of wolves living within YNP reported a significantly smaller effective population size than the censused population (vonHoldt et al., 2008), emphasizing the critical role of population connectivity to combat genetic drift, inbreeding, and erosion of heterozygosity (Allendorf et al., 2008; Gese & Mech, 1991; Jedrzejewski et al., 2005; Mech & Boitani, 2003; vonHoldt et al., 2008).

In the United States, grey wolves are managed as three populations with distinct demographic histories: northern Rocky Mountains, the western Great Lakes, and southwestern (explicitly for the Mexican wolf *C. l. baileyi* subspecies) regions. Grey wolves in the northern Rocky Mountains were extirpated by the 1920s and were listed under the Endangered Species Act (ESA) in 1973. As such, all grey wolves in the lower 48 United States range were listed as endangered, with the exception of grey wolves living in Minnesota that were listed as threatened. The northern Rocky Mountain Wolf Recovery Plan (NRMWRP) outlined grey wolf recovery by supporting natural colonization and translocation of 66 wolves from Alberta and British Columbia to central Idaho and Wyoming's YNP during the winters of 1995 and 1996 (59 FR 60266; U.S. Fish and Wildlife Service, 1987). Dispersers from YNP expanded into adjacent Montana, Idaho, and Wyoming counties (collectively referred to as the Greater Yellowstone Ecosystem), and dispersers from central Idaho expanded into adjacent Montana, Wyoming, and Oregon. Beginning in the late 1990s, periodic dispersing wolves from southern British Columbia and the northern Rocky Mountains were documented in the Pacific Northwest states of Washington, Oregon, and northern California. By 2011, the first wolf entered Oregon with confirmed reproduction in 2015.

The western Great Lakes population is composed of the eastern portion of the Dakotas, Minnesota, Iowa, Wisconsin, a northern portion of Illinois, and Michigan (lower and upper peninsula). Grey wolves in Minnesota were first protected under the ESA in 1974, with subsequent expansion into Wisconsin and Michigan by the early 1990s (Refsnider, 2009). The Timber Wolf Recovery Plan further considered the historic range to Minnesota eastward to Maine and south to the northern portion of Florida (Refsnider, 2009; U.S. Fish and Wildlife Service, 1992; Wisconsin, 1989; Wydeven et al., 2009). The southwestern population that encompasses the endangered Mexican grey wolf subspecies was not included in this study.

Effective in January 2021, the U.S. Fish and Wildlife Service (FWS) delisted grey wolves (excluding the Mexican wolf subspecies) everywhere in the lower 48 United States (final rule 85 FR 69778). By February 2022, ESA protections were restored for all grey wolves in the lower 48 United States except for the wolves of the northern Rocky Mountain region, where they remain under state-level management. The delisting decision relied in part on the lack of information from FWS that the western Great Lakes population could indeed be self-sustaining without federal protection. By January 2023, the Circuit Mediator issued an order for a scientific review of grey wolf status review to be conducted.

Our goal was to assess the temporal and spatial variations in genetic signatures over the recent decades of grey wolf protections and recovery across portions of North America and provide information to consider for long-term viability of grey wolves as it pertains to their ESA listing status in the United States. We conducted this genomic surveillance across the North American continent to showcase how demography and genomic signatures are intertwined. This assessment provides a contemporary assessment of genetic parameters important to genomic viability across geographic and regulatory scales for integration into conservation goals for a social carnivore species.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection and genomic library construction

We obtained archived blood or tissue samples collected from 482 grey wolves across their continental range in North America (Canada = 91, USA = 391) from state and federal partners, local trappers, and private genetic collections (Figure 1a; Table S1). Locations of sample origins varied, from regional identification to counties, parks, or states and provinces. We partitioned samples into two levels of geographic resolution, regional and U.S.-managed populations. For the U.S.-managed populations, we define the 'northern Rocky Mountains' (abbreviated as RM) as composed of samples that originated from California, Idaho, Montana, Washington, and Wyoming. We define Michigan, Minnesota, and Wisconsin to compose the 'western Great Lakes' (abbreviated as GL).

We extracted genomic DNA following manufacturer's protocol (Qiagen DNeasy Blood and Tissue kit). We used the Qubit fluorometer system for DNA quantification to standardize the input amount for use in the modified restriction-site-associated DNA sequencing (RADseq) capture protocol (Ali et al., 2015). Briefly, we digested genomic DNA with *SbfI* with a subsequent ligation of unique 8-bp barcoded biotinylated adapters to permit the pooling of 48 DNA samples into a single library. We randomly sheared each library to 400 bp in a Covaris LE220 followed by an enrichment for the adapter-ligated fragments using a Dynabeads M-280 streptavidin binding assay. We then prepared the enriched libraries for paired-end (2 × 150 nt) Illumina NovaSeq 6000 sequencing at Princeton University's Lewis-Sigler Genomics Institute core facility using the NEBnext Ultra II DNA Library Prep Kit (New England Biolabs). For any step of purifying or size selection of DNA, we used Agencourt AMPure XP magnetic beads (Beckman Coulter).

### 2.2 | Bioinformatic processing

We retained sequence read pairs that contained both our known unique barcodes and remnant *SbfI* recognition site, which were processed in *STACKS* v2.6 (Catchen et al., 2013; Rochette et al., 2019). We used the *process_radtags* module to rescue our barcoded reads with a 2 bp mismatch and excluded reads with a quality score < 10. We next removed PCR duplicates in the *clone_filter* module followed by mapping to the reference dog genome CanFam3.1 assembly (Lindblad-Toh et al., 2005) using *bwa-mem* (Li, 2013). We also included the Y chromosome (KP081776.1; Li et al., 2013) with the CanFam3.1 reference assembly. After alignment, we excluded mapped reads with MAPQ <20 and then converted the SAM files to BAM format in *Samtools* v0.1.18 (Li et al., 2009). We implemented the *gstacks* and *populations* modules in *STACKS* v2 with an increase in the minimum significance threshold in *gstacks* and used the maximum-likelihood marukilow model that incorporates uncertainties for low-coverage data (-vt-alpha and -gt-alpha with $p = .01$). We additionally used the flag -r 60 to retain only newly annotated sites found in at least 60% of the samples in the catalogue. In *VCFtools* v0.1.17 (Danecek et al., 2011), we estimated the pre-filtered sequence coverage and then subsequently filtered loci to exclude singleton and private doubleton alleles, removed loci with more than 90% missing data across all samples, and excluded individuals with more than 30% missing data. We removed loci with a minor allele frequency (MAF < 0.03) and required at least an 80% genotyping rate per locus (*-geno* 0.2) in *PLINK* v1.90b3i (Chang et al., 2015).

We used *VCFtools* for individual-level metrics of heterozygosity (observed, $H_O$; expected, $H_E$) and the two-sample Kolmogorov–Smirnov to test for statistical differences in data distributions and correlations in R (R Core Team, 2022). We then utilized the *populations* module in *STACKS* v2 to identify alleles private to each canid lineage. We further conducted a rarefaction method for private allele richness per locus while controlling for sample size variation in the number of genomes sampled in the programme *ADZE* (Szpiech et al., 2008) with the parameter G of sample size set to 100.

### 2.3 | Sex inference from sequence coverage of the Y chromosome

As we included the Y chromosome (KP081776.1; Li et al., 2013) with the CanFam3.1 reference assembly for read alignment, we used *t*-tests and the two-sample Kolmogorov–Smirnov to determine the sequence coverage differences between the sexes. This provided us an opportunity to establish a threshold of Y-specific sequence coverage to infer sex, with females inferred from falling below the threshold and males above. We then repeated analyses independently for each sex to explore the impact of sex-biased demography.

### 2.4 | Population structure and differentiation

For demographic analyses, we constructed a statistically neutral and unlinked dataset of SNPs by excluding sites within 50-SNP windows that exceeded genotype correlations of $r = .2$ (*-indep-pairwise* 50 5 0.2; a proxy for linkage disequilibrium or LD) and SNPs that

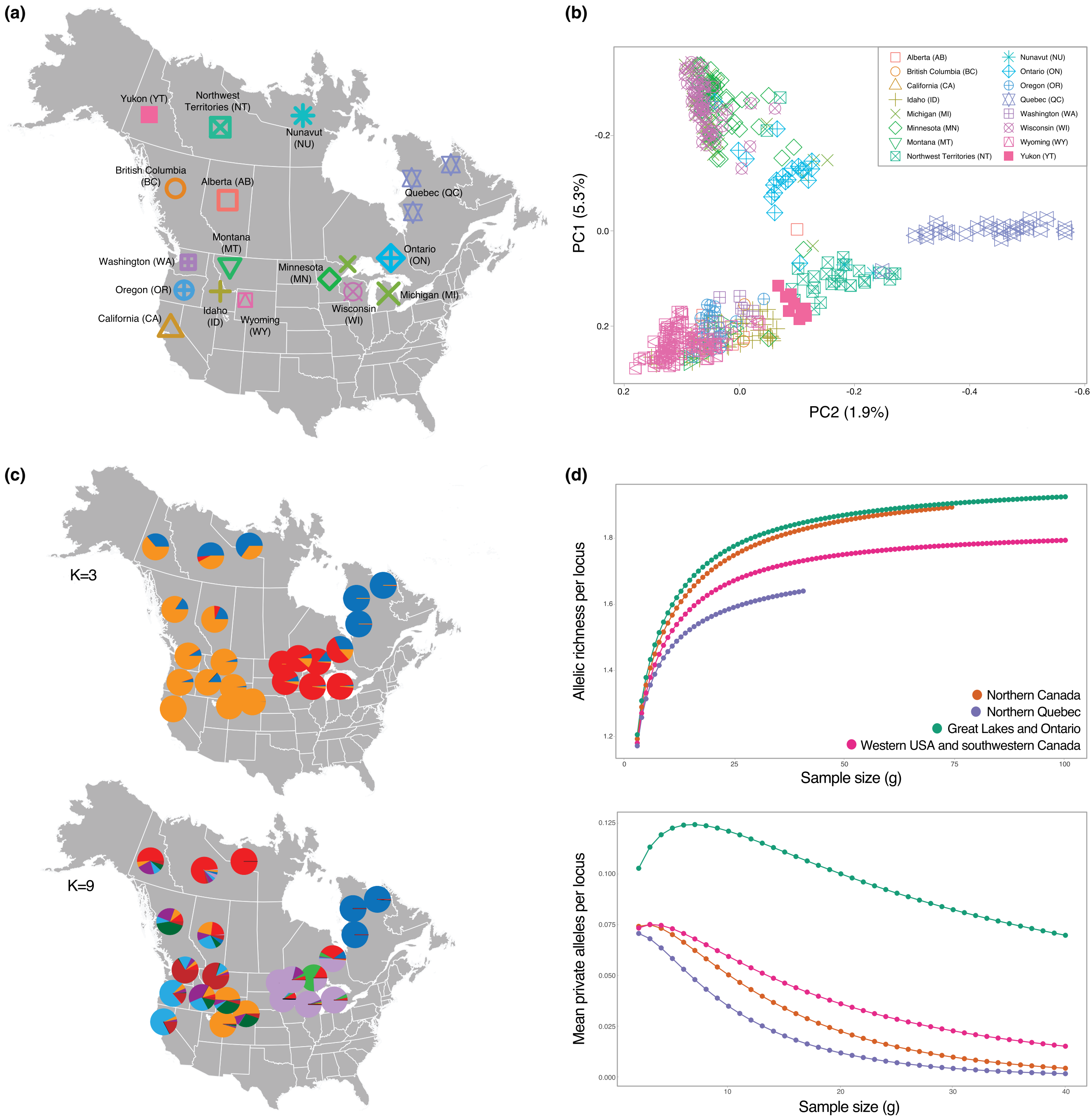


**FIGURE 1** Population genetic structure of 437 grey wolves from (a) North American populations genotyped at 81,595 statistically neutral and unlinked SNPs inferred from (b) principal component analysis (axes rotated to show geographic correspondence); and (c) a maximum-likelihood approach for three and nine partitions (map credit: Free Vector Maps WRLD-NA-01-0007). (d) Rarefaction of allelic richness and private alleles for each major geographic region of grey wolves (see Table S1).

significantly deviated from Hardy–Weinberg Equilibrium (HWE) with the argument *-hwe* 0.001. We conducted both non-model and model-based clustering analyses. We completed the former as a principal component analysis (PCA) in *FlashPCA v2.1* (Abraham et al., 2017) and the latter with an unsupervised maximum-likelihood framework with *Admixture* (Alexander et al., 2009). We analysed the fit of two to 10 partitions (*K*) with the cross-validation error (*cv*) flag. We also estimated inter-group pairwise genetic differentiation as Weir and Cockerham's $F_{ST}$ in *VCFtools* v0.1.17. We reported average $F_{ST}$ across the genome (autosomes and X chromosome combined).

## 2.5 | Inbreeding estimates from autozygosity

We analysed the minimally filtered SNP set separately for loci on the autosomes and X chromosome. These loci represented a total length ($L_{\text{genome}}$) of 2,202,059,258 and 123,842,264 nucleotides for autosomes and the X chromosome, respectively. The geographic region was used as an identifier for the function *homozyg* in *PLINK* v1.9 (Table S1). To detect autozygosity from runs of homozygosity (ROH), we used the following parameters for low-coverage data: homozyg-density 50, homozyg-gap 1000, homozyg-kb 300, homozyg-snp 50, homozyg-window-het 4, homozyg-window-missing 5, homozyg-window-snp 50, and homozyg-window-threshold 0.05 (Ceballos et al., 2018). We converted the ROH segments to an individual-level inbreeding coefficient ($F_{\text{ROH}}$) following Taboada et al. (2014):

$$F_{\text{ROH}} = \frac{\sum L_{\text{ROH}}}{L_{\text{genome}}},$$

where $L_{\text{ROH}}$ is the length of an ROH segment in an individual.

### 2.6 | Effective population size estimates

We estimated effective population ($N_e$) sizes and focused on recent (past 200 generations) estimations as presumed to be more accurate. Effective population size estimates extrapolate population parameters from genetic diversity metrics. Although dispersal and translocation events are known, the collection of genetic variation is the core of such inference and is bounded by how a population is defined in time and space. Here, we implemented the algorithm in *GONE* (Santiago et al., 2020), which is an LD-based method that accounts for drift (i.e. finite census size) and makes use of recombination rates but is influenced by both population structure and admixture. *GONE* leverages a genetic algorithm from Mitchell (1998) to search across sequences of possible historical effective population sizes that best explain the spectrum of observed LD values to minimize the sum of squares of the differences between observed and expected allelic covariances. We assumed unphased data, no MAF pruning, a maximum of 50,000 SNPs considered per chromosome, and ignored pairs of SNPs with recombination rate over 0.05, as recommended for the software. A constant rate of recombination of 1 cM per Mb was assumed across the genome. We estimated $N_e$ sizes at two levels: each major geographic region and population designations for management implications in the United States. However, resulting estimates for the wolf populations in Canada should be interpreted with caution given our limited genotype surveillance across the region. We estimated $N_e$ from autosomal SNP data and translated generations into years using 4 years per generation as the unit of time (Mech et al., 2016; vonHoldt et al., 2008). We believed that only the minimally filtered RADseq data (i.e. missingness and MAF) was appropriate for these estimates (Beichman et al., 2017). Finally, we were conservative when interpreting 'present-day effective population size' as the most recent four generations for $N_e$ are considered a single analytical block by GONE. Hence, we used the $N_e$ average of generations 1–8 to avoid biases from any lingering artefact in generations 1–4 (Novo et al., 2023). We also focus on reporting the results of the last 50 generations (approximately 200 years) as that is most pertinent to the recent population demography and conservation considerations.

We then assessed how well the effective population size estimates explain the expected decay in heterozygosity using the formula when $t = 8$:

$$f_t = 1 - \left(1 - \left(\frac{1}{2N_e}\right)\right)^t.$$

### 2.7 | Admixture is part of the history of the western Great Lakes grey wolf population

We rediscovered SNPs with the addition of BAM files from previously published canids: 106 reference western coyotes (*C. latrans*) from vonHoldt et al. (2022) and 30 reference eastern wolves (*C. lycaon*) from Heppenheimer et al. (2018) (Table S1b). The grey wolves in the Great Lakes region are known to have a history of admixture with both coyotes and eastern wolves (Heppenheimer et al., 2018; vonHoldt et al., 2011). The predominant signal described to date is that Great Lakes region grey wolves have partial coyote ancestry with grey wolves of southeastern Ontario carrying more partial ancestries of eastern wolves. These were merged with the BAM files from the population of northern Rocky Mountains and western Great Lakes samples to explore the impact of coyote and eastern wolf admixture on grey wolf genetic estimates. We followed the same analysis and filtering methods as described above to obtain a statistically unlinked and neutral set of SNP loci. We conducted an unsupervised assignment analysis for $K = 2$–10 in *ADMIXTURE* and complemented with genetic differentiation ($F_{ST}$) estimates using *VCFtools* v0.1.17.

### 2.8 | Reliable inferences from reduced representation low-coverage population-level genotype data

Population genomic studies can leverage the affordable technologies of reduced representation data collection methods, such as RADseq, to collect genotype data from hundreds or thousands of individuals. The drawbacks are obvious in terms of missing rare alleles or allele dropout rates due to the nature of the library preparation. Thus, studies have assessed the biases and challenges of low-coverage data (3–6×) compared to whole-genome sequence (WGS) and found that the former can be equally informative with careful adjustments to methods and inferences (Ceballos et al., 2018; Duntsch et al., 2021). It is known that some population metrics like ROH are expected to be biased. For example, low-coverage data likely underestimate the frequency of small and overestimate larger ROH fragments (Lavanchy & Goudet, 2023).

