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# Dispersal genetic relationships and landscape use by colonizing wolves in the central Rocky Mountains

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# DISPERSAL, GENETIC RELATIONSHIPS, AND LANDSCAPE USE BY COLONIZING WOLVES IN THE CENTRAL ROCKY MOUNTAINS

by

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Presented in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
University of Montana, 1997

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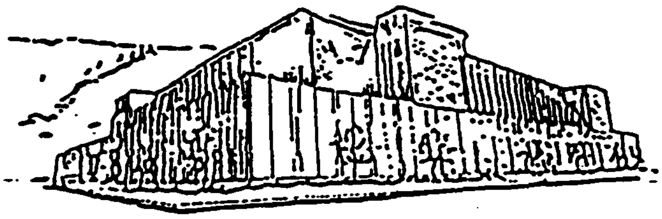

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Boyd-Heger, Diane K. Ph.D. October 1997 Fish and Wildlife Biology

Dispersal, Genetic Relationships, and Landscape Use by Colonizing Wolves in the Central Rocky Mountains (184 pp).

Director: Daniel H. Pletscher DHP

Wolves were eradicated from Montana in the 1930s and the adjacent Canadian Rockies by the 1950s, followed by recolonization in the 1980s. I studied wolf recovery in and near Glacier National Park, Montana, from 1979-1997. Wolves dispersed from the protected refugia of Glacier National Park and colonized areas in northwestern Montana, Idaho, southeastern British Columbia, and southwestern Alberta. During this time period 31 of 58 tagged wolves dispersed, and 3 of those dispersed twice. Most wolves (57%) did not conduct any exploratory forays 3 months prior to permanent separation from their natal pack. Wolves usually left their natal home range quickly (median = 4 days) once they separated from the pack. Mean dispersal distance was not significantly different for males (113 km) and females (78 km). Wolves tended to disperse in a northerly direction to areas of higher wolf density. January-February and May-June were peak months for dispersal. Mean dispersal age (male = 28.7 mos, females = 38.4 mos) was not correlated with maximum pack size. Twenty percent of dispersers were $\geq$ 57 months old at dispersal. Sex ratios of dispersers and captured wolves (both 71%F) were significantly different from parity. Survival for dispersers and biders did not differ. Eighty percent (n = 30) of wolf mortalities were caused by humans, with proportionately more dispersers (90%) than biders (60%) dying from human causes. Dispersers produced significantly more litters than biders. Effects of mountainous terrain on wolf dispersal are discussed.

Colonizing wolves had adequate genetic variation which was similar to that found in other wolf populations in North America. Sufficient gene flow occurred between the colonizing and source populations to minimize genetic divergence. Packs were founded by multiple, unrelated wolves from Canada, with no evidence of a population bottleneck. Reintroduced wolves were moderately genetically divergent from the colonizing population and have adequate genetic variation. I detected no evidence of hybridization between wolves and coyotes. Detection of non-native canids and management considerations are discussed.

I analyzed landscape attributes selected by 6 colonizing wolves including elevation, slope, aspect, distance to water, distance to roads, road density, and canopy cover. Wolves selected for landscapes with relatively lower elevation, flatter terrain, and closer to water and roads than expected based on availability inside and outside of their new home range. I built a logistic regression model using the univariately significant attributes (elevation, slope, and distance to roads) to predict wolf presence in areas of potential colonization. Impacts of habitat fragmentation and management considerations are discussed.

# Table of Contents

## *Dispersal, Genetic Relationships, and Landscape Use by Colonizing Wolves in the Central Rocky Mountains*

# LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

# LIST OF FIGURES

Chapter 2

Chapter 4

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Bob Ream and Dan Pletscher, are responsible for the initiation and completion, respectively, of my University of Montana research. I thank Bob Ream for initiating wolf monitoring in Montana in the 1970s and creating the Wolf Ecology Project. I reserve a very special thanks for Dan Pletscher, my Ph.D. advisor, who encouraged me to broaden my perspectives beyond the focus of field work. Dan's competence, dedication to conservation, and commitment to students is a model for wildlife professionals everywhere. Many thanks to my committee members Dan Pletscher, Fred Allendorf, Steve Fritts, Bob Ream, Roly Redmond, and Hans Zuuring; your expertise, time, and thorough editing were greatly appreciated. Dave Patterson and Dick Lane gave additional statistical advice.

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The genetic analyses were aided by a collaborative effort among many cooperators. Steve Forbes' meticulous work ensured integrity of analyses and provided an intelligible link between genetics and wildlife biology. Kristy Pilgrim conducted much of the lab work and was an enthusiastic asset to our efforts. Wolf samples were provided by Paul Paquet, Carolyn Callaghan, Banff Wolf Project; Cam McTavish, Kootenay Wolf Project; Steve Fritts, Ed Bangs, Joe Fontaine, Jim Till, Steve Fain, Rick Branzell, U.S. Fish and Wildlife Service; Mark Johnson, Yellowstone National Park; John Gunson and Bob McClymont, Alberta Wildlife Division; Mike Jimenez, University of Montana; Carter Neimeyer, USDA-APHIS-ADC; and Canadian hunters and trappers. Coyote samples were provided by Larry Handegard, Dave Nelson, John Bouchard, Graeme McDougal, Ted North, USDA-APHIS-ADC; and Wendy Arjo, University of Montana.

Landscape use analyses were conducted at the Wildlife Spatial Analysis Lab, University of Montana. Spatial data of Alberta were contributed by Craig Stewart, Crown of the Continent Ecosystem Atlas, and added to the existing data base at the Wildlife Spatial Analysis Lab. I thank Claudine Tobalske for her patience and technological skills during the GIS analyses. Judy Troutwine skillfully joined the U.S. and Canadian data bases into a digitally functional unit.

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My parents, Harold and Verna Boyd, deserve special thanks for their support. They fostered my independence and scientific curiosity, and enjoyed having a black sheep in the family. If Harold had lived long enough to witness the completion of this dissertation, I think he would have been proud.

And lastly, I thank my husband, Ed, for his love, patience, support, and editing skills. He has made this doctoral process much more enjoyable and my life much richer. When he moved to the North Fork as a bachelor and saw 3 wolves at his cabin one morning, little did he know the impact those canid messengers would have in his future. I owe that trio of wolves a big thanks.

***I dedicate this dissertation to Phyllis, Sage, Mojave, Aspen, and those yet to come.***

*In sustaining our images of objectivity,*
*we as biologists pretend that we study wild animals.*
*In reality, we inevitably study an interface*
*between ourselves and other species.*

*Harley Shaw, Soul Among Lions 1989*

# Chapter 1. Introduction and Synthesis of Dissertation

## BACKGROUND

Gray wolves (*Canis lupus*) were systematically extirpated from the western United States (US) between the late 1800s and early 1900s to eliminate wolf-human conflicts (Mech 1970). However, a change in public sentiment in the mid-1900s resulted in the anti-predator attitude being counter-balanced by increasingly pro-predator sentiment (Leopold 1949, Kellert 1985, McNaught 1987, Bath and Buchanan 1989, Tucker and Pletscher 1989). Land managers in the US have been mandated by the Endangered Species Act since 1973 to recover wolves. The combination of increasing public tolerance and legal protection set the stage for wolf recovery.

Wolves began colonizing northwestern Montana in the late 1970s (Ream and Mattson 1982, Ream et al. 1991) through dispersal from source populations in Canada. When these semi-isolated packs appeared progressively further from source wolf populations, questions arose regarding habitat suitability, landscape connectivity, gene flow, and loss of genetic variation. Successful colonization depends upon dispersers that find suitable habitat and produce reproductively successful offspring. Likewise, genetic relationships are intimately related to dispersal capabilities and subsequent reproductive success to enhance gene flow. Therefore, the integration of dispersal, genetics, and habitat are the biological foundation of wolf recovery and the focus of my dissertation.

Wolves began recolonizing the Glacier National Park, Montana, area (GNP) in the late 1970s (Ream and Mattson 1982, Ream et al. 1991). I monitored the growth of the GNP population from the first successful colonizer in 1979 to a population of approximately 35 wolves in 3 packs in 1997. I also monitored dispersals from the GNP population to disjunct areas, often several hundred kilometers from GNP, and subsequent colonization of Montana, Idaho, southeastern British Columbia, and southwestern Alberta.

Herein, I report on the dispersal movements, genetic relationships, and landscape use by colonizing wolves in the Central Rocky Mountains. I defined the Central Rockies as the geographic area centered on the junction of the Continental Divide, Canada, and the United States, and includes the landscape from Banff, Alberta, to Helena, Montana. My study of wolf colonization may provide insights on recovery that may be applied to other species. If habitat continues to become more fragmented and potentially unreachable as well as unsuitable, isolation and decreased gene flow may become more critical. Additionally, managed reintroductions of wolves to parts of their former range are an ongoing effort of restoration ecologists. Recent conservation efforts have focused on restoring populations at the edge of their range (Yellowstone National Park and central Idaho) and in isolated populations (Arizona and New Mexico). Wolves in the US have the benefit of Canada as a large source population, yet biologists should assess the role of landscape connections in maintaining adequate gene flow on an international scale. The colonization patterns I document may help predict the type of movements to expect as wolves expand their range, as well as address conservation concerns about effects of dispersal, gene flow, habitat fragmentation, and human influence on wolf recovery.

My overall objective was to evaluate the mechanisms of wolf colonization in the Central Rocky Mountains. My dissertation is composed of 3 chapters on various aspects of wolf colonization:

*Chapter 2* Dispersal Characteristics of Colonizing Wolves in the Central Rockies
*Chapter 3* Genetic Relationships of Colonizing Wolves in the Central Rockies
*Chapter 4* Landscape Use by Colonizing Wolves in the Central Rockies

When wolves appear in the western US far from occupied wolf range, questions arise regarding the origin of the animal. Managers and public alike are surprised when the proverbial "wolf at the door" becomes a reality in North Dakota (Licht and Fritts 1994) or Wyoming, as has happened in the past 5 years. What factors allow a disperser to be successful and subsequently colonize a new area? What role does an immigrant play in contributing new genes to the population? How do humans influence the survival of dispersers? Both survival and reproduction are critical components of the successful wolf colonization. How are dispersal, genetic variation, and landscape use linked in the process of wolf recovery?

## SYNTHESIS

Wolves have tremendous dispersal capabilities and, if tolerated by humans, can colonize areas far from source populations (Mech 1995). At the beginning of my study in 1979, the colonizing GNP population was the southernmost extension of the Rocky Mountain wolf population. As the GNP wolf population increased, it became the source population for colonization of other areas through dispersals. Dispersals covered a wide range of duration, distances, directions, seasons, and landscapes. Wolves apparently have sufficient behavioral flexibility to allow them to colonize unfamiliar areas under a variety of circumstances. Dispersal was apparently a successful strategy for colonization in terms of reproductive contribution: dispersers produced relatively more litters than did philopatric wolves. Survival of dispersers and biders of dispersal age was similar.

Gene flow occurs through genetically effective dispersals. Dispersal is important, especially in isolated populations, to benefit genetic exchange and reduce the probability of inbreeding depression (Allendorf and Leary 1986, McCaughley 1991). Published studies have not analyzed the relationship between wolf dispersal and genetics and the significance of this relationship to colonization. Slatkin (1985:426) reported that "detailed observations of dispersal are needed in more species for which adequate genetic data can be obtained, with particular attention paid to dispersal over long distances". My study presented a unique opportunity to combine more than a decade of dispersal data with genetic analyses to investigate their relationship. Adequate genetic variation was documented, and the minimum number and degree of relatedness of founders was determined in the colonizing population (Forbes and Boyd 1996). Additionally, genetic variation was examined in the reintroduced populations in Yellowstone National Park and central Idaho (Forbes and Boyd 1997). Forbes and Boyd (1996, 1997) demonstrated the need for landscape connectivity to maintain adequate gene flow through migration for both the naturally colonizing and reintroduced populations. We developed a test to differentiate wolf and coyote haplotypes using a restriction site and a length difference in the control region (D-loop) of mitochondrial DNA (Pilgrim et al., in press). We then examined samples of Rocky Mountain coyotes and wolves and found no evidence of hybridization.

Distance and frequency of dispersal may be affected by habitat quality and patchiness, population densities, social dynamics, and mating systems (Slatkin 1985). Habitat fragmentation, characterized by 1) reduction in amount of habitat available and 2) habitat division into smaller, more isolated patches, is the primary cause of extinctions in some species (Wilcox and Murphy 1985). Human modifications of the landscape have threatened the survival and caused the extinction of many species (Meffe and Carroll 1994). The differences between natural landscape patchiness and human-caused fragmentation have only recently been explored. Meffe and Carroll (1994) provided 3 defining distinctions: 1) naturally patchy landscapes have rich internal patch structure containing internal heterogeneity (e.g. different layers of vegetation), whereas landscapes fragmented by human activities tend to have simplified patches lacking internal heterogeneity (i.e. parking lots, mono-culture fields, tree farms); 2) a naturally patchy landscape has less contrast between adjacent patches than does a human-fragmented landscape; and 3) some features of human-fragmented landscapes pose threats to species existence, such as roads and human activities.

Wolf habitat use and the dynamics of colonization in mountainous terrain have not been previously studied. The rugged, heterogeneous nature of mountainous topography fragments habitat and separates packs (Fritts et al. 1994), causing a non-contiguous pack distribution. The patchiness of the landscape resulting from mountainous topography plus human activities may provide sufficient barriers to limit gene flow, thus hampering recovery. However, wolves are a highly mobile species with dispersals occasionally exceeding 600 km (Fritts 1983, Ballard et al. 1987, Boyd et al. 1995). I found that wolves often moved several hundred kilometers to colonize new areas. Colonizing wolves selected habitats that were lower in elevation, less steep, and closer to roads and water than expected based on availability. These 4 landscape attributes were highly correlated with each other. I developed a logistic regression model that predicted wolf presence (69% correct classification explained) in recently colonized areas as a function of elevation, slope, and distance to roads.

The Rocky Mountains have been viewed as a large mammal dispersal corridor between Canada and the US. However, wolves have demonstrated that the Central Rockies function as much more than a corridor of movement. This region provided suitable habitat patches along its length and into the foothills that wolves colonized. Wolf colonization occurred first in valley bottoms, with relatively long-distance linear separation between packs conforming to the heterogeneous nature of mountainous landscape (Fritts and Carbyn 1995, Fritts et al. 1994, my study). Valley bottoms are also the preferred habitat of humans for habitation, farming, and ranching, which may give rise to wolf-human conflicts. Humans caused 80% ($n$ =30) of wolf mortalities in the GNP area, and human-caused mortalities were significantly closer to roads than nonhuman-caused mortalities. Thus, there are increased risks associated with wolf habitation of lower elevation landscapes.

The present situation in the Central Rockies provided a timely opportunity to examine the relationship of dispersal, genetic variation, and landscape use in this colonizing wolf population. The combination of 17 years of field observations, genetic analyses, and landscape use coordinated over such a large geographic region lends insights into the conservation of wolves in the Central Rockies. Researchers often isolate and study only 1 of these issues, yet dispersal, gene flow, and landscape use are integrated ecological factors. Managers can best enhance recovery by examining all 3 factors and understanding their interdependence. Theoretical implications of this research may aid other sensitive species and further efforts to maintain biodiversity in an increasingly fragmented world.

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# Chapter 2: Characteristics of Dispersal in a Colonizing Wolf Population in the Central Rocky Mountains

**Abstract:** Wolves were eradicated from Montana in the 1930s and the adjacent Canadian Rockies by the 1950s, followed by recolonization in the 1980s. Wolf recovery was studied in and near Glacier National Park, Montana, from 1979-1997. During this period 31 of 58 tagged wolves dispersed. Most wolves (57%) did not make exploratory forays 3 months prior to permanent separation from their natal pack. Wolves usually left their natal home range quickly (median = 4 days, mode = 1 day) after separating from the pack. Mean dispersal distance was not significantly different for males (113 km) and females (78 km), excluding an unusually long dispersal of 840 km by a yearling female. Wolves tended to disperse in a northerly direction to areas of higher wolf density. January-February and May-June were peak months for dispersal. Mean dispersal age (male = 28.2 mos, females = 38.4 mos) was not correlated with maximum pack size. Twenty percent of dispersers were > 50 months old at dispersal. Sex ratios of dispersers and captured wolves (both 71% F) were significantly different from parity. Survival of dispersers and biders did not differ. Wolves killed by humans died significantly closer to roads (mean = 0.2 km) than wolves that died from other causes (mean = 0.8 km). Eighty percent ($n = 30$) of wolf mortalities were caused by humans, with proportionately more dispersers (90%) than biders (60%) dying from human-caused mortalities. Dispersers produced significantly more litters than biders. Effects of mountainous terrain and management on wolf recovery are discussed.

## INTRODUCTION

Wolves were extirpated from the western US by the 1930s (Young and Goldman 1944, USFWS 1987). Wolf populations were severely reduced in the Canadian Rocky Mountains of southeastern British Columbia and southwestern Alberta in the 1930s and again in the 1950s (Tompa 1983, Gunson 1983). By the late 1950s a viable wolf population ceased to exist between Jasper National Park, Alberta, and Glacier National Park (GNP), Montana (Day 1981, Ream and Mattson 1982, Boyd et al. 1995). In the 1970s wolves began recolonizing this region of Canada and northwestern Montana (Ream et al. 1991, Hayes and Gunson 1995), hereafter referred to as the Central Rocky Mountains. I defined the Central Rockies as the geographic area centered on the junction of the Continental Divide, Canada, and the United States, and includes the landscape from Banff, Alberta, to Helena, Montana.

This successful recolonization occurred through dispersal from source populations north of Banff National Park (BNP), Alberta (P. Paquet, pers. commun.; Boyd et al. 1995). Wolves are a highly mobile species with dispersals occasionally exceeding 600 km (Fritts 1983, Van Camp and Gluckie 1979, Ballard et al. 1987). Reports of long-distance movements are becoming more common with more wolves being tagged and improvements in telemetry technology. Wolves have substantial genetic variation across North America but relatively little differentiation between local populations (Kennedy et al. 1991, Wayne et al. 1992, Roy et al. 1994), additional evidence of frequent, long-distance dispersals and resulting gene flow.

In social animals such as wolves, dispersal is an important mechanism for population regulation (Lidicker 1975), genetic exchange (Forbes and Boyd 1996, Smith et al. 1997), social organization (Hamilton 1964, Zimen 1982, Greenwood 1980), and colonization (Gese and Mech 1991, Boyd et al. 1995). Other species may disperse in response to a variety of factors including competition for food and mating opportunities, environmental disruptions, social aggression, and habitat availability (Greenwood 1980, Waser 1985). Potential benefits of dispersal include increased reproductive success, decreased probability of inbreeding, release from intraspecific competition for resources, and expansion of a species range (Shields 1987). Potential costs of dispersal include increased mortality in unfamiliar habitat, outbreeding depression, and increased energetic demands during dispersal (Shields 1987). Population regulation and colonization will be enhanced by dispersal as long as benefits of dispersal for an individual exceed costs.

Several articles have been published containing information on wolf dispersal in North America. However, most dispersal data were collected incidental to other research topics and report on few dispersal events. Little information has been published on the role of dispersal in wolf recolonization (Fritts and Mech 1981), particularly in the Rocky Mountains. My objectives were to examine dispersal patterns, the outcome of dispersal events, and the role of dispersal in wolf recovery in the Central Rockies from 1979 to 1997.

## METHODS

### Study Area

The intensive study area included GNP and adjacent lands within 50 km of GNP, including southeastern British Columbia, southwestern Alberta, and northwestern Montana (Fig. 1). The core area is characterized by long narrow valley bottoms surrounded by rugged mountains, with elevations ranging from 1020-3600m. Dense coniferous forests dominate the GNP area, with meadow and riparian areas less common (Koterba and Habeck 1971). The few human residents were present mostly in summer. The extensive study area was defined by the movements of wolves dispersing from GNP, and extended from northern British Columbia south to Missoula, Montana, and east-west from the eastern Rocky Mountain Front to west-central Idaho (Fig. 2). The study area encompassed parts of 2 countries, 2 provinces, 2 states, and numerous subdivisions of jurisdictions within each of these (Pletscher et al. 1991), resulting in a mosaic of land management classes.

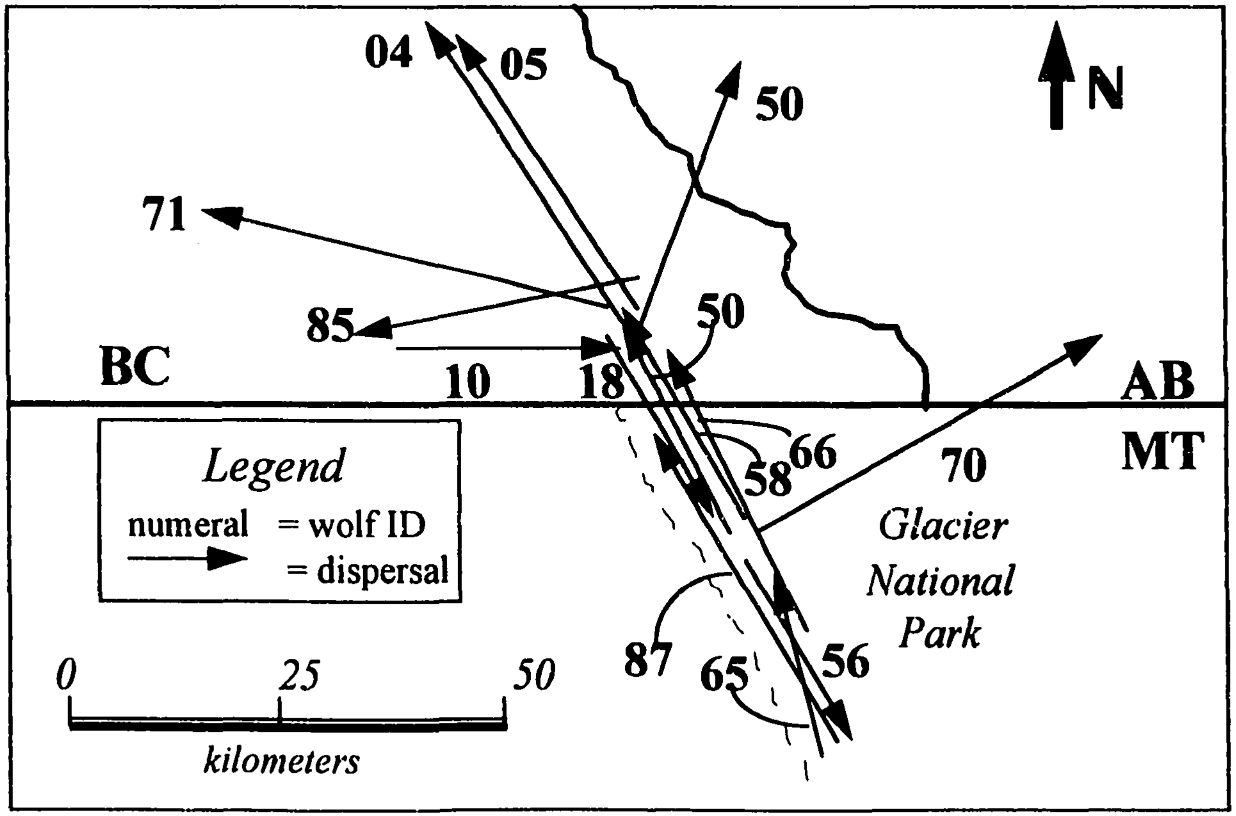

Figure 1. Dispersals < 50 km by 14 wolves from the Glacier National Park area, 1985-97.

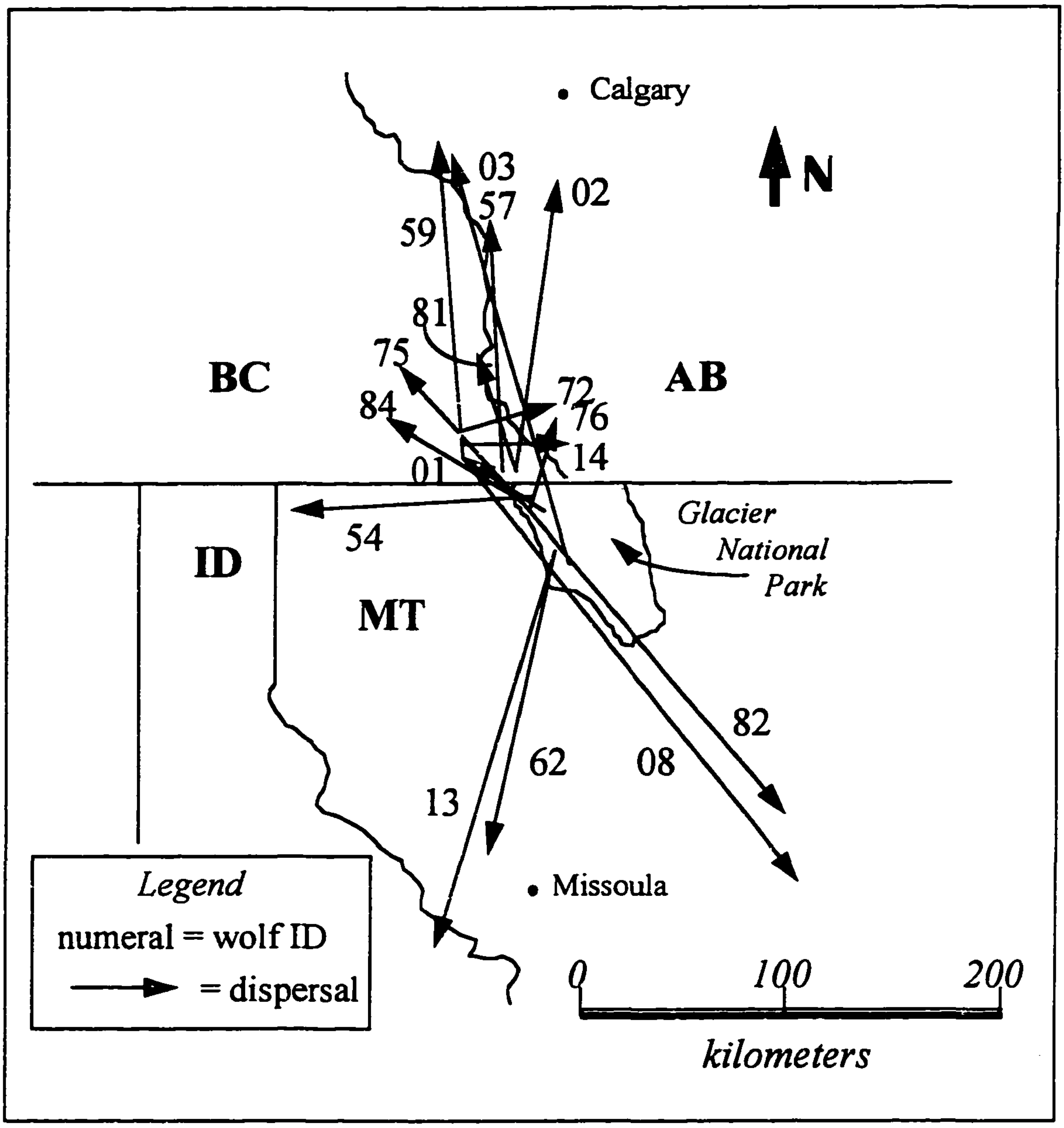

Figure 2. Dispersals > 50 km by 16 wolves from the Glacier National Park area, 1985-97.

## Captures

Wolves were captured with modified No. 4 Newhouse leghold traps and sedated as described by Mech (1974) and Ream et al. (1991). Pups < 20 kg were eartagged only; wolves > 20 kg were radiocollared and eartagged. Wolf age was estimated by dental eruption and wear patterns. Adult wolves of questionable age were assigned an age on the younger end of a reasonable range (e.g. a wolf 3-5 years old was called a 3 year-old), which may have biased age estimates downward.

## Telemetry and Dispersal

Radiocollared wolves were located approximately 3 times per week from the ground and once per week from an airplane. Immigration and emigration were often detected during aerial observations for 2 reasons: 1) Rocky Mountain wolves vary greatly in color from black to white, aiding identification of individuals, and 2) population dynamics of only a few packs were intensively monitored during the colonization period, enhancing visual familiarity with individuals. The first study wolf was tagged in 1979 but the first pre-dispersal movements weren't documented until 1985, and the actual first dispersal in 1986. Therefore, dispersal tables include data from 1985-1997.

If a radiocollared wolf was not detected during several location attempts, a search flight was conducted within 300 km of GNP at 3500-3700 m above sea level. When dispersed wolves were located outside of our study area, I passed their frequencies along to the regional biologists so they could monitor them. Frequencies of missing wolves were provided to biologists on other telemetry projects so they could listen for them. Dispersed wolves were monitored to determine their pack status, reproductive success, and fates. I analyzed many attributes of dispersal including distance, direction, season, age of disperser, sex ratio, temporal and spatial separation from pack, survivorship, and reproductive contribution. Data for all characteristics were not available for all dispersed wolves, so sample sizes differ for different analyses.

Wolves were protected as an endangered species in the US during the study, but were legally harvested in Alberta and British Columbia. I maintained contact with local residents and agencies which probably enhanced learning of mortalities of dispersed wolves. I investigated all mortalities of tagged wolves and called successful Canadian hunters to get accurate information on location, pack association, and reproductive condition of harvested wolves.

Shields (1987:4) defined *dispersal* as "the movement of an organism or propagule from its site or group of origin to its first or subsequent breeding site or group". Shields (1987) subdivided dispersal into: *natal dispersal* (the movement of a propagule between birthplace or natal group and first breeding site or group) and *breeding dispersal* (the movement between consecutive breeding sites or groups of adult breeders). Gese and Mech (1991) defined dispersal in wolves as having occurred when a wolf left its natal territory. I defined *natal pack* as the pack a wolf was a member of when it was captured, realizing that this was not necessarily the pack it was born in. Wolves that remained philopatric were classified as *biders* (Packard and Mech 1983).