## 3 | RESULTS

We sequenced 482 grey wolf samples from North America, collected between 1986 and 2021 when known, with an average fold sequence coverage of 7.3 (±3.4) to discover 1,099,764 raw, RAD loci that passed our *STACKS* filtering parameters but prior to population-level filtering (Table S1). We excluded 45 wolves due to high (>20%) missingness and repeated the filtering. The result is a dataset of 162,109 minimally filtered SNPs genotyped in 437 grey wolves from Canada ($n=92$) and the United States ($n=345$), with a subset of 81,595 loci referred to as the 'statistically neutral and unlinked' SNPs.

We inferred sex for individuals bioinformatically based on the depth of reads mapped to the Y chromosome. Of the 437 wolves, field-based observations identified 104 females and 118 males. When we presumed these samples having correct sex inference, the average sequence depth on the Y chromosome was significantly enriched in males (females = 3406.9, males = 25587.3, 1-tailed *t*-test of unequal variance $t=-17.99$, df = 219.7, $p<10^{-16}$) and these two distributions are significantly different (two-sample Kolmogorov–Smirnov $D=0.802$, $p<10^{-16}$) (Figure S1a). We inferred 205 females who had a sequence coverage <10,000× (average Y chromosome sequence coverage = 594.2) and 232 males with >18,000× (average coverage = 28,454.1), where these two inferred sequence coverage distributions were again statistically divergent (two-sample Kolmogorov–Smirnov $D=1.0$, $p<10^{-16}$) (Figure S1b).

## 3.1 | Grey wolves are genetically and geographically structured across North America

We presented two levels of genetic structure across the North American continent that reflect the geographic assignment probabilities for two cluster analyses: the PCA ($K=3$) and the best supported partition from maximum-likelihood inference ($K=9$) (Figure 1b,c; Figure S2). Three genetic clusters broadly represent three distinct demographic histories of western, central, and eastern regions of the continent. We divided the western cluster into two subclusters, one to reflect the shared demography of southwestern Canada and western USA through the translocation and colonization of wolves in the northern Rocky Mountains population, and the other representing northern Canada (Table S1). The other two clusters represent northern Quebec and the shared demography of Ontario and the western Great Lakes population (Table S1). Out of these four geographic groupings, we found that only two groups carried private alleles (western USA and southwestern Canada, $n=332$; Great Lakes and Ontario, $n=6801$) out of 162,109 SNPs. A rarefaction analysis mirrors the demographic history of each, with the Great Lakes and Ontario regional group showing the highest level of allele richness and mean number of private alleles per locus controlled for sample size differences (Figure 1d), likely due to their known history of coyote and eastern wolf admixture (Koblmüller et al., 2009; vonHoldt et al., 2016). Finer-scale clustering revealed a stronger role of geographic isolation, with more resolution of substructure within USA's northern Rocky Mountains and the Pacific Northwest regions (Figure 1c). The shared assignments across three genetic partitions reflect the shared genetic ancestry across large geographic distances due to the translocation of grey wolves in 1995 and 1996 (British Columbia, Alberta, and Montana) to central Idaho and the Greater Yellowstone Ecosystem (mean *Q*: partition 1 BC = 0.43, ID = 0.14, GYE = 0.22; partition 2 BC = 0.25, ID = 0.40, GYE = 0.07; partition 3 BC = 0.09, ID = 0.13, GYE = 0.65). Populations with shared demographic histories (northern Canada vs. western USA and southwestern Canada, $F_{ST}=0.034$) had the lowest levels of genetic differentiation while the highest was found between opposite coasts of the continent (western USA and southwestern Canada vs. northern Quebec, $F_{ST}=0.084$) (Table 1, Figure S3). We find that all genetic differentiation distributions are significantly distinct (Table S2). We assessed this metric for females and males separately for two geographic regions (western USA and southwestern Canada; Great Lakes and Ontario). While northern Rocky Mountain grey wolves showed variable levels of differentiation within the region ($F_{ST}$ genome = 0.0–0.13, X = 0.0–0.09), females were significantly higher levels of genome-wide differentiation to other females (female–female $F_{ST}=0.052$) than males (male–male $F_{ST}=0.032$, 1-tailed *t*-test of unequal variance $p=.01207$) (Figure S4a). In contrast, western Great Lakes grey wolves had much lower intra-region genetic differentiation ($F_{ST}$ genome = 0.0–0.03, X = 0.0–0.04), with no significant differences between males and females ($F_{ST}$ female–female = 0.017, male–male = 0.019, $p=.3242$) (Figure S4b).

## 3.2 | Genomic diversity and inbreeding coefficients are variable across continental North America

Northern Quebec grey wolves had the highest levels of observed and expected heterozygosity estimates ($H_O=0.284$), followed by equivalent levels found among northern/southwestern Canada and the western USA regions ($H_O=0.223$ and 0.220), and the Great Lakes

**TABLE 1** Average and weighted Weir and Cockerham estimates (above and below diagonal, respectively) of genetic differentiation ($F_{ST}$) across 81,595 SNPs between geographic regions of grey wolves (see Figure 1a for population abbreviations).

| Geographic group (*n*) | Population(s) | Northern Canada | Western USA and southwestern Canada | Northern Quebec | Great Lakes and Ontario |
|---|---|---|---|---|---|
| Northern Canada (42) | NT, NU, YT | – | 0.034 | 0.052 | 0.052 |
| Western USA and southwestern Canada (182) | AB, BC, CA, ID, MT, OR, WA, WY | 0.033 | – | 0.084 | 0.056 |
| Northern Quebec (24) | QC | 0.071 | 0.094 | – | 0.073 |
| Great Lakes and Ontario (189) | MI, MN, ON, WI | 0.054 | 0.065 | 0.090 | – |

and Ontario region carried the lowest ($H_O$ = 0.210) (Table 2a). Only northern Canada and Quebec significantly differed from expected, with the latter found to have significantly higher observed heterozygosity than expected (Table 2a). We further report the expected positive correlation between the number of autosomal ROH segments and inbreeding estimates ($R$ = .77), with a weaker yet similar trend for the X chromosome ($R$ = .44). Autosomal inbreeding levels were highest in the wolves of western USA and southwestern Canada ($F_{ROH}$ = 0.296), which were not significantly different from northern Canada ($F_{ROH}$ = 0.278) or northern Quebec ($F_{ROH}$ = 0.267). Wolves of the Great Lakes/Ontario ($F_{ROH}$ = 0.199) had significantly lower inbreeding levels ($F_{ROH}$ = 0.278) than the other geographic regions.

## 3.3 | The northern Rocky Mountain population is genetically distinct

To provide information relevant to ongoing management considerations and decisions, we partitioned the samples to analyse only those belonging to the populations identified in the United States, the northern Rocky Mountains ($n$ = 188) and the western Great Lakes ($n$ = 199). The preceding analysis identified the distinctiveness between the northern Rocky Mountains and western Great Lakes population segments as per their divergent assignment probabilities ($K$ = 3 and $K$ = 9) (Figure 1b,c, Figure S2). We found that six (4.5%) of the northern Rocky Mountains wolves had assignments to a cluster divergent from their geographic origins at $K$ = 3 (when $Q > 0.00001$, $Q$ = 0.01–0.25), all of which were individuals sampled in the Pacific Northwest. The misclassification of western Great Lakes wolves is more varied due to assignments to the proximate Canada wolf populations at $K$ = 3 ($Q$ = 0.01–0.86). This pattern continued at $K$ = 9, where the highest non-Rocky Mountains assignments were wolves assigned to Canada's Northwest Territories Province ($Q$ = 0.01–0.37), concordant with a shared demographic history. We identified seven western Great Lakes individuals with assignments (several samples in Isle Royale NP, $Q$ = 0.01–0.56) to Canada's Northwest Territories Province, two assigned to Idaho (sampled in MN and WI, $Q$ = 0.99), and one to Oregon/California (sampled in MN, $Q$ = 0.99), with several demographic processes to explain such signatures (e.g. admixture, shared ancestry, recent dispersal, statistical noise).

Although we found that the northern Rocky Mountains and western Great Lakes populations carried comparable observed heterozygosity levels ($H_O$, $H_E$ = [0.211, 0.224] and [0.211, 0.211], respectively), the per-state composition was quite variable (Table 2b). Estimations at the state level revealed that in the northern Rocky Mountains, the four samples from California were the most genetically diverse ($H_O$ = 0.562), followed by Montana (0.333), Washington (0.298), Oregon (0.285), Idaho (0.245), and Wyoming (0.238) (Table 2b). In the

TABLE 2 Average expected and observed heterozygosity ($H_E$ and $H_O$, respectively) and effective population size ($N_e$ from past 50 generations) estimates for each (a) major geographic location ($p$-values are from a Welch two-sample $t$-test of unequal variance between $H_E$ and $H_O$) and (b) regional population within the United States. Diversity estimates were derived from the statistically neutral SNP set while effective population size estimates from the minimally filtered SNP set.

| (a) | | | | |
|---|---|---|---|---|
| **Geographic group (*n*)** | **$H_O$** | **$H_E$** | **t, df, p** | **$N_e$** |
| Northern Canada (42) | 0.223 | 0.233 | $t = -3.96$, df = 41.5, $p = 2.879 \times 10^{-4}$ | 3050.1 |
| Western USA and southwestern Canada (182) | 0.220 | 0.222 | $t = -1.10$, df = 189.3, $p = .2721$ | 1240.5 |
| Northern Quebec (24) | 0.284 | 0.274 | $t = 4.82$, df = 23.7, $p = 6.777 \times 10^{-5}$ | 275.4 |
| Great Lakes and Ontario (189) | 0.210 | 0.211 | $t = -0.57$, df = 201.9, $p = .5682$ | 524.8 |
| **(b)** | | | | |
| **Population (*n*)** | **$H_O$** | **$H_E$** | **t, df, p** | **$N_e$** |
| Northern Rocky Mountains (176) | 0.211 | 0.224 | $t = -1.0$, df = 182.9, $p = .3127$ | 1274.3 |
| California (4) | 0.562 | 0.455 | $t = 18.9$, df = 3.2, $p = 2.058 \times 10^{-4}$ | |
| Idaho (43) | 0.245 | 0.243 | $t = 0.73$, df = 43.0, $p = 0.4678$ | |
| Montana (12) | 0.333 | 0.313 | $t = 1.9$, df = 11.0, $p = .9008$ | |
| Oregon (21) | 0.285 | 0.263 | $t = 6.6$, df = 21.8, $p = 1.403 \times 10^{-6}$ | |
| Washington (15) | 0.298 | 0.282 | $t = 2.3$, df = 14.5, $p = .03465$ | |
| Wyoming (81) | 0.238 | 0.235 | $t = 1.3$, df = 82.9, $p = .1958$ | |
| Western Great Lakes (168) | 0.211 | 0.211 | $t = -0.34$, df = 180.8, $p = .7351$ | 484.8 |
| Michigan (49)[a] | 0.219 | 0.226 | $t = -1.6$, df = 50.4, $p = .1082$ | |
| Minnesota (62) | 0.225 | 0.223 | $t = 1.0$, df = 67.5, $p = .306$ | |
| Wisconsin (57) | 0.231 | 0.223 | $t = 3.5$, df = 61.4, $p = 9.81 \times 10^{-4}$ | |

Abbreviation: $n$, sample size.

[a] Includes grey wolves from Isle Royale National Park in Lake Superior.

western Great Lakes, Michigan (including wolves on Isle Royale) had the lowest estimates ($H_O=0.219$) compared to Minnesota (0.225) and Wisconsin (0.231). We restricted the analysis to samples only with known years of sample collection between 1990 and 2020 within the population of the northern Rocky Mountains ($n=137$) and western Great Lakes ($n=86$) to survey changes in diversity over time. Using Pearson's product–moment correlation, we found that all heterozygosity estimates for the northern Rocky Mountains population significantly declined over the 30 years surveyed ($H_O$: $R=-.41$, $p=8.3\times10^{-7}$; $H_E$: $R=-.46$, $p=1.2\times10^{-8}$) (Figure 2a). Although the WGL population shows a similar albeit weaker pattern of decline, there was no statistical significance ($H_O$: $R=-.08$, $p=.47$; $H_E$: $R=-.12$, $p=.26$) (Figure 2b). Females in the northern Rocky Mountains population were significantly more differentiated from each other than males across the genome (mean $F_{ST}=0.052$ and 0.032, respectively; 1-tailed $t$-test of unequal variance $p=.01207$) and the X chromosome ($F_{ST}=0.051$ and 0.029; $p=.0051$) (Figure S4). This pattern was not found in the females of the western Great Lakes population (genome: $F_{ST}=0.017$ and 0.019; $p=.3242$; X chromosome: $F_{ST}=0.016$ and 0.012; $p=.1876$).

The northern Rocky Mountain grey wolves had significantly higher autosomal inbreeding coefficients compared to the western Great Lakes, which differences across the X chromosome were not significant ($F_{ROH}$, autosomes: RM$=0.299$, GL$=0.211$, $t=8.5$, df$=309.6$, $p=8.67\times10^{-16}$; X chromosome: RM$=0.076$, GL$=0.070$, $t=0.8$, df$=260.3$, $p=.4473$) (Figure S5). The outlier inbreeding coefficients for western Great Lakes can be attributed to the small and isolated grey wolf population living in Isle Royale National Park.

### 3.4 | Population effective size estimates show the continental history of extermination and recovery

We inferred population effective sizes for the past 50 generations (approximately 200 years) from autosomal SNPs for each of

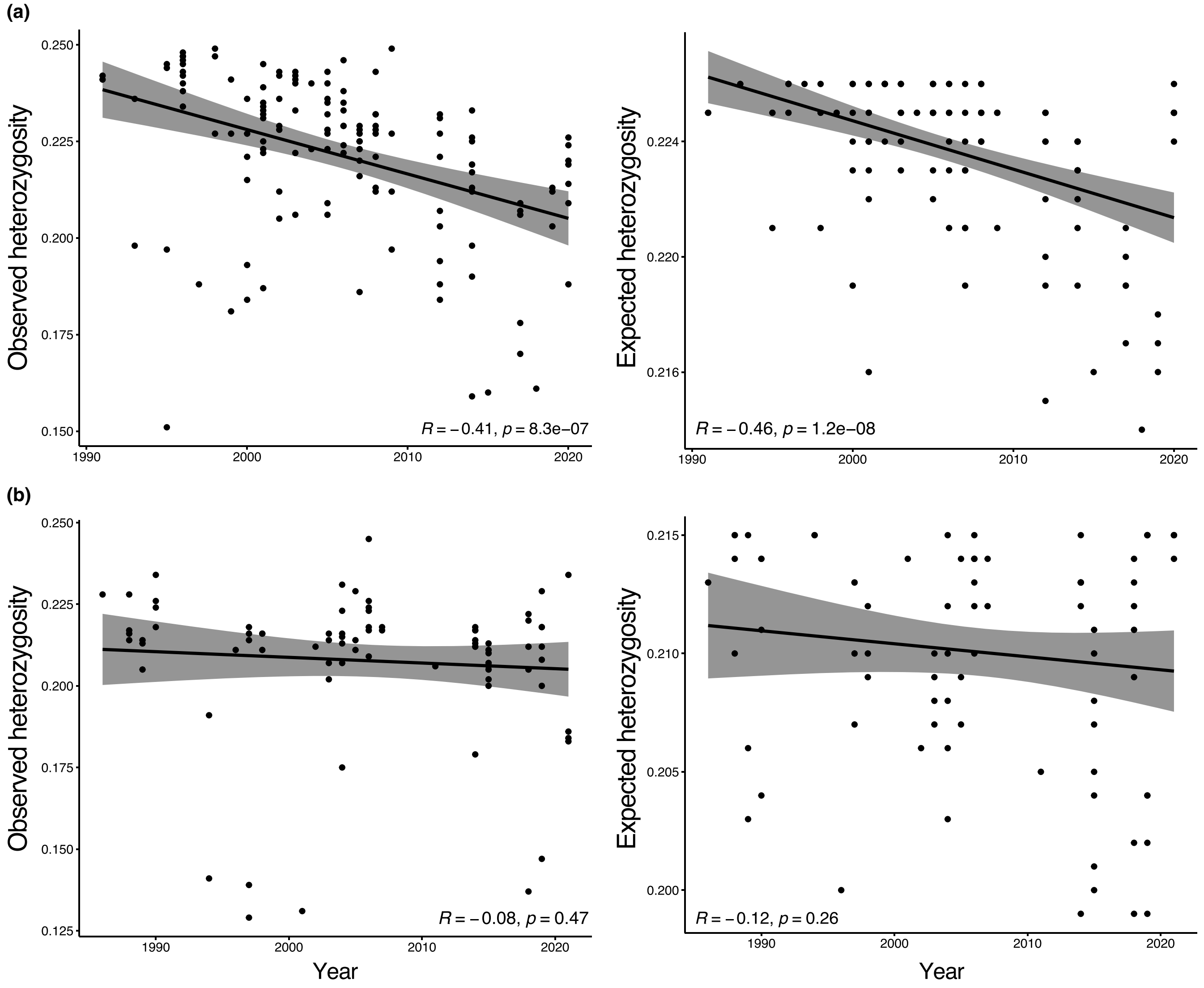


FIGURE 2 Heterozygosity (observed and expected) trends for the (a) northern Rocky Mountain ($n=137$) and (b) western Great Lakes ($n=86$) distinct population segments in the United States for a 30-year period between 1990 and 2020 (Y-axis). Pearson correlation coefficients and significance values are provided. Shaded area indicates the 95% confidence interval.

the four regional genetic clusters that carried genetic distinction. We estimated $N_e$ ranged between 63.0 and 3848.5 over the past 50 generations at a regional scale (Figure 3a; Table S3). Northern Canada had the highest historical size estimated at 3848.5 wolves 36 generations (144 years) ago, with western USA/southwestern Canada next largest for estimates of 1989.4 wolves 41 generations (164 years) ago, then Great Lakes/Ontario with 878.7 wolves (45 generations or 180 years ago), and finally northern Quebec at low estimates maxing at 464.8 wolves 46 generations (184 years) ago. We found a significant positive relationship between regional effective population size and number of generations before present (Pearson's product–moment correlation $R = .39$, $t = 7.3$, $df = 298$, $p = 2.03 \times 10^{-12}$) (Figure S6). When we restricted our analyses to the two populations, we found that the northern Rocky Mountains displayed a steep and rapid effective rate of loss ($m = -45.6$) per generation while the western Great Lakes population's decline was shallower ($m = -14.4$) (Figure 3a). The northern Rocky Mountains experienced a dramatic shift 20 generations ago losing 72.8 wolves per generation. In that same time frame, the western Great Lakes was losing 4.0 wolves per generation. Their current-day respective estimates are $N_{e\_RM} = 141.7$ and $N_{e\_GL} = 226.3$, after having effective population size estimates reduced by 1928.6 and 542.1 wolves, respectively (Table S3).