A wolf may remain in its natal home range but separate from the pack for days or weeks before actually dispersing out of the natal home range. I defined *temporal separation from pack* as the time a wolf permanently dissociated from its natal pack and remained in its natal home range before actual dispersal. I counted the days between the last time it was located with the pack and the date when it permanently left its natal home range. I defined *spatial separation* as movements of a pack member that were disjunct from other pack members but in the pack's home range.

I correlated dispersal rate and age at dispersal with maximum size of the pack (adults and pups) a disperser belonged to $\leq$ 12 months prior to the dispersal (an index of population density). I included only those dispersers that had been located with members of a pack $\geq$ 1 month prior to dispersal. I calculated dispersal rate by dividing the number of dispersers in a given year by the maximum number of tagged wolves in the same year (Gese and Mech 1991).

I used CALHOME (1994) software to estimate the center point of natal and dispersed home ranges for each wolf. I reduced my location data so that there was > 2 consecutive days between locations per wolf to attain independence between locations. I then ran the adaptive kernel method in CALHOME, plotting natal and dispersed home ranges separately to determine the center point of each with better resolution. I estimated the center of the home range based on the 25% isopleth. I used the Pythagorean theorem in EXCEL (Microsoft 5.0) to calculate the dispersal distance from the center point of the natal home range to the center point of the dispersed home range. The dispersal of 8 wolves was determined solely by a reported mortality location (radiocollar returned) but with little other post-dispersal data. I used the mortality location as the dispersal endpoint for these animals.

To determine direction dispersed, I connected the center point of the natal home range with the center point of the dispersal home range (or mortality point), and measured the resultant azimuth. I categorized the azimuths into 2 directional groups: north = 271 - 90° and south = 91-270°. Sample sizes were too small to divide azimuths into more categories or to compare dispersal direction differences based on gender.

*Month of dispersal* and *age at dispersal* were estimated by a date halfway between the date a wolf was last located in its natal home range and first located permanently away from its natal home range. Two exceptions to this were wolves eartagged as pups but not radiocollared. Wolf 8502 was shot a year after he was eartagged, so I estimated his dispersal date as halfway between 12 months of age and death. I used this estimate of dispersal date because the youngest dispersal age I documented was 12 months, so I presumed that was the earliest age a wolf would disperse. Wolf 8808 was first located 57 months after I eartagged him, so I omitted him from age and month of dispersal analyses.

I defined *bounce time* as the time period of post-dispersal movements after a wolf dispersed from its natal home range but before it settled into a new home range. Bounce time differed from "spatial separation from pack" (all locations within the natal home range) because it measured the time it took a wolf to establish a new home range once it left the old home range. Some wolves were "missing" for up to 55 months so I included only those wolves for whom I had nearly continuous telemetry data (i.e. less than 1 continuous month of missing location data) for estimating bounce time. This conservative approach may have underestimated bounce time for those dispersers with prolonged and long-distance movements who went undetected for several months at a time.

For estimating and comparing survivorship of dispersers and biders I assumed that 1) 12 months was the earliest possible age of dispersal, and 2) that biders at that age had the choice to disperse or remain biders. Assuming a date of birth as April 15, I calculated *days alive for dispersers and biders* as the number of days alive past 12 months of age to the last date of transmission or reported mortality, or until the end of this study (31 May 1997). For biders whose exact date of mortality was unknown, I chose the mortality date as halfway between the last known live location and the first confirmation of mortality (Pletscher et al. 1997). For dispersers I chose the mortality date as halfway between the dispersal date and the first confirmation of mortality (Pletscher et al. 1997). I then compared average number of days survived for dispersers and biders.

I determined breeding contribution for dispersers and biders based on several assumptions: 1) wolves would not reproduce until a minimum of 22 months of age (Mech 1970); 2) females were defined as breeders based on physical evidence of lactation upon capture

(Mech et al. 1993) and dominant or mating behaviors observed from aircraft during telemetry flights; 3) males were defined as breeders based on testicle size relative to other animals of known breeding status captured the same time of year, and dominant or mating behaviors aerially observed; 4) if a wolf A was breeding in year 1, remained with the same pack years 2 through *n*, no other breeders of the same sex were detected, and pups were whelped, I assumed wolf A was the breeder in years 2 through *n*; 5) den site attendance patterns relative to other pack members; and 6) parental exclusion through genetic analyses of family groups.

I compared reproductive contribution for dispersers and biders by wolf age. A male that sired pups or a female that whelped were considered to have "reproduced". Each year that a wolf reproduced was recorded as 1 reproductive event (1 litter). For example, male wolf 8401 reproduced at age 5 and age 6, so was recorded as 1 event in each of those age categories. He did not reproduce at age 2, 3, or 4, so these were recorded as non-reproductive events for those age categories. A wolf's reproductive status was omitted from analysis if it was unknown at a given age.

### Statistical Tests

All samples were assumed to be random and independent. Whenever possible, parametric tests (t-tests) were used to compare population means. Nonparametric tests (Pearson's Chi-square, Mann-Whitney U) were used when populations failed to meet assumptions of normality and homoscedasticity. All statistical tests were considered significant at $P \leq 0.05$.

## RESULTS

### Captures and Dispersals

Fifty-eight wolves were captured and tagged (17 males and 41 females) from 1979 to 1996 in the intensive study area. P. Paquet (Banff Wolf Project), personnel of the US Fish and Wildlife Service, and M. Jimenez (University of Montana) monitored some of our dispersed wolves. These personnel radiocollared > 30 additional wolves, and some of their dispersal data are cited anecdotally.

Thirty-one wolves dispersed once and 3 wolves dispersed twice, totaling 34 dispersals (Figs. 1-2). I designated 31 wolves as dispersers because they permanently left their natal home ranges and were subsequently located in an area disjunct from their former home range. Three additional wolves, 8550, 9065, and 9066, were classified as dispersers although they did not meet the above criteria. Females 8550 and 9065 dispersed from their natal packs, maintained a new home range which included a subset of their former home range, reproduced in the new home range, and were never again located with members of their natal packs. Wolf 9065 subsequently made pre-dispersal movements and her radiocollar ceased transmitting in the study area, and I assumed she dispersed a second time. Female 9066 dispersed from her natal pack at 12 months of age and joined a newly formed pair (of which neither was her parents) for 2 months. She exhibited pre-dispersal movements a few days prior to loss of her radio signal and was never located again. An additional 9 wolves were excluded from analysis because their radiocollars ceased transmitting prematurely and the wolves exhibited no pre-dispersal movements. This was a conservative approach to including dispersers because most likely some of the 9 excluded wolves dispersed (the majority of known dispersers showed no pre-dispersal movements.

## Pre-dispersal Behavior

### *Temporal separation from pack*

Most wolves dispersed relatively quickly after separating from their natal pack, with males dispersing more quickly than females after separation from the pack (P = 0.02, $n = 24$; Mann-Whitney U) (Table 1). For males and females combined, the range was 1-94 days, the median number of days separated was 4, the mean was 11.6 (SD = 20.5), and the mode was 1.

Table 1. Sex and number of days separated from pack before dispersal ($n = 24$ wolves) from the Glacier National Park area from 1985-97.

| Number of days separated from pack | Sex[1] and frequency |
|---|---|
| 1 | MMMMFFF |
| 2 | MF |
| 3 | FFF |
| 5 | F |
| 6 | FF |
| 7 | M |
| 10 | FFF |
| 17 | F |
| 18 | F |
| 29 | F |
| 46 | F |
| 94 | F |

### *Spatial separation from pack*

Forty-three percent ($n = 23$) of dispersing wolves made forays away from the pack and then rejoined the pack within 3 months of permanent separation from the pack. The remaining 57% (*n* = 23) were always located with other pack members 3 months prior to permanent separation. Most wolves remained spatially distant from pack members after permanent separation from the pack prior to dispersal. The typical pattern of pre-dispersal behavior was for a potential disperser to remain with the pack, then suddenly make a permanent break from the pack, wander around in the pack's home range for a few days, and then disperse to a new, disjunct home range.

### *Pack hopping and extraterritorial forays*

I classified wolves as pack hoppers when they made extraterritorial movements (Messier 1985) or were members of more than 1 pack in a given season. Packs in the study area accepted non-pack wolves into their group in 6 instances (Appendix 2). Five wolves were observed with more than 1 pack in a season and appeared to belong to multiple packs in a year's time (Appendix 2). I documented 2 incidents of extraterritorial movements by entire packs (Appendix 3).

## Dispersal Characteristics

### *Dispersal rate*

The number of wolves that dispersed was not correlated with the previous year's maximum pack counts for the disperser's pack (Pearson's $r = -0.04$, $P = 0.89$), suggesting that pack size does not influence dispersal rate.

Table 2. Wolf population estimates and frequency of tagged wolves ≥ 12 months old that dispersed from 1985-97 in the Glacier National Park area.

| Year | Maximum population[a] | Maximum pack size | % tagged wolves[b] (*n*) | % tagged wolves that dispersed[c] (*n*) |
|---|---|---|---|---|
| 1982 | 9 | 9 | 0 | 0[c] |
| 1983 | 8 | 8 | 0 | 0[c] |
| 1984 | 7 | 7 | 0 | 0[c] |
| 1985 | 14 | 13 | 14 (2) | 0[c] |
| 1986 | 14 | 13 | 36 (5) | 60 (3) |
| 1987 | 27 | 10, 9, 8 | 30 (8) | 38 (3) |
| 1988 | 33 | 14, 11, 3 | 33 (11) | 27 (3) |
| 1989 | 31 | 11, 10, 8 | 39 (12) | 17 (2) |
| 1990 | 33 | 13, 11, 8 | 39 (13) | 23 (3) |
| 1991 | 31 | 14, 9, 7, 3 | 52 (16) | 31 (5) |
| 1992 | 43 | 14, 11, 10, 9 | 30 (13) | 8 (1) |
| 1993 | 54 | 19, 18, 10, 6 | 41 (22) | 18 (4) |
| 1994 | 36 | 14, 11, 11 | 47 (17) | 29 (5) |
| 1995 | 40 | 16, 16, 7 | 28 (11) | 9 (1) |
| 1996 | 35 | 19, 10, 5 | 29 (10) | 20 (2) |

[a] does not include lone wolves

[b] wolves at least 12 months of age

[c] data were omitted from dispersal rate analyses because few or none of the population were tagged

*Distance dispersed*

Fifteen wolves dispersed ≤ 50 km (Fig. 1) and 19 wolves dispersed > 50 km (Fig. 2). Dispersal distances of males ($n$ = 10) and females ($n$ = 20) were not significantly different (P = 0.47, $n$ = 30, Mann-Whitney U, Table 3).

Table 3. Dispersal distances (km) from the Glacier National Park area for 30 wolves, by sex, 1985-97[1].

| Sex | *n* | $\bar{x}$ | SD | range |
|---|---|---|---|---|
| M | 10 | 113.3 | 94.7 | 16.6 - 247.2 |
| F | 20 | 77.7 | 65.1 | 15.6 - 254.9 |
| M + F | 30 | 89.6 | 76.5 | 15.6 - 254.9 |

[1] An additional female was omitted from the analysis to avoid skewing the results (dispersal distance = 840 km).

### *Direction dispersed*

Wolves tended to disperse in a northerly direction (Chi-square = 7.26, df = 1, P = 0.007) (Figs. 1-2); 74% (*n* = 31) of dispersals were northward.

### *Month of dispersal*

Table 4. Sex and month of 33 dispersals in the Glacier National Park area, 1985-97.

| Month dispersed | Sex and Frequency |
|---|---|
| Jan | MFFF |
| Feb | MFFF |
| Mar | MF |
| Apr | FFF |
| May | MMFF |
| Jun | MMFF |
| Jul | MMF |
| Aug | FF |
| Sep | FFF |
| Oct | FF |
| Nov | F |
| Dec | F |

Wolves dispersed in all months with a tendency to disperse in winter and spring (Table 4). Males tended to disperse the first half of the year (January-July) while females dispersed in all months.

### *Age at dispersal*

Dispersal age was not correlated with maximum size of the disperser's pack 12 months prior to dispersal (Pearson's r = 0.003, P = 0.998, *n* = 28). I found no difference between males and females for mean age of dispersal (P = 0.36, *n* = 33, Mann-Whitney U, Table 5).

Table 5. Wolf age (months) for 33 dispersals, by sex, from the Glacier National Park area, 1985-97.

| Sex | *n* | $\bar{x}$ | SD | median | range |
|---|---|---|---|---|---|
| M | 9 | 28.7 | 11.7 | 26 | 14- 49 |
| F | 24 | 38.4 | 22.2 | 33.5 | 12 - 90 |
| M+F | 33 | 35.7 | 20.2 | 33 | 11 - 90 |

Most wolves were tagged as pups or yearlings and did not disperse for several months or years, providing fairly accurate age estimates. Six wolves were relatively old compared with other reports (Gese and Mech 1991, Mech et al. in press) estimated as 57, 62, 72, 76, 77, and 90 months old when they dispersed.

The oldest known disperser, female 8550, had been the alpha female in 1985-86, but dispersed in late January of 1987, carved out the northerly portion from her former home range, and successfully produced pups in May 1987 (Boyd and Jimenez 1994). Shortly before denning, 4 members of her former pack joined her at the den site. Wolf 8550's pack disintegrated during the summer and fall of 1987 through human-caused mortalities ($n = 5$) and dispersals ($n = 3$). By November, 8550's pack consisted of herself and 1 surviving pup. A few months later the pup disappeared and 8550 subsequently dispersed north into the West Castle drainage of Alberta in 1989, where she lived another 3 years. She was easily identified because of her unusual white coloration and radiocollar (although non-functioning), and was frequently seen by local residents. She was at least 7 years old when she dispersed, and was 10+ years old when she was shot by a hunter. During her 3 years in Alberta, she associated briefly with wolves and dogs but failed to reproduce. Her last year of life was spent in and near a field camp, where she occasionally ate food set out for the camp's dogs, slept under buildings and in an abandoned shed. Although she was of wild origin in the GNP area, her behavior in later years could have been incorrectly interpreted as that of a captive-reared canid.

### *Sex ratios*

The sex ratio of wolves captured (17 M : 41 F; 71% F) differed from parity and favored females (Chi-square = 9.93, df = 1, P = 0.002, $n = 58$). The sex ratio for dispersals (10 M : 24 F; 71% F) also favored females (Chi-square = 5.77, df = 1, P = 0.016), and was not different from the sex ratio of captured wolves (Chi-square < 0.0001, df = 1, P = 0.99). Thus, males and females dispersed in proportion to the sex ratios of the tagged population.

### *Dispersal movements*

Locating wolves along their dispersal routes with our VHF radiocollars was extremely difficult because of the rapidity and long distances that wolves moved during dispersal. Despite the difficulty of monitoring dispersals, several unusual and noteworthy movements were documented (Appendices 2 - 4). Additionally, 2 incidents of sibling co-dispersal were documented with siblings 8703 and 8857, and also siblings 8962 and 9013:

Two siblings, male 8703 and female 8857, dispersed approximately 1 year apart from the same natal pack and were located together in a new pack 150 km north. Male 8703 dispersed from the Camas pack of GNP in March 1988. He was not located again until July 1989 and was found with the Highwood Pack. His sibling, female 8857, dispersed from the Camas pack in February 1989 and became the breeding female of the Highwood Pack in Alberta in spring 1989. These 2 wolves were found together for a few locations in 1989 before 8703 left the pack.

Female 8962 was last located with her natal Camas Pack in GNP on 19 January 1991. Approximately a week later, packmate male 9013 left the Camas Pack. The 2 were seen in the Ninemile area (R. Thisted, pers. commun.) in April 1991. Female 8962 stayed in the Ninemile area and became the breeding female (M.. Jimenez, pers. commun.) while 9013 moved on to Kelly Creek, Idaho. These 2 wolves were first observed in the Ninemile less than 2 weeks after the resident wolf pack had been removed for livestock depredations. Furthermore, 8962 was first observed in the rendezvous site of the previous pack, and assumed movements similar to that of the former pack.

## Post-dispersal Behavior and Fates

### *Bounce time*

Bounce time was often difficult to determine because wolves dispersed quickly and covered great distances in a short time, making their relocation difficult. Most dispersers had to be excluded from this analyses because after leaving their natal pack they often were not found for several months or years. The median bounce time was 10 days (mean = 36.6, range = 2 - 202, SD = 63.9) for 10 dispersed wolves.

### *Survival and mortalities*

Thirty mortalities of tagged wolves were recorded during the study period (Tables 6 and 7). Mortalities of several more untagged wolves were documented (Pletscher et al. 1997) but were omitted from these analyses because untagged wolf mortalities would include only biders.

Table 6. Month of wolf mortalities ($n = 30$) for dispersers and biders in the Glacier National Park area, 1985-97.

| Status | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Disperser | 2 | 1 | 2 | 2 | 0 | 0 | 1 | 2 | 2 | 3 | 3 | 2 |
| Bider | 0 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 0 |
| Total | 2 | 2 | 4 | 2 | 0 | 0 | 2 | 3 | 3 | 5 | 5 | 2 |

The distribution of month of mortality for dispersers and biders appears to be equal (Table 6) but sample sizes were too small for a Chi-square statistic. The mean number of days alive post-dispersal (785.3, $n = 30$) was not different from the mean number of days alive for biders (911.7, $n = 19$) ($t = -0.58$, $df = 47$, $P = 0.563$).

Eighty percent of all mortalities were caused by humans (Table 7). Humans caused 90% of the mortalities in dispersers and 60% of mortalities in biders. The majority of biders resided within protected GNP and the majority of dispersers lived on provincial and federal lands in Canada where wolves received less protection.

Table 7. Causes of mortalities for 30 tagged wolves in the Glacier National Park area, 1985-97.

| Status | Cause of Death<br>Human | <br>Other |
|---|---|---|
| Disperser | 18 | 2 (1 avalanche*, 1 killed by wolves) |
| Bider | 6 | 4 (1 elk, 1 unknown, 2 killed by wolves) |
| SUBTOTAL | 24 | 6 |
| TOTAL | 30 | |

*Boyd et al. 1992

I calculated the mean distance from an anthropogenic linear feature (road or seismic line) for each incident of human-caused mortalities (incidents where > 1 wolf was killed at the same time was counted as 1 incident). The mean distance from a human linear feature for human-caused mortalities was 0.2 km ($n = 25$, SD = 0.28) and non-human caused mortalities was 0.8 km ($n = 11$, SD = 0.91). This difference was significant (P = 0.0095 Mann-Whitney U). Seventy-five percent of human-caused wolf mortalities occurred within 250 m (potential shooting distance) of a road.

### *Reproductive contribution*

Fifty-seven percent ($n = 23$) of dispersing wolves found mates the first breeding season after dispersal, and all but 2 of these reproduced that first denning season. The number of known reproductive outcomes for dispersers ($n = 58$) and biders ($n = 61$) were similar (Table 8). Dispersers produced significantly more litters than biders (Chi-square = 16.95,

Table 8. Production of pups by wolves of various ages and sexes, for biders and dispersers in the Glacier National Park area, 1985-97.

| Sex and Wolf age (yr) | *Pups Produced?* Biders | | Dispersers | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| M 2 | 0 | 8 | 0 | 1 |
| F 2 | 2 | 16 | 2 | 1 |
| M 3 | 0 | 4 | 1 | 2 |
| F 3 | 4 | 7 | 4 | 3 |
| M 4 | 0 | 1 | 3 | 2 |
| F 4 | 3 | 5 | 3 | 2 |
| M 5 | 0 | 0 | 4 | 2 |
| F 5 | 5 | 0 | 4 | 2 |
| M 6 | 0 | 0 | 4 | 1 |
| F 6 | 3 | 1 | 5 | 0 |
| M 7 | 0 | 0 | 1 | 0 |
| F 7 | 0 | 2 | 2 | 1 |
| M 8 | 0 | 0 | 1 | 0 |
| F 8 | 0 | 0 | 2 | 1 |
| M 9 | 0 | 0 | 0 | 0 |
| F 9 | 0 | 0 | 1 | 1 |
| M 10 | 0 | 0 | 0 | 0 |
| F 10 | 0 | 0 | 1 | 1 |
| TOTAL M | 0 | 13 | 14 | 8 |
| TOTAL F | 17 | 31 | 24 | 12 |
| TOTAL M + F | 17 | 44 | 38 | 20 |

df = 1, $P < 0.001$ ). The outcome of reproductive events was known for more females ($n$ = 84) than males ($n$ = 35). Tagged females and males (dispersers and biders combined) produced proportionately similar numbers of litters (Chi-square = 0.77, df = 1, P = 0.38).

### Pack Formation

I documented the formation of 13 packs from 1982-1997 (Appendix 4). Ten of these packs (77%) became established in areas which had no breeding wolves, greatly expanding the recolonization process. Three (Sage Creek, South Camas, and Spruce Creek) of these 13 packs (23%) formed through pack splitting (Meier et al. 1995), whereby the new pack usurped a portion of the founders' natal home range and expanded into adjacent, unoccupied territory. Some intermingling of newly split packs and their former pack mates occurred within a few months after pack splitting, but thereafter the packs maintained separate territories.

## DISCUSSION

### Dispersal Characteristics

Ten comprehensive articles have been published documenting wolf dispersal (Fritts and Mech 1981, Peterson et al. 1984, Ballard et al. 1987, Mech 1987, Fuller 1989, Gese and Mech 1991, Boyd et al. 1995, Wydeven et al. 1995, and Ballard et al. 1997, Mech et al. in press). Five additional notes have been published documenting unusual wolf dispersal events (Van Camp and Gluckie 1979, Ballard et al. 1983, Fritts 1983, Van Ballenberghe 1983, Mech et al. 1995). I compared my data to the 8 most comprehensive dispersal articles in my analyses (Table 9) to evaluate differences between colonizing and established populations.

Table 9. Wolf dispersal data from North American studies.

|  |  |  | Dispersal Age[a] |  |  |  | disp sex | capt sex | M disp dist km | F disp dist km |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | Place | %Pups | %Yrlgs | %Adlts | *n* | status[b] | % F | % F | mean | mean |
| This study | GNP | 3 | 33 | 64 | 33 | I,S | 71 | 71 | 113 | 78 |
| Fritts & Mech 81 | MN | 0 | 88 | 12 | 8 | I | 63 | 59 | n/a | n/a |
| Peterson et al. 84 | AK | 6 | 61[e] | 33 | 18 | S | 41 | 58 | n/a | n/a |
| Ballard et al. 87 | AK | n/a | 47 | 53 | 38 | D | 26 | 50[d] | 84 | 114 |
| Fuller 89 | MN | 39 | 61[c] | n/a | 28 | I | n/a | 56 | n/a | n/a |
| Gese & Mech 91 | MN | 23 | 56 | 21 | 75 | D,S,I | 48 | 49 | 88 | 65 |
| Wydeven et al 95 | WI | 6 | 50 | 44 | 16 | D,I | 63 | 54 | 65 | 144 |
| Ballard et al. 97 | AK | 5 | 34 | 61 | 21 | I,D | 38 | 49 | 154 | 123 |
| Mech et al. 98 | AK | 4 | 47 | 49 | 51 | S | 48 | n/a | 83 | 83 |

[a] pups < 12 mos; yrlgs 12-24 mos; adlts > 24 mos

[b] D=decreasing; I=increasing; S=stable

[c] author combined yrlgs + adlts

[d] author reported even sex ratio for yrlgs + pups (listed above) but adlt sex ratios skewed toward large males when darting due to intentional selection of larger wolves (omitted)

[e] author combined yrlg + 2-yr olds

n/a data not reported

The proximate cause of wolf dispersal is not known. Wolves living in areas with inadequate prey may be subject to nutritional deficiencies and social stress, encouraging dispersal (Zimen 1982, Messier 1985, Ballard et al. 1987). I found no evidence of wolf starvation and no wolf-caused wolf mortalities prior to 1993. However, ungulate populations have been declining since at least 1993 in the study area (Kunkel 1997) and, subsequently, 4 incidents of wolves killing wolves (20% of 20 mortalities since 1993) have been documented. Interestingly, I observed a slight decrease in dispersal rate concurrent been documented. Interestingly, I observed a slight decrease in dispersal rate concurrent with decreases in the ungulate population since 1993 (Table 2 and Fig. 3), with a mean dispersal rate of 29.1% from 1986-92 and 19.0% from 1993-96, respectively (Chi-square = 0.28, $P > 0.50$, df = 1).

Wolves in northeastern Minnesota experienced nutritional shortages and often died of starvation (25% of known mortalities) or were killed by other wolves during incidents of interpack strife (25 % of known mortalities, Mech 1977a, 1994). These mortalities occurred in a saturated wolf population during a time of deer decline. Thirty-nine percent of known wolf mortalities in Denali National Park, Alaska, were caused by wolves killing other wolves, but no starvation was documented (Mech et al. in press). Although the saturated Denali population was apparently not food limited, the relatively high levels of intraspecific strife suggests that social stress may be a factor regulating the population. Peterson et al. (1984) found no incidents of intraspecific strife in the low-density, exploited wolf population of the Kenai Peninsula, Alaska. Fritts and Mech (1981) reported 4 mortalities (22% of known mortalities) caused by intraspecific strife in a colonizing population in northwestern Minnesota. Three of the 4 mortalities occurred after the population had increased, similar to trends I observed in the GNP area. Fritts and Mech (1981) did not find any deaths from malnutrition, suggesting the population was probably not food limited. Intraspecific strife accounted for 10% of known wolf mortalities in a colonizing wolf population in Wisconsin with an adequate prey base (Wydeven et al. 1995). This Wisconsin population is comprised of disjunct, small packs that may suffer from lack of suitable habitat outside of established territories. Summarily, mortalities caused by intraspecific strife tended to increase in wolf populations stressed by food or space limitations.

Wolves in my study area existed in disjunct packs, encompassing the ungulate winter ranges that were widely dispersed over the landscape. A dispersing wolf in the Central Rockies may have decreased chances of encountering prey or potential pack mates to assist with prey capture, than dispersers in the more homogeneous landscape of the Midwest. Wolf dispersal rates declined in my study area during times of lower ungulate densities. During times of food shortages, Minnesota wolves reduced movements to conserve energy in a high density wolf population with more evenly dispersed ungulate winter ranges (Mech 1977b). This need to conserve energy would be even greater in the Central Rockies where wolves are likely to have to travel further between more widely dispersed ungulate winter ranges to find prey. Elsewhere, increased resource competition (lower prey abundance) increased stress in saturated wolf populations, and subsequently wolf dispersal rates increased (Messier 1985, Ballard et al. 1987, Peterson and Page 1988, Gese and Mech 1991). These 4 cited study areas harbored high density wolf populations, with no wolf-free zones for dispersers to move to.

Mean dispersal distances in my study area were intermediate with those reported in other areas (Table 9). The longest dispersal (840 km) from my study area was made by a yearling female (Ream et al. 1991). Record dispersals from other studies range from 390-886 km, with longer dispersals tending to occur by males in more rugged terrain (Van Camp and Gluckie 1979, Fritts and Mech 1981, Berg and Kuehn 1982, Fritts 1983, Ballard et al 1987, Gese and Mech 1991, Mech et al. 1995, and Mech et al. in press). Great variation in long distance dispersals may be explained by differences in density of wolves, individual behavioral variation, prey, humans, and the patchiness of the landscape. Wolves traveling in mountainous landscapes or in areas of low wolf density may have to travel excessive distances to find suitable habitat, prey, or mates.

Several dispersals within and near the study area reported by other researchers have aided the recolonization effort in the Central Rockies. A yearling male dispersed from near Missoula, Montana, 280 km to northeastern Washington (M. Jimenez, pers. commun.). An adult female dispersed 470 km from Peter Lougheed Provincial Park, Alberta, to Deer Lodge, Montana (P. Paquet and J. Fontaine, pers. commun.). An adult female dispersed 250 km from Kootenay National Park, British Columbia, to Pincher Creek, Alberta (C.

McTavish, pers. commun.). An adult female fitted with a satellite transmitter in Peter Lougheed Provincial Park, Alberta, moved through southern Alberta, southeastern British Columbia, Browning, Montana, the GNP area, and Kellogg, Idaho (C. Callaghan and P. Paquet, pers. commun.). These individual anecdotal accounts are interesting individual events. But the greatest value in compiling them here is as an indicator of the tremendous movements that frequently occur by wolves in the Central Rockies, and their contribution to wolf recovery. If landscape linkages are maintained and human persecution is minimized, wolves have great potential to recolonize areas far from core wolf populations.

Most wolves (74%) in my study dispersed in a northerly direction toward higher density wolf populations in Canada (original source of GNP colonizers). The ability to detect direction of dispersal may have been affected by different management policies of wolves in Canada and the US. Wolves legally killed in Canada were more likely to be reported than those illegally killed as an endangered species in the US. Most wolf mortalities were reported to us by the hunter or agency locally responsible for wolf management. Nonetheless, 74% ($n = 34$) of our dispersal discoveries were the result of relocations of live animals with telemetry. The remainder were documented by contact from hunters or government officials when wolves were legally harvested. No dispersals were discovered through illegal mortalities. Another factor that may have effected dispersal direction was the availability of lands to wolves without conflicting human use. Wydeven et al. (1995) reported behavior in dispersers from a colonizing wolf population in Wisconsin to disperse to higher density wolf populations in Minnesota (the original source for Wisconsin colonizers).

Wolf dispersal peaked in January-February and again in May-June (Table 4). I observed 2 peak times of dispersal: January-February and May-June. January-February are times of courtship and breeding, and therefore a time of increased aggression (Zimen 1982, Packard et al. 1983), encouraging dispersal. Wolf dispersal may be catalyzed by increased stresses within the pack in response to food shortages, social aggression, competition for breeding, and weakening of social bonds during recruitment of new pups. Season of dispersal varies greatly in the literature, but most authors reported consistent dispersal peaks during the breeding season (Peterson et al. 1984, Ballard et al. 1987, Mech 1987, Fuller 1989, Gese and Mech 1991, Ballard et al. 1997, Mech et al. in press). However, Fritts and Mech (1981) reported 50% ($n = 8$) of dispersals occurred in fall (September - December) and 50% ($n = 8$) occurred in the breeding season (January - March). Ungulates are most vulnerable to wolves during late winter and early spring (March - April) which coincides with lower dispersal rates. This may reflect an abundance of available food resources and a time of relatively low stress between breeding and denning seasons. Potential dispersers may experience increased social distancing from the dominant animals during the early weeks of pup rearing (May - June) when parents are putting much social energy into provisioning pups. Potential dispersers may have begun distancing themselves socially from other pack members during the breeding season, and the additional distancing by the dominant animals rearing pups may be sufficient to launch a second pulse of dispersal.