We further compared population estimates for the northern Rocky Mountains and western Great Lakes populations obtained from management, agency, and public reports between 1982 and 2015 (Table S4). Both regional populations have a history of substantial expansion in census population sizes between 1982 and 2010 when the northern Rocky Mountains were estimated to have $N \sim 1723$ and western Great Lakes at $N \sim 4321$ wolves, remaining mostly stable to the present-day estimates of $N \sim 1881$ and 3025, respectively (Figure 3b). We estimated that the western Great Lakes effective population size has remained stable since 1990 with an average rate of growth larger than that of the northern Rocky Mountains (GL $m = 0.21$; RM $m = -0.05$), with significantly higher effective population size estimates for western Great Lakes ($N_e = 226.6$) than the northern Rocky Mountains ($N_e = 143.8$) (*t*-test unequal variance $p = 1.420 \times 10^{-11}$). Lastly, we estimated the temporal trend of $N_e/N$ collectively for the northern Rocky Mountains and the western Great Lakes and found the effective population size remained at 5.2–9.3% of the census size since mid-2000s (Figure 3b).

We estimated that the decay in heterozygosity for the northern Rocky Mountains had an initial level of $H_O \sim 0.235$ in 1991 and decayed to 0.208 by 2020 (approximately eight generations) (Figure 2a). When we use the estimated average effective population size $N_e = 141.7$ for the northern Rocky Mountains during that time (Table S3), we estimate that the observed heterozygosity should decay by 0.032 to $H_O = 0.203$, which is within the 95% confidence interval (Figure 2a). We found the same trend for the western Great Lakes ($H_O \sim 0.213$ and 0.213 in 1988 and 2020, respectively), estimated to decay by 0.016 to $H_O = 0.197$.

## 3.5 | Admixture with coyotes and eastern wolves is unique to the Great Lakes grey wolves

We created a second dataset that included western coyotes and eastern wolves to explore signatures of admixture in the grey wolves of the Great Lakes region. We discovered 163,314 genomic loci genotyped in 465 canids (179 grey wolves from the northern Rocky

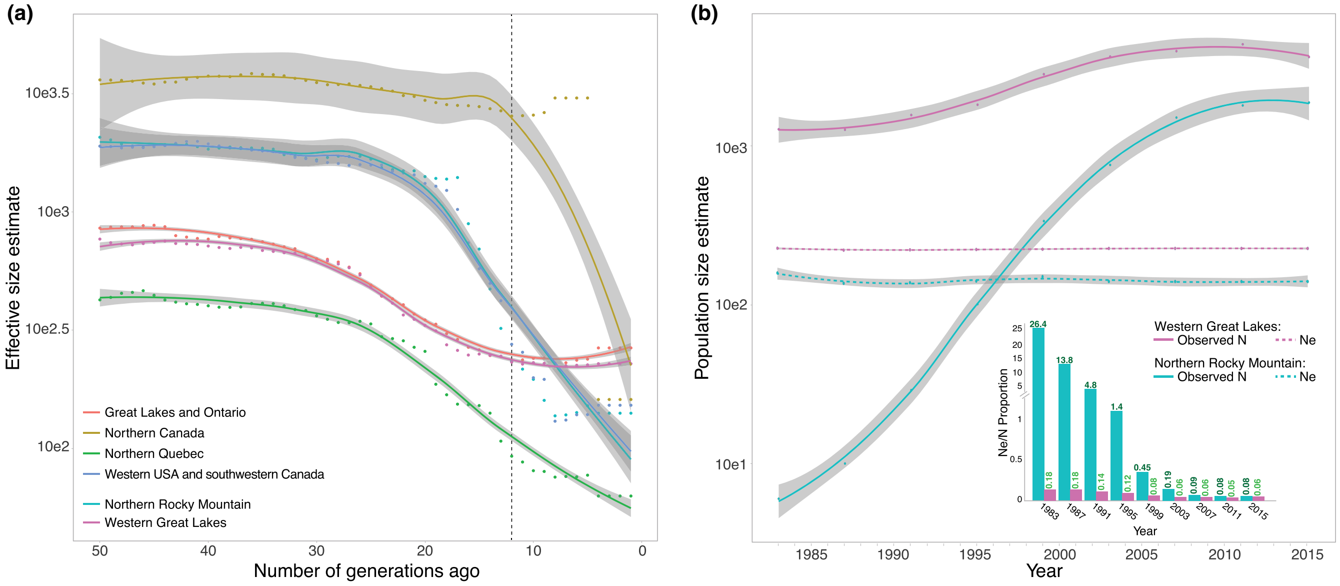


FIGURE 3 Locally estimated scatterplot smoothed (loess) trend lines of population effective size ($N_e$) histories for (a) each of the four identified regional genetic clusters and the regional populations in the United States. The vertical dashed line in each panel indicates the acceptance of the U.S. Endangered Species Act into law in 1973. (b) Observed ($N$) and inferred population effective size ($N_e$) histories for the northern Rocky Mountain and the western Great Lakes populations in the United States. We assumed 4 years per generation. The inset displays the ratio of $N_e$ to $N$ since 1982–2015 for each of the two populations with values included.

Mountains population, 184 from the western Great Lakes population, 74 western coyotes, and 28 eastern wolves). We also constructed a statistically neutral and unlinked dataset of 80,655 SNPs. At the highest level of partition ($K=10$), we found that grey wolves of the western Great Lakes population had the highest average (±sd) probability assignment to clusters of other Great Lakes grey wolves ($Q=0.64\pm0.4$) and <10% to any other wolf group (3.4±0.1% assignments to eastern wolves; <2% to Rocky Mountain grey wolves), with minimal assignments to western coyotes ($Q=0.01\pm0.1$) (Figure S7; Table S5). Rocky Mountain grey wolves similarly formed their own cluster ($Q>0.97$) with low, albeit detectable, partial assignments of Wyoming grey wolves with coyotes (<2%) and <1% to all other canid groups. The unsupervised cluster analysis was further supported by western Great Lakes population grey wolves having the lowest genetic differentiation estimates with eastern wolves ($F_{ST}=0.06$ and weighted $F_{ST}=0.08$) and western coyotes ($F_{ST}=0.09$ and 0.12), in contrast to the estimates between northern Rocky Mountains population grey wolves and eastern wolves ($F_{ST}=0.10$ and 0.10) or western coyotes ($F_{ST}=0.12$ and 0.15).

## 4 | DISCUSSION

An estimate of the effective population size provides a means by which conservation practitioners can accurately use theory to predict forward-in-time outcomes for various viability scenarios for an endangered species (Lacy, 1995). These estimates permit one to estimate the number of generations until gene flow is required to boost the genetic diversity and concomitantly reduce inbreeding coefficients. The application of this theory to wild endangered or threatened populations has remained challenging but is centrally needed for conservation planning and simulating evolutionary outcomes (Frankham et al., 2019). One complication in the interpretation of effective population sizes is the sensitivity of these estimates to population structure (Ellegren & Galtier, 2016). Grey wolves inhabiting North America represent a diversity of demographic histories and contemporary dynamics that manifest as distinct genomic signatures. Local adaptation, compounded with social structure of grey wolves, generates population structure and increases the rate at which random genetic drift depletes their genomic variation and evolutionary potential. When geographic regions experience local extinctions from over-exploitation, dispersals will re-populate the new vacancy and genetically homogenize across proximal subpopulations over time (Ausband & Waits, 2020). Despite these recent demographic events of reintroduction or re-population, observed heterozygosity is lower than expected with significant genetic structure across the continent. As per theory, this suggests that the effective population sizes calculated here for each grey wolf population are impacted (Ellegren & Galtier, 2016).

The comparison of the census and effective population sizes provides a more valuable metric beyond census size alone. For species with social organization, substructure, and non-random breeding, theory expects that effective population size will be a fraction of the census size (Ellegren & Galtier, 2016; Frankham, 1995). Although there are many field methods for estimating the ratio of census size to $N_e$, these are often challenging and require an immense effort in the field. For example, using wolf dispersal and density data on the Perch Lake pack ($N_m=5$, $N_f=5$) in Minnesota, Chepko-Sade et al. (1987) estimated effective population size with two methods: the root mean square (variance) method ($N_e=804$) and the 85th percentile distance of the original dispersal distribution method ($N_e=1660.7$). In comparison, we provided a genomic $N_e$ estimate of 222.6 wolves in 1987 for the western Great Lakes, roughly 13–28% of that derived from wolf dispersal and density data. Further, earlier population estimates from 26 microsatellite data of Yellowstone National Park wolves reported $N_e$ ranging between 6 and 22.6 for 1995–2004 and the respective census sizes of 21 and 80 (range $N_e/N=0.10$–0.37) (vonHoldt et al., 2008). Genomic-based inferences still face challenges albeit different from field-based inferences; regardless, estimates are critical for shaping appropriate conservation management plans. Understanding this relationship is important because management applies to actual populations which are observed and managed based on census size, not effective population size. Using genomic data from these populations, we show that this ratio is different in different parts of the distribution. Overall, the census and effective population sizes differ by approximately an order of magnitude.

We conducted a population genome-level survey of three genetic groups of grey wolves across North America and resolved deeper fine-scale resolution that was reflective of geography and demographic history. These groups correspond to the Great Lakes region, northern Quebec, and the western region of Canada and the United States. While all the populations we studied have a history of over-exploitation, each group has unique aspects to their population histories. The grey wolves of the Great Lakes carry a genetic signature of historic admixture (Heppenheimer et al., 2018; Koblmüller et al., 2009; Leonard & Wayne, 2008; Rutledge et al., 2010; vonHoldt et al., 2011, 2016), and habitat loss has been of consequence to wolves in northern Quebec (Larivière et al., 2000). The genetic cluster composed of the continent's western region is likely due to the shared ancestry when wolves were translocated from west-central Canada as founders for the populations in the northern Rocky Mountains with recent dispersal across the region (Hendricks, Schweizer, Harrigan, et al., 2019; vonHoldt et al., 2010).

### 4.1 | Northern Rocky Mountain grey wolves have declining genetic diversity

Grey wolves were restored in the northern Rocky Mountains through a reintroduction programme in the mid-1990s and a handful of dispersing wolves southward from Canada into northwestern Montana, which successfully established several populations that contributed towards the first of many delisting proposals for this population in 2003. A study by vonHoldt et al. (2010) provided the first evaluation of genetic structure, diversity, and connectivity over the initial 10-year recovery period (1995–2004) inferred from microsatellite markers and reported no immediate concerns for genetic variability. However, genome sequencing advances have provided the grey wolf with a plethora of new genetic methods that avoid some central and limiting concerns when using microsatellite markers (Väli et al., 2008). As such, we encourage genetic surveys of grey wolves to consider a genome-wide reduced representation or targeted sequence-based method for large-scale population studies, which is feasible for any sample type and is less prone to calibration and ascertainment concerns of microsatellites collected across facilities, platforms, and research groups (Bonin et al., 2004; Pompanon et al., 2005).

We found genetic evidence of dispersal patterns in the Pacific Northwest, where genetic signatures clearly identified that these western continental wolf populations relied upon male-mediated dispersal for gene flow. We also detected signatures that female wolves across the western USA and southwestern Canada were significantly more differentiated from each other than males. In contrast, this pattern was not found in the females of the Great Lakes and Ontario region, likely an interaction between the population never being fully eradicated and an evolutionary history of genetic admixture with coyotes. Further, we report evidence of both significantly lower levels of genomic diversity in the northern Rocky Mountains paired with eroding diversity and higher inbreeding coefficients since 1990, explained in part by our new effective population size estimates. This temporal decline in genetic diversity was not found in the western Great Lakes wolves. One limitation is that our genetic focus does not explore the fitness effects of such trends; however, such metrics are often central in conservation strategies. Although we currently do not report on fitness-related consequences, evaluations of such have been conducted on highly bottlenecked and inbred populations like Isle Royale and Scandinavia (Åkesson et al., 2022; Hagenblad et al., 2009; Robinson et al., 2019). The wolves of the northern Rocky Mountains currently have an increased mortality rate due to relaxed regulation. Notwithstanding, grey wolf life history of short time to sexual maturity, large litters, and dispersal can mitigate population-level risks from human-related mortality (Adams et al., 2008; Fuller et al., 2003). However, Cassidy et al. (2022) recently found significant effects of human-caused mortality on other important biological processes in wolves (e.g. pack persistence and pup production) that have implications for breeding and gene flow. Given the difficulty states have faced in meeting their goals of significant population reduction (e.g. Idaho's goal of 500 wolves with an estimated 1270 census size, Idaho Fish and Game Grey wolf management plan draft January 2023), the effective population size estimates are then interpreted to be strongly influenced by the number of breeding wolves and gene flow, less from census size. Current management actions that seek to reduce overall populations and permit hunting during the breeding season have the greatest potential to have negative consequences on effective population sizes.

## 4.2 | Great Lakes grey wolves have a unique demographic history

Following theoretical expectations, the level of genetic richness and uniqueness is correlated with the western Great Lakes wolf demographic history of colonization and admixture (Allendorf et al., 2001). In agreement with previous findings, western Great Lakes wolves carry the lowest levels of inbreeding and the highest levels of allelic richness and private alleles. This is explained by their historic genetic exchange with other sympatric canid lineages, supported by both genetic cluster analysis and the lowest genetic differentiation with eastern wolves ($F_{ST}=0.06$ and weighted $F_{ST}=0.08$) and western coyotes ($F_{ST}=0.09$ and 0.12), in contrast to the estimates between northern Rocky Mountains population grey wolves and eastern wolves ($F_{ST}=0.10$ and 0.10) or western coyotes ($F_{ST}=0.12$ and 0.15). This demography is unique and provides an immediate mechanism by which these populations can respond to a rapidly changing world in terms of both climate and anthropogenic activity (Carmichael et al., 2008; Kagawa & Seehausen, 2020; Ottenburghs, 2021; Pacheco et al., 2022; Rius & Darling, 2014; vonHoldt et al., 2017).

## 4.3 | Conservation decisions in light of effective size estimates

We compiled reported population sizes across the states that compose the northern Rocky Mountains and western Great Lakes population between 1982 and 2015 from public data and found that grey wolf effective population sizes were 5.2–9.3% of the census size. Peterson et al. (1998) used demographic models of $N_e$ for Isle Royale and estimated an $N_e/N$ ratio of 16%. Further, many wild canid species will avoid mating with relatives (Ausband, 2022; Geffen et al., 2011; Sparkman et al., 2012; vonHoldt et al., 2008), and this inbreeding avoidance mechanism will increase $N_e$. Our estimates are comparable to those for the cooperative breeding African wild dog (*Lycaon pictus*) where effective population sizes are 8.7–11.3% of the census size (Marsden et al., 2012). According to international conservation goals of the '50/500 rule', the genetic consequences of population subdivision are strongest in small ($N_e<500$) isolated populations where inbreeding depression occurs, and genomic diversity erodes due to drift. Thus, successful short-term conservation efforts can target $N_e \sim 50$ but should target $N_e>500$ for the long-term survival of a species (Caballero et al., 2017; Frankham et al., 2014; Jamieson & Allendorf, 2012; Pérez-Pereira et al., 2022). As per this rule, we show that grey wolves fall above minimum effective population sizes needed to avoid extinction due to inbreeding depression in the short-term but face long-term risk of extinction on their own given their present-day effective population sizes ($N_e \sim 142.7$–226.3).

A similar situation was also found for Scandinavian wolves, with realized $N_e$ below advised conservation goals (Laikre et al., 2016). Their ultimate suggestion was to increase $N_e$ and promote methods that would increase genetic exchange via 3–5 effective migrants per generation with neighbouring populations. Notably, such goals are clearly possible within the ESA framework which defines 'conservation' in section 3 to include 'the use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures provided pursuant to this Act are no longer necessary'. There are known dispersers, albeit unknown if they are effective dispersers, between southwestern Canada and the U.S. Rocky Mountains. Combined with the shared ancestry due to translocation from the western Canada and northern Rocky Mountain grey wolf populations, demography is a core feature that shapes conservation-relevant metrics. Further, wolves in North America can originate from dramatically different regions with distinct collections of local adaptations and ecotypes (Carroll et al., 2020; Hendricks, Schweizer, & Wayne, 2019; Schweizer et al., 2016). The suggested effective migrant strategy would require more consideration of regional signatures of adaptive variation (Carroll et al., 2020). We envision this study as a baseline for future assessments.

### 4.4 | Genetic conservation of grey wolves

Species recovery plans are constructed around a core conservation biology framework referred to as 'The Three R's' (representation, resiliency, and redundancy) for reducing the risk of extinction (Shaffer & Stein, 2000). Under the ESA, this can be satisfied by maintaining multiple large, genetically robust populations across the historic range that are self-sustaining. Grey wolves have already met many of these aspects, with several populations found across the United States, and natural dispersal occurring to help occupy portions of their historic range, although the species still only occupies approximately 10–15% of its historical range (Carroll et al., 2006). With fluctuating federal protection, populations can recover, be delisted, experience reductions through human-caused mortality, and then return to federal protection, thus restarting the cycle. In addition to jurisdictional issues within the United States (Smith et al., 2016), there are also international challenges. Both populations considered here are part of a larger grey wolf population that is distributed across the United States and Canada border, making their conservation status dependent upon biological and social conditions in both countries. Joint USA–Canada conservation plans and actions have been successfully executed in the past (Bangs & Fritts, 1996), but international coordination can be complicated to maintain (Quevedo et al., 2019). Any disruption of dispersal across this international line, or decline in one country, would impact the population viability of the wolves. The Assistant Secretary of the Interior is quoted, regarding the ESA that '...it is in the best interests of mankind to minimize the losses of genetic variations. The reason is simple: they are potential resources. They are keys to puzzles which we cannot solve, and may provide answers to questions we have not yet learned to ask' (H.R. Rep. No. 93-412, pp. 4–5, 1973). Such Congressional intent clearly displays the intent of including all means for the conservation of genetic variation. Further, human activity homogenizes the landscape on which endangered species rely, and such activities '... threaten their – and our own – genetic heritage. The value of this genetic heritage is, quite literately, incalculable' (93D Congress Report, 1st Session, No 93-412, page 143).

The minimum effective population size of 500 necessary to ensure long-term population viability has been difficult to apply in practice. There are many reasons for this. One reason is the abstractness—it can be hard for a manager to know what the effective population size of the population they are managing is when what they can count is the census size. In 2021, the northern Rocky Mountains had a census size estimated at 3354 and western Great Lakes at 4526. However, we can then translate these values to an effective population size ranging between 201 and 335 wolves for the northern Rocky Mountains and 272 and 453 for the western Great Lakes. Given the strong skew in the effective-to-census size ratio in grey wolves, larger wolf populations are necessary to ensure long-term adaptation and survival. Disperser success is an additional critical factor for long-term survival of the species, promoting gene flow that will reduce inbreeding and elevate effective population sizes through increased allelic variation and demographic rescue (Newmark et al., 2023). Dispersers are often challenged by utilizing lower quality corridors with high mortality risk to find suitable areas for establishing new territories (Oakleaf et al., 2010). The protection of grey wolf dispersers between wolf populations is thus important to improve their effective population sizes for long-term persistence.

## AUTHOR CONTRIBUTIONS

Bridgett M. vonHoldt, Daniel R. Stahler, and Robert K. Wayne conceived and designed the research study. Daniel R. Stahler, Marco Musiani, Rolf Peterson, John Stephenson, Kent Laudon, and Erin Meredith collected the samples. Bridgett M. vonHoldt generated the RADseq SNP data and analysed the data with guidance from Daniel R. Stahler, Kristin E. Brzeski, Rolf Peterson, and Kent Laudon. All authors contributed towards the preparation and writing of the manuscript.

## ACKNOWLEDGEMENTS

We acknowledge the following for samples used in this study: L. David Mech, Michel Crête, and the Michigan Department of Natural Resources. We are appreciative of comments provided by Stephen Gaughran, Armando Caballero, and all reviewers who greatly improved our manuscript. We are grateful to the Turner Endangered Species Fund for partial funding support. Daniel Stahler and Yellowstone Wolf Project efforts were funded by NSF DEB-0613730 and DEB-1245373, Yellowstone Forever, and their many donors, especially Valeria Gates and Annie and Bob Graham. This research was also implemented under the National Recovery and Resilience Plan (NRRP), Project title 'National Biodiversity Future Center -NBFC'. CUP J33C22001190001.

## CONFLICT OF INTEREST STATEMENT

Daniel R. Stahler is the leader of the Yellowstone Wolf Project. Michael Phillips is the director of the Turner Endangered Species Fund and advisor to Turner Biodiversity Divisions. John Stephenson is a wildlife biologist for the Grand Teton National Park. Kent Laudon is a senior environmental scientist specialist for the Northern Region of the California Department of Fish and Wildlife. Erin Meredith is a senior wildlife forensic specialist at the California Department of Fish and Wildlife.

## DATA AVAILABILITY STATEMENT

We have deposited all mapped and sorted BAM files on NCBI SRA under the accession PRJNA961038, and all accession numbers are listed per sample in Table S1.

## ORCID

*Bridgett M. vonHoldt* https://orcid.org/0000-0001-6908-1687
*Jennifer A. Leonard* https://orcid.org/0000-0003-0291-7819
*Robert K. Wayne* https://orcid.org/0000-0003-3537-2245

## REFERENCES

Abraham, G., Qiu, Y., & Inouye, M. (2017). FlashPCA2: Principal component analysis of biobank-scale genotype datasets. *Bioinformatics*, *33*(17), 2776–2778.

Adams, L. G., Stephenson, R. O., Dale, B. W., Ahgook, R. T., & Demma, D. J. (2008). Population dynamics and harvest characteristics of wolves in the Central Brooks Range, Alaska. *Wildlife Monographs*, *170*, 1–25.

Åkesson, M., Flagstad, Ø., Aspi, J., Kojola, I., Liberg, O., Wabakken, P., & Sand, H. (2022). Genetic signature of immigrants on their effect on genetic diversity in the recently established Scandinavian wolf population. *Conservation Genetics*, *23*, 359–373.

Alexander, D., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664.

Ali, O. A., O'Rourke, S. M., Amish, S. J., Meek, M. H., Luikart, G., Jeffres, C., & Miller, M. R. (2015). RAD capture (rapture): Flexible and efficient sequence-based genotyping. *Genetics*, *202*, 389–400.

Allendorf, F. W. (2016). Genetics and the conservation of natural populations: Allozymes to genomes. *Molecular Ecology*, *26*, 420–430.

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., & Ryman, N. (2008). Genetic effects of harvest on wild animal populations. *Trends in Ecology & Evolution*, *23*, 327–337.

Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problem with hybrids: Setting conservation guideliens. *Trends in Ecology & Evolution*, *16*, 613–622.

Ausband, D. E. (2022). Genetic diversity and mate selection in a reintroduced population of gray wolves. *Scientific Reports*, *12*, 535.

Ausband, D. E., & Waits, L. P. (2020). Does harvest affect genetic diversity in grey wolves? *Molecular Ecology*, *29*, 3187–3195.

Bangs, E. E., & Fritts, S. H. (1996). Reintroducing the gray wolf to Central Idaho and Yellowstone National Park. *Wildlife Society Bulletin*, *24*, 402–413.

Beichman, A. C., Phung, T. N., & Lohmueller, K. E. (2017). Comparison of single genome and allele frequency data reveals discordant demographic histories. *Genes Genomes Genetics*, *7*(11), 3605–3620.

Boitani, L. (2003). Wolf conservation and recovery. In L. D. Mech & L. Boitani (Eds.), *Wolves: Behavior, ecology, and conservation* (pp. 317–340). University of Chicago Press.

Bonin, A., Bellemain, E., Bronken, E. P., Pompanon, F., Brochman, C., & Taberlet, P. (2004). How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, *13*(11), 3261–3273.

Brainerd, S. M., Andrén, H., Bangs, E. E., Bradley, E. H., Fontaine, J. A., Hall, W., Iliopoulos, Y., Jimenez, M. D., Jozwiak, E. A., Liberg, O., Mack, C. M., Meier, T. J., Niemeyer, C. C., Pedersen, H. C., Sand, H., Schultz, R. N., Smith, D. W., Wabakken, P., & Wydeven, A. P. (2008). The effects of breeder loss on wolves. *Journal of Wildlife Management*, *72*, 89–98.

Caballero, A., Bravo, I., & Wang, J. (2017). Inbreeding load and purging: Implications for the short-term survival and the conservation management of small populations. *Heredity*, *118*, 177–185.

Carmichael, L. E., Krizan, J., Nagy, J. A., Dumond, M., Johnson, D., Veitch, A., & Strobeck, C. (2007). Northwest passage: Conservation genetics of Arctic Island wolves. *Conservation Genetics*, *9*, 879–892.

Carroll, C., Phillips, M. K., Lopez-Gonzalez, C. A., & Schumaker, N. H. (2006). Defining recovery goals and strategies for endangered species: The wolf as a case study. *Bioscience*, *56*(1), 25–37.

Carroll, C., Rohlf, D. J., vonHoldt, B. M., Treves, A., & Hendricks, S. A. (2020). Wolf delisting challenges demonstrate need for an improved framework for conserving intraspecific variation under the endangered species act. *Bioscience*, *71*(1), 73–84.

Cassidy, K. A., Smith, D. W., Stahler, D. R., Stahler, E., Metz, M., SunderRaj, J., Jackson, M., Binder, W., Meyer, C., Bland, T., Cassidy, B., Rabe, J., & Tatton, N. (2022). *Yellowstone National Park wolf project annual report 2021*. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, WY, USA, YCR-2022-04.

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140.

Ceballos, F. C., Hazelhurst, S., & Ramsay, M. (2018). Assessing runs of homozygosity: A comparison of SNP array and whole genome sequence low coverage data. *BMC Genomics*, *19*, 106.

Chang, C. C., Chow, C. C., Tellier, L. C. A. M., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, *4*, 7. https://doi.org/10.1186/s13742-015-0047-8

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, *10*, 783–796.

Chepko-Sade, B. D., Shields, W. M., Berger, J., Halpin, Z. T., Jones, W. T., Rogers, L. L., Rood, J. P., & Smith, A. T. (1987). The effects of dispersal and social structure on effective population size. In D. B. Chepko-Sade & Z. T. Halpin (Eds.), *Mammalian Dipsersal patterns: The effects of social structure on population genetics*. Chicago University Press.

Clutton-Brock, T. H. (2016). *Mammal societies*. Wiley-Blackwell.

Coltman, D. W. (2008). Molecular ecological approaches to studying evolutionary impact of selective harvesting in wildlife. *Molecular Ecology*, *17*, 221–235.

Coyne, J. A., Barton, N. B., & Turelli, M. (1997). Perspective: A critique of Sewall Wright's shifting balance theory of evolution. *Evolution*, *51*(3), 643–671.

Crow, J. F., & Kimura, M. (1970). *An introduction to population genetic theory*. Burgess Publishing.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R., Lunter, G., Marth, G., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158.

Duntsch, L., Whibley, A., Brekke, P., Ewen, J. G., & Santure, A. W. (2021). Genomic data of different resolutions reveal consistent inbreeding estimates but contrasting homozygosity landscapes for the threatened Aotearoa New Zealand hihi. *Molecular Ecology*, *30*(23), 6006–6020.

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics*, *17*, 422–433.

Fisher, R. A. (1930). *The genetical theory of natural selection*. Oxford University Press.

Fisher, R. A. (1958). *The genetical theory of natural selection* (2nd ed.). Dover Publishing.

Frankel, O. H., & Soulé, M. E. (1981). *Conservation and evolution*. Cambridge University Press.

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: A review. *Genetics Research*, *66*(2), 95–107.

Frankham, R. (2005). Genetics and extinction. *Biological Conservation*, *126*, 131–140.

Frankham, R., Ballou, J. D., Ralls, K., Eldridge, M. D. B., Dudash, M. R., Fenster, C. B., Lacy, R. C., & Sunnucks, P. (2019). *A practical guide for genetic management of fragmented animal and plant populations*. Oxford University Press.

Frankham, R., Bradshaw, C. J., & Brook, B. W. (2014). Genetics in conservation management: Revised recommendations for the 50/500 rules, red list criteria and population viability analyses. *Biological Conservation*, *170*, 56–63.

Franzmann, A. W., & Schwartz, C. (1997). *Ecology and management of the north American moose*. Smithsonian Institute Press.

Fuller, T. K., Mech, L. D., & Cochrane, J. F. (2003). Wolf population dynamics. In L. D. Mech & L. Boitani (Eds.), *Wolves: Behavior, ecology, and conservation*. University of Chicago Press.

Geffen, E., Kam, M., Hefner, R., Hersteinsson, P., Angerbjörn, A., Dalèn, L., Fuglei, E., Norèn, K., Adams, J. R., Vucetich, J., Meier, T. J., Mech, L. D., vonHoldt, B. M., Stahler, D. R., & Wayne, R. K. (2011). Kin encounter rate and inbreeding avoidance in canids. *Molecular Ecology*, *20*, 5348–5358.

Gese, E. M., & Mech, L. D. (1991). Dispersal of wolves (*Canis lupus*) in northeastern Minnesota, 1969-1989. *Canadian Journal of Zoology*, *69*, 2946–2955.

Hagenblad, J., Olsson, M., Parker, H. G., Ostrander, E. A., & Ellegren, H. (2009). Population genomics of the inbred Scandinavian wolf. *Molecular Ecology*, *18*(7), 1341–1351.

Hedrick, P. W., & Kalinowski, S. T. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, *31*, 139–162.

Hendricks, S. A., Schweizer, R. M., Harrigan, R. J., Pollinger, J. P., Paquet, P. C., Darimont, C. T., Adams, J. R., Waits, L. P., vonHoldt, B. M., Hohenlohe, P. A., & Wayne, R. K. (2019). Natural re-colonization and admixture of wolves (*Canis lupus*) in the US Pacific northwest: Challenges for the protection and management of rare and endangered taxa. *Heredity*, *122*, 133–149.

Hendricks, S. A., Schweizer, R. M., & Wayne, R. K. (2019). Conservation genomics illuminates the adaptive uniqueness of North American gray wolves. *Conservation Genetics*, *20*, 29–43.

Heppenheimer, E., Harrigan, R. J., Rutledge, R. Y., Koepfli, K. P., DeCandia, A. L., Brzeski, K. E., Benson, J. F., Wheeldon, T., Patterson, B. R., Kays, R., Hohenlohe, P. A., & vonHoldt, B. M. (2018). Population genomic analysis of north American eastern wolves (*Canis lycaon*) supports their conservation priority status. *Genes*, *9*(12), 606.

Hoffmann, A. A., Sgro, C. M., & Kristensen, T. N. (2017). Revisiting adaptive potential, population size, and conservation. *Trends in Ecology & Evolution*, *32*, 506–517.

Jamieson, I. G., & Allendorf, F. W. (2012). How does the 50/500 rule apply to MVPs? *Trends in Ecology & Evolution*, *27*, 578–584.

Jedrzejewski, W., Branicki, W., Veit, C., MeDugorac, I., Pilot, M., Bunevich, A. N., Jedrzejewska, B., Schmidt, K., Theuerkauf, J., Okarma, H., Gula, R., Szymura, L., & Förster, M. (2005). Genetic diversity and relatedness within packs in an intensely hunted population of wolves *Canis lupus*. *Acta Theriologica*, *50*, 3–22.

Kagawa, K., & Seehausen, O. (2020). The propagation of admixture-derived adaptive radiation potential. *Proceedings of the Royal Society B: Biological Sciences*, *287*, 20200941.

Keller, L., & Reeve, H. K. (1994). Partitioning of reproduction in animal societies. *Trends in Ecology & Evolution*, *9*, 98–103.

Koblmüller, S., Nord, M., Wayne, R. K., & Leonard, J. A. (2009). Origin and status of the Great Lakes wolf. *Molecular Ecology*, *18*, 2313–2326.

Kolenosky, G. B., & Standfield, R. (1975). Morphological and ecological variation among gray wolves (*Canis lupus*) of Ontario, Canada. In M. W. Fox (Ed.), *The wild canids: Their systematics, behavioural ecology and evolution* (pp. 62–72). Van Nostrand Reinhold.

Lacy, R. (1995). Clarification of genetic terms and their use in the management of captive populations. *Zoo Biology*, *14*, 565–578.

Laikre, L., Olsson, F., Jansson, E., Hössjer, O., & Ryman, N. (2016). Metapopulation effective size and conservation genetic goals for the Fennoscandian wolf (*Canis lupus*) population. *Heredity*, *117*, 279–289.

Lanfear, R., Kokko, H., & Eyre-Walker, A. (2014). Population size and the rate of evolution. *Trends in Ecology & Evolution*, *29*, 33–41.

Larivière, S., Jolicoeur, H., & Crête, M. (2000). Status and conservation of the gray wolf (*Canis lupus*) in wildlife reserves of Québec. *Biological Conservation*, *94*, 143–151.

Lavanchy, E., & Goudet, J. (2023). Effect of reduced genomic representation on using runs of homozygosity for inbreeding characterization. *Molecular Ecology Resources*, *23*(4), 787–802.

Leonard, J. A., & Wayne, R. K. (2008). Native Great Lakes wolves were not restored. *Biology Letters*, *4*(1), 95–98.

Li, G., Davis, B. W., Raudseep, T., Wilkserson, A. J. P., Mason, V. C., Ferguson-Smith, M., O'Brien, P. C., Waters, P. D., & Murphy, W. J. (2013). Comparative analysis of mammalian Y chromosomes illuminates ancestral structure and lineage-specific evolution. *Genome Research*, *23*, 1486–1495.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*. https://arxiv.org/abs/1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*, 2078–2079.

Lindblad-Toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, E. K., Jaffe, D. B., Kamal, M., Clamp, M., Change, J. L., Kulbokas, E. J., III, Zody, M. C., Mauceli, E., Xie, X., Breen, M., Wayne, R. K., Ostrander, E. A., Ponting, C. P., Galibert, F., Smith, D. R., DeJong, P. J., … Lander, E. S. (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*, *438*, 803–819.

López-Cortegano, E., Pouso, R., Labrador, A., Pérez-Figueroa, A., Fernández, J., & Caballero, A. (2019). Optimal management of genetic diversity in subdivided populations. *Frontiers in Genetics*, *10*, 843.

Marsden, C. D., Woodroffe, R., Mills, M. G. L., McNutt, J. W., Creel, S., Groom, R., Emmanuel, M., Cleaveland, S., Kat, P., Rasmussen, G. S. A., Ginsberg, J., Lines, R., André, J. M., Begg, C., Wayne, R. K., & Mable, B. K. (2012). Spatial and temporal patterns of neutral and adaptative genetic variation in the endangered African wild dog (*Lycaon pictus*). *Molecular Ecology*, *21*, 1379–1393.