Messier (1985), Ballard et al. (1987), and Gese and Mech (1991) reported a higher rate of yearling dispersal during times of lower prey density. Wolves in northeastern Minnesota (Mech 1977a) dispersed from a saturated wolf population at a younger age than colonizing wolves in my study. Gese and Mech (1991) stated that dispersal of adults was unaffected by changes in the saturated wolf population level. Fritts and Mech (1981) found that most dispersers from a colonizing wolf population in northwestern Minnesota were yearlings. This area had abundant prey and unoccupied wolf range for new packs to colonize (Fritts and Mech 1981). These relatively young dispersers were maximizing their fitness by dispersing early in life during a time of favorable environmental conditions.
In my study area, a higher percentage of dispersing wolves were adults than was reported elsewhere (Table 9). The most similar pup:yearling:adult disperser ratios were reported by Ballard et al. 1997 in a low density, exploited wolf population. If adequate prey are available, younger wolves may remain with the pack longer if benefits of biders (security)

outweigh the hazards of dispersal (unknown risks and potentially increased mortality). Bider benefits may include carcass defense against other packs and grizzly bears (*Ursus arctos horribilus*), ease of capturing large prey by cooperative hunting, and advantage in numbers during interpack trespass encounters.

The sex ratio favored females for both captures and dispersers (Table 3), but I found no sex-bias in the dispersal rate of wolves (assuming the sex ratio of the captured sample represents the population at large). This trend was also observed in other colonizing or increasing wolf populations in Minnesota (Fritts and Mech 1981, Fuller 1989) and Wisconsin (Wydeven et al. 1995). Sex ratios that were even or favored males were reported in stable or decreasing wolf populations (Peterson et al. 1984, Gese and Mech 1991, Ballard et al. 1987, Ballard et al. 1997, and Mech et al. in press) (Table 9). Mech (1975) suggested that wolves in saturated populations (stable to decreasing) with marginal nutrition have a disproportionate number of male pups, and wolves from populations with lower wolf density had equal sex ratios or a disproportionate number of female pups. Assuming equal survivorship of males and females, and equal capture vulnerability, the sex ratios of captured wolves of all age groups should reflect pup sex ratios. Thus, the sex ratios reported in the present study and others (Table 9) support Mech's hypothesis.

## Movements and Dispersal Behavior

Ray et al. (1991) postulated the theory of conspecific attraction whereby animals disperse preferentially to sites occupied by conspecifics. This may explain why most dispersers from GNP moved northward to higher density wolf populations. Wolves find other wolves by long-term communications using scent-marks and short-term communications with howling (Mech 1970, Peters and Mech 1975, Harrington and Mech 1979). Wolves often travel well-established routes and may detect scent marks long after deposition. Wolves from the same pack dispersed months or years apart, and ended up in the same area (3 times in this study; Gese and Mech 1991). Male 8703 and female 8857 dispersed in March 1988 and February 1989 from the North Camas Pack, GNP, and ended up occasionally together in the Highwood Pack, Alberta, in 1989-91. Female 8859 dispersed from the Wigwam Pack, British Columbia (pack immediately west of the North Camas Pack ) in October 1988 and was occasionally also found with the Highwood Pack in 1990-91. Female 8962 and male 9013 dispersed 5 days apart, in January 1991, from adjacent packs in GNP (North Camas and South Camas) and were seen together in the Ninemile (R. Thisted, pers. commun.) in April 1991. Additionally, single dispersing wolves usually found other wolves soon after they left their natal home range (mean = 66 days, range = 2-202) despite non-contiguous pack distribution and a landscape-scale, low-density wolf population. Fritts and Mech (1981) reported the mean time from dispersal to pairing was 16.5 days (range = 8-30) in a colonizing population. Gese and Mech (1991) reported mean time from dispersal to pairing as 112 days (range = 2.2 to 5.1 months) in a saturated population. Finding unoccupied territories may be easier in colonizing populations with relatively low wolf density and an adequate prey base.

Messier (1985) described dispersal as a lengthy dynamic process that occurred over many months or years in a saturated wolf population in relatively homogeneous terrain. I observed the opposite: wolves engaged in very few extraterritorial movements relative to other studies. Once wolves permanently separated from their pack they dispersed out of the area relatively quickly. Fritts and Mech (1981) also observed rapid (≤ 30 days) dispersal and rapid pairing in a colonizing population in northeastern Minnesota. Recolonizing, low-density wolf populations may present dispersers with lower probabilities of finding potential mates. However, the probability of encountering hostile resident packs is also lower. Additionally, there may be an increased number of unoccupied potential wolf territories with adequate prey. The combination of these factors may encourage more rapid dispersal in colonizing populations. The rugged nature of mountainous terrain with non-contiguous pack distribution may encourage dispersing wolves to keep traveling once movement has begun. If a disperser is seeking a mate, he or she may have to travel long distances to encounter another wolf. The heterogeneous, linear nature of mountainous terrain causes patchy distribution of ungulates. This clustered prey base may cause wolves to move further and more quickly through the landscape in search of food.

### *Was Dispersal a Successful Strategy?*

Wolf colonization in the Central Rockies occurred through dispersal and was of paramount importance to recovery efforts. Prey abundance, social strife, and breeding opportunities undoubtedly influenced dispersal attempts and their outcomes. These proximate factors may vary between ecosystems in severity and consequences. However, the ultimate purpose of dispersal, increased reproductive success, remains the same for dispersers overall. Potential bias may have been introduced favoring detection of breeding status for dispersed wolves because packs newly formed by a disperser were generally smaller than natal packs so reproductive activity may be easier to detect for dispersers. Confirming reproductive status is easier to ascertain for females than males because 1) females have stronger den site fidelity than males due to lactation demands and 2) permanence of mammary development post-lactation whereas testicle size varies with season and status. However, pseudolactation may complicate the evaluation of female reproduction (Mech and Seal 1987, Mech et al. 1993).

Usually only the alpha male and female in a wolf pack breed (Mech 1970). If mortality rates are relatively high for dispersers or breeding opportunities are limited, an individual can increase inclusive fitness by helping provision related offspring. However, I found no significant differences between the number of days alive post-dispersal for dispersers and biders, and dispersers produced significantly more offspring than did biders (Table 8). It would seem advantageous for wolves to disperse younger to increase their number of reproductive years. However, wolves may have honed their survival and pup-rearing skills in their familiar natal home range in the protected habitat of GNP by remaining home longer before dispersing. If the pre-dispersal learning time were shortened, perhaps the post-dispersal survival would be lower.

Wolf recovery is continuing in the Central Rockies, with dispersing wolves recently repopulating areas of British Columbia, Alberta, Idaho, and Montana from which they had been extirpated. Recolonizing wolves were often tolerant of visiting non-pack members, a situation uncommon in established wolf populations (Mech 1970). However, Meier et al. (1995) documented 8 cases of strange wolves joining established packs in a stable wolf population in Denali National Park, Alaska. This unusual behavior may have occurred in the saturated Denali population because wolves existed at low density with adequate prey, thereby reducing resource competition. Colonizing wolves may have a less rigid social system so foreign wolves may be allowed to visit or become pack members more easily (Ballard et al. 1987). Dispersing wolves joined packs or found mates relatively quickly after leaving their former home range, augmenting wolf recovery.

Southwestern Alberta is an example of a recolonized area becoming a source and then a sink for wolves. Wolf populations in southwestern Alberta greatly increased during the 1980s and became a source for dispersals to Montana. Anti-wolf sentiment grew rapidly in southwestern Alberta during the early 1990s, resulting in removal of approximately 95% of the population between Peter Lougheed Provincial Park and GNP (45 wolves killed 1994-95; C. Callaghan and P. Paquet, pers. commun.). Concurrently, wolf densities in Montana increased to the point that Montana became a source to recolonize Alberta. This heavily exploited region of Alberta became a sink for dispersing wolves from Montana and British Columbia. However, numerous long-distance dispersals and successful reproduction have allowed continued recolonization throughout the Central Rockies.

Colonization outward from the GNP area expanded as the study progressed. By 1996, 9 wolf packs had established home ranges in western Montana through dispersal (2 in GNP and 7 elsewhere). Additionally, 35 Canadian wolves were reintroduced to central Idaho and 31 were reintroduced to YNP in 1995-1996 (Bangs and Fritts 1996, Fritts et al. 1997). The combination of colonization through dispersal and artificial reintroduction should greatly enhance the rate of wolf recovery. Dispersal must occur between packs to maintain genetic variation and promote population viability. Dispersal at the present time is apparently adequate to maintain genetic variation (Forbes and Boyd 1996, 1997), but landscape connections must be maintained and human persecution minimized to continue this trend.

## CONCLUSIONS

Dispersal was a successful strategy for wolf colonization in the Central Rockies. Biders in and dispersers from the GNP area survived equally well. Furthermore, dispersers produced relatively more offspring than did biders. Colonizing wolves moved over large-scale landscapes and these rather indeterminate connections are critical to continued recovery.

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# Appendix 1

Dispersal distance, direction, age and month for 34 dispersals in the Glacier National Park area, 1985-97.

| wolf # | dispersal distance | dispersal azimuth | dispersal age | month disperse |
|---|---|---|---|---|
| 8401 | 67.6 | 315 | 49 | 5 |
| 8502 | 171.7 | 5 | 15 | 7 |
| 8703 | 236.0 | 340 | 23 | 3 |
| 8704 | 40.8 | 330 | 26 | 6 |
| 8705 | 41.3 | 330 | 14 | 6 |
| 8808 | 247.2 | 140 | unk | unk |
| 8910 | 22.4 | 90 | 39 | 7 |
| 9013 | 222.8 | 190 | 33 | 1 |
| 9014 | 67.0 | 90 | 22 | 2 |
| 9318 | 16.6 | 155 | 37 | 5 |
| 8550* | 25.6 | 330 | 57 | 1 |
| 8550* | 34.8 | 15 | 90 | 10 |
| 8551 | 840.0 | 330 | 20 | 12 |
| 8654 | 89.8 | 260 | 23 | 3 |
| 8756 | 20.6 | 140 | 36 | 4 |
| 8857 | 151.6 | 355 | 22 | 2 |
| 8858 | 28.3 | 335 | 42 | 9 |
| 8859 | 186.6 | 350 | 30 | 10 |
| 8962 | 169.2 | 190 | 33 | 1 |
| 9065* | 15.6 | 320 | 72 | 4 |
| 9065* | unk | unk | 77 | 9 |
| 9066* | 41.3 | 330 | 12 | 4 |
| 9066* | unk | unk | 14 | 6 |
| 9167 | unk | unk | 34 | 2 |
| 9270 | 48.0 | 55 | 21 | 1 |
| 9271 | 47.5 | 290 | 76 | 8 |
| 9272 | 55.9 | 60 | 20 | 11 |
| 9375 | 110.3 | 320 | 62 | 6 |
| 9376 | 55.9 | 15 | 34 | 2 |
| 9381 | 78.6 | 350 | 41 | 9 |
| 9482 | 254.9 | 150 | 15 | 7 |
| 9484 | 72.4 | 300 | 28 | 8 |
| 9485 | 28.8 | 250 | 25 | 5 |
| 9587 | 37.4 | 325 | 37 | 5 |

* wolf dispersed twice

# Appendix 2

## Extraterritorial Forays and Pack Hopping

Adult female 9271 apparently joined the Spruce Creek Pair during denning season in 1992. She remained with the Spruce Creek breeding pair in the vicinity of the den and pups until her dispersal in August. The Spruce Creek Pack was formed 2 years prior and consisted of the breeding pair who had no surviving offspring from the previous year. Wolf 9271 was quite old, based on tooth wear and had not been seen previously in this pack's home range.

Female 9066 was radiocollared as a 5 month-old pup during a time of pack splitting in 1990. For the next 7 months she moved between the South Pack, North Pack, and Spruce Creek Pack, changing associations with breeding females approximately every 2 weeks. On April 22 she joined the Spruce Creek Pack and ceased pack hopping. She remained with the Spruce Creek Pack for the next 2 months before she apparently dispersed out of the study area.

Female 8755 was lactating when captured in May 1987. She was observed at the den sites of the North Pack and the Sage Creek Pack, playing with pups at both sites in 1987. The dens were 40 km apart. She was shot in September 1987 so her eventual pack association could not be determined.

Female 8654 was captured as a pup of the Camas Pack in October 1986. She dispersed to the Sage Creek Pack in spring of 1987 and remained with them until fall 1987. Canadian hunters harvested 5 of the 8-member pack during September-October 1987. Wolf 8654 dispersed out of the Sage Creek Pack during the pack reduction and returned to the Camas Pack in October. She remained with the Camas Pack until her separation on 5

January 1988. She traveled alone in the Camas Pack's home range until she dispersed 31 March 1988 to the Yaak River area, Montana.

Female 8858 was a member of the Camas Pack until her disappearance 19 January 1988. She was located on 26 January 1988 near Highwood Pass, Alberta, nearly 200 km north of her previous location. She remained in the Kananaskis and Elk Lakes area south of Banff National Park, Alberta, for nearly 2 months, occasionally associating with members of the Spray Pack and Highwood Pack (P. Paquet, pers. commun.). She returned to the Camas Pack on 25 March 1988.

Female 8961 was a member of the North Pack when captured in 1989. In 1990 she visited pups of the North and South Packs in 1990 (the dens were 22 km apart) and freely associated with adults of both packs. She remained with the North Pack after 30 January 1991.

# Appendix 3

## Extraterritorial movements by packs in the Glacier National Park area, 1985-97

An 30 km extraterritorial movement by the Camas Pack occurred on 28 January 1989. The pack passed through the territory of the Headwaters Pack and was located near Morrissey Provincial Park, British Columbia. They returned to Glacier National Park 6 days later.

The 17 member South Pack trespassed into the 7 member North Pack's territory at a deer wintering area during December 1995. The encounter resulted in the death of at least 2 wolves.

# Appendix 4

## Observations of Pack Formation

*Magic Pack:* A newly established pair of wolves produced pups in 1982 in the Flathead area, founding the Magic Pack. This pack eventually underwent fission when 2 females produced pups, and formed the Sage Creek and the Camas Packs (the name "Magic Pack" was dropped).

*Sage Creek:* Alpha female 8550 separated from the pack she founded, apparently replaced by another female. Wolf 8550 usurped the northern end of her former range, denned 1 month later than usual (Boyd et al. 1993), and founded the Sage Creek Pack. Five adult wolves eventually joined her during the denning period. This pack dissolved within a year when it suffered 63% mortality during the 1987 hunting season in British Columbia.

*Wigwam Pack:* Male 8401 roamed over a 2000 km$^2$ area prior to restricting his home range to the Wigwam drainage in 1986. He paired with a black female and they founded the Wigwam Pack and produced pups 2 consecutive years before his radiocollar ceased transmitting.

*South Pack:* Female 8756 left her natal pack in early April when the alpha female denned. Wolf 8756 moved to the south half of her pack's territory, whelped, and remained separate from her natal pack. Two females from her natal pack occasionally joined her, but a male was not seen with 8756 all spring and early summer. Wolf 8756 produced pups at least 6 years.

*Headwaters Pack:* Two males, 8704 and 8705, dispersed together in June from the Sage Creek Pack to the Headwaters area. They were observed together on all flights June-

November. In November they were joined by female 8963. This threesome founded the Headwaters pack and produced pups at least 4 years before all 3 were shot or poisoned.

*Spruce Creek*: Male 8910 and female 8858 (from different packs) went on numerous extraterritorial forays to the same home area during June-August 1990. During this time they overlapped spatially but were separate temporally. Wolf 8858 returned to her natal pack; 8910 followed her and was located with her pack in September 1990. The pair traveled north together, founded the Spruce Creek Pack, and from that point on remained a resident pack, producing pups at least 6 years before their radiocollars ceased transmitting in 1996.

*Highwood Pack:* Female 8857 dispersed north from the Flathead area in February 1989 and wandered around southeastern British Columbia. She was observed alone in February-April then traveled to Alberta in May where she was subsequently observed with at least 4 other wolves. Wolf 8857 was the founding female of this pack and produced pups for at least 6 years before she was killed by a trapper.

*Sawtooth Pack:* Male 8808 dispersed from the Wigwam area (where he had been eartagged in 1988) and founded the Sawtooth Pack with a black female. He was radiocollared by the biologists from the US Fish and Wildlife Service in 1993 near Augusta, Montana (J. Fontaine, pers. commun.). They produced pups at least 2 years before 8808's radiocollar fell off.

*Ninemile:* Female 8962 left the North Camas Pack in January 1991 and established her new home range in the Ninemile area in April 1991 just 2 weeks after the former Ninemile Pack had been removed for livestock depredations. Female 8962 found a mate and founded the new Ninemile Pack. She successfully reproduced in 1992-94 before she was killed.

*Belly River:* Female 9270 dispersed from the North Camas Pack in January 1993 and was located in Waterton National Park, Alberta, in February 1993. She and her new mate founded the Belly River Pack, successfully producing pups in 1993 and 1994 before she was killed.

*Carbondale:* Female 9272 dispersed from the Headwaters Pack in November 1993 and was located in the Carbondale, Alberta, area in February 1994. She and her new mate successfully reproduced in 1994 and founded the Carbondale Pack. She was shot and killed prior to the 1995 breeding season.

*Beauvais:* Female 9376 dispersed from the North Pack in February 1994, found a mate shortly there after, and founded the Beauvais Pack in Beauvais Provincial Park, Alberta. They produced pups in 1994. She was shot and killed late in 1994.

# Chapter 3: Conservation Genetics of Wolves in the Central Rockies: a Synopsis

**Abstract:** This chapter is a synopsis of 2 published papers and a third manuscript presently in review that examine genetic relationships of colonizing wolves in northwestern Montana, southeastern British Columbia, and southwestern Alberta (Fig. 1) (manuscripts are attached as appendices). Parameters investigated include genetic variation (both heterozygosity and allelic diversity), effective population size, founder effect, genetic bottleneck, relatedness, parentage, gene flow, and detection of non-native canids. Management considerations are discussed.

## INTRODUCTION

Recent research in conservation genetics, greatly enhanced by advances in molecular techniques, has significantly contributed to conservation management of wild populations. The greatest contribution of conservation genetics is to generate information necessary to preserve genetic diversity and potential for future adaptation. Molecular genetics has been used to investigate taxonomy and hybridization, and to formulate species conservation plans (O'Brien 1994). Genetic analysis has become a powerful tool for biologists and managers to investigate questions about the genetic relationships of individuals and populations, to assess reproductive contribution, metapopulation structure, genetic variation (within individuals, among individuals within a population, and differences among populations), hybridization, effective population size ($N_e$), population bottlenecks, inbreeding, and migration (Amos and Hoelzel 1992, Hedrick and Miller 1992).

The peninsular expansion of wolves (*Canis lupus*) down the Rocky Mountains from Canada to the northwestern United States (US) provided a rare opportunity to use molecular techniques to investigate some of the above topics. Potential for a genetic bottleneck and loss of genetic variation through drift may increase as wolves move further south from the source population in Canada. Loss of genetic variation could cause decreased fitness and hinder recovery in the US; therefore, I focused on assessing gene flow in my genetic analyses. Additionally, I hoped to gain insight into wolf demographics, breeding behavior, dispersal, and effective population size through genetic analyses. Forbes and Boyd (1996, 1997) used microsatellite markers to examine genetic relationships of a colonizing wolf population in northwestern Montana, southeastern British Columbia, and southwestern Alberta, hereafter referred to as the "colonizing" population of the Central Rocky Mountains. I defined the Central Rockies as the geographic area centered on the junction of the Continental Divide, Canada, and the United States, and includes the landscape from Banff, Alberta, to Helena, Montana.

The objective of this chapter is to synthesize the available genetic information on wolves of the Central Rocky Mountains and to provide management recommendations incorporating this information. I investigated migration rates ($N_em$), parentage, genetic variation, gene flow, extent of a founding population bottleneck, relatedness, dispersal, and detection of non-native animals through the synergetic combination of DNA analyses and field studies. The combination of field observations and genetic analyses produced a more thorough documentation of the colonization process than either technique alone could do. My work resulted in the publication of 3 papers on Rocky Mountain wolf genetics (Forbes and Boyd 1996, Appendix A; and Forbes and Boyd 1997, Appendix B), and a third paper on testing for wolf-coyote hybridization (Pilgrim et al., Appendix C).

## Background of Wolf Genetics

Until recently, the traditional view of social structure and breeding strategies of wolves, based upon field studies, was that wolves lived in packs composed of the dominant breeding pair, their offspring of the year, and a few offspring from previous years, bound together by a dominance hierarchy (Mech 1970, Kleiman and Eisenberg 1973, Moehlman 1989). This led to the conclusion by some that wolves may be locally inbred without deleterious effects (Mech 1970, Woolpy and Eckstrand 1979, Shields 1983). Shields (1983:91) stated "for wolves, inbreeding does not appear to be an anathema, but rather an adaptive mode of reproduction in their natural environment".

The advent of new molecular techniques (protein electrophoresis, mitochondrial DNA, minisatellites, and microsatellites) has enabled scientists to test genetic relationships and question previous beliefs about social and reproductive behavior. Since 1990, geneticists studying wolves have found little evidence of inbreeding in wild populations; their findings have included the discovery of 1-3 unrelated individuals per pack and more than 1 breeding female in a pack (Kennedy et al. 1991, Lehman et al. 1992, Meier et al. 1995, Forbes and Boyd 1996, 1997). The exception to this is the disjunct population of Isle Royale wolves which apparently descended from a founding pair with no subsequent immigration and have lost 50% of their allozyme heterozygosity (Wayne et al. 1991). The Isle Royale wolves suffered loss of genetic variation due to a low $N_e$ and isolation, rather than inbreeding by choice.

Wild wolves have evolved several behaviors to avoid inbreeding, including selection of a unrelated or distantly related mate (Smith et al. 1997) and long distance dispersal. Ethiopian wolves (*Canis simensis*) have responded to severe habitat loss, curtailed dispersal options, and declining wolf numbers by remaining philopatric and engaging in extra-pair copulations with non-pack members, a unique way to avoid inbreeding (Sillero-Zubiri et al. 1996). Some captive wolves with few outbreeding opportunities have suffered a significant loss of genetic variability resulting in increased occurrence of hereditary blindness, and reductions in reproductivity, juvenile weights, and longevity (Laikre and Ryman 1991). These authors cumulatively suggest that wolves are not adapted to inbreeding as Shields (1983) stated.

Geneticists have examined variation among and within gray wolf populations throughout North America and Europe (Kennedy et al. 1991, Lehman et al. 1992, Wayne et al. 1992, Roy et al. 1994, Meier et al. 1995, Ellegren et al. 1996, Smith et al. 1997). Wolves have substantial genetic variation across North America, but relatively little differentiation between local populations. Lack of genetic differences between populations reflects a nearly panmictic population connected by extensive movements of individuals. Some movement between packs is essential to decrease allelic drift to fixation and inbreeding depression. The present wolf population in western North America extends south from the arctic to Yellowstone National Park (YNP), aided by recent reintroduction efforts in central Idaho and YNP (Forbes and Boyd 1997). Because of non-random mating and social hierarchies, the $N_e$ for wolves is much less than actual population size ($N$). Thus, potential population bottlenecks in relatively isolated wolf subpopulations along the southern edge of wolf distribution (e.g. Montana) are possible.

## Genetic Analyses of Wolves of the Central Rocky Mountains

Radio telemetry and DNA microsatellite markers were used to assess movement of wolves between packs in the Central Rockies (Forbes and Boyd 1996, 1997). Monitoring migration with telemetry had some drawbacks: 1) only a small proportion of the wolf population were radiocollared, 2) fewer than half of the radiocollared animals were known to disperse, and 3) packs appeared in the study area founded by animals of unknown origins. Moreover, estimating migration through microsatellite analyses also had some drawbacks: 1) a small proportion of the wolf population was sampled, 2) we were unable to sample the breeding male in most packs (due to capture equipment biases that excluded the larger males) although the breeding female was usually sampled, and 3) due to statistical limitations parentage and pack composition could not be unequivocally determined from genetic analyses. However, Forbes and Boyd (1996) were able to better determine relationships among wolves and packs by combining the knowledge gathered from 17 years of field work with the genetic samples.

Forbes and Boyd (1996) found that genetic variation (measured as both heterozygosity, *H*, and allelic diversity, *A*) of the colonizing population in the Central Rockies study area is similar to that found in other populations in North America (Table 1). Forbes and Boyd (1996) detected no evidence of a founding population bottleneck in the colonizing population.

Table 1. Estimated genetic variation for 9-10 microsatellite loci in wolves.

| Location | mean sample size per locus | genetic variation $H_o^1$ | $H_e^2$ | $A^3$ | Source |
|---|---|---|---|---|---|
| Vancouver Island | 12.6 | 0.421 | 0.566 | 3.4 | Roy et al. 1994 |
| Mexican (Certified) | 20.9 | 0.437 | 0.503 | 2.5 | Garcia-Moreno et al. 1996 |
| Minnesota | 19.8 | 0.532 | 0.686 | 6.3 | Roy et al. 1994 |
| Kenai Peninsula | 18.9 | 0.536 | 0.581 | 4.1 | Roy et al. 1994 |
| NW Territories | 20.9 | 0.547 | 0.721 | 6.4 | Roy et al. 1994 |
| Banff Nat'l Park | 32.0 | 0.553 | 0.581 | 4.4 | Forbes and Boyd 1996 |
| Hinton | 33.0 | 0.579 | 0.628 | 4.5 | Forbes and Boyd 1997 |
| Fort St. John | 41.0 | 0.588 | 0.589 | 4.5 | Forbes and Boyd 1997 |
| Alberta | 18.2 | 0.605 | 0.668 | 4.5 | Roy et al. 1994 |
| Central Rockies (through 1995) | 66.0 | 0.606 | 0.606 | 4.1 | Forbes and Boyd 1997 |
| Central Rockies (through 1987) | 13.0 | 0.685 | 0.619 | 3.8 | Forbes and Boyd 1996 |

[1] $H_o$ = observed heterozygosity
[2] $H_e$ = expected heterozygosity
[3] $A$ = allelic diversity (mean number of alleles per locus)

Genetic variation ($A$ and $H_e$) of wolves from the colonizing population was 1) greater than that of the more isolated wolf populations of Vancouver Island, Kenai peninsula, and captive Mexican wolves, 2) approximately equal to wolf populations in southwestern

Canada, and 3) slightly less than values reported in the Northwest Territories where wolf populations had not been extirpated by people. Sufficient gene flow occurred between the colonizing and source populations to minimize genetic divergence. The degree of allele sharing and genetic differentiation indicated a lack of inbreeding in the founding colonizers (Forbes and Boyd 1996, 1997). In most populations $H_o < H_e$ (Table 1) suggesting a decrease in heterozygosity due to very slight inbreeding. The only exception to this is the apparently outbred 1987 colonizing population where $H_o$ (.685) > $H_e$ (.619), and reaches equality ($H_o = H_e = .606$) in the 1995 colonizing population.

Wolves from Fort St. John and Hinton (selected for the reintroduction in central Idaho and YNP) had slightly higher $A$ (4.5) than the colonized population (4.1). Additionally some alleles from the reintroduced wolves were rare or absent in the colonizing populations and vice versa (Forbes and Boyd 1997) so migration between the native and reintroduced populations may increase genetic variation. However, loss of genetic variation will depend on the initial rate of reproduction and survivorship. Heterozygosity will be lost at a rate of $1/(2N_e)$ (Wright 1969) but high reproduction and survivorship should minimize loss of $H$. In May 1997, YNP wolf biologists reported that 8 packs produced at least 11 litters of pups (D. Smith, pers. commun., YNP Wolf Project), which far exceeded reproductive expectations.

Forbes and Boyd (1997) calculated migration rates (migrants per generation; $N_em$) using the $F_{ST}$ estimator theta with the program GENEPOP (Raymond and Rousset 1995). This model assumes that the population is continuously distributed without discrete boundaries between packs, which is in concordance with our dispersal information and that of Nowak (1983).

Table 2. Migration estimate ($N_em$), in migrants per generation, for wolves in North America.

| Geographic areas compared | Distance apart (km) | $N_em$ |
|---|---|---|
| Fort St. John & Hinton | 600 | 2.7[a] |
| Northwest Territories & Fort St. John | 1000 | 1.6[a] |
| Northwest Territories & Hinton | 1200 | 2.3[a] |
| Colonizing | < 840 | ~ 2.0[b] |

[a] estimated using $F_{ST}$ estimator theta

[b] estimated from field data because colonizing populations are not likely to be in equilibrium and, therefore, violate assumptions

Wright (1931) estimated that 1 migrant (e.g. disperser with genetic contribution) per generation, regardless of population size, is needed to prevent fixation due to genetic drift. However, Mills and Allendorf (1996) concluded that 1 to 10 migrants per generation would be a better management guideline for genetic purposes, because natural populations often violate Wright's assumptions for ideal populations. Fewer than 1 migrant per generation may cause a loss of genetic variation within a subpopulation and ultimately increase chances for extinction. More than 10 migrants per generation may result in too much gene flow, thereby reducing diversity among subpopulations. This would tend to make subpopulations less able to differentially respond to environmental variation, thereby making the population more vulnerable to extinction. We documented sufficient migration in the colonizing population to maintain genetic variation approximating that of the Canadian source population.