Mech, L. D., Barber-Meyer, S. M., & Erb, J. (2016). Wolf (*Canis lupus*) generation time and proportion of current breeding females by age. *PLoS One*, *11*(6), e0156682.

Mech, L. D., & Boitani, L. (2003). Wolf social ecology. In L. D. Mech & L. Boitani (Eds.), *Wolves: Behavior, ecology, and conservation* (pp. 1–34). The University of Chicago Press.

Mitchell, M. (1998). *An introduction to genetic algorithms*. MIT Press.

Newmark, W. D., Halley, J. M., Beier, P., Cushman, S. A., McNeally, P. B., & Soulé, M. (2023). Enhanced regional connectivity between western north American national parks will increase persistence of mammal species diversity. *Scientific Reports*, *13*, 474.

Novo, I., Pérez-Pereira, N., Santiago, E., Quesada, H., & Caballero, A. (2023). An empirical test of the estimation of historical effective population size using *Drosophila melanogaster*. *Molecular Ecology Resources*, *23*(7), 1632–1640.

Oakleaf, J. K., Murray, D. L., Oakleaf, J. R., Bangs, E. E., Mack, C. M., Smith, D. W., Fontaine, J. A., Jimenez, M. D., Meier, T. J., & Niemeyer, C. C. (2010). Habitat selection by recolonizing wolves in the northern Rocky Mountains of the United States. *Journal of Wildlife Management*, *70*(2), 554–563.

Ottenburghs, J. (2021). The genic view of hybridization in the Anthropocene. *Evolutionary Applications*, *14*, 2324–2360.

Pacheco, C., Stronne, A. V., Jedrzejewska, B., Plis, K., Okhlopkov, I. M., Mamaev, N. V., Drovetski, S., & Godinho, R. (2022). Demography and evolutionary history of grew wolf populations around the Bering Strait. *Molecular Ecology*, *31*, 4851–4865.

Parker, G. (1995). *Eastern coyote: The story of its success*. Nimbus Publishing Limited.

Pérez-Pereira, N., Wang, J., Quesada, H., & Caballero, A. (2022). Prediction of the minimum effective size of a population viable in the long term. *Biodiversity and Conservation*, *31*, 2763–2780.

Peterson, R. L. (1955). *North American moose*. University of Toronto Press.

Peterson, R. O., Thomas, N. J., Thurber, J. M., Vucetich, J. A., & Waite, T. A. (1998). Population limitation and the wolves of isle Royale. *Journal of Mammalogy*, *79*(3), 828–841.

Pompanon, F., Bonin, A., Belleman, E., & Taberlet, P. (2005). Genotyping errors: Causes, consequences and solutions. *Nature Reviews Genetics*, *6*(11), 847–859.

Quevedo, M., Echegaray, J., Fernández-Gil, A., Leonard, J. A., Naves, J., Ordiz, A., Revilla, E., & Vilà, C. (2019). Lethal management may hinder population recovery in Iberian wolves. *Biodiversity and Conservation*, *28*, 415–432.

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Reed, D. H., & Frankham, R. (2003). Correlation between fitness and genetic diversity. *Conservation Biology*, *17*, 230–237.

Refsnider, R. L. (2009). The role of the endangered species act in midwest wolf recovery. In A. P. Wydeven, T. R. Van Deelen, & E. J. Heske (Eds.), *Recovery of gray wolves in the Great Lakes region of the United States* (pp. 311–330). Springer Science+Business Media, LLC.

Rick, J. A., Moen, R. A., Erb, J. D., & Strasburg, J. L. (2017). Population structure and gene flow in a newly harvested gray wolf (*Canis lupus*) population. *Conservation Genetics*, *18*, 1091–1104.

Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the success of colonizing populations? *Trends in Ecology & Evolution*, *29*, 233–242.

Robinson, J. A., Räikkönen, J., Vucetich, L. M., Vucetich, J. A., Peterson, R. O., Lohmueller, K. E., & Wayne, R. K. (2019). Genomic signatures of extensive inbreeding in isle Royale wolves, a population on the threshold of extinction. *Science. Advances*, *5*(5), easu0757.

Rochette, N. C., Givera-Colon, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology*, *28*, 4737–4754. https://doi.org/10.1111/mec.15253

Rowe, G., & Beebee, T. J. C. (2004). Reconciling genetic and demographic estimators of effective population size in the anuran amphibian *Bufo calamita*. *Conservation Genetics*, *5*, 287–298.

Rutledge, L. Y., Garroway, C. J., Loveless, K. M., & Patterson, B. R. (2010). Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. *Heredity*, *105*, 520–531.

Santiago, E., Novo, I., Pardinas, A. F., Saura, M., Wang, J., & Caballero, A. (2020). Recent demographic history inferred by high-resolution analysis of linkage disequilibrium. *Molecular Biology and Evolution*, *37*(12), 3642–3653.

Schweizer, R. M., vonHoldt, B. M., Harrigan, R., Knowles, J. C., Musiani, M., Coltman, D., Novembre, J., & Wayne, R. K. (2016). Genetic subdivision and candidate genes under selection in north American grey wolves. *Molecular Ecology*, *25*, 380–402.

Shaffer, M. L., & Stein, B. (2000). Safeguarding our precious heritage. In B. A. Stein, L. S. Kutner, & J. S. Adams (Eds.), *Precious heritage: The status of biodiversity in the United States* (pp. 301–322). Oxford University Press.

Smith, D. W., White, P. J., Stahler, D. R., Wydeven, A., & Hallac, D. E. (2016). Managing wolves in the Yellowstone area: Balancing goals across jurisdictional boundaries. *Wildlife Society Bulletin*, *40*(3), 436–445.

Sparkman, A. M., Adams, J. R., Steury, T. D., Waits, L. P., & Murray, D. L. (2012). Pack social dynamics and inbreeding avoidance in the cooperatively breeding red wolf. *Behavioral Ecology*, *23*, 1186–1194.

Szpiech, Z. A., Jakobsson, M., & Rosenberg, N. A. (2008). ADZE: A rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics*, *24*, 2498–2504.

Taboada, X., Hermida, M., Pardo, B. G., Vera, M., Piferrer, F., Viñas, A., Bouza, C., & Martínez, P. (2014). Fine mapping and evolution of the major sex determining region in turbot (*Scophthalmus maximus*). *G3: Genes, Genomes, Genetics*, *4*, 1871–1880.

Treves, A., Krofel, M., & McManus, J. (2016). Predator control should not be a shot in the dark. *Frontiers in Ecology and the Environment*, *14*(7), 380–388.

U.S. Fish and Wildlife Service. (1987). *Northern Rocky Mountain wolf recovery plan*. U.S. Fish and Wildlife Service.

U.S. Fish and Wildlife Service. (1992). *Recovery plan for the eastern timber wolf*. U.S. Fish and Wildlife Service, Twin Cities.

Väli, Ü., Einarsson, A., Waits, L., & Ellegren, H. (2008). To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? *Molecular Ecology*, *17*(17), 3808–3817.

vonHoldt, B., Stahler, D. R., Bangs, E. E., Smith, D. W., Jimenez, M. D., Mack, C. M., Niemeyer, C. C., Pollinger, J. P., & Wayne, R. K. (2010). A novel assessment of population structure and gene flow in grey wolf populations of the northern Rocky Mountains of the United States. *Molecular Ecology*, *12*, 4412–4427.

vonHoldt, B. M., Brzeski, K. E., Aardema, M. L., Schell, C. J., Rutledge, L. Y., Fain, S. R., Shutt, A. C., Linderholm, A., & Murphy, W. J. (2022). Persistence and expansion of cryptic endangered red wolf genomic ancestry along the American gulf coast. *Molecular Ecology*, *31*(21), 5440–5454.

vonHoldt, B. M., Brzeski, K. E., Wilcove, D. S., & Rutledge, L. Y. (2017). Redefining the role of admixture and genomics in species conservation. *Conservation Letters*, *11*, e12371.

vonHoldt, B. M., Cahill, J. A., Fan, Z., Gronau, I., Robinson, J., Pollinger, J. P., Shapiro, B., Wall, J., & Wayne, R. K. (2016). Whole-genome sequence analysis shows that two endemic species of north American wolf are admixtures of the coyote and gray wolf. *Science Advances*, *2*(7), e1501714.

vonHoldt, B. M., Pollinger, J. P., Earl, D. A., Knowles, J. C., Boyko, A. R., Parker, H., Geffen, E., Pilot, M., Jedrzejewski, W., Jedrzejewska, B., Sidorovich, V., Greco, C., Randi, E., Musiani, M., Kays, R., Bustamante, C. D., Ostrander, E. A., Novembre, J., & Wayne, R. K. (2011). A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Research*, *21*, 1294–1305.

vonHoldt, B. M., Stahler, D. R., Smith, D. W., Earl, D. A., Pollinger, J. P., & Wayne, R. K. (2008). The genealogy and genetic viability of reintroduced Yellowstone grey wolves. *Molecular Ecology*, *17*(1), 252–274.

Wade, M. J., & Goodnight, C. J. (1998). Perspective: The theories of Fisher and Wright in the context of metapopulations: When nature does many small experiments. *Evolution*, *52*(6), 1537–1553.

Wang, J., Santiago, E., & Caballero, A. (2016). Prediction and estimation of effective population size. *Heredity*, *117*, 193–206.

Wisconsin, D. N. R. (1989). *Wisconsin timer wolf recovery plan. Wisconsin Endangered Resources Report 50*. Wisconsin Department of Natural Resources.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, *16*, 97–156.

Wright, S. (1943). Isolation by distance. *Genetics*, *28*, 114–138.

Wright, S. (1965). The interpretation of population structure by f-statistics with special regard to the system of mating. *Evolution*, *19*, 395–420.

Wydeven, A. P., Van Deelen, T. R., & Heske, E. J. (2009). Wolf recovery in the Great Lakes region: What have we learned and where will we go now? In A. P. Wydeven, T. R. Van Deelen, & E. J. Heske (Eds.), *Recovery of gray wolves in the Great Lakes region of the United States* (pp. 331–337). Springer Science+Business Media, LLC.

Young, S. P., & Goldman, E. A. (1944). *The wolves of North America, Part I*. American Wildlife Institute.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** vonHoldt, B. M., Stahler, D. R., Brzeski, K. E., Musiani, M., Peterson, R., Phillips, M., Stephenson, J., Laudon, K., Meredith, E., Vucetich, J. A., Leonard, J. A., & Wayne, R. K. (2024). Demographic history shapes North American gray wolf genomic diversity and informs species' conservation. *Molecular Ecology*, *33*, e17231. https://doi.org/10.1111/mec.17231

ELSEVIER

Biological Conservation 86 (1998) 243–255

BIOLOGICAL
CONSERVATION

# Predictors of avian and mammalian translocation success: reanalysis with phylogenetically independent contrasts

C. Magdalena Wolf[a,*], Theodore Garland, Jr[b], Brad Griffith[c,†]

[a]Departments of Zoology and Wildlife Ecology, 430 Lincoln Drive, University of Wisconsin, Madison, WI 53706-1381, USA
[b]Department of Zoology, 430 Lincoln Drive, University of Wisconsin, Madison, WI 53706-1381, USA
[c]US National Biological Service, 101 12th Avenue, Box 15, Fairbanks, AK 99701, USA

Received 27 November 1996; received in revised form 20 October 1997; accepted 23 November 1997

---

## Abstract

We use the phylogenetically based statistical method of independent contrasts to reanalyze the Wolf, C.M., Griffith, B., Reed, C., Temple, S.A. (1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology 10, 1142–1154). translocation data set for 181 programs involving 17 mammalian and 28 avian species. Although still novel in conservation and wildlife biology, the incorporation of phylogenetic information into analyses of interspecific comparative data is widely accepted and routinely used in several fields. To facilitate application of independent contrasts, we converted the dichotomous (success/failure) dependent variable (Wolf et al., 1996; Griffith, B., Scott, J.M. Carpenter, J.W., Reed, C., 1989. Translocations as a species conservation tool: status and strategy. Science 245, 477–480) into a more descriptive, continuous variable with the incorporation of persistence of the translocated population beyond the last release year, relative to the species' longevity. For comparison, we present three models: nonphylogenetic multiple logistic regression with the dichotomous dependent variable (the method used by Wolf et al. 1996 and Griffith et al. 1989), nonphylogenetic multiple regression with the continuous dependent variable, and multiple regression using phylogenetically independent contrasts with the continuous dependent variable. Results of the phylogenetically based multiple regression analysis indicate statistical significance of three independent variables: habitat quality of the release area, range of the release site relative to the historical distribution of the translocated species, and number of individuals released. Evidence that omnivorous species are more successful than either herbivores or carnivores is also presented. The results of our reanalysis support several of the more important conclusions of the Wolf et al. (1996) and Griffith et al. (1989) studies and increase our confidence that the foregoing variables should be considered carefully when designing a translocation program. However, the phylogenetically based analysis does not support either the Wolf et al. (1996) or Griffith et al. (1989) findings with respect to the statistical significance of taxonomic class (bird vs mammal) and status (game vs threatened, endangered, or sensitive), or the Griffith et al. (1989) findings with respect to the significance of reproductive potential of the species and program length.

*Keywords:* Birds; Comparative method; Mammals; Phylogenetically independent contrasts; Reintroduction; Translocation

---

## 1. Introduction

Numerous methodological, environmental, species-specific, and population-level factors influence whether the intentional release of wild-caught or captive-reared animals into new locations will result in the successful establishment, re-establishment, or augmentation of a wild population. Because specific causal factors and their relative importance vary widely among such release programs (herein referred to as translocations, following Griffith et al., 1989; see also Wolf et al., 1996), it is difficult to identify general trends associated with success. Nevertheless, both theoretical considerations and empirical evidence suggest that some methodological and biological factors are of general importance. For example, such methodological details as raise and release procedures, number and composition of animals released, and choice of source stock for the released animals were shown to influence translocation outcomes in single-species studies (Beck et al., 1991; Allen et al., 1993; Reed et al., 1993; Bright and Morris, 1994; Veltman et al., 1996). Environmental factors perceived as

---

* Corresponding author at: P.O. Box 52918, Riverside, CA 92517.

† Present address: Alaska Cooperative Fish and Wildlife Research Unit, 216 Irving I Building, University of Alaska, Fairbanks, AK 99775.

0006-3207/98/$19.00
PII: S0006-3207(97)00179-1

important to success include general habitat quality and climatic conditions (Lindenmayer, 1994; Veitch, 1994), as well as the absence of predators or competitors (Crawley, 1986; Short et al., 1992). Some species-specific and population characteristics considered favorable for successful invasion of a new location include a relatively high reproductive potential, an omnivorous diet, small body mass, and high genetic diversity (Mayr, 1965; Laycock, 1966; Berger, 1972; Smith et al., 1976; Crawley, 1986; Ehrlich, 1986; O'Connor, 1986). The question remains: how universal is the influence of such methodological and biological factors? Also, in light of substantial case-by-case variations, are generalizations across a range of taxa possible?

Griffith et al. (1989) (see also Griffith et al., 1990, 1993) used a comparative approach to test for general patterns underlying the success vs failure of translocations among species of birds and mammals. They conducted surveys of translocation programs throughout North America, Australia, and New Zealand; coded the outcomes as either a success (reported establishment of a self-sustaining population), a failure, or incomplete; and used multiple logistic regression to identify seven statistically significant predictors of success: (1) taxonomic class (bird vs mammal), (2) legal status of the translocated species (native game vs threatened, endangered, or sensitive species), (3) habitat quality of the release area (excellent, good, or fair/poor), (4) location of the release area relative to the historical range of the species (core vs periphery or outside), (5) number of animals released ($\log_{10}$ transformed), (6) program length (number of years over which releases occurred), and (7) potential productivity of the translocated species (high vs low). Wolf et al. (1996) conducted a follow-up survey, in which they up-dated the status of the translocations in the Griffith et al. study, increased the number of programs available for multiple regression analyses from 155 to 181, and tested additional variables as predictors of success. Their results, using comparable analyses, were largely consistent with the findings of Griffith et al. (1989). Wolf et al. (1996) produced a model which contained the first five of the Griffith et al. variables (as listed earlier) plus adult diet of the species in the wild (herbivorous vs omnivorous vs carnivorous).

As was abundantly demonstrated in the last decade, interspecific comparisons are potentially compromised by statistical non-independence of species values (Felsenstein, 1985; Harvey and Pagel, 1991; Garland et al., 1993; Martins and Hansen, 1996). A simplified consensus view of the problem can be summarized as follows. Species are related to greater or lesser degrees, as indicated by their phylogenetic (evolutionary) relationships. Closely related species possess many characteristics, and sometimes much of their selective regime, that were inherited from common ancestors. As a result of such inheritance, data for a series of species may contain hierarchical resemblances (e.g. snakes look like snakes, elephants look like elephants) and cannot be assumed to represent independent data points, a key assumption of both traditional parametric and nonparametric statistical methods. Detailed discussions of why closely related species tend to be similar are available elsewhere (Grafen, 1989; Brooks and McLennan, 1991; Harvey and Pagel, 1991; Eggleton and Vane-Wright, 1994; Martins, 1996a). In general, common effects of violating the assumption of independence include an inflation of Type I error rates when hypothesis testing (claiming statistical significance when none actually exists) and poor estimation of relationships among variables (and hence diminished predictive accuracy). The reality of these adverse statistical effects was demonstrated both analytically and by computer simulation studies (Felsenstein, 1985; Grafen, 1989; Martins and Garland, 1991; Garland et al., 1992, 1993; Pagel, 1993; Purvis et al., 1994; Díaz-Uriarte and Garland, 1996, in press; Martins, 1996b).