$F_{ST}$ (the proportion of genetic variation that is due to differences between subpopulations) is a measure of genetic drift whereby increasing drift causes increasing divergence, increasing $F_{ST}$ values (Nei 1977). $F_{ST}$ of the Central Rocky Mountain populations agreed closely with values reported in other North American populations (Table 3), indicating there is adequate gene flow between subpopulations (Forbes and Boyd 1997). Nei's genetic distance, *D*, confirmed that there are small but significant differences between the Alberta source population and the colonizing (Forbes and Boyd 1996).

Table 3. $F_{ST}$ values of wolves in North America.

| Geographic source | level of comparison | # populations | $F_{ST}$ |
|---|---|---|---|
| NW Canada[a] | subpopulations | 8 | 0.074 |
| North America[b] | population | 5 | 0.168 |
| Central Rockies[c] | subpopulations | 7 | 0.074 |

[a] Kennedy et al. 1991
[b] Roy et al. 1994
[c] Forbes and Boyd 1997

Levels of genetic diversity are determined by the opposing forces of gene flow (migration) and genetic drift. Isolated subpopulations tend to diverge due to drift, increasing differentiation among subpopulations. Gene flow via migration between subpopulations tends to homogenize variation on the larger metapopulation scale. The best way to maintain long-term, large scale genetic diversity lies in the middle ground between completely isolated subpopulations and a homogeneous metapopulation. Managers should strive to minimize loss of genetic variation within subpopulations, but allow moderate genetic divergence among subpopulations (Mills and Allendorf 1996).

Wolves are relatively prolific breeders and are capable of colonizing new areas quickly (Mech 1970, 1995). The high dispersal rates and long dispersal distances typical of wolves (Gese and Mech 1991, Boyd et al. 1995) enhance gene flow between subpopulations, thereby maintaining high genetic variation within the metapopulation. Forbes and Boyd (1996, 1997) determined that ample genetic variation exists in the colonizing and reintroduced populations. Demographics and stochastic events in semi-isolated populations may be a more serious concern at this point in recovery. Lack of animals of suitable breeding age, disparate sex ratios, disease, or environmental disaster may lead to the extinction of a small, isolated population. "It is simply not possible to separate genetic factors from environmental ones when addressing concerns over small population persistence" (Mills 1996:315).

## Management Choices: Natural Recovery or Reintroduction

Natural recovery (engineered by wolves) and reintroduction (engineered by humans) are different strategies presently occurring in wolf recovery. Natural recovery occurs through dispersal and colonization events over a broad landscape scale with connectivity, but with little opportunity for humans to manage for genetic considerations. Reintroduction allows managers to select genetic stock in founders, and then release the animals into often disjunct populations (e.g. red wolves, YNP wolves). The synergy of these 2 methods may present the best scenario for management of genetic diversity. However, this is a rather simplistic view that may disregard ecological, social, and political considerations. Extrinsic factors (e.g. tolerance by local human residents, adequate prey, access, political persuasion of governing bodies) have historically played a more critical role in wolf survival than genetic considerations.

A wolf that successfully "runs the gauntlet" from a northern source population to become a southern colonizer has probably avoided humans, livestock, and vehicles. These behaviors that minimize wolf-human conflicts may be passed on socially and genetically to their offspring. Wolves that are reintroduced have not passed through this behavioral filter and may be more inclined to cause problems once they leave the protection of parks and wilderness areas. However, wolves exhibit a wide range of behavioral variability and learn relatively quickly, allowing potential habituation to human activities regardless of wolf origins. The critical issue is that landscape connectivity must be maintained between recolonizing and reintroduced populations to allow some degree of gene flow.

Dispersal data and microsatellite analyses indicate that wolves have the capability to disperse at least 840 km, resulting in genetic exchange between northern British Columbia and YNP and probably beyond (Ream et al. 1991). The study population inhabits 2 countries, 2 provinces, 3 states, and numerous smaller management districts within each province and state, complicating the coordination of wolf management across the area. Optimum wolf management would include better coordination among agencies and also between agencies and local residents. The reintroductions have aided the wolf population expansion demographically and genetically, but managers must consider both genetics and extrinsic factors to maximize successful recovery.

### Wolf-like Canids

Gray wolves, coyotes, domestic dogs, and red wolves are known to hybridize in captivity and in the wild (Mech 1970, Mengel 1971, Schmitz and Kolenosky 1985, Lehman et al. 1991, Wayne and Jenks 1991, Boitani and Cuicci 1993, Wayne 1993, Clutton-Brock et al. 1994, Gottelli et al. 1994). This hybridization has caused problems for conservationists seeking to maintain the genetic integrity of local wolf populations. With the aid of increasing molecular resolution, hybridization has been detected in wolf populations previously considered "pure" (e.g. the Great Lakes region and the red wolf) (Wayne and Jenks 1991, Lehman et al. 1991, Wayne 1993) creating a potential political dilemma for protection under the Endangered Species Act. Captive wolves and wolf-dog hybrids escape confinement or are intentionally released by their owners into wolf habitat, complicating management decisions. Managers often expect a simple answer to the origin of wolf-like canids that appear in unexpected places. How reliably can present molecular techniques identify the origins of these wolf-like canids?

A subpopulation of wild wolves will have only a subset of all possible canid microsatellite alleles. Non-native wolf-like canids (dogs, wolf-dog hybrids, wolf-coyote hybrids, and released captive wolves) are likely to have different alleles than the local native wolf population. examined samples from 172 Rocky Mountain wolves, 4 non-native wolves, and 13 dogs at 10 microsatellite loci. Forbes and Boyd (1997, unpubl. data). The 17 non-native wolf-like canids had 0-5 alleles not found in the Rocky Mountain wolf population, which excluded all but 1 of these animals from the native population in genotype likelihood calculations. We concluded that a wolf-like canid of questionable origin can be excluded from the native wolf population with fair reliability.

Non-native wolves and dogs were equally different from native wolves, so identifying the origins of a non-native wolf-like canid as wolf, dog, or wolf-dog hybrid is impossible with present microsatellite techniques and databases. This is in agreement with S. Fain (USFWS, pers. commun.) who analyzed samples of hundreds of wolves and dogs at 32 microsatellite loci and concluded there is no infallible wolf-dog marker that differentiates the two (unpubl. data). Furthermore, Fain found as much mtDNA variability among wolves as between wolves and dogs, and considers dog DNA as a subset of wolf genetic variation. Dogs were domesticated from wolves approximately 14,000 years before present (ybp), determined through archeological evidence (Morey 1994). This is a relatively short separation on a geologic time scale and has not allowed enough time for mutations and drift to distinguish dogs and wolves. Vila et al. (1997) stated that 14,000 ybp would be a minimum domestication age based on mtDNA, and it may be as much as 135,000 ybp based on a divergent monophyletic clade that shared no sequences with wolves. Vila et al. (1997) suggested that dogs and wolves may have continued to interbreed after dogs were domesticated from wolves, further clouding the issue. In summary, present molecular techniques do not allow complete forensic distinction between native wolves and other wolf-like canids.

Coyotes and wolves shared a common ancestor about 2 million ybp (Wayne 1993) allowing enough time to evolve detectable divergence between the 2 species. Coyote-wolf hybridization is more easily detected than dog-wolf hybridization with mtDNA haplotypes.

Coyote haplotypes were commonly found (>50%) in the wolves of the Great Lakes region, but wolf haplotypes were not found in sympatric coyote populations (Lehman et al. 1991, Roy et al. 1994). Pilgrim et al. (in press) analyzed mtDNA of 90 wolves and 30 coyotes and found no evidence of coyote introgression into Rocky Mountain wolves.

## CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

1. Adequate genetic variation currently exists among colonizing wolves in the Central Rockies, with no evidence of a population bottleneck during the colonization process.
2. We must maintain landscape linkages between the US and Canada (source-sink dynamics) and between natural and reintroduced populations. This is critical to maintain genetic variation in the wolves of the Central Rockies.
3. Demographics may be more of a problem for isolated wolf subpopulations than genetic impoverishment, so minimizing wolf mortalities in recovering populations will enhance long-term population viability.
4. Ideally, wolves should be managed as 1 metapopulation from the Yukon to Yellowstone, coordinating efforts between various managing factions.
5. Molecular techniques do not exist at the present time to reliably determine origins of wolf-like canids. More research is needed in this area.
6. Managers should evaluate 3 criterion when trying to ascertain the origin of a wolf-like canid: morphology, behavior, and genetics.

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# APPENDIX A

Forbes, S.H., and D.K. Boyd. 1996. Genetic variation of naturally colonizing wolves in the Central Rocky Mountains. Conservation Biology 10(4): 1082-1090.

**Abstract:** Recovery of gray wolf (*Canis lupus*) populations in North America depends on minimizing human-caused mortality and enhancing migration from stable source populations to suitable habitat unoccupied by wolves. We used a combination of field observation and DNA microsatellite genotyping to examine natural wolf colonization of Glacier National Park, Montana, and surrounding lands. We found high amounts of genetic variation in the colonizing population, showing that these packs were founded by multiple unrelated wolves from Canada. High dispersal rates, long dispersal distances and lack of a founding population bottleneck indicate that wolves in the U.S. and Canada should be viewed and managed as a single population. Restoration in the U.S. by artificial transplants from Alberta to Yellowstone National Park and central Idaho began this past winter. The transplanted wolves will likely aid demographic recovery, but permanently retaining the high genetic variation already present in U.S. wolves will require assuring gene flow throughout the Central Rocky Mountains.

## Introduction

Gray wolves (*Canis lupus*) in the Rocky Mountains of Canada and the U.S. provide an excellent case study for restoration and conservation of a keystone predator. Since wolves were extirpated from much of the Rocky Mountains, several decades of wolf research and dramatic changes in public attitudes towards predators have occurred, resulting in a potentially more favorable environment for wolf recovery.

Wolves have remarkable ability to recolonize their former range when they are protected from persecution by man (Mech 1995). The wolf was listed as an endangered species in the lower 48 United States in 1973 (U.S. Fish and Wildlife Service 1987). Recovery in the Great Lakes region has been successful (Mech 1995), but in the western U.S. it has been slower. Wolves were extirpated from the Rocky Mountains in and south of Banff National Park, Alberta, by about 1930, while viable but declining populations persisted in

Northern Alberta (Gunson 1983). Wolves increased in Northern Alberta and returned to most of the southern Canadian Rockies by the 1940s but they were again exterminated in and south of Banff National Park in the 1950s (Fig. 1; Gunson 1983; Boyd et al. 1995).

In the 1970s solitary wolves were again reported in the protected areas of Banff National Park and Glacier National Park, Montana, and in 1979 a lone female established a home range that included the northwest corner of Glacier National Park (Ream et al. 1991). This female bred in 1982 and in 1985 in Canada within 10 km of the U.S. border, and 1986 she was the first wolf to den in Montana in over 50 years (α-female 8550 of the Magic pack; Figs. 1 and 2; Ream et al. 1989). Fragmented wolf habitat exists throughout western Montana, but potential obstacles to wolf survival (highways, agricultural development, poachers) are numerous. The colonizing population of wolves that began with the Magic pack has been monitored for 15 years (Pletscher et al. 1991; Ream et al. 1991; Boyd & Jimenez 1994; Boyd et al. 1994, 1995; Fig. 1), and in Montana it now contains about 60 wolves in 7 packs (S.H. Fritts, U.S. Fish and Wildlife Service, pers. comm.).

New insights into wolf population genetics are possible through use of DNA microsatellite loci cloned from the domestic dog (*C. familiaris*; Ostrander et al. 1993; Gottelli et al. 1994; Roy et al. 1994). Microsatellites are hypervariable, single locus, genetic markers that can be analyzed from minuscule tissue samples using the polymerase chain reaction (PCR). Microsatellites are valuable for parentage and relatedness studies because they generally have large numbers of co-dominant Mendelian alleles (Queller et al. 1993; Morin et al. 1994; Craighead et al. 1995), and for population-genetic studies because of the large number of variable loci available (e.g. Schlotterer et al. 1991; Ellegren 1992; Bowcock et al. 1994; Taylor et al. 1994; Morin et al. 1994; Paetkau and Strobeck 1994; Paetkau et al. 1995).

Roy et al. (1994) found ample genetic variation at microsatellite loci in wolf populations throughout Canada and Alaska, including a sample from Alberta, and there is little concern about genetic diversity in North American wolves generally. However, loss of genetic variation is a concern in certain isolated wolf populations not included in the above study (Wayne et al. 1992; Randi et al. 1993; Gottelli et al. 1994; Fritts & Carbyn 1995).

Conservation genetics addresses the effects of inbreeding in recolonized or reintroduced populations founded by a limited number of individuals (Leberg 1990). In a captive population in Sweden, wolves were found to be susceptible to inbreeding depression (Laikre & Ryman 1991). Inbreeding depression is more often demonstrated in captivity than in the wild, but a recent study of *Peromyscus leucopus* showed that deleterious effects of inbreeding were greater in wild than in captive populations, likely due to the more rigorous environment in the wild (Jimenez et al. 1994).

In the recolonizing Montana wolf population, field data alone were inadequate to assess possible inbreeding in the founding packs. There were very few wolves in southern British Columbia and Alberta when the Magic pack first denned in Montana (Ream et al. 1989, 1991). Several subsequent U.S. packs were founded by Magic pack dispersers, but occasional new breeding wolves appeared whose relatedness to the Magic pack was unknown. Founders descended from a single Canadian pack would constitute a bottleneck of 2 animals, and a string of such packs extending southward from Banff National Park to Glacier National Park and beyond would result in a colonized population with especially low genetic variation. Alternatively, colonizers from many packs would approach the management ideal of multiple unrelated founders (Leberg 1990).

Little is known of the effects of long-distance dispersal and social structure on genetic variation in wolves, especially wolves occupying new territory. Occasional single wolves travel more than 600 km in search of territory and mates (VanCamp & Gluckie 1979; Fritts 1983; Boyd et al. 1995), and dispersal rates of young are greater in increasing populations (Gese & Mech 1991; Boyd & Jimenez 1994). Long-distance dispersal potentially enhances large-scale effective population size ($N_e$) by increasing migration between subpopulations (Fritts & Carbyn 1995). However, wolf $N_e$ is limited by a social structure where usually only a single pair (an α-female and α-male) breeds in each large territory (Chepko-Sade et al. 1987). Lehman et al. (1992) used DNA fingerprinting to show that short-distance dispersal was common in permanent wolf populations, but this did not address long-distance migration or colonization.

We used the 8-year period (1986-1994) of natural colonization in Montana to examine genetic variation in an expanding, disjunct wolf population. The results are relevant to concerns about inbreeding during wolf recovery and they add to our knowledge of wolf dispersal. They also address the relative importance of genetic and demographic factors in the Rocky Mountain wolf reintroduction program currently in progress.

## Methods

We sampled and genotyped 59 colonizing wolves, including members of all known breeding wolf packs (Fig. 1). We also genotyped 32 wolves from the Banff National Park, Alberta, area as a reference for gene frequencies in potential transboundary migrants. Blood samples were taken from live-trapped animals (Ream et al. 1991; Boyd et al. 1995) and muscle samples were taken from wolves found dead. Genomic DNA was purified by protease K digestion and phenol/chloroform extraction (Ausubel et al. 1989). Alternatively, 5 mg of tissue or 2 ul of blood were prepared by autoclaving for 5 minutes in 200 ul 5% Chelex 100 resin (Bio Rad Laboratories) and pelleting the resin in a microcentrifuge. DNA microsatellite loci were amplified using the polymerase chain reaction (PCR). Ten dinucleotide repeat (AC)n loci (Ostrander et al. 1993) were chosen from those previously used in wolves by Roy et al. (1994) except that we used locus 200 instead of locus 213, and our locus 2 is their locus 200. Primers were labeled with $^{32}P$ using polynucleotide kinase (Ausubel et al. 1989). Ten-ul PCR reactions contained 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 2 ug/ml BSA, 0.2 mM each dNTP, 4 pmoles of each unlabeled primer, 0.2 pmoles of labeled primer, 0.5 U Taq DNA polymerase (Perkin-Elmer Cetus) and either 50-100 ng purified genomic DNA or 1 ul Chelex tissue preparation. The thermal profile was 94°C for 3 minutes, followed by 35 cycles of 92°C for 1 min. and the selected annealing temperature (55°C or 58°C) for 1 min. with no extension step. Samples were run on a 7% acrylamide, 11 M urea sequencing gel in 1X TBE buffer (Ausubel et al. 1989), and the gel was and exposed to x-ray film for 4-20 hours. Allele sizes were scored using the M13 phage control DNA in the Sequenase$^{TM}$ DNA sequencing kit (United States Biochemical).

Population genetic data were calculated using BIOSYS-1 (Swofford and Selander 1989). We tested allele frequency differences using the Monte Carlo simulation in the REAP computer program package (McElroy *et al.* 1991). Heterozygosity differences between samples were tested using a paired $t$ test on $H$ values paired at individual loci (Nei 1987; Leberg 1992). When $H$ was lower in a colonizing group than in the reference population, a one-tailed test was used. Tests for deviations from binomial expected (Hardy-Weinberg) genotype proportions were made using 2 different chi-square tests: pooling all alleles except the most common into a single class (Swofford & Selander 1989) and pooling all genotypes into 2 classes, heterozygotes and homozygotes (Lessios 1992).

## Results and Discussion

### Genetic Variation

Heterozygosity in the colonizing wolves ($H = 0.607$) was slightly higher than in the Alberta reference sample ($H = 0.581$; Table 1). However, the difference in these values is not significant ($p > 0.1$) and its sign is opposite to that expected with a founding bottleneck.

Genotype proportions did not indicate marked population subdivision. The Alberta sample and the total sample showed only slight heterozygote deficits compared to binomial expectations (Table 1). Only a few single locus tests showed significant deviations after correcting for the number of tests (see Methods). The colonizing wolves had 1 locus with a heterozygote excess by both tests, the Alberta wolves had 1 locus with a deficit using 1 of the tests, and the combined sample had deficits at 2 loci using both tests. Thus, in Alberta and in the combined sample, non-random mating within subpopulations was detectable but not strong. The heterozygote excesses in single packs (Table 1) may have a different cause. Excesses are expected in founding cohorts because of chance differences in allele frequencies between male and female parents (Robertson 1965).

Allelic diversity (*A*) is more sensitive to founder events than *H*, and it is more indicative of future adaptive potential (Nei et al. 1975; Leberg 1992). In the earliest packs (wolves born through 1987) *A* was predictably lower than in Alberta, but by 1989 there was already a maximum of 41 different alleles, or 93 percent of the 44 alleles in Alberta (Table 1). Thus, the presence of a majority of the reference population alleles in the founding population after 2 generations also indicates lack of a serious founding bottleneck. Because rare alleles affect *A* but have little effect on *H*, it is reasonable that heterozygosity is high in the first founding packs despite the lower number of alleles. With only 3 exceptions all alleles in the colonizing wolves were found in the reference Alberta population, consistent with natural immigration of wolves from Canada (Table 2). Two exceptions were alleles (2-F and 377-B) absent in our Alberta sample but found there at low frequency by Roy et al. (1994). Another unique allele (123-F) in 1 Browning (BG) wolf may also be rare in Alberta.

We sampled 3 wolves that were found farther south than any known breeding pack, and whose relationship to the colonizing population was unknown. Two of these (2150 from near Cardwell, Montana; 9203 from south of Yellowstone National Park, Wyoming; Fig. 2) were genetically consistent with birth in the colonizing population, but not with descent from any sampled breeding female. They could be offspring of an unsampled breeding female or they could be long distance migrants from Canadian packs. However, the third wolf of unknown origin (from near Deerlodge Montana) had 5 alleles not otherwise present in our Alberta or Montana data. We inferred that this animal was not born in the colonizing population or in the native Alberta population, and we omitted it from this report. However, the ability to detect such animals is an argument for continued genetic screening. The appearance of multiple non-native alleles in known breeding wolf packs would indicate hybridization with released captive wolves or other wild, feral, or domestic canids (e.g. Gottelli et al. 1994).

### Pack Relationships and Number of Founding Wolves

Relationships of the colonizing wolves known from field observation (Ream et al. 1991; Pletscher et al. 1991; Boyd & Jimenez 1994; Boyd et al. 1994, 1995) are shown in Fig. 2. Wolves were assumed offspring of the pack α-female unless this was disproved by age or genetic exclusion (see below). The chart includes the α-females of most packs, but fewer α-males because relatively few adult males were captured. However, the study includes genes from all known breeding pairs because pups were sampled from packs where 1 or both parents were not sampled.

Field data collected over several generations indicated that many of the 15 packs were closely related. Seven packs showed matrilineal descent from the MG pack founding female (8550), based on dispersal and breeding of tagged or otherwise individually known female pups. Two additional packs were related to the MG pack through males: the HD pack through a male disperser (8704) and the WG pack through a male born in the Canadian MG pack prior to 1985 (8401). Thus only 3 pack lineages did not have a field-documented relationship to the MG pack through at least 1 parent (BG, FO/TR, MA/NM1).

Two different estimation methods indicated the number of founders to be about 8. First, 5 breeding females were identified as founders because they were too old to have been born in colonizing packs. Four of these females were sampled and genotyped (MG, HD, BG, and MA packs; Fig. 2). Excepting the 2 males cited above as born within colonizing packs (WG and HD packs), the fathers of the other 3 founding packs were assumed to be founders as well. Added to the 5 founding females, this gives an upper estimate of 8 possibly unrelated founders.

The appearance of new alleles also provides an estimate of the number of founders. In 1989 there were 41 different alleles at the 10 loci (Table 1). Thirty-five of these alleles first appeared in the 4 sampled female founders. The 6 additional alleles first appeared in pups, and they were attributable to unsampled parents: the α-males of the MG and BG packs, and both parents of the 1987 WG pack. Added to the 4 founding females, this gives a minimum estimate of 8 founders.

## Genetic Accuracy of the Pedigree

Based on genetic parentage exclusion, assignment of pups to packs was accurate. All wolves judged to be young enough to have been born in the pack where they were trapped had genotypes consistent with their putative α-female or α-male parent when the parent was sampled (Fig. 2). By inferring partial paternal genotypes from genotypes of pups and mothers, we found several cases where pups born in successive years were attributable to different unsampled fathers (data not shown).

Wolves are difficult to age after their first year, and they may emigrate to non-natal packs (Lehman et al. 1992). Five wolves found by genetic exclusion not to be offspring of the pack α-female were also excluded by estimated age, and were assumed to be immigrants. For example, NC pack wolves 9378 and 9379 were too old (3-4 years old when trapped in 1993) to have been born to the NC α-female. However, they were genetically consistent with birth in the adjacent and closely related SC pack.

Failure to exclude a given pup as offspring is not proof of maternity, and indeed some pups were consistent with more than 1 mother. However, high genetic consistency of mothers and pups is a test of accuracy of the field data. The exclusion efficiency (the frequency of parentage exclusion for pups known by field observation and genetic compatibility to be born in another pack) was highest (97 percent) for non-offspring of the 7 sampled adults defined as founders. However, exclusion efficiency declined as new packs were formed by offspring of founders and the number of sampled breeding adults related as parent-offspring or as siblings increased (92 percent overall).

## Genetic Inference of Founder Relatedness

The number of founders tells little about wolf dispersal without knowledge of their relatedness. Changes in *H* and *A* values expected due to a bottleneck depend on the assumption that founders are randomly selected from the source population, and inbreeding would cause predictable deviations from these expectations. In fact, all 3 measures discussed below (*H* and *A* in the founding packs and allele sharing between the founding adults) differed from expected values in the direction opposite to that expected if the founders were closely related.

First, heterozygosity (*H*) in the first packs was high (Table 1). As long as parents of colonizing packs are unrelated founders and not progeny of related colonizing packs, binomial expected *H* will remain at the source population level, because loss of *H* due to inbreeding does not occur until the next generation (Robertson 1965). In fact, 3 of the first 4 and 4 of the first 7 packs had higher (but not significantly higher) $H$ than the Alberta sample.

Two packs that formed following the initial colonizing event had lower heterozygosity than the others, suggesting a local, transitory founder effect. The NC and SC packs had the lowest $H$ compared to Alberta (Table 1), and the differences approached significance ($p = 0.04$ and 0.07 respectively). The α-females of these packs were second-generation descendants of the earliest founding female (8550, MG pack; Fig. 2). The fathers of these packs were not identified or sampled, but the lower heterozygosity suggests that the α-males were also direct descendants of founders, and thus that they were relatively closely related to their mates. The fact that lower $H$ values occurred only in 2 packs formed after the initial colonization supports the inference that high heterozygosity in the first packs indicated lack of founder relatedness.

Allelic diversity ($A$) in the founding packs was also higher than expected. The expected loss of alleles after a founding event of $n$ individuals is
where $n$ is the starting number of alleles, $n'$ is the number remaining after 1 generation, and $p_j$ is the frequency of the $j_{th}$ allele (Denniston 1978). Using $n = 2$ and the observed allele frequencies in Alberta gives an expected $A$ of 2.26 in single packs. The mean observed value for pups in each of 7 sampled litters was slightly higher ($A = 2.54$). Similarly, assuming the 4 packs sampled by 1987 are a founding population of $n = 8$, the observed $A$ (3.80) is again higher than the expected (3.54). Closely related parents would give $A$ values lower than expected.

We also examined founder relatedness using allele sharing as a measure of genetic similarity between pairs of individuals (Fig. 3). Allele sharing is simply the number of alleles in 2 individuals that match at each locus, summed over loci. In our case identical genotypes would share 20 alleles at the 10 loci. This parameter is not highly informative about particular relationships with the observed amount of variation and number of loci

(Chakraborty & Jin 1993). Nevertheless, allele sharing was predictably higher among colonizing packmates than among unrelated colonizing wolves or randomly selected Alberta wolves (Fig. 3). Most importantly, mean allele sharing among the 4 sampled female founders was lower than the mean for all 3 other groups: the Alberta sample (which include packmates and unrelated animals), pairs compared between packs in the colonizing population, and colonizing packmates. Although the small number of sampled founders gives little statistical power in these comparisons, the low mean allele sharing among the founders suggests once again that they were not closely related.

## Population Structure, Gene Flow and Heterozygosity

The north-to-south gene flow indicated during colonization should result in genetically similar Canadian and U.S. wolf populations. This is confirmed by a Nei's standard genetic distance (Nei 1987) between the Alberta and colonizing samples of $D = 0.116$. This is 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). However, $D$ is significantly greater than zero, because allele frequencies differ significantly between the Alberta and colonizing samples (9 out of 10 single locus tests were significant at $p < 0.05$). $G_{ST}$, the proportion of heterozygosity due to population differences, between these populations is 0.037, a moderate value. Despite low genetic divergence between Alberta and Montana due to substantial gene flow, the 2 regions cannot be viewed as an absolutely panmictic population.

The high genetic variation in the founding packs and the apparently low relatedness of the founding wolves indicate high dispersal rates, and perhaps behavioral avoidance of inbreeding. An additional cause, however, may be population structure on a level that our sampling does not detect. That is, our Banff-centered reference sample likely does not perfectly represent the pool of potential transboundary migrants. The best way to maintain genetic variation is in multiple, semi-isolated breeding lines or subpopulations

(Denniston 1978; Leberg 1990). In the present case, wolf populations on the east front of the Rocky Mountains may be semi-isolated from those in the intermountain valleys of British Columbia. High dispersal into unoccupied territory on the southern periphery of this area (near the U.S. border) would tend to bring together unrelated breeding pairs.

The current estimate of about 60 wolves in the colonizing population is likely to be accurate. Much of the landscape uninhabited by humans is unavailable to wolves because it is high elevation rock and snow, and devoid of prey. Wolf territories are in roaded, accessible valley bottoms (Fritts et al. 1994) with concentrations of prey, reducing the difficulty of sampling a high percentage of the population.

Field data supported by genetics gave a founding population estimate of 5 breeding pairs and the current estimate is about 7 pairs. The reported 60 percent heterozygosity cannot be maintained in so small a population without continued immigration. With mutation rates estimated from about $\mu = 10^{-4}$ to $10^{-3}$ (Dietrich et al. 1992; Weissenbach et al. 1992), equilibrium heterozygosity this high would require an effective population size ($N_e$) in the range of 1000-10,000 under a stepwise mutation model ($H = 1 - [1/(1 + 8N_e\mu)^{0.5}]$) (Ohta & Kimura 1973). Historical wolf populations were apparently large enough and sufficiently connected by migration to maintain this much genetic variation (Roy et al. 1994). The present population throughout Canada and Alaska is estimated at 60-70,000 (Fritts & Carbyn 1995), and mitochondrial DNA data indicate that historical continent-wide gene flow was substantial (Wayne et al. 1992). However, $H$ is expected to be lost in a finite population due to genetic drift. The recolonized Alberta and Montana wolf populations will only retain the historical effective size indicated by their high genetic variation if there is continued migration among packs.

## Conclusions

Genetic variation in the colonizing packs compared favorably with amounts in Alberta and throughout North America (Table 1; Roy et al. 1994). Only a few, rare Canadian alleles were apparently lost during colonization (Table 2), and rare alleles have little effect on the total genetic variance needed for long-term viability and future adaptation (Allendorf 1986). Loss of genetic variation is clearly not an immediate concern in Rocky Mountain wolves.

The genetic data indicated dispersal of a greater number of unrelated wolves than was revealed by field observation alone. In addition, recent artificial relocations promise to further aid dispersal of wolves throughout the Rocky Mountains. Concurrent with natural wolf colonization of western Montana, the U.S. Fish and Wildlife Service began in early 1995 to move wolves from west-central Alberta to Yellowstone National Park and central Idaho (U.S. Fish and Wildlife Service 1987; Fritts & Carbyn 1995). Principle reasons for the reintroduction program are that it will speed up the process of colonization, and that it will help ensure ample genetic variation. Our findings indicate that the demographic argument is the more compelling, because genetic variation in Montana wolves is already high. A goal for U.S. Rocky Mountain wolves of 30 breeding packs established in 3 years is deemed adequate for demographic recovery (U.S. Fish and Wildlife Service 1987).

Even a population meeting this recovery goal will not permanently retain the founding genetic variation without ongoing gene flow from Canada. However, adequate genetic exchange by natural migration appears possible. The combined evidence from radiotelemetry and genetics indicates high dispersal rates and long dispersal distances in Rocky Mountain wolves. Some of the wolves transplanted to Idaho have already traveled to within 65 km of the colonizing NM pack den site (S.H. Fritts, U.S. Fish and Wildlife Service, pers. comm.), and interbreeding with the natural colonizers is imminent. Wolves appear to differ from other large mammals such as grizzly bears (Allendorf et al. 1991) in that wolves may be more able to sustain genetic viability without artificial transplants between subpopulations.

Despite their great dispersal ability and their adequate genetic variation, Rocky Mountain wolves face daunting challenges to their permanent recovery. No single U.S. reserve is large enough to support a genetically viable wolf population in isolation, so that wolves must use lands where they are not protected from human-caused mortality (Fritts & Carbyn 1995; Mech 1995). Our study documents extensive use by wolves of such lands for both residence and migration. Wolves will best flourish in the Rocky Mountains if public tolerance and legal protection allow continued natural migration throughout the region.

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**Table 1.** Genetic variation at 10 microsatellite loci in selected groups of Alberta and colonizing wolves.

| Population | $n$ | $A$ | $H_o$ | $H_e$ |
|---|---|---|---|---|
| Alberta (total through 1994) | 32 | 4.4 | 0.553 | 0.581 |
| Colonizing: | | | | |
| Four Female Founders | 4 | 3.5 | 0.675 | 0.686 |
| (Bred 1986-1989) | | | | |
| MG Pack 1985 | 4 | 2.5 | 0.625 | 0.536 |
| BG Pack 1987 | 4 | 2.5 | 0.725 | 0.586 |
| CA Pack 1987 | 4 | 2.7 | 0.725 | 0.582 |
| Four Packs 1987 | 13 | 3.8 | 0.685 | 0.619 |
| HD Pack 1989 | 6 | 2.9 | 0.800 | 0.636 |
| Seven Packs 1989 | 24 | 4.1 | 0.667 | 0.639 |
| SC Pack 1990 | 5 | 2.6 | 0.600 | 0.496 |
| NC Pack 1991 | 4 | 2.1 | 0.550 | 0.432 |
| SP Pack 1991 | 4 | 2.5 | 0.775 | 0.589 |
| Eight Packs 1991 | 41 | 4.1 | 0.639 | 0.611 |
| Colonizing (total through 1994) | 59 | 4.1 | 0.634 | 0.607 |
| Combined Alberta and colonizing | 91 | 4.7 | 0.605 | 0.616 |

$n$ = sample size, $A$ = mean alleles per locus, $H_o$ = observed heterozygosity, and $H_e$ = binomial (Hardy-Weinberg) expected heterozygosity (unbiased estimate; Swofford and Selander 1989). Listings for single packs each include 1 sampled parent and the pups from 1 breeding year. Pack codes: MG = Magic, BG = Browning, CA = Camas, HD = Headwaters, SC = South Camas, NC = North Camas, SP = Spruce.

**Table 2.** Allele frequencies at 10 microsatellite loci in Central Rocky Mountain wolves.

| Locus and Allele | Alberta | Colonizing |
|---|---|---|
| 2: | | |
| D | .766 | .670 |
| F | .000 | .144 |
| H | .031 | .000 |
| I | .203 | .186 |
| 109: | | |
| A | .094 | .034 |
| B | .203 | .178 |
| C | .281 | .153 |
| D | .031 | .288 |
| E | .375 | .305 |
| G | .016 | .042 |
| 123: | | |
| E | .562 | .687 |
| F | .000 | .008 |
| G | .172 | .203 |
| I | .063 | .000 |
| J | .203 | .102 |
| 172: | | |
| H | .141 | .280 |
| I | .859 | .720 |
| 200: | | |
| E | .657 | .364 |
| I | .031 | .246 |
| J | .156 | .314 |
| L | .155 | .076 |
| 204: | | |
| A | .281 | .348 |
| B | .344 | .127 |
| D | .344 | .322 |
| E | .031 | .203 |
| 225: | | |
| B | .078 | .237 |
| C | .500 | .263 |
| D | .141 | .051 |
| E | .281 | .449 |
| 250: | | |

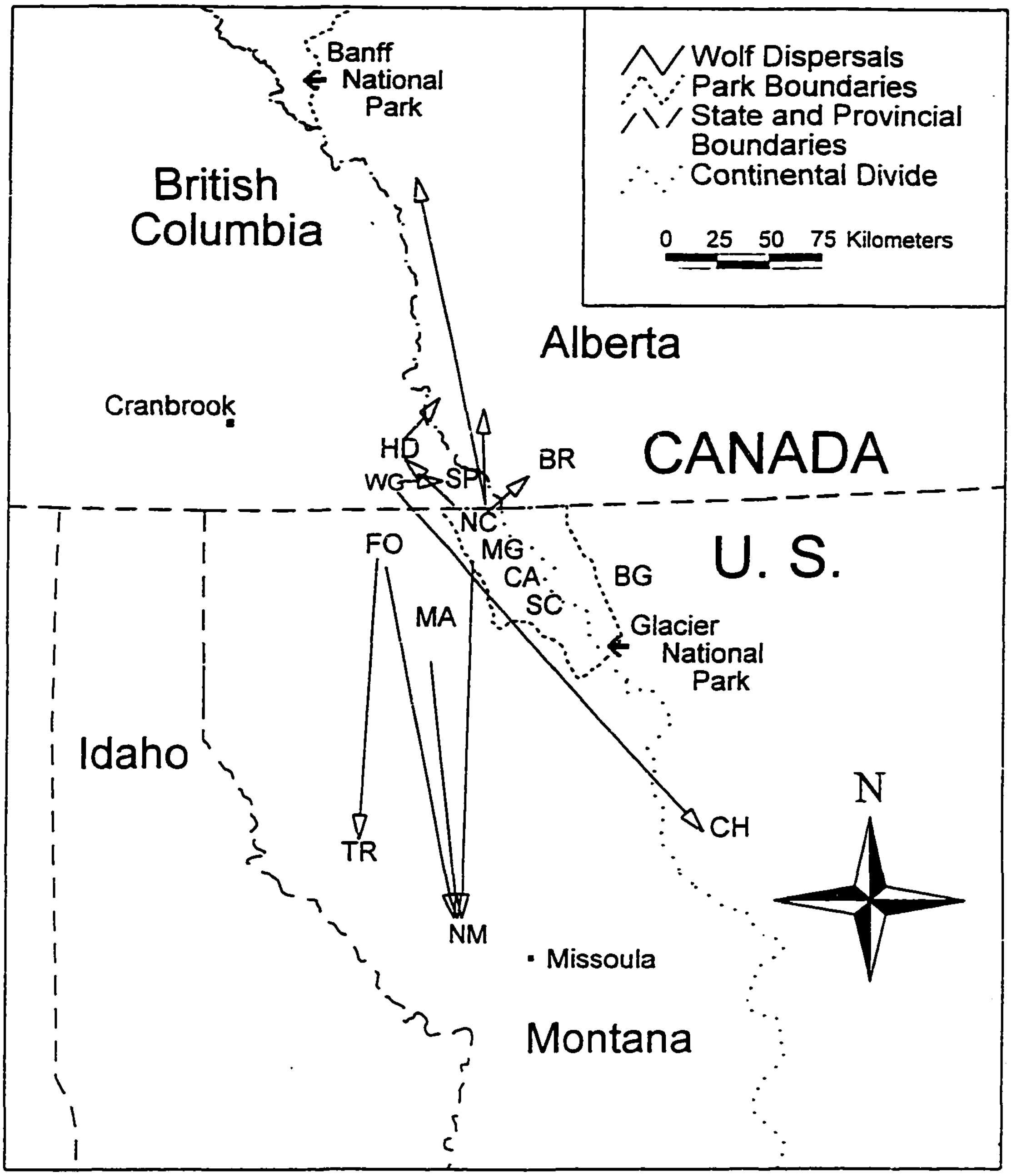
| | | |
|---|---|---|
| E ....... | .063 | .000 |
| F ....... | .250 | .212 |
| G ....... | .047 | .059 |
| H ....... | .312 | .085 |
| I ....... | .063 | .220 |
| J ....... | .265 | .424 |
| 344: | | |
| A ....... | .734 | .703 |
| D ....... | .063 | .042 |
| E ....... | .172 | .254 |
| F ....... | .031 | .000 |
| 377: | | |
| B ....... | .000 | .025 |
| C ....... | .140 | .551 |
| G ....... | .094 | .000 |
| H ....... | .016 | .051 |
| J ....... | .203 | .110 |
| K ....... | .063 | .051 |
| L ....... | .437 | .212 |
| O ....... | .047 | .000 |

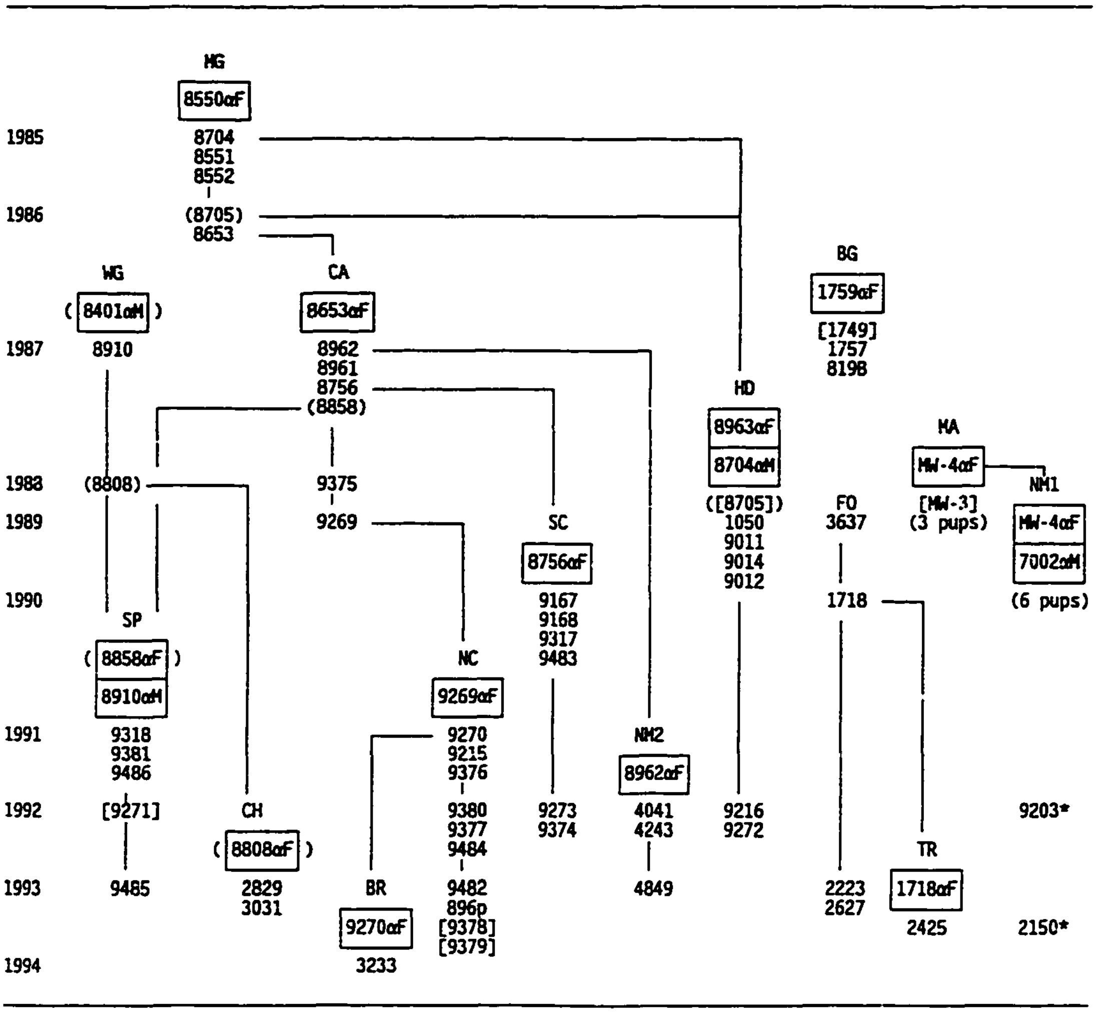
Letter codes designate 2-basepair allele size increments. Sample sizes for all loci are $n$ = 32 (Alberta) and $n$ = 59 (Colonizing).

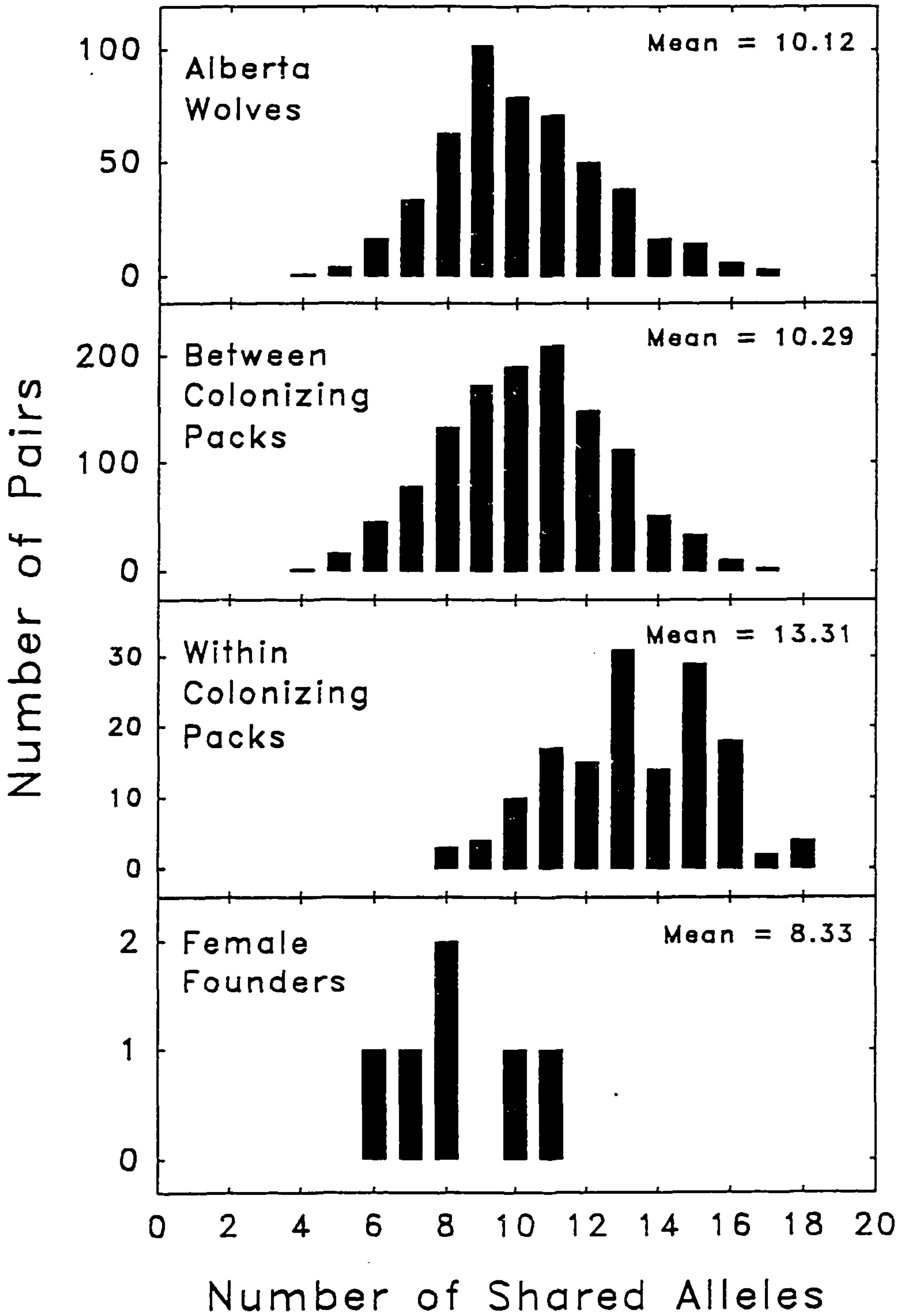
**Fig. 1.** Map of the study area. Colonizing wolf packs are marked with 2 letter codes. Dispersals that resulted in new breeding packs are marked with arrows, except for those within the core Glacier National Park population (Fig. 2). Pack codes not shown in Table 1: WG = Wigwam, BR = Belly River, FO = Fortine, MA = Marion, TR = Thompson River, NM = Ninemile, CH = Choteau.

**Fig. 2.** Relationships of colonizing Glacier National Park area and Montana wolves established by field observation. Vertical lines follow single α-female wolves through successive breeding years. Boxed numbers identify breeding adults (αF = α-female; αM = α-male). Bracketed numbers indicate adult pack members that were inconsistent by age and genetics with α-female maternity, listed by year of appearance. Wolves with numbers not boxed or bracketed are offspring listed by estimated year born. Individuals found alone and of unknown origin are starred (*). Parentheses show wolves tagged but not sampled for genetic study. Two-letter codes are pack name abbreviations (see Table 1, Fig. 1 for definitions).

**Fig. 3.** Allele sharing distributions using 10 microsatellite loci in Alberta wolves and colonizing wolves. For the within- and between-pack comparisons breeding dispersers were listed in their breeding pack but not in their natal pack. Pack identities are not accurately known for all the Alberta wolves, but they include packmates and unrelated animals. The 4 sampled female founders are 8550, 8963, 1759, and MW-4 (Fig. 2).


Wolf Dispersals
Park Boundaries
State and Provincial Boundaries
Continental Divide
0 25 50 75 Kilometers
Banff National Park
British Columbia
Alberta
Cranbrook
HD
WG
SP
BR
CANADA
NC
U. S.
FO
MG
CA
SC
BG
MA
Glacier National Park
Idaho
CH
N
TR
NM
Missoula
Montana


1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
MG
8550αF
8704
8551
8552
(8705)
8653
WG
( 8401αM )
8910
(8808)
CA
8653αF
8962
8961
8756
(8858)
9375
9269
SP
( 8858αF )
8910αM
9318
9381
9486
[9271]
9485
CH
( 8808αF )
2829
3031
NC
9269αF
9270
9215
9376
9380
9377
9484
9482
8960
[9378]
[9379]
BR
9270αF
3233
SC
8756αF
9167
9168
9317
9483
9273
9374
NM2
8962αF
4041
4243
4849
HD
8963αF
8704αM
([8705])
1050
9011
9014
9012
9216
9272
BG
1759αF
[1749]
1757
8198
FO
3637
1718
2223
2627
MA
MW-4αF
[MW-3]
(3 pups)
TR
1718αF
2425
NM1
MW-4αF
7002αM
(6 pups)
9203*
2150*


Alberta Wolves
Mean = 10.12
100
50
0
Between Colonizing Packs
Mean = 10.29
200
100
0
Within Colonizing Packs
Mean = 13.31
30
20
10
0
Female Founders
Mean = 8.33
2
1
0
0 2 4 6 8 10 12 14 16 18 20
Number of Shared Alleles
Number of Pairs

# APPENDIX B

Forbes, S.H., and D.K. Boyd. 1997. Genetic structure and migration in native and reintroduced Rocky Mountain wolf populations. Conservation Biology 11:1226-1234.

**Abstract:** Gray wolf (*Canis lupus*) recovery in the Rocky Mountains of the U.S. is proceeding both by natural recolonization and managed reintroduction. We have used DNA microsatellite analysis of wolves transplanted from Canada to study population structure in native and reintroduced wolf populations. We found that gene flow due to migration between regions in Canada is substantial, and all 3 recovery populations in the U.S. have high genetic variation. Because they came from allopatric source populations, the reintroduced founders are moderately genetically divergent from the naturally colonizing population. These findings corroborate that the reintroduction more than meets generally accepted genetic guidelines. However, maintaining this variation 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 2 transplanted populations. Evidence from field observation and genetic studies show 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.

## Introduction

Wild canid populations worldwide vary in status from very secure to fragmented, isolated, hybridized, or locally extinct. Canid conservation genetics has benefited 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.

Wolves in the Central Rocky Mountains have a history of persecution and tenuous recovery (Gunson 1992; Boyd et al. 1995; here 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 7 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 numerous in large parts of Canada. The area of natural recolonization in Montana is 1 of 3 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 2 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).

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 6 Rocky Mountain wolf samples differ in population history and sampling structure. The 4 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 1980's (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 2 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, Table A1) includes all the transplanted wolves and adds 7 new wolves to the Montana sample. In both reintroduction years 9 family groups of wolves were collected. In YNP, wolves were held in pens and released as family groups based on their pack membership in Canada, while 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). 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 2 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 $\theta$ (theta; Cockerham and 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 and 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 subpopulations (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 2 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 6 Rocky Mountain samples compared at the same 9 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 2 sources (Fort St. John and Hinton). Observed heterozygosity is approximately the same in the source groups and in the mixed transplant groups (all $H_o = 0.579\text{-}0.591$; 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 due to allele frequency differences between the source populations (the Wahlund effect).

### Population Structure

Random mating (panmixia) is a proper null hypothesis for population structure. However, realistically 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 4 native (non-reintroduced) Canadian and Montana samples in all pairwise tests (all $P<0.001$ when combined over 10 loci). However, significant allele frequency differences are compatible with substantial levels of gene flow (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 3 Canadian populations and among all 4 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 and 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 8 wolf subpopulations from northwestern Canada assayed at 5 polymorphic allozyme loci. In another study using microsatellites, wolves from 5 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 4 native populations (Fort St. John, Hinton, Banff, Montana) showed significant deviations only in the Hinton sample. In the Hinton wolves 2 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 sub-populations 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 9 loci in common between the studies. For the 4 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 8 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).

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. However, the range within which this is true 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 numerous 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 (see below); 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 ($N_em$) 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: $N_em$ = 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 percent (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) 2 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 2 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 and 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 3 recovery areas are likely, and that 2 migrants per generation between large, permanent wolf populations is possible. Distances between the population centers of the 3 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 2 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. However, the long-distance movements described here 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 and Carbyn 1995).

Conservation planning includes enhancing genetic exchange among recovery areas by management for migration corridors. However, the effectiveness of corridors 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 in press). 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 and Carbyn 1995; Noss et al. 1996). In the Rocky Mountains these connections are diminishing but apparently adequate at present. However, the critical amount of landscape development and persecution that would stop such movements entirely is hard to predict.

## 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. 1996), and the result has been beneficial from a genetics perspective. The 2 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, since 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 due to dispersal of genetically sufficient numbers of animals from stable population centers. We conclude that none of the 3 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.

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

| 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)[a] | 31.0 | 4.7 | 0.591 | 0.635 |
| Idaho (founders)[b] | 35.0 | 4.6 | 0.589 | 0.636 |
| Total[c] | 172.0 | 5.4 | 0.587 | 0.641 |

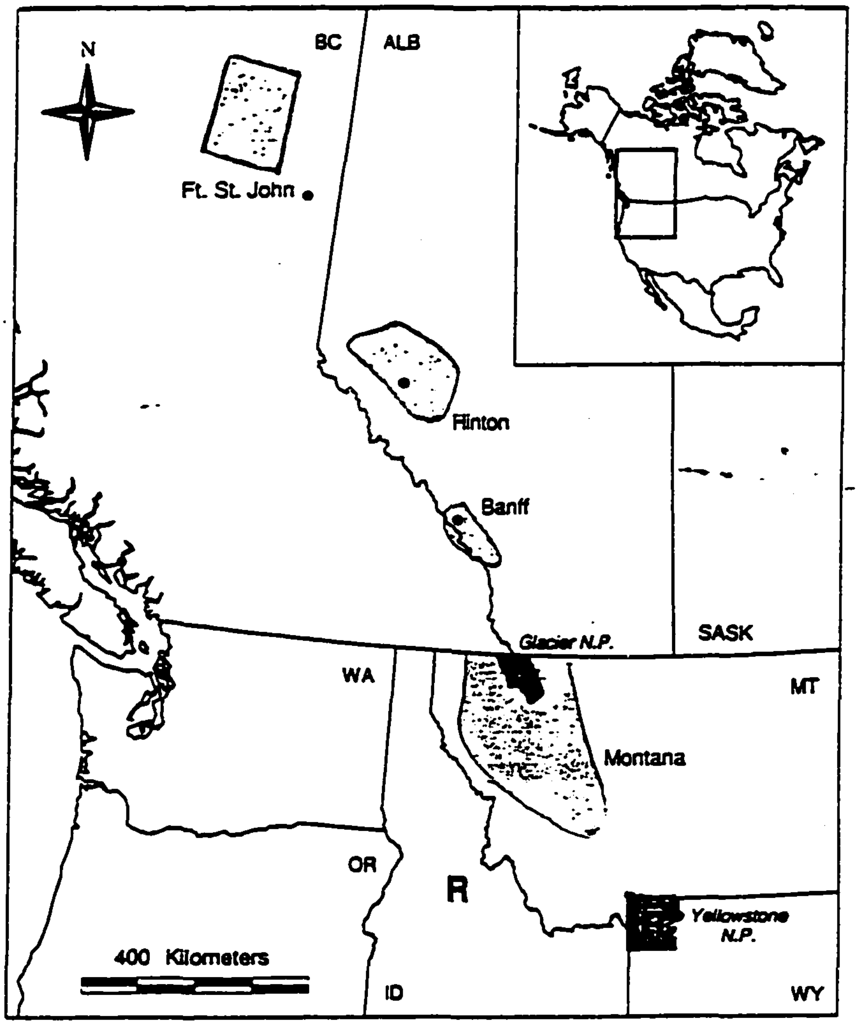
$n$ = mean sample size per locus, $A$ = mean number of alleles per locus, $H_o$ = observed heterozygosity, and $H_e$ = binomial (Hardy-Weinberg) expected heterozygosity (unbiased estimate). Eight wolves sampled at Fort St. John and Hinton were released and not transported to the U.S. [a]14 wolves from Hinton (1995) and 17 from Fort St. John (1996). [b]15 wolves from Hinton (1995) and 20 from Fort St. John (1996). [c]106 wolves from Canada and 66 from Montana.

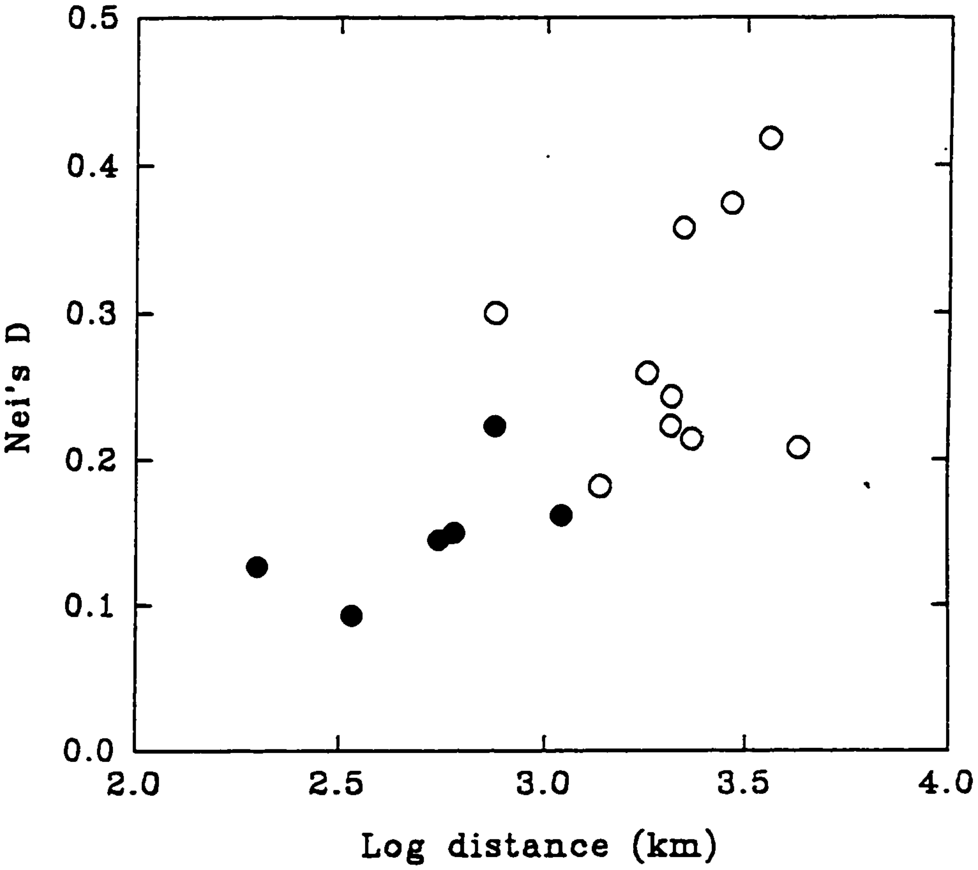
**Table 2.** Pairwise genetic distances among Rocky Mountain wolf populations (unbiased standard genetic distance; Nei 1978).

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

**Fig. 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.

**Fig. 2.** Pairwise comparisons of genetic differentiation ($F_{ST}$ estimator $\theta$) and geographic distance at 9 microsatellite loci among 8 wolf populations: the 4 Rocky Mountain samples from the present study, and 4 more distantly spaced populations (Vancouver Island, Kenai Peninsula, Northwest Territories, and Quebec; Roy et al. 1994). The open circles are the comparisons among the 4 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 4 nearest neighbors, which are the 4 Rocky Mountain samples.