Inclusion of phylogenetic information in statistical analyses is now routine in such fields as behavioral and physiological ecology (Miles and Dunham, 1993; Garland and Carter, 1994; Losos and Miles, 1994; Díaz et al., 1996; Martin and Clobert, 1996; Martins, 1996a; Reynolds and Lee, 1996; Ricklefs and Starck, 1996; Williams, 1996; Abouheif and Fairbairn, 1997; Bauwens and Díaz-Uriarte, 1997; Price, 1997; Clobert et al., 1998). Although an historical, evolutionary approach may not initially seem germane to questions pertaining to contemporary wildlife translocation success, *all* interspecific (and many interpopulation) comparisons are potentially subject to phylogenetic influence in statistical tests. To illustrate, one might expect a translocation of bighorn sheep in Nevada to share inherent similarities with a translocation of bighorn sheep conducted elsewhere. Part of this similarity would be caused by general capture and release methodology, whereas part of this similarity would also be caused by biological characteristics of bighorn sheep (e.g. reproductive potential, social systems, disease susceptibility). Likewise, one might expect similarities between translocations conducted with closely related species (e.g. Prairie Chicken and Sharp-tailed Grouse), as compared with translocations involving more distantly related species (e.g. Canada Goose).

Although comparative biologists have long recognized that species should not be treated as independent data points, early proposed solutions to this problem, such as averaging within genera or choosing only one species per genus, are inadequate because they ignore potentially informative variation within phylogenetic lineages (clades), ignore hierarchical relationships among clades, and diminish statistical power (review in Harvey and Pagel, 1991). State-of-the-art phylogenetically based analyses allow incorporation of phylogenetic information without loss of statistical power (Martins and Garland, 1991; Garland and Adolph, 1994; Purvis et al., 1994; Martins, 1996b).

The present study, therefore, employs phylogenetically based statistical methods to reanalyze the Wolf et al. (1996) data set (104 avian and 77 mammalian translocation programs, representing 28 avian and 17 mammalian species). Felsenstein's (1985) method of phylogenetically independent contrasts, the first fully phylogenetic method to be proposed, is the best understood of available methods (Grafen, 1989; Martins and Garland, 1991; Garland et al., 1992; Pagel, 1993; Purvis and Garland, 1993; Purvis et al., 1994; Díaz-Uriarte and Garland, 1996, in press; Martins, 1996b; Martins and Hansen, 1996). Moreover, several free computer programs are available which implement the method (e.g. Joe Felsenstein's PHYLIP package, the PDAP package (Garland et al., 1993), CAIC (Purvis and Rambaut, 1995), COMPARE (Martins and Hansen, 1996)). In brief, the method of independent contrasts uses phylogenetic information (topology and branch lengths) to transform interspecific data (i.e. estimates of mean values for a series of species) into values (standardized independent contrasts) that, in principle, are independent and identically distributed, and hence can be analyzed with standard statistical methods (see Methods section for more details).

To facilitate application of phylogenetically independent contrasts, we used a more detailed indicator of translocation outcome as a dependent variable, rather than the dichotomous measure (success vs failure) used previously (Griffith et al., 1989; Wolf et al., 1996). We developed a continuous outcome variable that was a composite (see Methods section) of the following information: (1) the translocated population persistence (in years) in the field; (2) the classification of the population as self-sustaining, declining, or gone; and (3) the maximum potential life span (years) of each species as a scaling factor. The continuous dependent variable better meets assumptions of phylogenetically independent contrasts, and the inclusion of population persistence information provides a more quantitative measure of the population's ability to persist through time. In principle, this more inclusive dependent variable should increase statistical power to detect significant predictors of 'success.'

## 2. Methods

### 2.1. The data

The up-dated avian and mammalian translocation data used by Wolf et al. (1996) in the multiple logistic regression models were also used in this study. Of the 421 targeted avian and mammalian translocations throughout North America, Australia, and New Zealand, 181 programs were used by Wolf et al. (1996) in the logistic analyses. Within the sample of 181 translocations, 122 were classified as successful and 59 as unsuccessful; 104 involved translocations of birds and 77 of mammals; and 24 programs released captive-reared animals, 141 released wild-caught animals, and 16 released a mixture of captive-reared and wild-caught animals. Ninety translocations were common to both the Griffith et al. (1989) and Wolf et al. (1996) multiple regression model-building samples.

Both the application of phylogenetically independent contrasts and ordinary multiple regression analyses generally require a continuous dependent variable (Felsenstein, 1985; Grafen, 1989; Garland et al., 1992; but see simulation results in Ridley and Grafen, 1996). We therefore developed a composite, continuous dependent variable representing the outcome of the translocation programs. Because the successful establishment of a population ultimately depends upon its ability to persist through time, the composite dependent variable incorporated the following two features of the translocated population: (1) its persistence (years) in the field since the year of the last release of animals; and (2) its classification (by the survey respondent) as self-sustaining, declining, or gone (following the criteria for the dichotomous dependent variable used by Griffith et al. (1989) and Wolf et al. (1996), with the further distinction of the unsuccessful programs into 'declining' and 'gone' categories). The persistence of a translocated population was divided by the maximum potential life span (years) of an individual of the species in the wild to scale for the wide variability (5–50 yr) in species' longevity. To be conservative in our scaling of the dependent variable, we used maximum (as opposed to mean) life expectancy in the wild to account for the longest possible time that the original released individuals could have remained in the area. Therefore, populations which persist beyond the maximum expected life span are likely sustaining through reproduction (or through ingress from other populations).

Actual construction of the outcome variable was as follows. After dividing the population persistence by the longevity estimate for the species, the resulting ratios were arranged in ascending order within each of the three status categories (self-sustaining, declining, and gone). To eliminate overlap between the three categories (e.g. self-sustaining populations could have the same persistence/longevity ratios as populations which are now gone), the highest persistence/longevity ratio in the 'gone' group was added to all of the ratios in the 'declining' group. In this way, programs with low persistence ratios and declining populations have a higher outcome score than populations that have already disappeared. Then, the highest ratio for the 'declining' group (which now includes the highest value for the 'gone' group) was added to all of the ratios in the 'self-sustaining' group.

### 2.2. Phylogenetic 'transformation'

Phylogenetically based statistical methods require specification of the topology (branching order) and branch lengths of the phylogenetic tree for the species in the study. The interspecific topology and branch lengths depicted in Fig. 1 is a composite of information drawn from a variety of studies in the literature, representing molecular, morphological, and paleontological information (predominant sources include Li et al., 1990; Sibley and Ahlquist, 1990; Wayne et al., 1991; Garland et al., 1993; see Appendix for a detailed description of the tree derivation).

In the analyses, each tip on the phylogenetic tree represented a separate translocation program (as indicated by separate questionnaires). Where more than one translocation was conducted with the same species, we represented the within-species branching order as a polytomy (multifurcation) of the distinct translocated populations. In effect, intraspecific populations were assumed to have diverged from a common ancestral population more-or-less instantaneously (termed a 'hard' polytomy; see Purvis and Garland, 1993). This is appropriate because many programs combined more than one source stock of animals. For some programs, mixing of the source stock was done intentionally in an effort to 'increase the genetic diversity' of the translocated animals.

Felsenstein's (1985) method of phylogenetically independent contrasts is intended to transform comparative data so as to make them independent and identically distributed, as is assumed by most conventional statistical methods. The method works as follows (see Felsenstein, 1985; Garland et al., 1992 for more details; and Garland and Adolph, 1994 for worked examples). Firstly, for a given variable, the datum at a given tip of the phylogenetic tree is subtracted from the value for its closest relative in the data set. For example, we might subtract the value of the outcome variable for the bird *Telespiza ultima* from the value for *T. cantans* (Fig. 1). The direction of subtraction is arbitrary (Garland et al., 1992). This difference would constitute one 'contrast'. The reason for computing contrasts is to transform the data to make them independent in the statistical sense. The rationale is that any difference between, say, *T. ultima* and *T. cantans* depends only on evolutionary events that have occurred since they diverged from their last common ancestor and is independent of other such differences (e.g. between the two *Anas* species).

Secondly, a contrast is standardized by division by the square root of the sum of its branch lengths (its standard deviation, under the assumptions that evolution was similar to a Brownian motion process and that the available branch lengths are proportional to expected variance of trait evolution). The standardization is intended to bring all contrasts to a common variance, such that they can be considered 'identically distributed', another common assumption of most statistical procedures. The rationale for standardizing by branch lengths (i.e. computing a ratio; see Garland, 1992) is that contrasts involving relatively long divergence times (e.g. *Nyctanassa violacea* and *Puffinus puffinus*) are likely to show greater phenotypic divergence than are contrasts that involve recent divergences (e.g. *T. ultima* and *T. cantans*).

Thirdly, the foregoing computations would be repeated for all such pairs of tip species (e.g. for the two *Anas* species, for *N. violacea* and *P. puffinus*). For many of the 45 species shown as tips in Fig. 1, the species is actually represented in our data set by multiple translocation programs (e.g. two programs for the mouse *Peromyscus polionotus*, 10 programs for the otter *Lutra canadensis*, 35 programs for the wild turkey *Meleagris gallopavo*). As noted earlier, for the actual analyses, these conspecific programs were represented as polytomies (i.e. small 'stars' of equal-length branches descending from the tip node). Computations of contrasts within these 'hard' polytomies were done following Felsenstein (1985) and Purvis and Garland (1993). It is important to note that independent contrasts applied to a 'star' phylogeny result in estimates of correlations, regressions, etc., that are *exactly* the same as conventional correlations or regressions (Purvis and Garland, 1993).

Lastly, after contrasts are computed for pairs of tip species (or for programs within a given species), contrasts between internal nodes (branching points) are computed. Values at internal nodes are estimated as the weighted (by branch lengths) mean of the descendants of that node, whether they be two tip species (measured values), one tip value and one estimated nodal value, or two estimated nodal values (see Felsenstein (1985) for details). (Branches below internal nodes in the phylogenetic tree are lengthened to reflect the fact that values at nodes are estimates, not data, and so contrasts involving internal nodes should be devalued [receive lower weight] in statistical analyses; see Felsenstein, 1985). This procedure continues all the way down to the root (basal node) of the tree, and ultimately produces $n-1$ contrasts, where $n$ is the number of original tip data points (181 for the present study). Note that the contrast at the very bottom of the tree (the root or basal contrast — not shown in Fig. 1) constitutes a general comparison between the birds and mammals (Garland et al., 1993; Clobert et al., 1998). Thus, a test of its statistical significance by use of a 0–1 dummy variable is equivalent to a test comparing the mean translocation success of birds and mammals (after adjusting for other variables in the model).

The logic behind phylogenetically based analytical methods, of which independent contrasts is the best understood (review in Martins and Hansen, 1996), is

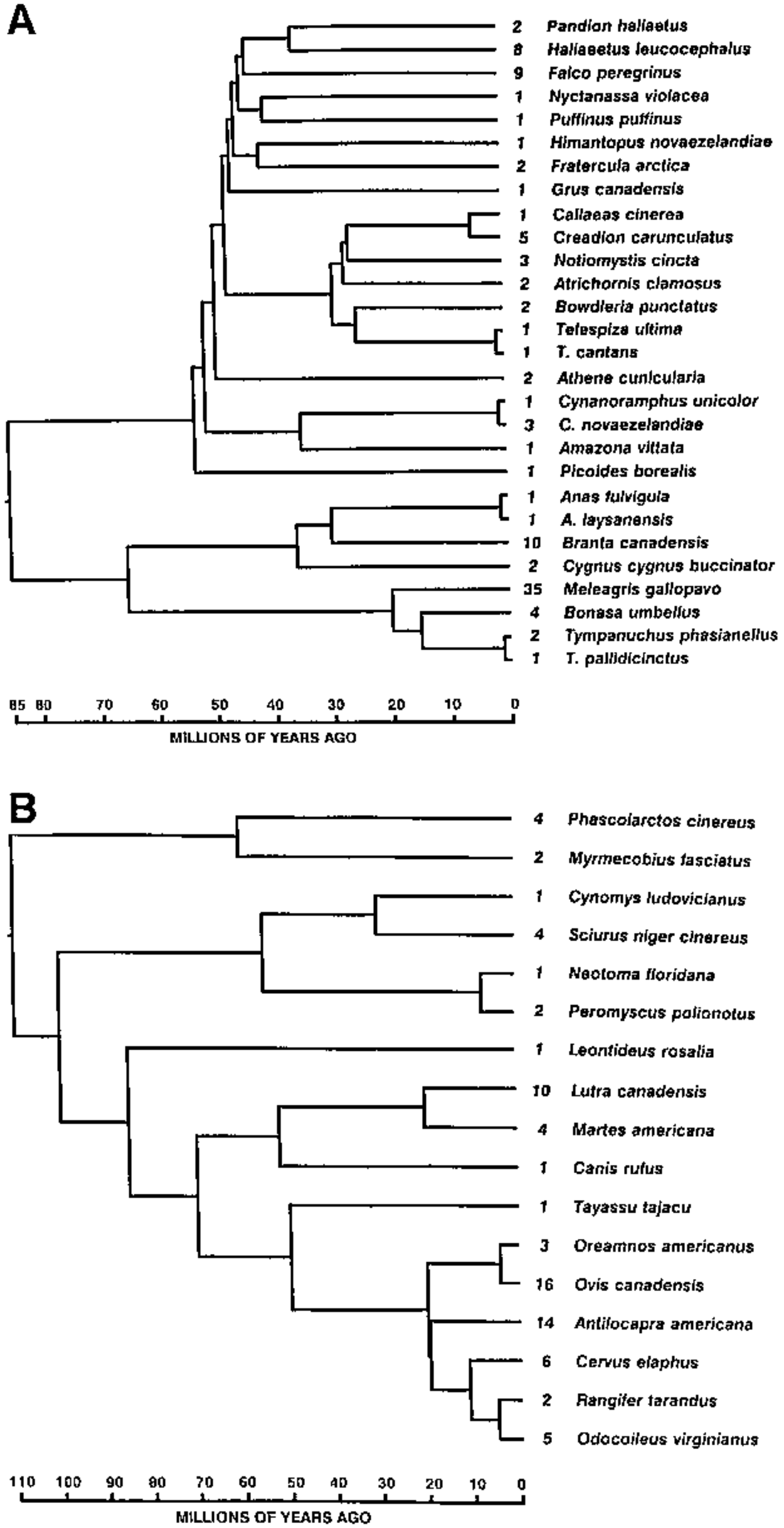


Fig. 1. Composite hypothesis of phylogenetic relationships for the 28 avian and 17 mammalian species (181 translocation programs) represented in our data set. Appendix provides a description of and the sources for the compilation of this phylogeny. Numbers at tips represent the number of translocation programs in our data set for a given species. Not shown are the polytomous relationships between conspecific translocations, which, for computation of phylogenetically independent contrasts (see Methods section), were achieved by setting internodal branch lengths to zero (see Felsenstein, 1985; Purvis and Garland, 1993) and tip branch lengths equivalent to a divergence time of 1 yr. This arbitrary, small number signifies an expectation of similarity among conspecific translocations relative to programs conducted with different species. Also not shown is a connecting branch between the birds and mammals (their divergence, from ancestral reptiles, was estimated at 270 million years ago).

that species inherit genotypes, phenotypes, and to some extent habitats from their ancestors. Thus, biological variables (e.g. body mass) will tend to 'follow phylogeny'. However, many of the independent variables used in the present analysis are not biologically inherited: (1) the purpose of the translocation as either the establishment of a new population, the reestablishment of an extirpated population, or the augmentation of a critically small population; (2) the range of the release area in relation to the historical distribution of the species; (3) the status of the translocated species; (4) the number of animals released; (5) the number of separate release events; (6) the presence or absence of potential competitors on the release site; (7) the source of the translocated animals; (8) program length; (9) whether the translocated animals were released the same day as transport or some time later; (10) whether the release was 'hard' or 'soft'; (11) whether or not the release was onto an island; (12) the quality of the habitat into which the release occurred; (13) and whether or not the habitat was artificially improved prior to release. For such variables, it makes no sense to place them on a hierarchical phylogeny. Instead, for non-biological independent variables, we collapsed (by setting internal branches to zero length) the phylogenetic tree shown in Fig. 1 to create one large polytomy (a 'star' phylogeny) for all 181 translocations. Because non-species-specific variables are neither genetically based nor inherited through phylogeny, a polytomy signifies, in effect, equal-relatedness and, hence, independence among the translocations for those variables. The phylogenetic topology still exists within this polytomy (see Felsenstein, 1985; Purvis and Garland, 1993), which thus preserves contrast computation order and allows incorporation of such variables into an analysis with other variables whose contrasts were computed on a non-star, hierarchical phylogeny.

A non-star, hierarchical phylogeny was used to calculate the independent contrasts for the dependent and biologically based (phylogenetically inherited) independent variables (i.e. taxonomic class, adult diet in the wild, potential productivity, mean adult body mass, and migratory behavior). Branch lengths were checked for statistical adequacy as described by Garland et al. (1992); see also Díaz-Uriarte and Garland, 1996, in press). These graphs indicated that contrasts computed with all (non-zero) branch lengths equal to one yielded adequate standardization for each of the species-specific variables. Thus, contrasts for the dependent variable and all phylogenetically inherited variables were computed with equal-length branches, not those shown in Fig. 1.

PDTREE (Version 2) of the Phenotypic Diversity Analysis Program (PDAP, Garland et al., 1993) was used for computation of the independent contrasts. These values were then input to SPSS/PC+ version 5.0 (Norusis, 1992a,b) for subsequent statistical analyses (e.g. multiple regression through the origin).

### 2.3. Statistical analyses

To facilitate comparison with the previous study (Wolf et al., 1996) and to understand differences between the analytical modes, we emphasized three models: nonphylogenetic multiple logistic regression with the original dichotomous dependent variable, nonphylogenetic multiple regression with the new continuous dependent variable, and multiple regression using phylogenetically independent contrasts with the continuous dependent variable. Each of the models was for the entire data set (i.e. all 181 avian and mammalian programs). Eighteen candidate-independent variables were evaluated in the multiple regression analyses, including all but one of the independent variables considered by Wolf et al. (1996). (The species' potential maximum life span was not used as a candidate independent variable because this information was included in the new composite dependent variable.)