N
BC
ALB
Ft. St. John
Hinton
Banff
Glacier N.P.
SASK
WA
MT
Montana
OR
R
Yellowstone N.P.
400 Kilometers
ID
WY


0.5
0.4
0.3
0.2
0.1
0.0
Nei's D
2.0
2.5
3.0
3.5
4.0
Log distance (km)

**Appendix: Table A1.** Allele frequencies at 10 microsatellite loci in Rocky Mountain wolves .

| | | Population (sample size) | | | | | |
|---|---|---|---|---|---|---|---|
| Locus and Allele | *BP* | FSJ (41) | Hint (33) | Banff (32) | MT (66) | YNP (31) | ID (35) |
| 2 | | | | | | | |
| D | 213 | .500 | .409 | .766 | .705 | .387 | .486 |
| E | 215 | .061 | .015 | .000 | .000 | .048 | .043 |
| F | 217 | .402 | .030 | .000 | .129 | .274 | .229 |
| H | 221 | .000 | .167 | .031 | .000 | .048 | .114 |
| I | 223 | .037 | .379 | .203 | .167 | .242 | .129 |
| 109 | | | | | | | |
| A | 143 | .012 | .348 | .094 | .030 | .226 | .129 |
| B | 145 | .427 | .136 | .203 | .212 | .274 | .314 |
| C | 147 | .024 | .227 | .281 | .159 | .161 | .086 |
| D | 149 | .305 | .106 | .031 | .250 | .161 | .271 |
| E | 151 | .122 | .061 | .375 | .303 | .048 | .086 |
| F | 153 | .012 | .000 | .000 | .000 | .016 | .000 |
| G | 155 | .098 | .121 | .016 | .045 | .113 | .114 |
| 123 | | | | | | | |
| E | 145 | .780 | .727 | .563 | .712 | .758 | .729 |
| F | 147 | .000 | .000 | .000 | .008 | .000 | .000 |
| G | 149 | .037 | .061 | .172 | .182 | .016 | .086 |
| H | 151 | .183 | .015 | .000 | .000 | .097 | .114 |
| I | 153 | .000 | .000 | .063 | .000 | .000 | .000 |
| J | 155 | .000 | .197 | .203 | .098 | .129 | .071 |
| 172 | | | | | | | |
| H | 155 | .488 | .485 | .141 | .288 | .516 | .457 |
| I | 157 | .512 | .515 | .859 | .712 | .484 | .543 |
| 200 | | | | | | | |
| E | 123 | .268 | .485 | .656 | .333 | .387 | .343 |
| I | 131 | .268 | .091 | .031 | .235 | .161 | .214 |
| J | 133 | .195 | .303 | .156 | .318 | .194 | .286 |
| K | 135 | .012 | .030 | .000 | .000 | .048 | .000 |
| L | 137 | .256 | .091 | .156 | .114 | .210 | .157 |
| 204 | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 202 | .049 | .197 | .281 | .318 | .097 | .143 |
| B | 204 | .085 | .242 | .344 | .129 | .177 | .157 |
| D | 206 | .317 | .333 | .344 | .318 | .339 | .300 |
| E | 208 | .549 | .227 | .031 | .235 | .387 | .400 |
| 225 | | | | | | | |
| B | 160 | .354 | .424 | .078 | .235 | .452 | .314 |
| C | 162 | .378 | .379 | .500 | .288 | .355 | .443 |
| D | 164 | .244 | .000 | .141 | .045 | .113 | .143 |
| E | 166 | .024 | .197 | .281 | .432 | .081 | .100 |
| 250 | | | | | | | |
| E | 134 | .000 | .000 | .063 | .000 | .000 | .000 |
| F | 136 | .244 | .197 | .250 | .182 | .258 | .171 |
| G | 138 | .183 | .273 | .047 | .053 | .290 | .200 |
| H | 140 | .232 | .348 | .313 | .076 | .161 | .386 |
| I | 142 | .000 | .015 | .063 | .205 | .000 | .000 |
| J | 144 | .232 | .136 | .266 | .485 | .226 | .143 |
| L | 148 | .110 | .030 | .000 | .000 | .065 | .100 |
| 344 | | | | | | | |
| A | 156 | .913 | .818 | .734 | .697 | .883 | .871 |
| D | 162 | .050 | .030 | .063 | .061 | .050 | .043 |
| E | 164 | .013 | .152 | .172 | .242 | .050 | .071 |
| F | 166 | .000 | .000 | .031 | .000 | .000 | .000 |
| G | 168 | .025 | .000 | .000 | .000 | .017 | .014 |
| 377 | | | | | | | |
| B | 146 | .073 | .076 | .000 | .023 | .000 | .129 |
| C | 148 | .098 | .136 | .141 | .500 | .145 | .114 |
| G | 156 | .049 | .000 | .094 | .000 | .032 | .029 |
| H | 158 | .037 | .045 | .016 | .045 | .048 | .014 |
| I | 160 | .000 | .045 | .000 | .000 | .032 | .014 |
| J | 162 | .134 | .439 | .203 | .144 | .242 | .314 |
| K | 164 | .122 | .061 | .063 | .045 | .129 | .086 |
| L | 166 | .488 | .197 | .438 | .242 | .371 | .300 |
| O | 172 | .000 | .000 | .047 | .000 | .000 | .000 |

Letter codes designate 2-basepair allele size increments that match the codes in Roy et al. (1994). *BP* = the size of the PCR product for each allele in DNA basepairs.

FSJ = Fort St. John; Hint = Hinton; Banff = Banff National Park; MT = Montana;
YNP = Yellowstone National Park; ID = Idaho

# APPENDIX C

Pilgrim, K.L., D.K. Boyd, and S.H. Forbes. In press. Testing for Wolf-Coyote Hybridization in the Rocky Mountains Using Mitochondrial DNA. Journal of Wildlife Management 62(2):000-000.

**Abstract:** We used mitochondrial DNA to evaluate potential gray wolf (*Canis lupus*) - coyote (*Canis latrans*) hybridization in wolf populations in the western U.S., Alberta and British Columbia, including wolves reintroduced into Yellowstone National Park and central Idaho. A restriction site and a length difference in the control region (D-loop) of mitochondrial DNA (mtDNA) was used to differentiate wolf and coyote haplotypes. All 90 wolves tested had wolf haplotypes. We conclude that wolf populations in the Rocky Mountain region have not hybridized with coyotes as they have in the Great Lakes region. This method could be used to test other wolf populations for wolf-coyote hybridization and monitor the translocated Yellowstone and Idaho populations in the future.

## Introduction

Evidence of hybridization in the wild among species of Canidae has been found using molecular genetics (Lehman et al. 1991, Gottelli et al. 1994, Roy et al. 1994). Hybridization between gray wolves (*Canis lupus*) and coyotes (*Canis latrans*) has occurred in the Great Lakes region of the United States and Canada (Lehman et al. 1991, Wayne et al. 1991, Wayne et al. 1992, Roy et al. 1994). In this area of introgressive hybridization, coyote haplotype frequency in wolf populations was >50%, yet wolf haplotypes were not found in coyote populations (Lehman et al. 1991). Multiple coyote mitochondrial DNA (mtDNA) types found in wolves indicate at least 6 hybridization events occurred in the wild (Lehman et al. 1991). The unidirectionality of wolf-coyote matings, (a male wolf mating with a female coyote) is not well understood, yet viable hybrids apparently backcross to wolves but not coyotes (Lehman et al. 1991, Roy et al. 1994, Wayne 1996).

Coyote mtDNA introgression into wolf populations has been documented in the Great Lakes region, but it has not been extensively tested in the Rocky Mountain region. An initial study included 6 wolves from Montana, 4 from Alberta and 2 from Manitoba, none of which had coyote haplotypes (Lehman et al. 1991). Differentiating wolves and coyotes genetically and detecting coyote genes in wolf populations is valuable for management. The re-establishment of pure gray wolf populations in the Rocky Mountain region is important for the eventual de-listing of this endangered species. Introgressive hybridization between gray wolves and coyotes would threaten the recovery of locally endangered gray wolf populations and complicate management.

Wolf recovery in Montana, southwest Alberta and southeast British Columbia has been slow yet dramatic following repeated extirpations in the 1930's and 1950's (Boyd et al. 1995). Wolves began to naturally recolonize the Banff National Park, Alberta (BNP), and Glacier National Park, Montana (GNP) areas through dispersal from populations further north in Canada during the 1970's and 1980's. The current wolf population estimate in western Montana is about 80-100 wolves with 8-10 breeding pairs (Bangs and Fritts 1996). The chances for survival of the gray wolf in the Rocky Mountains are favorable due to its ESA listing as endangered in the lower 48 states in 1973 (U.S. Fish and Wildlife Service 1987), changing public attitudes (Mech 1995), and the recent reintroductions of wolves into Yellowstone National Park (YNP) and central Idaho (Fritts et al. 1995, Fritts et al. 1997). However, given that coyote introgression has occurred in wolf populations in other areas, the possibility of interbreeding is an ongoing concern.

Genetic markers vary in their ability to detect species differences. Allozyme studies are unlikely to detect wolf-coyote hybridization because relatively little genetic variation is found within wolves (Kennedy et al. 1991, Wayne et al. 1991), and there is little allozyme differentiation between wolves and coyotes (Ferrell et al. 1978, Wayne and O'Brien 1987). Allozyme analyses also require relatively large amounts of tissue and potential sacrifice of individuals.

In contrast, polymerase chain reaction (PCR) (Mullis et al. 1994) techniques require minuscule amounts of blood or tissue and can utilize numerous, polymorphic genetic markers. Analysis of mtDNA is useful for investigating genetic differences between closely related species (Kocher et al. 1989, Avise 1994), provided thorough testing of haplotype distributions has been conducted. Due to its maternal inheritance and lack of recombination, mtDNA can be used to detect past hybridization events. The non-coding control region (D-Loop) of mtDNA is especially valuable for population genetic studies in mammals (e.g. Randi et al. 1994, Slade et al. 1994, Taberlet et al. 1995). In mammals, this region mutates faster than the rest of the mtDNA molecule or single-copy nuclear DNA (Avise 1994), and is therefore often more polymorphic and informative for detecting species differences.

MtDNA has been used previously to detect introgressive hybridization in wolves in the Great Lakes region (Lehman et al. 1991). To determine whether introgressive hybridization detected with mtDNA was an ancient event or is ongoing, biparentally inherited nuclear markers such as microsatellite loci (hypervariable, tandem repeats) are needed. A previous microsatellite study on wolves and coyotes in the Great Lakes region demonstrated nuclear gene introgression, with allele frequencies affected in wolf but not coyote populations (Roy et al. 1994). This finding further supports the use of mtDNA as an adequate marker for detecting introgressive hybridization between these 2 species, given the behavioral pattern of hybridization. A recent microsatellite study on wolves from the Rocky Mountain region (Forbes and Boyd 1996, 1997) will provide baseline nuclear data for future monitoring of these wolf populations should introgression be detected with mtDNA.

We used the mtDNA control region (D-loop) to investigate whether the same pattern of wolf-coyote hybridization found in the Great Lakes is evident in Rocky Mountain wolves. We sequenced the control region in wolves and coyotes from the Rocky Mountain region to detect diagnostic genetic differences between wolf and coyote mtDNA. We compared our sequences to wolf and coyote mtDNA sequence data from throughout each species' range to verify the global conservation of species-specific sequences. We then tested for coyote mtDNA introgression in wolves from populations in Montana, Alberta and British Columbia, as well as wolves recently translocated to YNP and central Idaho .

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## Study Area and Methods

We analyzed mtDNA of 90 wolves (46 from northwestern Montana, 19 from the BNP area, 13 from near Hinton, Alberta, and 12 near Fort St. John, British Columbia) previously studied using DNA microsatellites (Forbes and Boyd 1996, 1997). The Hinton and Fort St. John areas were sources of wolves trapped for translocation to YNP and central Idaho in 1995 and 1996 (Fritts et al. 1997). Our sample represents wolves from 14 of the 18 packs used for translocation. Thirty coyotes were sampled from northwestern and central Montana.

Blood was collected from live, wild wolves and muscle samples were taken from wolves and coyotes found dead (Ream et al. 1991, Boyd et al. 1995). Genomic DNA was isolated using either genomic DNA or Chelex preparations (Forbes and Boyd 1996). PCR was used to amplify about 400 base pairs (bp) of the control region using universal mtDNA primers L15905 and H16517 (Gottelli et al. 1994). Ten-ml PCR reactions contained 10 mM Tris-HCl pH 8.3, 50 mM KCL, 4.0 mM $MgCl_2$, 2 mg/ml BSA, 0.2mM of each dNTP, 4 pmcles of each primer, 0.5 U Taq DNA polymerase (Perkin-Elmer Cetus) and either 50-100 ng purified genomic DNA or 1 ml Chelex tissue preparation. The PCR profile for the universal mtDNA primers was 35 cycles at 92°C for 1 minute, 45°C for 1 minute, 72°C for 30 seconds.

PCR products from the universal mtDNA primers were run on a 2.5% agarose gel containing ethidium bromide in TAE buffer (Ausubel et al. 1989). Direct automated sequencing (Applied Biosystems Inc.) was performed on PCR products purified from the agarose gel using the GENECLEAN Kit (BIO 101 Inc.). Our wolf and coyote sequences (Genbank No. AF020699, AF020700) and a dog mtDNA control region sequence (Slade et al. 1994, Genbank No. U03575) were aligned, and restriction sites were identified using the computer program BACHREST from the FSAP package (Fristensky et al. 1982). The restriction enzyme *Mva* I (Boehringer Mannheim) was used to digest the PCR products at 37°C for 3 hours, and restriction fragments were run on a 2.5% agarose gel containing ethidium bromide in TAE buffer (Ausubel et al. 1989).

Canid-specific PCR primers were designed from the wolf and coyote sequence data (Fig. 1). The PCR profile for the canid-specific primers was 94°C for 3 minutes and 35 cycles of 92°C for 1 minute, 55°C for 1 minute, 72°C for 30 seconds. Both *Mva* I cut and uncut PCR products from the canid-specific primers were run on a 7% denaturing poly-acrylamide gel and visualized using a Hitachi FMBIO-100 fluorescent imager.

## Results

The universal mtDNA primers produced a PCR product of approximately 440 bp in both wolves and coyotes (Fig. 2). The canid-specific primers produced species-specific bands of 164 bp for wolves and 160 bp for coyotes which could be resolved on a 7% denaturing poly-acyrlamide gel.

We sequenced the mtDNA control region in 3 wolves and 2 coyotes from northwestern Montana. Removing insertions and deletions to align the sequences revealed that wolves and coyotes differed by 6.8% of the nucleotide sequence (Fig. 1). The published dog sequence differed from the wolf sequence by only 2.3%, and differed from the coyote sequence by 6.3% (data not shown). Wolves and coyotes were estimated to share a common ancestor about 2 mya and differ in mtDNA sequence by approximately 4% (Lehman et al. 1991, Wayne 1993). We found a greater sequence divergence due to the rapidly evolving nature of the mtDNA control region.

Aligning sequences, we identified a restriction site present in wolves but lacking in coyotes due to a 2 bp deletion (Fig. 1) To test for wolf or coyote haplotypes, we used restriction enzyme *Mva* I. *Mva* I cuts at cc(a,t)gg and cuts wolf but not coyote mtDNA. Universal mtDNA primer products from all 90 wolves were cut into 2 fragments of approximately 280 and 160 bp (Fig. 2). Canid-specific primer products from 7 wolves were cut into 2 fragments of 49 and 113 bp. MtDNA remained uncut in all 30 coyotes tested with both primer sets. Thus all 90 wolves tested had the restriction site present in wolves that is absent in coyotes. The canid-specific primers were more efficient than the universal mtDNA primers in amplifying low-quality wolf and coyote samples. This may be because the target DNA template is shorter and possibly because these primers match the DNA template sequences better. Due to this efficiency, and the ability to distinguish wolf and coyote uncut PCR products based on size on an acrylamide gel, these primers may be more useful for forensic purposes.

In addition to our samples, we examined wolf and coyote mtDNA control region sequences collected by other labs from throughout each species' range. The *Mva* I restriction site was found to be perfectly conserved in 150 European wolf mtDNA sequences (E. Randi, personal communication), and in an additional 162 wolf mtDNA sequences from throughout Europe, Asia, and areas of North America where wolves have had no recent contact with coyotes (C. Vila, personal communication). Our wolf sequences perfectly matched wolf haplotype W22 (Vila et al. 1997) which was found in 5 wolves from Alberta, Montana and the Canadian Northwest Territories. The *Mva* I restriction site was absent in 13 coyote mtDNA sequences from Mexico, California, Florida, Louisiana, Michigan, Washington, Utah, Manitoba and 4 unknown locations (C. Vila, personal communication).

## Discussion

### Genetics

We found that wolf and coyote mtDNAs were distinct and could be differentiated both by a PCR product length difference and by a single mtDNA restriction site. Using *Mva* I restriction enzyme, mtDNA from animals with the wolf haplotype gave 2 distinct products while mtDNA from coyotes remained uncut. The deletion at the restriction site in coyotes assured the test could not be confounded by a single nucleotide difference between species. Therefore, coyotes would not be able to gain this restriction site by a single base-substitution mutation.

While mtDNA is extremely informative for detecting hybridization between species, there are also limitations. MtDNA is inherited clonally from the mother and does not undergo recombination. Therefore, a mtDNA haplotype could persist in a population for many generations. Consequently, while mtDNA is often able to detect even an ancient hybridization event, a concomitant study of nuclear markers would be needed to detect ongoing hybridization, and to assess the degree of introgression at nuclear genes.

## Hybridization

Introgressive hybridization between wolves and coyotes has previously been found to be unidirectional, affecting wolves but not coyotes (Lehman et al. 1991, Roy et al. 1994). Coyote mtDNA haplotypes found in wolves suggest initial mating of male wolves with female coyotes, followed by back-crossing of the female progeny to male wolves or to other F1 hybrids.

While the exact ecological causes of past hybridization are not known, it has occurred in areas where deforestation and a conversion to agriculture has taken place (Lehman et al. 1991, Wayne 1996). Coyotes are opportunistic predators that colonize disturbed habitats where wolf populations have been decimated. In such areas wolves may be unable to find conspecific mates when population densities are very low creating a potential Allee effect for wolves (Allee et al. 1949). In addition to low wolf density, coyotes may greatly outnumber wolves in these areas and a lone male wolf may more easily find a female coyote to mate with than another wolf.

Absence of initial matings between female wolves and male coyotes may be due to the evolution of different prey selection, social structure, mating strategy, and dispersal behavior for coyotes and wolves. Coyotes are highly adaptable, medium-sized carnivores that most commonly capture prey smaller than themselves and therefore do not need other pack members to aid prey capture. Wolves are approximately 3-4 times the weight of coyotes and usually capture prey larger than themselves, facilitated by cooperative hunting in relatively large packs (Mech 1970, Moehlman 1989). The reproductive success of a female wolf is greatly increased by other pack members helping with pup rearing (Harrington et al. 1983, Moehlman 1989). A female wolf may perceive a male coyote as a poor investment and refuse to mate with him.

A common alternative for wolf pack formation involves pack splitting where more than 1 female in a pack may conceive during a breeding season (Mech and Nelson 1989, Mech 1991). The pregnant, subordinate female may leave the pack and den by herself, or may take a small portion of the pack members with her (Boyd et al. 1993, Boyd and Jimenez 1994). The mate selection process by the pregnant female occurred while she was still a pack member, eliminating the potential for hybridization with coyotes.

## Management Implications

We found that there has not been an introduction of coyote mtDNA haplotypes into wolf populations in Alberta, British Columbia, or the recolonized Montana population. It is therefore unlikely that introgressive hybridization between gray wolves and coyotes has occurred in the Rocky Mountain region as it has in the Great Lakes region.

Wolf numbers are increasing in western Montana and the translocated populations in YNP and Idaho are persisting. These areas contain suitable wolf habitat with a sufficient prey base, appropriate denning sites and reduced human encounters (U.S. Fish and Wildlife Service 1987). Wolves and coyotes co-exist in these regions and as long as habitat is preserved and wolf numbers are maintained, hybridization with coyotes seems unlikely. However, hybridization may yet occur in this region as a result of ongoing anthropogenic habitat alterations. We have demonstrated a diagnostic mtDNA test that can detect introgressive hybridization between wolves and coyotes given the behavioral pattern of hybridization between these 2 species. We recommend that mtDNA analyses continue to be conducted on wolves and coyotes throughout the Rocky Mountain Region to monitor for possible future introgressive hybridization.

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**Fig. 1.** Aligned wolf and coyote mtDNA sequences amplified using universal mtDNA PCR primers. Numbers on the right-hand margin indicate numbers of DNA base pairs. The canid-specific PCR primers were designed from these sequences and are indicated by the dotted arrows. The *Mva* I restriction enzyme cut site is indicated by asterisks.

**Fig. 2.** Agarose gel showing mtDNA fragments from 2 wolves and a coyote amplified using universal mtDNA PCR primers. The first 3 lanes after the standard show the uncut PCR products, and the last 3 lanes are the products cut with *Mva* I from the same individuals. First and last lanes are 1 kb DNA size standards.

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Wolf      GAGTAACCGCCCTCCCTAAGACTCAAGGAAGAAGCTCTTGCTCCACCATCAGCACCCAAA 60
          |||||| | |||||||||||||||||||||||||||||||||||||||||||||||||||
Coyote    GAGTAATCACCCTCCCTAAGACTCAAGGAAGAAGCTCTTGCTCCACCATCAGCACCCAAA 60

Wolf      GCTGAAATTCTTCTTAAACTATTCCCTGA-CACCCCTACATTCATATATTGAATCACCCC 119
          |||||||||||||||||||||||||||||   ||||||||||||||||||||||||||||
Coyote    GCTGAAATTCTTCTTAAACTATTCCCTGATGCCCCCTACATTCATATATTGAATCACCCC 120

                                Mva1
                                *****
Wolf      TACTGTGCTATGTCAGTATCTccaggTAAA-CCCTTCTTCCCTCCCCTATGTACGTCGTG 178
          |||||||||| |||||||||||||   |||  |||||| |   |||||||||||||||||
Coyote    TACTGTGCTACGTCAGTATCTCCA---AAAATCCTTCTCC---CCCCTATGTACGTCGTG 174

Wolf      CATTAATGGTTTGCCCCATGCATATAAGCATGTACATAATATTATATTCTTACATAGGAC 238
          ||||||||| |||||||||||||||||||||||||||||||||||||  |||||||||||
Coyote    CATTAATGGCTTGCCCCATGCATATAAGCATGTACATAATATTATATCTTTACATAGGAC 234

Wolf      ATATCAACTCAATCTCACAATTCATTGATCTATCAACAGT-AATCAAATGCATATCACTT 297
          |||   ||| || || ||| |||| |||||| ||||||||   | ||||||||||||| |
Coyote    ATACTGACTTAACCTTACAGTTCACTGATCT-TCAACAGTTGGTTAAATGCATATCACCT 293

Wolf      AGTCCAATAAGGGCTTAATCACCATGCCTCGAGAAACCATCAACCCTTGCTCGTAATGTC 357
          ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Coyote    AGTCCAATAAGGGCTTAATCACCATGCCTCGAGAAACCATCAATCCTTGCTCGTAATGTC 353

Wolf      CCTCTTCTCGCTCCGGGCCCATACTAACGTGGGGGTTACTATCAT                402
          |||||||||||||||||||||||  ||||||||||| |||||||
Coyote    CCTCTTCTCGCTCCGGGCCCATATCAACGTGGGGGTTTCTATCAT                398
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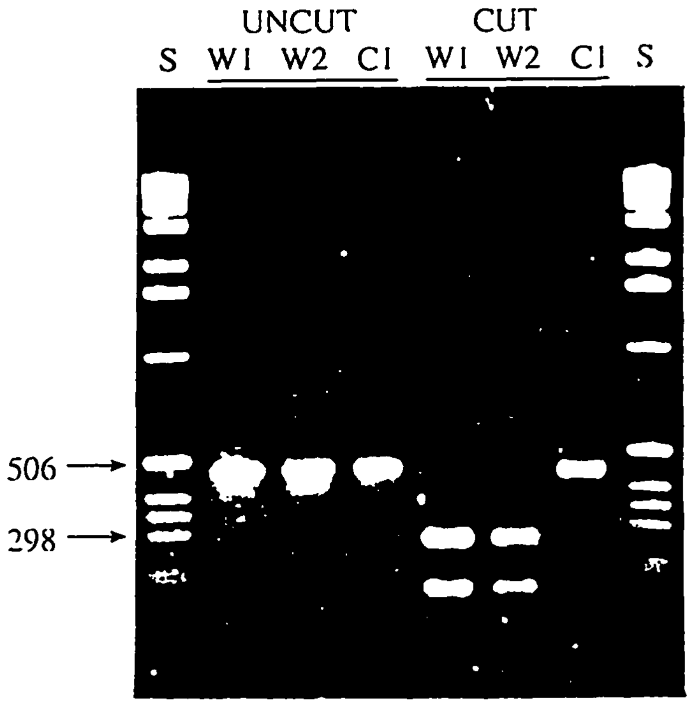
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# Chapter 4: Landscape Use by Colonizing Wolves in the Central Rocky Mountains

**Abstract:** Wolves have recently dispersed from protected refugia and colonized areas in northwestern Montana, southeastern British Columbia, and southwestern Alberta. I analyzed landscape attributes selected by 6 colonizing wolves including elevation, slope, aspect, distance to water, distance to roads, road density, and canopy cover. Wolves selected for landscapes with relatively lower elevation, flatter terrain, and closer to water and roads than expected based on availability inside and outside of their new home range. I built a logistic regression model using elevation, slope, and distance to roads to predict wolf presence in areas of potential colonization. Impacts of habitat fragmentation and management considerations are discussed.

## INTRODUCTION

Gray wolves (*Canis lupus*) were systematically extirpated from the western United States earlier this century for socioeconomic reasons. Similar wolf control programs in southeastern British Columbia and southwestern Alberta resulted in the extirpation of wolves in the Canadian Rockies south of Jasper National Park, Alberta, in the 1930s and again in the 1950s (Cowan 1947, Gunson 1983). Since then, recent public attitudes toward predators have become more positive (Kellert 1985, McNaught 1987, Bath and Buchanan 1989, Tucker and Pletscher 1989, Kellert et al. 1995, Mech 1995) and the passage of the Endangered Species Act federally protected the wolf in the lower 48 states in 1973. Subsequently, the wolf population began a peninsular expansion south to Banff National Park, Alberta (BNP), and Glacier National Park (GNP), Montana (Day 1981; Ream and Mattson 1982; Boyd et al. 1995). Resident wolves became established in the GNP area in the 1980s (Ream et al. 1991) with subsequent dispersers from GNP colonizing areas farther from the GNP source population (Boyd et al. 1995, Forbes and

Boyd 1996). Putative non-collared native wolves dispersed as far south as Yellowstone National Park, Wyoming (Fritts et al. 1995, Forbes and Boyd 1996). Although the population of wolves in Canada seems secure and dispersal corridors appear fairly intact, anthropogenic landscape modifications and the inherent patchiness of mountainous landscapes may affect the outcome of colonization attempts.

Habitat fragmentation, characterized by 1) reduction in amount of habitat available and 2) habitat division into smaller, more isolated patches, is the primary cause of extinctions in some species (Wilcox and Murphy 1985). In addition to physical changes of the landscape, human attitudes are also an important dimension of habitat. A large habitat patch may contain all the required physical landscape features for wolves (e.g. prey, space, den sites), but if humans in the area feel negative about wolves and act accordingly, wolves will not be allowed to exist. Large carnivores require vast areas for their persistence, and the fragmentation of landscape into smaller refugia in a matrix of civilization may jeopardize their survival. Leopold (1949:276-277) recognized this nearly 50 years ago and stated "The parks are certainly too small for a far-ranging species as the wolf. Many animal species, for reasons unknown, do not seem to thrive as detached islands of population." Since Leopold's time, principles of island biogeography (MacArthur and Wilson 1967), conservation biology (Soule and Wilcox 1980, Soule 1986, Meffe and Carroll 1994), and conservation genetics (Frankel and Soule 1981, Schonewald-Cox et al. 1983, Allendorf and Leary 1986) have explored why isolated populations have a decreased ability to persist.

The relationship between patch size, distribution, and connectivity between patches (corridors) may significantly impact species' distribution. The value of corridors to species conservation has been debated by researchers (Noss 1987, Simberloff and Cox 1987, Soule and Gilpin 1991, Beier 1993). The Central Rocky Mountains (I defined the

Central Rockies as the geographic area centered on the junction of the Continental Divide, Canada, and the United States, and includes the landscape from Banff, Alberta, to Helena, Montana) have often been viewed as a dispersal corridor connecting Canadian and US large mammal populations. However, wolves have demonstrated that the Central Rocky Mountains function as much more than a corridor of movement. This region provides suitable habitat patches along and adjacent to its length that wolves have colonized. Sandstrom (1996) made a distinction for grizzly bears (*Ursus arctos horribilis*) that corridors are areas of movement between habitat fragments, whereas linkage zones are areas between habitat fragments suitable for low levels of occupancy as well as movements (Servheen and Sandstrom 1993). The linkage zone concept may be more appropriate for wolves than the concept of corridors. Boyd et al. (1995; Boyd, unpubl. data) and Forbes and Boyd (1996) have shown that most of western Montana, southeastern British Columbia, southwestern Alberta, and northern Idaho are indeed landscape linkage zones for wolves and not merely corridors.

Research on habitat use and colonization by wolves has been limited. Wolf colonization has been studied in the more homogeneous, relatively flat terrain of Minnesota (Fritts and Mech 1981), Wisconsin (Thiel 1993, Wydeven et al. 1995), Isle Royale, Michigan (Mech 1966, Allen 1979, Peterson 1977), and Michigan mainland (Hammill 1995). Mladenoff et al. (1995, 1997) reported on wolf recolonization and habitat selection in the Great Lake states using a Geographic Information System (GIS). In midwestern landscapes, colonization most often occurred by slowly extending occupied wolf range with relatively short-distance dispersals, although long-distance dispersals have been reported (Fritts and Mech 1981, Gese and Mech 1991, Mech et al. 1995).

Relatively little information has been published on colonization and landscape selection by wolves in the Rocky Mountains. Matteson (1992) analyzed habitat characteristics of wolf den sites in the Central Rockies. Kunkel (1997) examined landscape attributes connected with wolf predation on ungulates in the newly recolonized GNP area. Singleton (1995) evaluated winter habitat selection by wolves near GNP. Paquet and Callaghan (unpubl. data) and Paquet et al. (1996) have analyzed habitat selection by a recently colonized wolf population in the BNP area. Evidence suggests that wolf colonization will occur in the Central Rockies first in valley bottoms, with relatively long-distance linear separation between packs conforming to the heterogeneous nature of mountainous landscape (Fritts and Carbyn 1995, Fritts et al. 1994); therefore, landscape features in the mountains are of paramount importance to wolf recovery.