Following Wolf et al. (1996), the multiple regression models were derived using a stepwise selection procedure (forward selection with an option for removal; significance was evaluated at $p \leq 0.05$ for entry and $p \leq 0.051$ for removal). All first-order interaction terms of the model parameters were checked for statistical significance. For the phylogenetically based model, regressions were computed through the origin, as required by independent contrasts (Garland et al., 1992). Assumptions (e.g. normality, heteroscedasticity) of the multiple linear regressions were checked. Normal probability and detrended normal probability plots of the deviances were used to examine the distribution of the residuals (Draper and Smith, 1981). Plots of Cook's distance statistic and centered leverage values against case numbers allowed for identification of influential points. (Note: influential or 'leverage' points may not be identified by residual diagnostics alone (Draper and Smith, 1981)). All pairwise correlations (calculated through the origin for the independent contrasts), variance inflation factors (VIF), and eigenvalues were examined for evidence of multicolinearity (Draper and Smith, 1981; Montgomery and Peck, 1992). We also conducted tests for different slopes and mean values between the birds and mammals (see Garland et al., 1992, 1993; Clobert et al., in press). Final model selection was based on the statistical significance of model parameter estimates and whether the regression coefficients were robust following identification of influential points.

To allow direct comparisons, the three models are presented with the same independent variables; thus, we forced into each model any variable that emerged as significant in any one of the other two model types. In general, comparisons between the two nonphylogenetic models (logistic and ordinary multiple regressions) should indicate the effects of our rescoring of the success variable, whereas comparisons of the nonphylogenetic ordinary regression and the regression using independent contrasts should indicate the effects of incorporating phylogenetic information.

In addition to the forced inclusion of two variables, the nonphylogenetic logistic regression model was modified from the combined avian and mammalian model presented by Wolf et al. (1996), their table 3) in the following ways: the golden lion tamarin translocation (a statistically influential point) was retained, indicator (as opposed to deviation) coding was used (Norusis, 1992a), and the variable for habitat quality was treated as continuous (0 = poor, 1 = good, 2 = excellent). These modifications change the appearance of the model, including the presentation of the coefficients, but, with one exception, do not alter the general interpretations of the model presented in Wolf et al. (1996), their table 3). As a result of these modifications, animals with a carnivorous diet appear to be as successful as those with an omnivorous diet (the difference in the partial regression coefficients is negligible, $B_{\text{carnivorous}} - B_{\text{omnivorous}} = 0.062$), both being significantly more successful than herbivores.

## 3. Results

After converting the dichotomous dependent variable (success vs failure) into a composite, continuous variable (see Methods section), the nonphylogenetic ordinary stepwise regression produced a model with five main-effect variables and one first-order interaction term: habitat quality ($B = 0.252$, $p = 0.0176$, where $B$ equals the partial regression coefficient), migratory behavior ($B = -0.504$, $p = 0.0021$), status ($B = 0.551$, $p = 0.0002$), range ($B = 2.332$, $p < 0.0001$), number of animals ($B = 0.954$, $p < 0.0001$), and range × number of animals ($B = -0.920$, $p = 0.0003$) (model $r^2 = 0.31$, $F = 12.911$, $p < 0.0001$). Thus, this model contained four of the six variables included in the multiple logistic regression model of Wolf et al. (1996). Although taxonomic class (bird vs mammal) and diet were not included in the initial model using the stepwise algorithm, they had statistically significant coefficients when forced into the model (Table 1, second column; combined $p$ for the two diet dummy variables was determined using the TEST subcommand in SPSS/PC+). Migratory behavior had a negative coefficient sign, suggesting that nonmigratory animals were more likely to establish self-sustaining populations when translocated; however, this variable was statistically nonsignificant with diet and class forced into the model (Table 1, second column). Thus, the two nonphylogenetic models, one with a dichotomous and the other with a continuous dependent variable, were highly consistent.

The initial phylogenetically based model, derived from ordinary stepwise regression (through the origin) with the independent contrasts, contained three main-effect variables and one first-order interaction term: omnivory ($B = 0.857$, $p = 0.0332$), range ($B = 0.450$, $p = 0.0008$), number of animals ($B = 0.226$, $p = 0.0191$), and range × number of animals ($B = 0.382$, $p = 0.0347$) (model $r^2 = 0.13$, $F = 6.688$, $p < 0.0001$). For comparability with the derivation of the logistic regression model (where the SPSS/PC+ procedure automatically codes indicator variables and assesses their fit as a unit), the indicator variable for carnivory was then forced into the model. With both the omnivory and carnivory indicator variables in the phylogenetically based model, the combined influence of diet was no longer statistically significant ($p = 0.0756$, as determined using the TEST subcommand in SPSS/PC+). With the subsequent removal of both diet indicator variables from the model, habitat quality entered into the model as a statistically significant predictor, producing the model: habitat quality ($B = 0.184$, $p = 0.0421$), range ($B = 0.418$, $p = 0.0020$), number of animals ($B = 0.214$, $p = 0.0266$), and range × number of animals ($B = 0.474$, $p = 0.0094$).

The foregoing indicates that three variables entered by stepwise model-building procedures into all three multiple regression models (logistic, ordinary, phylogenetically independent contrasts): the number of animals released, the range of the release area, and habitat quality. For more direct comparison of the three models, they are presented in Table 1 containing the same eight independent variables. Forcing variables into the models alters the partial regression coefficients, $p$ values, and model-fit statistics; however, this enables comparison of the relative size of the $p$ values for specific independent variables and, to a certain degree, permits interpretation of the relative importance of variables across the different models. Notable differences between the two nonphylogenetic models and the phylogenetic model are the lack of significance of taxonomic class, diet, and status (Table 1). (Although omnivory alone was initially significant in the phylogenetically based model, the combined influence of omnivory plus carnivory was statistically nonsignificant.)

Of the 45 species represented in our data set (see Fig. 1), five were represented by a sufficient number of programs ($\geq 10$) to evaluate intraspecific correlations of translocation success. Of those five species, however, only two exhibited sufficient variation for both independent and dependent variables. In the logistic regression model for bighorn sheep ($n = 16$ programs), type of release was a significant predictor of translocation success ($B = -5.804$, $p = 0.0032$); that is, ‘hard’ releases (no provisioning of food or water) were more successful for this species. For pronghorn ($n = 14$ programs), logistic

Table 1
Model coefficients and statistics of combined avian and mammalian translocations using nonphylogenetic multiple logistic regression, nonphylogenetic ordinary multiple regression (with a composite, continuous dependent variable), and ordinary multiple regression with phylogenetically independent contrasts[a]

| | Nonphylogenetic | | | | Regression of Phylogenetically Independent Contrasts[c] | |
|---|---|---|---|---|---|---|
| | Logistic Regression[b] | | Ordinary Regression[c] | | | |
| Variable | *B* | *p* | *B* | *p* | *B* | *p* |
| Taxonomic Class | 1.181 | 0.0231 | 0.413[d] | 0.0262 | 0.028[d] | 0.9735 |
| Diet | | 0.0003 | | 0.0414 | | 0.1135 |
| Carnivorous | 2.070 | | 0.275[d] | | −0.558[d] | |
| Omnivorous | 2.008 | | 0.551[d] | | 0.539[d] | |
| Migratory | −0.532[d] | 0.2936 | −0.332 | 0.0746 | −0.140[d] | 0.5360 |
| Status | 1.967 | 0.0001 | 0.513 | 0.0055 | 0.093[d] | 0.4651 |
| Habitat Quality | 0.817 | 0.0098 | 0.209 | 0.0500 | 0.176 | 0.0570 |
| # Animals ($\log_{10}$) | 2.512 | 0.0001 | 1.018 | $<0.0001$ | 0.190 | 0.0592 |
| Range | 3.847 | 0.0119 | 2.269 | $<0.0001$ | 0.400 | 0.0033 |
| Range × # Animals | −1.415[d] | 0.1023 | −0.907 | 0.0003 | 0.415 | 0.0296 |
| Constant | −7.777 | | −0.413 | | — | |
| Model Statistics | | | | | | |
| $X^2$ | 74.637 (9df) | | | | | |
| *p* value | $<0.0001$ | | | | | |
| $r^2$ | | | 0.3365 | | 0.1599[e] | |
| *F* | | | 9.638 | | 3.617 | |
| *p* value | | | $<0.0001$ | | 0.0004 | |

[a] $n = 181$ translocation programs or 180 phylogenetically independent contrasts.

[b] Compare with Wolf et al. (1996); regression coefficients and statistics differ for reasons discussed in text.

[c] Produced with a composite, continuous dependent variable.

[d] Not an original variable for this model; forced into the model for comparison with the other two models. Parameter estimates without footnote were statistically significant ($p \leq 0.05$) prior to the forced inclusion of additional variables.

[e] Regression of independent contrasts is computed through the origin; therefore, the $r^2$ is not comparable with the nonphylogenetic model $r^2$.

regression indicated that habitat improvement was negatively associated with success ($B = -10.491$, $p = 0.0073$). These intraspecific results appear to be nonintuitive and suggest that further investigations are necessary to understand the relationship between the dependent and independent variables. Hence, although it is possible to identify general predictors of translocation success, practitioners are cautioned that such results may or may not apply to any particular species.

## 4. Discussion

Complicated biological questions and associated data sets can be analyzed in many different ways. Often, little consensus exists as to the 'best' way to analyze a particular type of data. The application of different analytical methods to a single data set is, therefore, prudent. To the extent that different analytical techniques lead to similar conclusions, then we may have enhanced confidence in those conclusions.

With respect to interspecific comparative studies, a flurry of research activity since Felsenstein's (Felsenstein, 1985) seminal paper has led to a consensus that phylogenetic information should be incorporated into analyses (Brooks and McLennan, 1991; Harvey and Pagel, 1991; Eggleton and Vane-Wright, 1994; Martins, 1996a). We have, therefore, reanalyzed the data set of Wolf et al. (1996) with the method of phylogenetically independent contrasts. Our reanalysis supports several of the more important conclusions of Wolf et al. (1996), and of the original paper by Griffith et al. (1989), but also indicates some important differences.

Table 1 shows that habitat quality at the release site, the number of animals released, and release into an area within the core of the species' historical distribution were positively associated with success in avian and mammalian translocation programs, irrespective of the type of multiple regression analysis employed. All three of these independent variables are under the control of translocation practitioners. Thus, our phylogenetic analysis increases our confidence that these variables should indeed be considered carefully when designing an avian or mammalian translocation program.

Habitat quality was emphasized as an essential component of translocation success by innumerable other authors (Dodd and Seigel, 1991; Jackson, 1994; Lindenmayer, 1994; Wilson and Stanley Price, 1994;

Armstrong and McLean, 1995; Veltman et al., 1996). Indeed, suitable habitat quality (and quantity) relative to the requirements of the translocated species was the factor most often cited as influencing translocation outcome by our survey respondents (Wolf et al., 1996, their table 4). In contrast, 64% of the Wolf et al. (1996) survey respondents reported that they based their evaluation of the habitat quality on their subjective opinion alone. (The remaining 36% of the respondents incorporated some quantitative assessment of the habitat quality.) Nevertheless, given the consistency of habitat quality as an important correlate of successful translocations, we agree with Lindenmayer (1994), Armstrong and McLean (1995), and others who recommend that translocation protocols should incorporate rigorous, quantitative assessments of habitat quality.

The total number of animals released over the duration of a translocation program also remained a consistent predictor of translocation success, irrespective of analytical technique (Griffith et al., 1989; Wolf et al., 1996; this study). Although populations were established with an extremely small number of individuals (see examples in Copley, 1994), the consistency of our results supports the notion that adverse demographic and environmental stochastic effects are more prevalent in small populations. As discussed by Wolf et al. (1996), however, determination of some minimum viable number of animals to release will depend on the unique circumstances of each translocation. Computer programs that analyze population viability under diverse conditions may provide helpful minimum population size estimates. One admonition to translocation practitioners is not to mistake a high density of animals present as necessarily indicating a self-sustaining population. By including duration of a population's persistence in our outcome variable, we presumably have reduced potential effects of over-reporting of self-sustaining translocation populations by respondents. This is an important consideration because the average population of translocated animals reported on by survey respondents had not persisted (since the last release) longer than the maximum expected life span of the translocated species in the wild.

Release of translocated animals into the core of the species' historical range was the third consistent predictor of success in both nonphylogenetic and phylogenetic analyses. As noted in Wolf et al. (1996), our findings appear to contradict Lomolino and Channell (1995) recommendations, which were derived from their observation that declining terrestrial mammals tend to persist on the periphery of their historical range. However, our findings do support a study by Nathan et al. (1996), in which they conclude that peripheral bird populations are more vulnerable to extinction. With all else held equal (e.g. habitat quality), our results suggest that animals released into the core of their historical distribution should have a greater chance of persisting and establishing a self-sustaining population.

Unlike the previous analyses (Griffith et al., 1989; Wolf et al., 1996), the phylogenetically based statistical model indicated that translocations involving mammals did not have statistically higher average success rates than those which involved birds. This difference cannot be attributed to our recoding of success into a composite, continuous variable, because the conventional multiple regression (Table 1, second column) also indicated taxonomic class as a significant predictor of success. Instead, the difference was likely caused by the fact that mammals and birds constitute two distinct clades, and hence all of the information pertaining to a possible difference in mean value was encapsulated in a single independent contrast connecting the roots of the mammalian and avian phylogenetic trees (Garland et al., 1993). The drastic difference in $p$ values between the nonphylogenetic and phylogenetic analyses with respect to taxonomic class (Table 1) is consistent with a methodological paper in which home range areas of mammalian Carnivora and ungulates were compared (Garland et al., 1993; and see Reynolds and Lee, 1996, for a similar example). Whenever single evolutionary events are involved (e.g. the ancient divergence between birds and mammals), it may be exceedingly difficult to demonstrate statistically significant effects (see Garland and Adolph, 1994; Martins and Hansen, 1996; Ricklefs and Starck, 1996).

Also, unlike the previous nonphylogenetic analysis (Wolf et al., 1996 and Table 1, first column), neither diet nor status was a statistically significant predictor of translocation success in the phylogenetic model. However, the omnivory indicator variable exhibited statistical significance ($p < 0.05$) in an early-stage phylogenetic model without the complementary carnivory indicator variable, thus providing some evidence that omnivores were generally more successful than animals of the other two diet categories. Moreover, the significance level for diet in the phylogenetic model ($p = 0.1135$) was not that much different from the ordinary regression ($p = 0.0414$). The difference between the nonphylogenetic logistic regression and the phylogenetically based regression with respect to status again cannot be attributed to our recoding of success, because the conventional multiple regression also indicated statistical significance (Table 1).

Significant model parameters in the Griffith et al. (1989) study, program length (number of years over which releases occurred) and species' reproductive potential were not found to be significant predictors of translocation outcome in either the phylogenetic or nonphylogenetic multiple regressions in Wolf et al. (1996) or this study (see Wolf et al. (1996) for further discussion).

We close by noting an important caveat concerning the present data set: it is not necessarily representative of birds and/or mammals in general. Extant birds and mammals comprise over 9 000 and 4 000 recognized species, yet were represented here by only 28 (0.3%) and 17 (0.4%) species, respectively. Many major lineages were entirely absent, such as hummingbirds, flycatchers, bats, cats, and whales. Moreover, some species were represented by multiple programs, most notably the wild turkey, which accounted for 19% of the 181 data points. These 35 data points can have a considerable impact on the statistical models. For example, when turkey programs were omitted from the non-phylogenetic ordinary multiple regression analysis (Table 1, second column), both diet ($p=0.4198$) and status ($p=0.1383$) became statistically nonsignificant. In the logistic regression (Table 1, first column), both variables also became less significant (diet $p=0.0170$; status $p=0.0553$). Because we were constrained for reasons of comparability with Griffith et al. (1989) and Wolf et al. (1996) and because many translocations in the original surveys had undetermined outcomes, many species were not included in our model building data set. Future comparative papers on translocations should, therefore, focus on improving the phylogenetic 'evenness' of species represented.

## 5. Conclusion

Although routinely employed in other disciplines, phylogenetically based statistical analyses are virtually nonexistent within such fields as conservation biology and wildlife management. Yet conservation and wildlife biologists often make interspecific comparisons. The same resemblances among closely related species that are often exploited by wildlife biologists for guidance in developing methodological protocols can confound traditional correlational and regression analyses of multi-species data sets. Species are related to greater or lesser degrees, depending on their phylogeny, and so do not necessarily offer independent or identically distributed data points, both of which are assumptions of conventional statistical analyses. Although conventional statistical analyses can be used to suggest important predictor variables in an interspecific comparative data set (Griffith et al., 1989; Wolf et al., 1996), phylogenetically based procedures are theoretically more reliable in terms of both hypothesis testing and parameter estimation. In any case, the different statistical methods presented in this study yielded congruent results (see also Ricklefs and Starck, 1996; Price, 1997) with respect to three variables associated with translocation success: the habitat quality of the release location, the number of individuals released, and the range of the release area relative to the historical distribution of the species.

Although still somewhat experimental, phylogenetic methods are continually being developed and refined, and our understanding of comparative statistical methods has risen dramatically in the past decade. We strongly encourage conservation and wildlife professionals to consider application of phylogenetically based statistical methods in future analyses of comparative data sets.

## Acknowledgements

The authors thank M.R. Dohm for assistance with statistical analyses and comments on manuscript drafts; R.E. Bleiweiss, J.A.W. Kirsch, and S. Moore helped with derivation of the phylogeny. Also greatly appreciated were C.M.W's. graduate committee members, P. Arcese, T.C. Moermond, and S.A. Temple. This study was funded with grants from the Zoological Society of Milwaukee County, the Chicago Zoological Society, and the Idaho Department of Fish and Game; T.G. is supported by National Science Foundation grants IBN-9157268 and DEB-9509343.

## References

Abouheif, E., Fairbairn, D.J., 1997. A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. American Naturalist 149, 540–562.

Allen, D.H., Franzreb, K.E., Escano, R.E.F., 1993. Efficacy of translocation strategies for red-cockaded woodpeckers. Wildlife Society Bulletin 21, 155–159.

Armstrong, D.P., McLean, I.G., 1995. New Zealand translocations: theory and practice. Pacific Conservation Biology 2, 39–54.

Bauwens, D., Díaz-Uriarte, R., 1997. Covariation of life-history traits in lacertid lizards: a comparative study. American Naturalist 149, 91–111.

Beck, B.B., Kleiman, D.G., Dietz, J.M., Castro, I., Carvalho, C., Martins, A., Rettberg-Beck, B., 1991. Losses and reproduction in reintroduced golden lion tamarins *Leontopithecus rosalia*. Dodo 27, 50–61.

Berger, A.J., 1972. Hawaiian Birdlife. University of Hawaii Press, Honolulu, Hawaii.

Bleiweiss, R., Kirsch, J.A.W., LaPointe, F.J., 1994. DNA hybridization-based phylogeny for higher nonpasserines: reevaluating a key portion of the Avian family tree. Molecular Phylogenetics and Evolution 3, 248–255.

Bleiweiss, R., Kirsch, J.A.W., Shafi, N., 1995. Confirmation of a portion of the Sibley–Ahlquist "tapestry". Auk 112, 87–97.

Bright, P.W., Morris, P.A., 1994. Animal translocation for conservation: performance of dormice in relation to release methods, origin and season. Journal of Applied Ecology 31, 699–708.

Brooks, D.R., McLennan, D.A., 1991. Phylogeny, Ecology, and Behavior: a research program in comparative biology. University of Chicago Press, Chicago, Illinois.

Catzeflis, F.M., Dickerman, A.W., Michaux, J., Kirsch, J.A.W., 1993. DNA hybridization and rodent phylogeny. In: Szalay, F.S., Novacek, M.J., McKenna, M.C. (Eds.), Mammal Phylogeny, Vol. II. Placentals, Springer-Verlag, New York, pp. 159–172.

Clobert, J., Garland, T., Jr, Barbault, R., 1998. The evolution of demographic tactics in lizards: a test of some hypotheses concerning life history evolution. Journal of Evolutionary Biology 11, 329–364.

Copley, P.B., 1994. Translocations of native vertebrates in South Australia: a review. In: Serena, M. (Ed.), Reintroduction Biology of Australian and New Zealand fauna. Surrey Beatty and Sons, Chipping Norton, Australia, pp. 35–42.

Crawley, M.J., 1986. The population biology of invaders. Philosophical Transactions of the Royal Society of London B314, 711–731.

Díaz, J.A., Bauwens, D., Asensio, B., 1996. A comparative study of the relation between heating rates and ambient temperatures in lacertid lizards. Physiological Zoology 69, 1359–1383.

Díaz-Uriarte, R., Garland, T., Jr, 1996. Testing hypotheses of correlated evolution using phylogenetically independent contrasts: sensitivity to deviations from Brownian motion. Systematic Biology 45, 27–47.

Díaz-Uriarte, R., Garland, T. Jr, In press. Effects of branch lengths errors on performance of phylogenetically independent contrasts. Systematic Biology.

Dodd, C.K., Jr, Seigel, R.A., 1991. Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica 47, 336–350.

Draper, N., Smith, H., 1981. Applied Regression Analysis, 2nd edition. John Wiley and Sons, New York.

Easteal, S., 1990. The pattern of mammalian evolution and the relative rate of molecular evolution. Genetics 124, 165–173.

Eggleton, P., Vane-Wright, R.I. (Eds.), 1994. Phylogenetics and Ecology. Linnean Society Symposium Series Number 17. Academic Press, London.

Ehrlich, P.R., 1986. Which animal will invade? In: Mooney, H.A., Drake, J.A. (Eds.), Ecology of Biological Invasions of North America and Hawaii. Springer-Verlag, New York, pp. 79–95.

Felsenstein, J., 1985. Phylogenies and the comparative method. American Naturalist 125, 1–15.

Garland, T., Jr, 1992. Rate tests for phenotypic evolution using phylogenetically independent contrasts. American Naturalist 140, 509–519.

Garland, T., Jr, Adolph, S.C., 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. Physiological Zoology 67, 797–828.

Garland, T., Jr, Carter, P.A., 1994. Evolutionary physiology. Annual Review of Physiology 56, 579–621.

Garland, T., Jr, Harvey, P.H., Ives, A.R., 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology 41, 18–32.

Garland, T., Jr, Dickerman, A.W., Janis, C.M., Jones, J.A., 1993. Phylogenetic analysis of covariance by computer simulation. Systematic Biology 42, 265–292.

Grafen, A., 1989. The phylogenetic regression. Philosophical Transactions of the Royal Society of London B326, 119–157.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C., 1989. Translocations as a species conservation tool: status and strategy. Science 245, 477–480.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C., 1990. Translocations of captive-reared terrestrial vertebrates, 1973–1986. Endangered Species UPDATE 8, 10–14.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C., 1993. Animal translocations and potential disease transmission. Journal of Zoo and Wildlife Medicine 24, 231–235.

Hafner, D.J., 1984. Evolutionary relationships of the Nearctic Sciuridae. In: Murie, J.O., Michener, G.R. (Eds.), The Biology of Ground-Dwelling Squirrels. University of Nebraska Press, Lincoln, Nebraska, pp. 3–23.

Harvey, P.H., Pagel, M.D., 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.

Jackson, J.A., 1994. The red-cockaded woodpecker recovery program: professional obstacles to cooperation. In: Clark, T.W., Reading, R.P., Clarke, A.L. (Eds.), Endangered Species Recovery: Finding the Lessons, Improving the Process. Island Press, Washington, D. C., pp. 157–181.

Janke, A., Feldmaier-Fuchs, G., Thomas, W.K., von Haeseler, A., Pääbo, S., 1994. The marsupial mitochondrial genome and the evolution of placental mammals. Genetics 137, 243–256.

Johnsgard, P.A., 1973. Grouse and Quails of North America. University of Nebraska Press, Lincoln, Nebraska.

Johnson, J.I., Kirsch, J.A.W., Reep, R.L., Switzer III, R.C., 1994. Phylogeny through brain traits: more characters for the analysis of mammalian evolution. Brain, Behavior and Evolution 43, 319–347.

Kurtén, B., Anderson, E., 1980. Pleistocene Mammals of North America. Columbia University Press, New York.

Laycock, G., 1966. The Alien Animals. Natural History Press. Garden City, New York.

Li, W.-H., Gouy, M., Sharp, P.M., O'hUigin, C., Yang, Y.-W., 1990. Molecular phylogeny of Rodentia, Lagomorpha, Primates, Artiodactyla, and Carnivora and molecular clocks. Proceedings of the National Academy of Science, USA 87, 6703–6707.

Lindenmayer, D.B., 1994. Some ecological considerations and computer-based approaches for the identification of potentially suitable release sites for reintroduction programmes. In: Serena, M. (Ed.), Reintroduction biology of Australian and New Zealand fauna, Surrey Beatty and Sons, Chipping Norton, Australia, pp. 1–5.

Livezey, B.C., 1986. A phylogenetic analysis of Recent anseriform genera using morphological characters. Auk 103, 737–754.

Lomolino, M.V., Channell, R., 1995. Splendid isolation: patterns of geographic range collapse in endangered mammals. Journal of Mammalogy 76, 335–347.

Losos, J.B., Miles, D.B., 1994. Adaptation, constraint, and the comparative method: phylogenetic issues and methods. In: Wainwright, P.C., Reilly, S.M. (Eds.), Ecological Morphology: Integrative Organismal Biology. University of Chicago Press, Chicago, Illinois, pp. 60–98.

Martin, T.E., Clobert, J., 1996. Nest predation and avian life history evolution in Europe versus North America: a possible role of humans?. American Naturalist 147, 1028–1046.

Martins, E.P. (Ed.), 1996a. Phylogenies and the Comparative Method in Animal Behavior. Oxford University Press, Oxford.

Martins, E.P., 1996. Phylogenies, spatial autoregression and the comparative method: a computer simulation test. Evolution 50, 1750–1765.

Martins, E.P., Garland, T., Jr, 1991. Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. Evolution 45, 534–557.

Martins, E.P., Hansen, T.F., 1996. The statistical analysis of interspecific data: a review and evaluation of comparative methods. In: Martins, E.P. (Ed.), Phylogenies and the Comparative Method in Animal Behavior. Oxford University Press, Oxford, pp. 22–75.

Mayr, E., 1965. The nature of colonizations in birds. In: Baker, H.G., Stebbins, G.L. (Eds.), The Genetics of Colonizing Species. Academic Press, New York, pp. 29–43.

Miles, D.B., Dunham, A.E., 1993. Historical perspectives in ecology and evolutionary biology: the use of phylogenetic comparative analyses. Annual Review of Ecology and Systematics 24, 587–619.

Montgomery, D.C., Peck, E.A., 1992. Introduction to Linear Regression Analysis, 2nd Edition. John Wiley and Sons, New York.

Nathan, R., Safriel, U.N., Shirihai, H., 1996. Extinction and vulnerability to extinction at distribution peripheries: an analysis of the Israeli breeding avifauna. Israel Journal of Zoology 42, 361–383.

Norusis, M.J., 1992a. SPSS/PC+ Advanced Statistics. SPSS, Chicago, Illinois.

Norusis, M.J., 1992b. SPSS/PC+ Base System User's Guide, Version 5.0. SPSS, Chicago, Illinois.

Novacek, M.J., 1992. Fossils, topologies, missing data, and the higher level phylogeny of eutherian mammals. Systematic Biology 41, 58–73.

Novacek, M.J., 1992. Mammalian phylogeny: shaking the tree. Nature 356, 121–125.

O'Connor, R.J., 1986. Biological characteristics of invaders among bird species in Britain. Philosophical Transactions of the Royal Society of London B314, 583–598.

Pagel, M., 1993. Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. Journal of Theoretical Biology 164, 191–205.

Price, T., 1997. Correlated evolution and independent contrasts. Philosophical Transactions of the Royal Society of London B352, 519–529.

Purvis, A., Garland, T., Jr, 1993. Polytomies in comparative analyses of continuous characters. Systematic Biology 42, 569–575.

Purvis, A., Rambaut, A., 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. Computer Application Biosciences 11, 247–251.

Purvis, A., Gittleman, J.L., Luh, H.-K., 1994. Truth or consequences: effects of phylogenetic accuracy on two comparative methods. Journal of Theoretical Biology 167, 293–300.

Reed, C.E.M., Nilsson, R.J., Murray, D.P., 1993. Cross-fostering New Zealand's black stilt. Journal of Wildlife Management 57, 608–611.

Reynolds, P.S., Lee III, R.M., 1996. Phylogenetic analysis of avian energetics: passerines and nonpasserines do not differ. American Naturalist 147, 735–759.

Ricklefs, R.E., Starck, J.M., 1996. Applications of phylogenetically independent contrasts: a mixed progress report. Oikos 77, 167–172.

Ridley, M., Grafen, A., 1996. How to study discrete comparative methods. In: Martins, E.P. (Ed.), Phylogenies and the Comparative Method in Animal Behavior. Oxford University Press, Oxford, pp. 76–103.

Rowe, T., 1993. Phylogenetic systematics and the early history of mammals. In: Szalay, F.S., Novacek, M.J., McKenna, M.C. (Eds.), Mammal Phylogeny, Vol. I. Mesozoic differentiation, Multituberculates, Monotremes, early Therians, and Marsupials. Springer-Verlag, New York, pp. 129–145.

Sarich, V.M., 1985. Rodent macromolecular systematics. In: Luckett, W.P., Hartenberger, J.L. (Eds.), Evolutionary Relationships Among Rodents: a Multidisciplinary Analysis. Plenum Press, New York, pp. 423–452.

Short, J., Bradshaw, S.D., Giles, J., Prince, R.I.T., Wilson, G.R., 1992. Reintroduction of macropods (Marsupialia: Macropodoidea) in Australia — a review. Biological Conservation 62, 189–204.

Sibley, C.G., Ahlquist, J.E., 1990. Phylogeny and Classification of Birds: a Study in Molecular Evolution. Yale University Press, New Haven, Connecticut.

Smith, M.H., Hillestad, H.O., Manlove, M.N., Marchinton, R.L., 1976. Use of population genetics data for management of fish and wildlife populations. Transactions of the North American Wildlife and Natural Resources conference 41, 119–133.

Veitch, C.R., 1994. Habitat repair: a necessary prerequisite to translocation of threatened birds. In: Serena, M. (Ed.), Reintroduction Biology of Australian and New Zealand Fauna. Surrey Beatty and Sons, Chipping Norton, Australia, pp. 97–104.

Veltman, C.J., Nee, S., Crawley, M.J., 1996. Correlates of introduction success in exotic New Zealand birds. American Naturalist 147, 542–557.

Wayne, R.K., VanValkenburgh, B., O'Brien, S.J., 1991. Molecular distance and divergence time in carnivores and primates. Molecular Biological Evolution 8, 297–319.

Williams, J.B., 1996. A phylogenetic perspective of evaporative water loss in birds. The Auk 113, 457–472.

Wilson, A.C., Stanley Price, M.R., 1994. Reintroduction as a reason for captive breeding. In: Olney, P.J.S., Mace, G.M., Feistner, A.T.C. (Eds.), Creative Conservation: Interactive Management of Wild and Captive Animals. Chapman and Hall, London, pp. 243–264.

Wolf, C.M., Griffith, B., Reed, C., Temple, S.A., 1996. Avian and mammalian translocations: update and reanalysis of 1987 survey data. Conservation Biology 10, 1142–1154.

## Appendix

Table A1
Sources used to derive the topology and divergence times of the phylogenetic tree in Fig. 1[1]

| Lineage Divergence | Date (MYA) | Source[1] |
|---|---|---|
| Aves/Mammalia | 270 | Li et al., 1990 |
| Avian Taxa | | |
| *Callaeas/Creadion* | 5 | (arbitrary date) |
| *Telespiza ultima/T. cantans* | 1 | (arbitrary date) |
| *Cynanoramphus unicolor/C. novaezelandiae* | 1 | (arbitrary date) |
| *Anas fulvigula/A. laysanensis* | 1 | (arbitrary date) |
| *Meleagris/Bonasa*, *Tympanuchus* | 20 | (arbitrary date) topology, Johnsgard, 1973 |
| *Bonasa/Tympanuchus* | 15 | Johnsgard, 1973<br>(mid-Miocene for earliest fossil of *Tympanuchus*) |
| *Tympanuchus pallidicinctus/T. phasianellus* | 1 | (arbitrary date) |
| Mammalian Taxa | | |
| Marsupialia/Eutheria | 110 | J.A.W. Kirsch, pers. comm. (100–110 MYA)<br>Novacek, 1992b (130 MYA)<br>Rowe, 1993 (95–100 MYA) |
| Phascolaretidae/Myrmecobiidae | 60 | J.A.W. Kirsch, pers. comm. |
| Rodentia/Primates,Carnivora,Artiodactyla[2] | 100 | Li et al., 1990 (81–100 MYA)<br>Easteal, 1990 (topology)<br>Janke et al., 1994 (114 ± 15 MYA) |
| Sciuridae/Muridae | 55 | Sarich, 1985 |
| *Peromyscus/Neotoma* | 7.3 | Catzeflis et al., 1993 |
| *Cynomys/Sciurus* | 30 | Hafner, 1984 (near the end of the Oligocene) |
| Primates/Carnivora,Artiodactyla | 85 | Li et al., 1990 (65–85 MYA)<br>Janke et al., 1994 (93 ± 12 MYA) |
| *Lutra/Martes* | 20 | Kurtén and Anderson, 1980<br>(lower Miocene for earliest occurance of *Martes*) |

[1] Sources reported are those other than Sibley and Ahlquist (1990) and Garland et al. (1993). The DNA–DNA hybridization study of Sibley and Ahlquist (1990) served as the primary source for the avian taxa. Branch lengths were based on the mean $\Delta$ $T_{50}H$ values provided by Sibley and Ahlquist (1990); each $\Delta$ $T_{50}H$ value was multiplied by a calibration constant between 2 and 4.5 for conversion to an estimated divergence time. Calibration constants vary because the rate of DNA evolution differs among lineages; those used for Fig. 1 were approximated to accommodate the $\Delta$ $T_{50}H$ branching order and the tentative rates of evolution for specific lineages and the fossil record as discussed by Sibley and Ahlquist (1990). Bleiweiss et al. (1994, 1995) supported our use of the Sibley and Ahlquist phylogeny for the branching order between the Passeriformes, Strigiformes, Piciformes, Anseriformes, and Galliformes. Although Livezey (1986) hypothesized a conflicting phylogenetic tree for the Anseriform genera based on 120 morphological characters (showing *Branta* more closely related to *Cygnus* than with *Anas*), we chose to use the molecularly-based analyses of Sibley and Ahlquist (1990). Garland et al. (1993) and sources therein as the primary source for the mammalian taxa. If divergence times were not available from the literature, we assigned arbitrary divergence times of 1 MYA (million years ago) between congeneric species and 5 or 20 MYA between species within the same family. The estimated divergence times were used to test for the adequacy of the branch lengths for statistical analyses (see Garland et al., 1992). The virgule (/) represents the tree node separating the taxon (a) on its right and left (refer to Fig. 1).

[2] For contradicting branching order, see also Novacek, 1992a,b and Johnson et al. (1994).