The ongoing expansion of the wolf population in the Central Rocky Mountains provides a timely opportunity to investigate these phenomena. This region is a critical connection for persistence of large carnivores, like wolves and grizzlies, between source populations in Canada and the semi-isolated subpopulations in the northwestern US. These analyses may provide insights to other wolf recovery areas such as YNP and Idaho. Furthermore, the metapopulation of wolves in the Central Rockies may serve as a good model for the effects of habitat fragmentation on dispersal and genetics of other species. Meffe and Carroll (1994:244) stated "large carnivores and other wide-ranging animals are typically among the species most threatened by habitat fragmentation, in part because small areas fail to provide enough prey, but also because these animals are vulnerable to mortality due to humans and vehicles when they attempt to travel through fragmented landscapes."

My objective was to determine how landscape features and various human influences impact habitat selection by colonizing wolves. Attributes selected for analyses included natural factors (elevation, slope, aspect, distance to water, canopy cover) and anthropogenic factors (distance to roads, road density). Theoretical implications of this research may aid other threatened and endangered species and enhance efforts to maintain biodiversity in an increasingly fragmented world.

## STUDY AREA

The study area was defined by the movements of wolves dispersing from GNP and extended along the Central Rockies from BNP south to Missoula, Montana, and east-west from the eastern Rocky Mountain Front to central Idaho and southeastern British Columbia (Fig. 1). Wolf dispersals 840 km north of GNP (Ream et al. 1991) have been documented, but these outliers were not included in my analysis due to a lack of location data in the interim after leaving the natal home range and a mortality location in the new home range.

The study area is characterized by long, narrow valley bottoms surrounded by rugged mountains. GNP elevations range from 1,020 m in the valleys to 3,600 m along the Continental Divide. Dense coniferous forests dominate the GNP area, with meadow and riparian areas less common (Koterba and Habeck 1971). Landscapes that dispersers colonized were fairly diverse in vegetation and terrain, but all colonizers selected new home ranges that included mountains, valley bottoms, coniferous forests, and a substantial elevation gradient. The study area is a mosaic of numerous land management

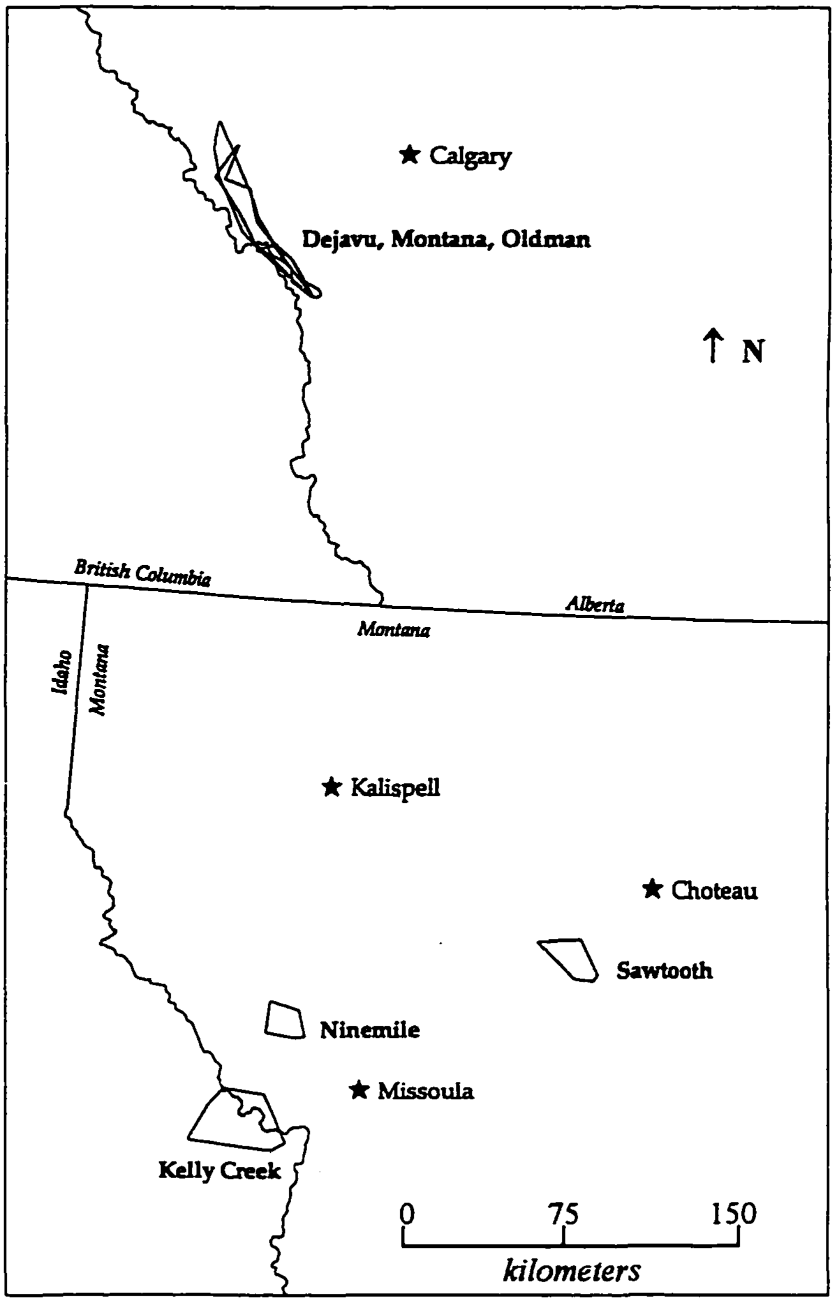

Figure 1. Map of the study area including home ranges of wolves Kelly, Ninemile, Sawtooth, Kananaskis, Highwood, and Oldman.

classifications, including 2 countries, 2 states, 2 provinces, and numerous subdivisions of jurisdiction within each of these (Pletscher et al. 1991).

## METHODS

### Capture and telemetry

Wolves weighing $\geq$ 20 kg were captured and radiocollared in the GNP area following techniques described by Mech (1974) and Ream et al. (1991). Wolves weighing < 20 kg were ear-tagged only. As the colonizing wolf population expanded, wolves dispersed along the Rocky Mountains between BNP and GNP, across western Montana, and northeastern Idaho. Subsequently, several wolves were tagged by the US Fish and Wildlife Service and the Banff Wolf Project in these regions and location information was exchanged. Radiocollared wolves were located approximately 3 times per week and information on dispersers from the study areas was shared. Aerial locations were used instead of ground triangulation whenever possible to enhance accuracy of location. Ground triangulated locations were used only if the error polygon was < 0.25 km$^2$ and if there were not enough aerial locations ($n$ < 30) for a particular wolf. I selected wolf locations for analysis to evenly distribute year-round use and to maximize independence between locations. This involved some non-random selection to delete concentrations of locations at kill sites, den sites, and rendezvous sites.

### Spatial Analyses

Six of 34 wolves that dispersed from the GNP study area had more than 30 locations distributed throughout all months of the year in a non-natal home range, the minimum number adequate for home range determination (Fuller and Snow 1988). These 6 colonizing wolves selected for analyses were named Kelly, Ninemile, Sawtooth, Kananaskis, Highwood, and Oldman, named after geographic descriptors of where they settled. Kananaskis, Highwood, and Oldman overlapped spatially but not temporally and, therefore, were considered to be independent. Coarse scale landscape attributes for these 6 colonizing wolves were examined using the GIS software Arc/Info 7.0.4. (ESRI 1996) on a Unix workstation. Canadian digital data were obtained from the Crown of the Continent Ecosystem Atlas and added to the database at the Wildlife Spatial Analysis Lab, University of Montana.

Landscape attributes were compared at 2 levels: 1) smaller scale of use vs. availability within home range (third order selection, Johnson 1980) and 2) larger scale use vs. availability outside of a home range (second order selection, Johnson 1980). The combination of evaluating resource selection at both scales involves elements of Design II and Design III of Manly et al. (1993). A total of 34 true wolf location points (lowest common denominator) were selected for each wolf to determine colonizing home ranges for all wolves (as a measure of use). Actual wolf home ranges were delineated with a minimum convex polygon (Appendices A - C). To sample availability outside the home range, this boundary was then duplicated twice, once upstream and once downstream from the actual home range, to create an artificial home range in areas containing landscape characteristics similar to that of the actual home range (Appendices A -C). However, elevation, slope, and aspect data (DEM's) were not available for BNP so adjustments had to be made for the 3 Canadian wolves that abutted BNP on its south border: 1 artificial home range with 34 location points was located south of the actual home range for each of these wolves (Appendices A - C). Two artificial home ranges staggered to the south would have resulted in a major change in the terrain features, habitat types, or been partially located in British Columbia with unavailable databases. Random locations were obtained within each artificial home range for each wolf by creating a 1 km regular grid, and randomly selecting grid intersection coordinates. For each wolf, 17 intersections were selected in both of the artificial home ranges (or 34 points in the single combined artificial home range for the Canadian wolves), and 34 intersections were selected in the actual home range. Points were coded as follows: actual wolf locations in home range = wolf.loc (*n* = 34), random points outside true home range = rand.out (*n* = 34), and random points inside true home range = rand.in (*n* = 34).

Data sources for the US locations were as follows:

- * elevation, slope, and aspect were determined from 7.5 minute USGS Digital Elevation Models and 1:20,000 Alberta Resource Data Division (DEM), raster files, grid cell size of 30 m.;
- * hydrography was determined from 1:100,000 USGS Digital Line Graphs (DLG), vector files;
- * roads were determined from 1:24,000 scale USFS Cartographic Feature Files (CFF), vector files.
- * canopy closure was determined from classification of Landsat satellite imagery (Redmond et al. 1996).

The following data were obtained for each wolf location and random point:

1. Elevation (meters): the elevation in the 30x30m raster cell corresponding to each point.
2. Slope (degrees): derived from the DEM; slope was obtained for the 30x30m raster cell corresponding to each point.
3. Aspect (degrees): derived from the DEM; aspect was obtained for the 30x30m raster cell corresponding to each point. Aspects were divided into 8 categories: N = 338-22°, NE = 23-67°, E = 68-112°, SE = 113-157°, S = 158-202°, SW = 203-247°, W = 248-292°, and NW = 293-337°.

4. Distance to water (meters): computed as the linear distance to the closest stream, river or lake, without taking topography into account.
5. Distance to road (meters): computed as the linear distance to the closest road (all roads that can be driven on, whether paved or not, excluding trails), without taking topography into account.
6. Road density (km/km$^2$): computed by assigning the density of roads within a 1 km circle to the 30x30m raster cell at the center of the circle, and moving the circle so that each cell in turn is at the center (Sandstrom 1996). The densities were obtained for the random points by reading density values in the corresponding raster cell, yielding integer values of km road/km$^2$ land.
7. Canopy cover (category integers): classified only for US wolves with 0 = open (no canopy), 1 = low (1-39%), 2 = medium (40-69%), and 3 = high (70-100%).

### Statistical Tests

All samples were assumed to be random and independent at their appropriate scales. Whenever possible, parametric tests were used to compare sample means for significance. Nonparametric tests were used when populations failed to meet assumptions of parametric tests: normal distributions were determined primarily through stem and leaf diagrams and secondarily through K-S Lilliefors test for normality, $P < 0.10$; and homogeneity of variances as evaluated primarily with whiskered box plots and secondarily with Levene's test for equality of variances, $P < 0.10$ (Sokal and Rohlf 1995). I attempted to normalize non-normal data using natural log (ln) and square root (sqrt) transformations.

If data were normally distributed and reasonably homoscedastic, I used t-tests, one-way ANOVA, or two-way ANOVA (whichever was most appropriate and maximized robustness) to compare means of 2-3 groups of independent samples (Sokal and Rohlf

1995) with significance level $P < 0.05$. The experimental unit was wolf (*n* = 6), wolves were the replicates, and the treatment was code (*n* = 3) in a crossed experimental design. The thirty-four points for each wolf per code were subsamples (not replicates) of wolf. The variable "wolf" is a random effect and the variable "code" is a fixed effect, requiring a mixed Model II ANOVA for analysis (Sokal and Rohlf 1995; D. Patterson, pers. commun.). This avoided problems of pseudoreplication (Hurlbert 1984). Evaluation of the main effects (code and wolf) of the two-way ANOVA pooling all 6 wolves was precluded if the interaction term was significant ($P < 0.05$); significance was determined through analysis of each wolf separately using a series of t-tests. I used the Dunn-Sidak method to adjust for the increased probability of a type I error over a set of multiple comparisons (experimentwise error rate) of t-tests: $a' = 1 - (1 - \alpha)^{1/k}$ where $k$ = number of pairs to be tested, which in all cases was 3 ($P < 0.017$), or a combination of a one-way ANOVA and a Bonferonni test (which incorporates adjustments for multiple comparisons, significance level $P < 0.05$) (Sokal and Rohlf 1995).

If the populations were not normally distributed, I checked for overall significance among codes by wolf with the Kruskal-Wallis test. A nonsignificant (but close to significant) Kruskal-Wallis analysis may contain significant pairwise comparisons when the grouped Kruskal-Wallis is broken down into paired comparisons (Sokal and Rohlf 1995). Therefore, if the Kruskal-Wallis probability value was near significance ($P < 0.20$) I used the Mann-Whitney U test to compare mean ranks of 2 groups and determine significance within code pairs. Mean rank values for Kruskal-Wallis and Mann-Whitney U tests (slope, road density, canopy cover), and mean values for transformed data of t-tests (distance to water, distance to roads) are the basis of interpretation and discussion. However, tables in the text use the untransformed raw data, providing a more lucid context for the reader (e.g. mean slope vs. mean rank slope). Probability levels were adjusted using the Dunn-Sidak method previously described to compensate for multiple comparisons. I used the

Chi-square statistic to determine significant relationships ($P < 0.05$) between categories of aspect, and a standardized residual ($Z = (\text{Observed} - \text{Expected})/\text{Expected}^{0.5}$) to determine significance of cells. The probability value for each row was then multiplied by the number of aspect categories ($n = 8$) to give a final P value. Similar multiplications were applied to Z scores. Correlations were used to determine the strengths of linear associations between significant landscape attributes.

## Logistic Regression

I conducted logistic regression analyses on significant dependent variables for 1) individual wolves, and 2) pooled wolves. I standardized pooled wolves to remove variability between wolves at both scales of analysis as follows:

For second order selection (larger scale) (wolf.loc and rand.out) for each wolf:

$X_P/(X_L - X_S)$ where for each significant variable (elevation, slope, distance to roads):

$X_P = X_1$ through $X_N$ ($n = 34$) for wolf.loc

$X_L$ = the largest value of the pooled variable (wolf.loc and rand.out)($n = 68$)

$X_S$ = the smallest value of the pooled variable (wolf.loc and rand.out)($n = 68$)

For third order selection (smaller scale) (wolf.loc and rand.in) for each wolf:

Same as described above, but substituting values of rand.in for rand.out

Significant variables were entered into a forward stepwise likelihood-ratio logistic regression model for individual wolves, and forced entry for pooled wolves ($P > 0.05$) (SPSS Advanced Statistics, 6.1). The logistic regression model for more than 1 independent variable is written as:

$$\text{Prob (event)} = 1/(1 + e^{-Z})$$

$$\text{where } Z = B_0 + B_1X_1 + B_2X_2 + \ldots + B_pX_p$$

e is the base of the natural logarithms (~ 2.718)
$B_0$ through $B_p$ are coefficients estimated from the data
$X_0$ through $X_p$ are the independent variables

## RESULTS

### Wolf Distribution

Fifty-six wolves were captured in the GNP area between 1979-96, and 29 of the tagged wolves dispersed. The 6 wolves selected for analyses became permanent residents of new home ranges 29-232 km from their natal home ranges. All wolves, with the exception of Kelly, who remained a loner, became founding members of new packs. Male Kelly colonized the relatively high elevation, low human density, unroaded Kelly Creek drainage in eastern-central Idaho. Female Ninemile colonized the Ninemile drainage near Missoula, Montana, with a fairly high density of humans and roads, and became the breeding female. Male Sawtooth colonized the Choteau and Sun River Game Range of Montana, along the Rocky Mountain Front, utilizing both mountains and prairies, and was the founding male breeder. Kananaskis (non-breeding male), Highwood (founding breeding female), and Oldman (non-breeding female) colonized mountainous areas of southwestern Alberta between BNP and Crowsnest Pass.

## Landscape Attributes

### *Elevation*

Data for elevation were normally distributed and homoscedastic, so no transformations were necessary. The two-way ANOVA interaction term for code*wolf was significant ($P < 0.001$) so wolves were analyzed separately (not pooled) for wolf response to elevation using t-tests.

Table 1. Mean elevation (m) for wolves by use (wolf.loc) and availability (rand.out and rand.in).

| Wolf | Wolf.loc | Rand.out | Rand.in |
|---|---|---|---|
| Kelly | $1571^{ab}$ | $1526^{a}$ | $1703^{b}$ |
| Ninemile | $1252^{a}$ | $1313^{ab}$ | $1428^{b}$ |
| Sawtooth | $1540^{a}$ | $1939^{b}$ | $1825^{b}$ |
| Kananaskis | $1822^{a}$ | $1700^{a}$ | $1989^{b}$ |
| Highwood | $1708^{a}$ | $1920^{b}$ | $1950^{b}$ |
| Oldman | $1735^{a}$ | $1685^{a}$ | $2063^{b}$ |

[a,b] Pairs with similar letters within a wolf pack are not significantly different; if the letters are not the same the pairs are significantly different ($P \leq 0.05$) (Appendix A1).

All 6 wolves selected lower elevations than expected based on availability within their home range (Table 1). Two (Sawtooth and Highwood) wolves selected lower elevations than expected based on availability outside their home range. Elevational differences between random points (availability) inside and outside home ranges were significant for 3 wolves (Kelly, Kananaskis, Oldman) with points inside their home ranges being significantly higher in elevation than outside their home ranges. No wolves, at any scale, selected higher elevations than expected based on availability. Summarily, most wolves selected lower elevational landscapes inside (third order) and outside (second order) their home range.

### *Slope*

No transformations produced normality or homogeneity of variances for values of slope, so I used the nonparametric Mann-Whitney U test on paired observations.

Table 2. Mean slope (degrees) for wolves by use (wolf.loc) and availability (rand.out and rand.in).

| Wolf | Wolf.loc | Rand.out | Rand.in |
|---|---|---|---|
| Kelly | $15.4^a$ | $21.6^b$ | $21.4^b$ |
| Ninemile | $11.8^a$ | $20.7^b$ | $17.1^b$ |
| Sawtooth | $10.2^a$ | $20.4^b$ | $16.8^b$ |
| Kananaskis | $13.1^a$ | $15.9^a$ | $19.9^a$ |
| Highwood | $8.8^a$ | $16.3^b$ | $17.7^b$ |
| Oldman | $10.9^a$ | $19.6^b$ | $20.4^b$ |

$^{a,b}$ Pairs with similar letters within a wolf pack are not significantly different; if the letters are not the same the pairs are significantly different ($P \leq 0.05$) (Appendix A2).

Five of 6 wolves (Kananaskis being the exception) selected areas of flatter terrain than expected based on availability both outside and inside their home range (Table 2). There was no significant difference in slope of random points (availability) inside and outside the home range.

### *Aspect*

Aspect data could not be normalized through transformations. There were no significant values in Kruskal-Wallis tests ($P > 0.117$) for any of the 6 wolves in all comparisons of use and availability. The Chi-square test showed a significant difference among all categories examined (Chi-square = 18.59, df = 7, P = 0.0096), indicating a non-random assortment of aspects. However, when I used the Bonferroni test to determine which aspect category was selected or avoided, no 2 groups were significantly different at the 0.05 level. This indicates that values among all categories were significant, but values within the categories were not quite significant. The most significant categories, E (Z = 3.03, P = 0.019), NW (Z = -2.12, P = 0.272), and N (Z = -1.54, P = 0.989) showed a preference for east facing slopes and non-significant values for other aspects. There was not a strong selection for aspect by wolves compared to random points, but there was a slight tendency for wolves to select east-facing slopes.

### *Distance to Water*

The distance to water variable was transformed using natural logarithms to normalize the distribution and equalize the variances. The code*wolf interaction term in the two-way ANOVA was nonsignificant (P = 0.728), so use/availability was compared using t-tests for the pooled 6 wolves. The main effects variables of code and wolf were both significant (P = 0.02 and 0.001, respectively).

Table 3. Mean distance to water (m) for pooled wolves by use (wolf.loc) and availability (rand.out and rand.in).

| | Wolf.loc | Rand.out | Rand.in |
|---|---|---|---|
| Mean distance to water | 253[a] | 307[a] | 309[a] |

[a,b] Pairs with similar letters are not significantly different; if the letters are not the same the pairs are significantly different ($P \leq 0.05$) (Appendix A3).

Wolves selected for water more than expected based on availability inside and outside of their home range, although the t-test probability values were not quite significant after adjusting for multiple comparisons (table 3). This near significance indicates a weak selection for water compared to availability. The mean distance to water was similar for all random points (availability) sampled inside and outside the home range ($P = 0.925$).

### *Distance to Roads*

The distance to roads data were transformed using natural logarithms to normalize data and equalize variances. The two-way ANOVA interaction term for code*wolf was significant ($P < 0.001$) so wolves were analyzed separately for wolf response to roads using t-tests.

Table 4. Mean distance to roads (m) for wolves by use (wolf.loc) and availability (rand.out and rand.in).

| Wolf | Wolf.loc | Rand.out | Rand.in |
|---|---|---|---|
| Kelly | 4404$^{a}$ | 3581$^{ab}$ | 1977$^{b}$ |
| Ninemile | 229$^{a}$ | 434$^{a}$ | 339$^{a}$ |
| Sawtooth | 1125$^{a}$ | 6105$^{b}$ | 1709$^{a}$ |
| Kananaskis | 917$^{a}$ | 3020$^{b}$ | 1486$^{ab}$ |
| Highwood | 885$^{a}$ | 1673$^{a}$ | 1662$^{a}$ |
| Oldman | 940$^{a}$ | 2818$^{a}$ | 2043$^{a}$ |

$^{a,b}$ Pairs with similar letters within a wolf pack are not significantly different; if the letters are not the same the pairs are significantly different ($P \leq 0.05$) (Appendix A4).

Four (Sawtooth and Kananaskis were significant, Ninemile and Oldman were nearly significant) of 6 wolves selected roads more than expected based on availability outside their home range (Table 4). Two wolves (Kelly was significant, Ninemile was nearly significant) were found closer to roads than expected based on availability inside the home range. Wolves generally were found closer to roads than was expected based on availability at both scales analyzed. No wolves were found significantly further from roads than expected based on availability. Random points (availability) outside the home range were not significantly further from roads than random points inside the home range for 5 of 6 wolves (Sawtooth being the exception) suggesting that distribution of roads in the 2 areas was comparable.

*Road Density*

Road density was categorized to the nearest whole integer value of km/km$^2$, so the nonparametric Mann-Whitney U test was used for analysis of these data.

Table 5. Mean road density (km/km$^2$) for wolves by use (wolf.loc) and availability (rand.out and rand.in).

| Wolf | Wolf.loc | Rand.out | Rand.in |
|---|---|---|---|
| Kelly | 0.677$^a$ | 0.500$^a$ | 1.000$^a$ |
| Ninemile | 2.794$^a$ | 2.147$^a$ | 3.177$^a$ |
| Sawtooth | 0.559$^a$ | 0.177$^a$ | 0.529$^a$ |
| Kananaskis | 0.912$^a$ | 1.088$^a$ | 0.500$^a$ |
| Highwood | 0.912$^a$ | 0.618$^{ab}$ | 0.412$^b$ |
| Oldman | 0.882$^a$ | 0.529$^a$ | 0.618$^a$ |

$^{a,b}$ Pairs with similar letters within a wolf pack are not significantly different; if the letters are not the same the pairs are significantly different ($P \leq 0.05$) (Appendix A5).

Two (Sawtooth, Oldman) of 6 wolves selected higher road density than expected based on availability outside their home range, although the values were not quite significant (Table 5). The remaining 4 wolves showed weak selection (nonsignificant but detectable) for higher road density than expected based on availability outside their home range). One (Kelly) of 6 wolves selected (nearly significant) areas of lower road density than expected based on availability within their home range. Four of 6 wolves (Appendix A5) showed weak selection (nonsignificant) for higher road densities than expected based on availability within their home range. Summarily, wolves showed weak selection for areas of higher road density than expected based on availability at both scales analyzed. There was not a significant difference in road densities of random points (availability) outside vs. inside the home range.

Table 6. Mean polygon road densities (km/km$^2$) of wolf home range and artificial home ranges (random points) adjacent to the wolf home range.

| Wolf | Wolf Home Range | Adjacent Artificial Home Range |
|---|---|---|
| Kelly | 0.61 | 0.77 |
| Ninemile | 1.89 | 1.77 |
| Sawtooth | 0.34 | 0.13 |
| Kananaskis | 0.25 | 0.14 |
| Highwood | 0.24 | 0.22 |
| Oldman | 0.29 | 0.22 |

This measure of road density (Table 6) differs from the previous section (Table 5) in that it measures overall road density within the 3 polygons (wolf.loc, rand.out, and rand.in), and not road densities at the selected 34 points in each of the 3 polygons. Wolves generally selected home ranges with road densities equal to or slightly greater than road densities of the surrounding landscapes, but the differences were not significant (Table 6; Wilcoxon P = 0.2489, $n = 6$ pairs).

### *Canopy Cover*

Data for canopy cover were categorical so a Mann-Whitney U test was used for analysis on observations.

Table 7. Mean categorical canopy cover (0 = none, 1 = 1-39%, 2 = 40-69%, and 3 = 70-100%) for wolves by use (wolf.loc) and availability (rand.out and rand.in).

| Wolf | Wolf.loc | Rand.out | Rand.in |
|---|---|---|---|
| Kelly | 1.74[a] | 2.00[a] | 1.76[a] |
| Ninemile | 1.85[ab] | 1.32[a] | 2.00[b] |
| Sawtooth | 1.23[a] | 0.91[a] | 1.09[a] |

[a,b] Pairs with similar letters within a wolf pack are not significantly different; if the letters are not the same the pairs are significantly different ($P \leq 0.05$) (Appendix A6).

The only significant value was detected in canopy cover availability for Ninemile, with significantly more canopy cover available in the home range than in habitats adjacent to the home range (Table 7). Ninemile selected (nearly significant) for more canopy cover than expected based on availability outside the home range. In contrast, Kelly selected (nearly significant) for less canopy cover than was expected based on availability outside the home range. Very few comparisons were significant or nearly significant, and those were for contrasting canopy coverages. This suggests that canopy cover was not significantly affecting wolf use of the landscape.

### *Correlation Between Distance to Water, Distance to Roads, Elevation, Slope, Code, and Wolf*

Table 8. Correlation coefficients of distance to water (disthyd), distance to roads (distrd), elevation (elev), slope, code, and wolf ($n = 612$ for all variables).

| | | Landscape Attributes | | | | Grouping Variables | |
|---|---|---|---|---|---|---|---|
| | | disthyd | distrd | elev | slope | code | wolf |
| disthyd | r | 1.000 | 0.146 | -0.014 | 0.041 | 0.076 | -0.356 |
| | P | - | 0.000* | 0.737 | 0.311 | 0.059[a] | 0.000* |
| distrd | r | | 1.000 | 0.288 | 0.123 | -0.031 | -0.094 |
| | P | | - | 0.000* | 0.002* | 0.447 | 0.020* |
| elev | r | | | 1.000 | 0.112 | 0.268 | 0.397 |
| | P | | | - | 0.006* | 0.000* | 0.000* |
| slope | r | | | | 1.000 | 0.228 | -0.082 |
| | P | | | | - | 0.000* | 0.042* |
| code | r | | | | | 1.000 | 0.000* |
| | P | | | | | - | 1.000 |
| wolf | r | | | | | | 1.000 |
| | P | | | | | | - |

* values are significant

[a] nearly significant

\- a coefficient cannot be computed

The r values for many of these comparisons were small but significant due to the large sample size (Table 8). Most of the landscape attributes were correlated. This is not surprising because in mountainous terrain, roads are usually built along streams and rivers, at lower elevation, and in less steep terrain.

## Logistic Regression

Table 9. Logistic regression significance, percent correct classification explained (forward stepwise selection, likelihood-ratio method), and coefficients of significant variables for wolf presence (rd = distance to road; el = elevation; sl = slope).

| WOLF | CODEPAIR | VARIABL INCLUD | SIGNIFICANCE | % EXPL | COEFFICIENTS *B* (CONSTANT + VARS) |
|---|---|---|---|---|---|
| kelly | wolf.loc/rand.out | sl | 0.0072 | 63.24 | -1.4964+0.0814(sl) |
| kelly | wolf.loc/rand.in | rd | 0.0008 | 70.59 | |
| kelly | wolf.loc/rand.in | rd, el | .0002, .0023 | 75.00 | |
| kelly | wolf.loc/rand.in | rd, el, sl | .0002, .0041, .0067 | 76.47 | -7.5447 -0.0005(rd) +0.0044(el)+0.1093(sl) |
| | | | | | |
| ninemile | wolf.loc/rand.out | sl | 0.0024 | 66.18 | -1.2172 +0.0756(sl) |
| ninemile | wolf.loc/rand.in | el | 0.0141 | 63.24 | |
| ninemile | wolf.loc/rand.in | el, sl | .0125, .0201 | 75.00 | -4.8909+0.0029(el) +0.0731(sl) |
| | | | | | |
| sawtooth | wolf.loc/rand.out | el | 0.0001 | 85.29 | |
| sawtooth | wolf.loc/rand.out | el, sl | .0002, .0024 | 82.53 | |
| sawtooth | wolf.loc/rand.out | rd, el, sl | .0354, .0124, .0063 | 91.18 | -16.3206 +0.0004(rd) +0.0076(el)+0.1481(sl) |
| sawtooth | wolf.loc/rand.in | el | 0.0001 | 76.47 | -11.1177 +0.0067(el) |
| | | | | | |
| kananask | wolf.loc/rand.out | rd | 0.0037 | 69.12 | |
| kananask | wolf.loc/rand.out | rd, el | .0008, .0021 | 88.24 | 7.5896 +0.0009(rd) -0.0052(el) |
| kananask | wolf.loc/rand.in | el | 0.0195 | 61.76 | -4.4294 +0.0023(el) |
| | | | | | |
| highwood | wolf.loc/rand.out | el | 0.0004 | 69.12 | |
| highwood | wolf.loc/rand.out | el, sl | .0022, .0117 | 79.41 | |
| highwood | wolf.loc/rand.out | rd, el, sl | .0275, .0034, .0067 | 82.35 | -12.2724 + 0.0007(rd) +0.0055(el)+0.1313(sl) |
| highwood | wolf.loc/rand.in | el | 0.0018 | 72.06 | |
| highwood | wolf.loc/rand.in | el, sl | .0023, .0022 | 75.00 | -9.6802 +0.0046(el) +0.1017(sl) |
| | | | | | |
| oldman | wolf.loc/rand.out | rd | 0.0042 | 70.59 | |
| oldman | wolf.loc/rand.out | rd, el | .0001, .0007 | 79.41 | |
| oldman | wolf.loc/rand.out | rd, el, sl | .0007, .0001, .0027 | 88.24 | 12.0193 +0.0012(rd) - 0.0094(el) +0.1332(sl) |
| oldman | wolf.loc/rand.in | el | 0.0001 | 77.94 | |
| oldman | wolf.loc/rand.in | rd, el | .0034, .0001 | 80.88 | -14.9546 +0.0009(rd) +0.0073(el) |

The dependent variables slope, distance to road, and elevation consistently came up significant for most wolves at both levels of use-availability (Table 9). The logistic regression models built for all individual wolves were different from one another because wolves inhabited different landscapes. Data from Table 9 suggest that elevation plays the most significant role in predicting landscape use by wolves producing the least misclassification error, followed by distance to roads and slope. All 3 variables were important in correctly classifying most cases and should be included in the logistic regression model. A dilemma arose in selecting the best model of wolf presence because 2 levels of habitat selection were analyzed for 6 wolves, resulting in 12 models with different coefficients as follows:

Prob (wolf presence) = $1/(1 + e^{-(Z)})$ for landscapes with:

1) rugged, high elevation, roadless areas, and low human density (Kelly):

$Z = (-1.4964 + 0.0814{*}\text{slope})$, wolf home range and outside home range

$Z = (-7.5447 + -0.0005{*}\text{road} + 0.0044{*}\text{elev} + 0.1093{*}\text{slope})$, wolf home range and within home range

2) moderate ruggedness, high road density, and relatively high human density (Ninemile):

$Z = (-1.2172 + 0.0756{*}\text{slope})$, wolf home range and outside home range

$Z = (-4.8909 + 0.0029{*}\text{elev} + 0.0731{*}\text{slope})$, wolf home range and within home range

3) the Rocky Mountain Front, including prairie and foothills, low to moderate ruggedness, low road density, low human density, (e.g. Sawtooth):

$Z = (-16.3206 + 0.0004{*}\text{road} + 0.0076{*}\text{elev} + 0.1481{*}\text{slope})$, wolf home range and outside home range

$Z = (-11.1177 + 0.0067{*}\text{elev})$, wolf home range and within home range

4) the foothills and mountains, moderate ruggedness, low human density, low to moderate road density (Kananaskis, Highwood, Oldman):

$Z = (7.5896 + 0.0009*\text{road} + -0.0052*\text{elev})$, wolf home range and outside home range

$Z = (-12.2724 + 0.0007*\text{road} + 0.0055*\text{elev} + 0.1313*\text{slope})$, wolf home range and outside home range

$Z = (12.0193 + 0.0012*\text{road} + -0.0094*\text{elev} + 0.1332*\text{slope})$, wolf home range and outside home range

$Z = (-4.4294 + 0.0023*\text{elev})$, wolf home range and within home range

$Z = (-9.6802 + 0.0046*\text{elev} + 0.1017*\text{slope})$, wolf home range and within home range

$Z = (-14.9546 + 0.0009*\text{road} + 0.0073*\text{elev})$, wolf home range and within home range

For most wolves at most scales of analyses, the general trend in univariate analyses indicated that wolves generally selected for lower elevation, flatter terrain, and roads. I believed it made sense statistically and biologically to combine the 6 wolves for each scale, and analyzed the standardized pooled data using the forced entry method of logistic regression (Table 10). When I ran the analysis on the pooled wolves data with the forward stepwise, likelihood-ratio method, I found that elevation was removed from the larger scale selection, and distance to roads was removed from the smaller scale selection (values were not significant so were deleted from the model based on specified selection criteria). Therefore, I used the forced entry method instead of the forward stepwise, likelihood-ratio method for the pooled wolves model because I wanted to retain all 3 variables in the final simplified model for both scales of analysis.

Table 10. Logistic regression significance, (forced entry method) percent correct classification explained, and coefficients of significant variables for pooled wolf presence (rd = distance to road; el = elevation; sl = slope).

| WOLF | CODEPAIR | VARIABL INCLUD | SIGNIFICANCE | % EXPL | COEFFICIENTS *B* (CONSTANT + VARS) |
|---|---|---|---|---|---|
| all 6 | wolf.loc/rand.out | el, rd, sl | 0.8002, 0.0001, < 0.00001 | 68.87 | -1.6483 + 0.1634(el) + 3.1552(rd) + 3.7466(sl) |
| | | | | | |
| all 6 | wolf.loc/rand.in | el, rd, sl | < 0.00001, 0.0730, < 0.00001 | 68.87 | -4.8155 + 4.2697(el) - 1.0418(rd) + 3.8122(sl) |

## DISCUSSION

The heterogeneity of habitat in the Central Rockies is a result of 2 processes acting on the landscape: 1) anthropogenic habitat fragmentation due to human development, and 2) natural patchiness inherent in mountainous terrain caused by elevational and climatic gradients. The flatter Midwestern landscapes of the US may be more homogeneous due to a relative lack of natural patchiness: habitat in Wisconsin had less variation in elevation, slope, water availability, and road distribution (Mladenoff et al. 1995) than the Central Rockies. Roads, water, and ungulate winter ranges may be more patchily distributed in the Central Rockies than in the Midwest, which may differentially affect wolf distribution.

### Anthropogenic Habitat Fragmentation

Wolves have typically recolonized areas of the Central Rockies in valley bottoms where humans build roads and homes, raise livestock, and recreate, nullifying our typical perception of the wolf as a wilderness species (Fritts et al. 1994, Fritts and Carbyn 1995, M. Jimenez, pers. commun.). This wolf-human cohabitation may result in increasing wolf-human conflicts. Recent expansion of wolves into these areas inhabited by humans shows a remarkable adaptability by wolves and an increasing tolerance by humans toward wolves. However, humans remain the main cause of wolf mortality in the majority of areas where wolves and humans coexist (Fritts et al. 1995, Wydeven et al. 1995, Paquet and Callaghan 1996, Weaver et al. 1996, Pletscher et al. 1997). Humans caused 85% of the wolf mortalities in the GNP area (Pletscher et al. 1997) and Central Rockies (Bangs et al. 1995).

Roads have been used as a measure of negative human influence for a variety of large carnivores including coyotes (*Canis latrans*) (Gibeau and Heuer 1996), cougars (*Felis concolor*) (Gibeau and Heuer 1996, Evink 1996), grizzly bears (McLellan 1989, McLellan and Shackleton 1989, Sandstrom 1996), black bears (*Ursus americanus*) (Kasworm and Manley 1990, Gibeau and Heuer 1996, Gilbert and Wooding 1996), and wolves. The home ranges of 5 of 6 successful colonizers in the Central Rockies had road densities < 0.62 km/km$^2$. The exception was the Ninemile wolf whose territory road density was 1.89 km/km$^2$, a value greatly exceeding road densities where wolves cease to exist elsewhere. The Ninemile pack's territory was > 50 km from the nearest wolf pack (prior to the 1995-96 reintroductions in central Idaho) and yet Ninemile wolves have persisted and consistently produced offspring since 1990 in an area of relatively high human density (M. Jimenez, pers. commun). The survival of these wolves may be due to intensive management and research efforts including feeding roadkills to orphaned pups, anti-depredation efforts, efficient removal of livestock killing wolves, and working with the local residents to increase human tolerance of wolf presence.

Researchers have reported varying response of wolves to roads (Thiel 1985; Jensen et al. 1986; Mech et al. 1988; Mech 1989; Thurber et al. 1994; Mladenoff et al. 1995, 1997; Paquet and Callaghan 1996), but most agreed that increased road densities reduce wolf survival. Generally, wolves existed in higher densities in areas with lower road densities (Table 11). Several researchers reported that a maximum road density above 0.6 km/km$^2$ jeopardized wolf survival. Two exceptions to this were reported by Mech (1989) and Fuller et al. (1992) (Table 11). Mech (1989) concluded that a low-density wolf population may exist in an area of higher road densities (e.g. 0.76 km/km$^2$) if adjacent areas had a reservoir wolf population and low road densities. Fuller et al. (1992) concluded that 88% of wolf packs in Minnesota occurred in areas with <0.70 km roads/km$^2$. However, this estimate may be biased toward higher road densities because records "were obtained on or adjacent to roads and thus biased towards areas with relatively higher densities of roads and humans" (Fuller et al. 1992:48).

Table 11. Mean road densities (km/km$^2$) at various geographic locations and wolf pack distribution patchiness.

| | | Wolf Pack Distribution | | |
|---|---|---|---|---|
| Source | Location | Disjunct | Contiguous | No wolves |
| Thiel 1985[a] | WI | ---- | 0.53 | ---- |
| Thiel 1985[b] | WI | 0.74 | ---- | >0.59 |
| Thiel 1985[c] | WI | 0.42 | ---- | ---- |
| Jensen et al. 1986 | ON/MI | 0.38 | 0.60 | 0.93 |
| Mech et al. 1988 | MN | <0.58 | 0.36 | >0.83 |
| Mech 1989 | MN | ---- | 0.76[d] | ---- |
| Fuller 1989 | MN | ---- | 0.15 - 0.72 | >0.72 |
| Fuller et al. 1992 | MN | ---- | <0.50-0.70 | >0.70 |
| Mladenoff et al. 1995 | WI/MI | 0.23 | ---- | >1.0 |

[a] before 1940 (prior to extirpation)
[b] after 1949 (during extirpation)
[c] 1980-83 (post extirpation, recolonization)
[d] adjacent to a large, roadless area with high wolf density

Roads in mountainous terrain are most commonly built along the rivers and streams because this presents the route of least resistance for vehicles and wolves alike. Roads were correlated with water and elevation (Table 8). Wolves in my study area selected for roads more frequently than expected based on availability both inside and outside their home range. Lower elevation riparian areas are relatively uncommon in mountainous terrain and are productive habitat supporting the higher ungulate densities on which wolves depend. This correlation between roads, water, elevation, and prey probably explains why wolves apparently selected for roads. This is contrary to findings reported in other studies in flatter terrain (Mladenoff et al. 1995, 1997).

Roads may be used as travel routes or avoided as barriers, depending on species and scale. Human encounters with wolves and potential mortality is greatly increased by the presence of roads. Seventy-five percent of human-caused wolf mortalities in my study area occurred within 250 m (potential shooting distance) of a road. Although wolves in my study generally selected for roads, there was an increased probability of human-caused mortality associated with road use. Most roads in the study area were unpaved (sometimes impassable to vehicles) or low grade highways with relatively low volumes of traffic. Wolves often used them year-round as travel corridors because they were easier travelways than the densely forested habitat. Singleton (1996) found that GNP wolves selected for closed roads for winter travel routes. Thurber et al. (1994) reported that wolves avoided open oilfield access roads in Alaska but were attracted to gated pipeline access roads and secondary gravel roads with limited access. Roads that are not plowed in winter may become snowmobile highways or ski trails that enhance wildlife travel. Trails packed by snowmobiles in Manitoba and Alberta became travel corridors for wolves, giving them access to remote elk herds that had not previously experienced wolf predation and were easy prey (P. Paquet, pers. commun.). Lower levels of human traffic are commonly documented in areas where wolves preferentially use roads as travel corridors. In these situations, wolf-vehicle collisions are unlikely, but roads create access for people who may kill wolves (Fuller 1989, Mech 1989, Paquet and Callaghan 1996).

Animals may avoid heavily used roads in areas of developed transportation corridors. For animals that attempt crossings, high-use highways and railways may become effective barriers to movements through roadkill and linear habitat fragmentation. Paquet and Callaghan (1996) reported that the Trans Canada Highway 1 (TCH-1) (4 lanes with an average daily traffic volume of 11,000 vehicles) and railroad (approximately 20 trains per day) combined accounted for more than 90% of the wolf mortalities in the Bow Valley of BNP. Highway underpasses constructed to encourage animal movements through the TCH-1 corridor have proven ineffective for most of the large carnivores (Page et al. 1996). The coyote is an extremely adaptable species and has a fairly high tolerance of human-developed landscapes. Yet, in a 20-month period, 21 of 24 known coyote mortalities in BNP were animals killed on the TCH-1 (Gibeau and Heuer 1996). This amounts to an annual highway mortality rate of 25% for adult coyotes which is typical of a harvested population, not a protected population in a national park (Gibeau and Heuer 1996).

In contrast, the less traveled 2-lane Highway 1A did not impede wolf movement in the Bow Valley. In Montana, Interstate 90 essentially forms the south boundary of the Ninemile pack's territory. Ninemile wolves rarely cross the 4-lane I-90 (9,000 vehicles per day, Montana Department of Transportation, pers. commun.), but 2 Ninemile wolves have been killed on I-90 since 1990 (M. Jimenez, pers. commun.). Despite collisions, several dispersers have successfully crossed major transportation corridors, indicating that linkages are not yet sufficiently degraded to prohibit all wolf movement. Interstate 90 is the only major interstate highway that traverses Montana, but there are several 2-lane highways that may be potential obstacles to animal movement. However, as human densities and road densities increase and roads are upgraded, increasing traffic will likely cause wolf mortalities to increase. The level at which this becomes critical for wolves is unknown. One transportation corridor may not prohibit animal movement, but cumulative encounters may sufficiently decrease crossing survival to effectively block movement.

Towns and development corridors may reduce or halt wolf movement between habitats. Hundreds of elk have become residents in the Banff townsite (5,000 people) apparently seeking the town as a refuge from predators. This elk immigration has caused conflicts with humans, often resulting in injured people and dead elk. The Banff townsite stops east-west movement of wolves in the Bow Valley, removing a valuable source of elk prey from potential wolf predation (P. Paquet, pers. commun.). However, the town of Polebridge (< 50 people) along GNP's western boundary does not block wolf movement along the Flathead River corridor in the GNP area. The scale of the town development in relation to the width of valleys is critical to permeability of wolf movement. The long-term existence of wolves in the Rockies will depend on the level of human influence on the landscape: at low levels wolves may adapt through habituation, but at higher levels wolves may cease to exist in viable numbers.

Wolves are great dispersers with a high reproductive potential which enhances their colonization opportunities. This is not necessarily true for many of the mid- and large-sized carnivores with access to the same landscape linkages wolves use. Habitat fragmentation has prevented grizzly bears in small, isolated populations in YNP and the Cabinet Mountains from connecting with larger source populations, yet wolves apparently can successful navigate between these areas. Landscape barriers may act as a permeable filter, sorting individuals based on species or individuals within a species. Wolves exhibit great behavioral plasticity with some individuals reticent to approach or cross development corridors while conspecifics may cross without problems (Boyd unpublished data, Paquet and Callaghan 1996).

## Natural Patchiness

Habitat patchiness caused by the heterogeneous nature of rugged terrain is inherent in mountainous landscapes. Landscape attributes such as habitat heterogeneity may exert significant influence on wolf distribution and abundance (Paquet et al. 1996). Natural landscape features such as aspect and canopy had little effect on habitat use by colonizing wolves in my study area. The correlated parameters of elevation, slope, hydrography, and roads (Table 8) were better predictors of wolf presence. Valley bottoms are important linkages for wolf movement in mountainous terrain (Singleton 1996, Paquet et al. 1996). Some topographic features may create physiographic barriers to dispersal that may retard wolf movement. However, I found no evidence that the Continental Divide or hydrologic features compromised wolf movement. A female wolf translocated for livestock depredation apparently swam the Hungry Horse Reservoir (Fritts 1993, Bangs et al. 1995).

The natural landscape patterns of mountainous terrain create a linear mosaic of habitats, aligning along drainages. An area of only a few square kilometers may contain a vast difference in landscape types, ranging from lowland riparian areas to high elevation permanent snowfields. The elevational gradient results in climatic and vegetational gradients that may greatly effect seasonal animal movements. Low elevation habitats contain winter ungulate ranges (Peek 1984, Rachel 1992, Bureau 1992, Kunkel 1997) which effect predator movements. Unfortunately, sufficient data do not exist for estimating ungulate densities or winter ranges throughout the study area, so I could not include these attributes in my analyses. Nonetheless, wolf habitat use is linked to ungulate distribution and abundance (e.g. Fuller 1989, Huggard 1992, Weaver 1994). Moreover, winter ranges for white-tailed deer (Peek 1984) and elk (Bureau 1992, Kunkel 1997) are usually located along river bottoms and riparian areas, so measuring attributes such as elevation, distance to water, and slope may serve as an index to ungulate presence.

## Dynamics of Colonization and Landscape

Since the early 1980s, wolves in the Central Rockies dispersed from source populations in protected parks to mostly low elevation, highly productive lands of mixed federal, state, and private ownership (Boyd et al. 1995, Fritts et al. 1995). These lands are the matrix connecting the isolated refugia of public wild lands, and are a critical component of wolf habitat if wolves are to survive as a viable population in the contiguous US. Isolated, protected parks do not contain enough land to maintain viable wolf populations (Fritts and Carbyn 1995). The wolf is a landscape generalist (Mech 1970, Mech 1993, Fritts et al. 1994), but it may be a habitat specialist within the larger landscape, selecting attributes (e.g. water, slope, roads, prey) disproportionately to their availability (Fuller 1989; Mladenoff et al. 1995, 1997; Weaver 1994; Singleton 1995; Paquet et al. 1996; Callaghan and Paquet, unpubl. data). The 2 most critical habitat elements for wolves are attributes based not upon vegetation type, but rather: 1) an adequate supply of wild ungulates and 2) freedom from excessive persecution from humans (Fritts et al. 1994).

In the previous sections I discussed dual landscape fragmentation in the Rocky Mountains: 1) the rugged terrain tends to naturally dissect the landscape into linear drainages, defined by low elevation valley bottoms and high elevation, inhospitable linear mountain ranges; and 2) anthropogenic effects that tend to further fragment landscapes in scattered patches (e.g. towns, ranches) and linear corridors (developments along transportation systems). This fragmentation has not prohibited dispersers from moving between suitable habitats, however, it may have slowed the rate (Paquet et al. 1996, Pletscher et al. 1997). A small percentage of mountainous landscape is suitable wolf habitat, so dispersing wolves tend to travel relatively long distances before finding other wolves or finding suitable habitat (Callaghan, pers. commun.; Paquet, pers. commun.; Boyd et al. 1995; Forbes and Boyd 1996). Typical maps of wolf pack distribution in the more homogeneous terrain of the midwest (Fritts and Mech 1981, Fuller 1989, Mech 1973) and the hills/forested lowlands of Denali National Park, AK (Meier et al. 1995) show wolves contiguously distributed with little, if any, unoccupied habitat between packs. Mech et al. (1991) noted that the mountainous and glacial areas adjacent to their lower elevation study area were devoid of wolves and prey in Denali National Park, AK. My study in the more rugged terrain of the Central Rockies indicated that most packs have a disjunct distribution over the landscape, selecting the scattered areas of lower elevation and higher prey density.

The present disjunct pack distribution of the Rockies is apparently an artifact of landscape heterogeneity, more so than a gauge of early-stage colonization. The growth of the wolf population in the GNP area stabilized in the early 1990s (Pletscher et al. 1997, Kunkel 1997, Boyd unpubl. data, Paquet pers. commun.) with suitable habitat apparently saturated with wolves. Subsequent colonization of areas further from the GNP area greatly expanded the wolf distribution in Montana. Managers may better serve the goals of wolf recovery by working with humans to increase tolerance of wolves in the landscape matrix surrounding wolf subpopulations. Minimizing wolf-human conflicts may be a more effective management tool than delineating and protecting potential corridors.

### Cooperative Management

Our data and those of others (Boyd et al. 1995; Forbes and Boyd 1996; E. Bangs and J. Fontaine, USFWS, pers. commun.) have shown that a single wolf population exists between BNP and YNP based on dispersal and genetics data. In the Central Rockies wolf status varies from a fully protected endangered species in the lower 48 states to an unprotected predator in Alberta. Wolf recovery efforts will be significantly enhanced by coordinated efforts to maintain reservoir source populations, landscape linkages, and reduction in human-wolf conflicts. In the western US and western Canada, laws governing large carnivores may not specifically protect these species but may be used to protect their habitat (Keiter and Locke 1996). Collaborative legislation to conserve wolves has not been initiated between states or between provinces, let alone between countries. The wolf has proven to be a fairly resilient colonizing species due to 1) its high reproductive rate, 2) long distance dispersal capabilities, 3) behavioral flexibility, 4) increasingly pro-predator human attitudes, and 5) availability of potentially suitable habitat. However, wolf recovery is dependent primarily upon public tolerance, and secondarily upon ecological capabilities.

## CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

1) Aspect, canopy cover, and road density were not significant attributes for predicting wolf presence.

2) Wolves appeared to select for landscapes with relatively lower elevation, flatter terrain, and closer to water and roads at both smaller and larger scales.

3) Elevation, distance to roads, distance to water, and slope, were correlated.

4) Land managers should use a perspective appropriate for mountainous landscapes when managing for western wolf recovery, and not rely on guidelines developed in the flatter terrain of the Midwest.

5) Roads, linear development corridors, and human habitation, should be assessed by biologists and managers to enhance wolf conservation efforts.

6) Manage for maintenance of large-scale linkage zones between wolf packs, rather than narrow linear corridors.

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# Appendix A.

## Paired Use-Availability Comparisons for Wolves and Significant Variables.

**A1.** Significant probabilities (*) of paired use-availability comparisons for wolves and mean elevation, determined by Mann-Whitney U tests.

| Wolf | Wolf.loc & Rand.out | Wolf.loc & Rand.in | Rand.out & Rand.in |
|---|---|---|---|
| Kelly | 0.548 | 0.019[a] | 0.017* |
| Ninemile | 0.271 | 0.008* | 0.108 |
| Sawtooth | 0.000* | 0.000* | 0.109 |
| Kananaskis | 0.053[a] | 0.014* | 0.000* |
| Highwood | 0.000* | 0.000* | 0.641 |
| Oldman | 0.404 | 0.000* | 0.000* |

[a] Value is nearly significant.

A2. Significant probabilities (*) of paired use-availability comparisons for wolves and mean rank slope, determined by Mann-Whitney U tests.

| Wolf | Wolf.loc & Rand.out | Wolf.loc & Rand.in | Rand.out & Rand.in |
|---|---|---|---|
| Kelly | 0.008* | 0.008* | 0.912 |
| Ninemile | 0.003* | 0.017* | 0.156 |
| Sawtooth | 0.000* | 0.013* | 0.091 |
| Kananaskis | 0.332 | 0.104 | 0.397 |
| Highwood | 0.000* | 0.001* | 0.956 |
| Oldman | 0.000* | 0.008* | 0.976 |

A3. Nearly significant probabilities (*) of paired use-availability comparisons for pooled wolves and natural log of mean distance to water, determined by t-tests.

|  | Wolf.loc & Rand.out | Wolf.loc & Rand.in | Rand.out & Rand.in |
|---|---|---|---|
| Prob value | 0.040* | 0.031* | 0.925 |

**A4.** Significant probabilities (*) of paired use-availability comparisons for wolves and natural log mean distance to roads, determined by Mann-Whitney U tests.

| Wolf | Wolf.loc & Rand.out | Wolf.loc & Rand.in | Rand.out & Rand.in |
|---|---|---|---|
| Kelly | 0.398 | 0.016* | 0.084 |
| Ninemile | 0.022[a] | 0.040[a] | 0.657 |
| Sawtooth | 0.000* | 0.192 | 0.000* |
| Kananaskis | 0.009* | 0.101 | 0.219 |
| Highwood | 0.265 | 0.187 | 0.783 |
| Oldman | 0.021[a] | 0.095 | 0.527 |

[a] values are nearly significant

**A5.** Significant probabilities (*) of paired use-availability comparisons for wolves and mean rank road density, determined by Mann-Whitney U tests.

| Wolf | Wolf.loc & Rand.out | Wolf.loc & Rand.in | Rand.out & Rand.in |
|---|---|---|---|
| Kelly | 0.926 | 0.049[a] | 0.033[a] |
| Ninemile | 0.230 | 0.696 | 0.143 |
| Sawtooth | 0.035[a] | 0.685 | 0.097 |
| Kananaskis | 0.115 | 0.046[a] | 0.804 |
| Highwood | 0.110 | 0.013* | 0.465 |
| Oldman | 0.026[a] | 0.076 | 0.744 |

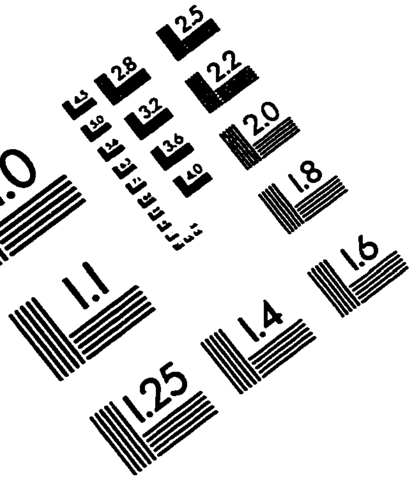
[a] values are nearly significant.

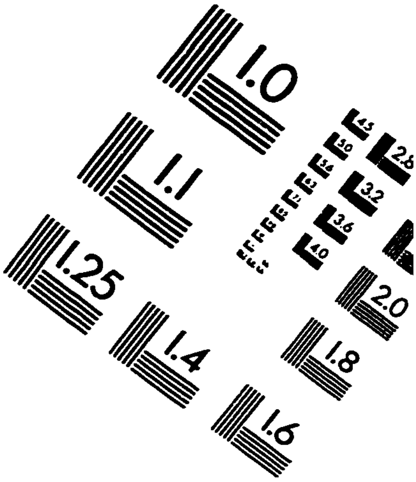
A6. Significant (*) paired use-availability comparisons for wolves and mean rank canopy cover, determined by Mann-Whitney U tests.

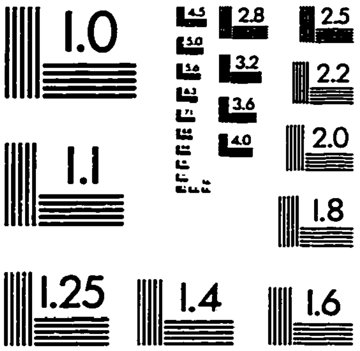
| Wolf | Wolf.loc & Rand.out | Wolf.loc & Rand.in | Rand.out & Rand.in |
|---|---|---|---|
| Kelly | 0.0462[a] | 0.7633 | 0.2071 |
| Ninemile | 0.0228[a] | 0.2103 | 0.0051* |
| Sawtooth | 0.1125 | 0.3602 | 0.5085 |

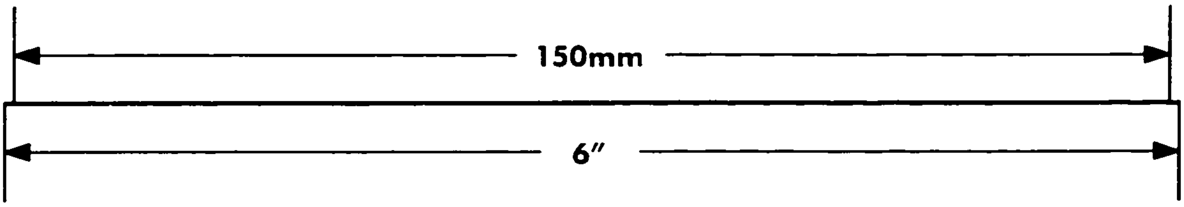
[a] values are nearly significant.

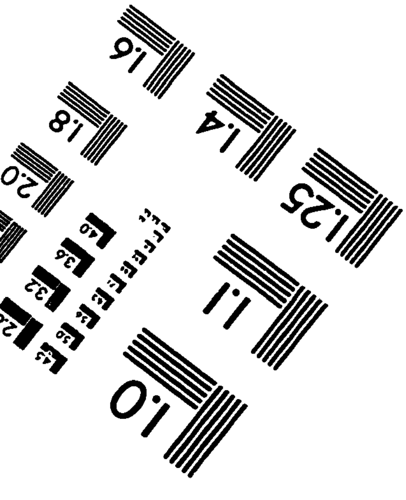
# IMAGE EVALUATION TEST TARGET (QA-3)

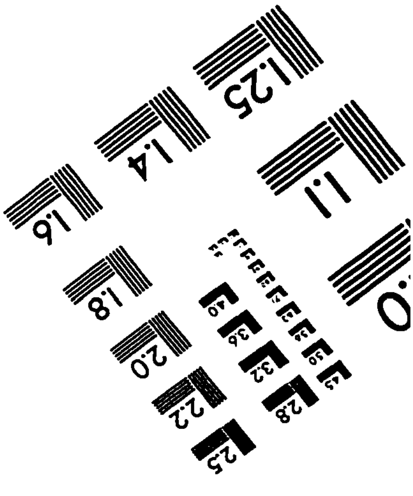












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