University of Alberta

Population Structure and Dispersal of Wolves (*Canis lupus*) in the Canadian Rocky Mountains

By

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Ecology and Environmental Biology

Department of Biological Sciences

Edmonton, Alberta

Spring 2007

Your file Votre référence
ISBN: 978-0-494-30033-6
Our file Notre référence
ISBN: 978-0-494-30033-6

Abstract

Wolves (*Canis lupus*) are highly mobile within their territories and during periods of dispersal. Quantifying wolf dispersal using traditional radio or GPS collaring methods is time consuming and costly. By using a combination of invasive and non-invasive DNA collection methods and genetic analysis I was able to identify subpopulations of wolves and dispersers between them. Population structure occurred at the three levels I examined: individual, pack, and subpopulation. Four subpopulations were identified and appeared to be divided along naturally occurring and anthropogenic features. However, the cause of some divisions was unknown, and may have been due to prey specialization or other factors. Rates of dispersal between subpopulations were sufficiently low to allow genetic differentiation between geographic regions. Dispersal between some subpopulations was asymmetric and may be indicative of source – sink dynamics. Subpopulations are likely to become more vulnerable to extirpation and further isolated from one another as habitat modification and human habitation increases. Movement corridors must be maintained to allow recolonization of areas that may become extirpated.

Acknowledgements

This research project was much bigger than me, and could not have been completed without the cooperation and assistance of a large number of people and organizations. I was very fortunate to have received funding from a number of sources. The majority of funding for this study came from the Banff Field Unit of Parks Canada, and I would like to thank Tom Hurd and Cliff White for being so generous with the funding and logistical support. Tom and Cliff were key to the success of this project from the start to very finish and I am thankful for the opportunity to work with them. I received a grant from the Alberta Sport, Parks, Recreation, and Wildlife trust fund. The Columbia Basin Fish and Wildlife Compensation Program provided funding for a portion of the B.C. research. Evelyn Merrill and Nate Webb were extremely generous in providing me with a portion of their Yellowstone to Yukon Conservation Grant to work on the samples they had collected. The Alberta Cooperative Conservation Research Unit (ACCRU) provided much of the necessary field equipment for very reasonable or no cost. The ACCRU equipment kept us safe and efficient while in the field. Grants from the FGSR and Department of Biological Sciences allowed me to travel to several conferences to present my research.

The field portion of the study was very demanding, but made enjoyable by Nathan DeBruyn and Rebecca Rothgeb in 2003/2004 and Dave Garrow in 2004/2005. I cannot say enough about their competence and dedication. Nathan's 'miracle tracking' still amazes me to this day and he consistently logged the longest tracking sessions no matter what the terrain. Rebecca's attention detail and ability to tolerate the cold hours spent crouching over bed sites picking hairs with bare fingers undoubtedly resulted in many wolves being identified that otherwise may have gone undetected. Dave was always one step ahead of me, literally and figuratively, and being able to trust in his knowledge of wolves and backcountry travel made my life much easier. I thank them heartily and wish I could have paid them what they truly deserved. Zulima Tablado-Almela and Seth Cherry volunteered their time and there help was much appreciated. I want to thank the folks that helped with the skull cleaning portion of the study; it was a dirty job, but they managed it with a smile on their faces.

I was able to include wolves, living and dead, from such a large area thanks to the assistance of fellow students, biologists and hunters and trappers. Banff and Jasper National Parks have been studying wolves for many years and were kind to allow me use of historic and current samples they had collected. From the University of Alberta Mark Hebblewhite, Layla Neufeld, Shannon Stotyn, and Nate Webb all provided me with samples they had collected during live-trapping as well as samples they collected non-invasively. Casey Black at the Northern Lights Wildlife Center in Golden, B.C. provided me with scats and hair samples

from captive wolves, as well as amazing photos that I used in many presentations. In southern Alberta, regional biologist Carita Bergman went out of her way to acquire samples and provide information on the wolf packs in the region, as well as lending telemetry equipment to locate collared wolf packs in the area. In both Alberta and B.C., I received tremendous support and interest in the research from the Alberta Trappers Association and the East Kootenay Trappers Association. The trappers knowledge of the areas, submission of samples, and dedication to conservation was a welcome addition to the study. The importance of the samples provided to me cannot be underestimated in terms of cost, effort, and dedication, and I am grateful for the generosity of everyone who allowed me to use their samples.

My field seasons involved travel to many remote locations and frequent relocations of housing. Throughout the two winters of sample collection housing was generously provided for free or a small cost by a wide array of individuals and organizations. Thanks to Alan Dibb from Banff District West who set us up at the Saskatchewan River Crossing warden station. Tom Daniels of Sunpine Forest Products allowed the crew to stay at their bunkhouse near Rocky Mountain House. A comfortable month was spent in a strategically perfect location at the SRD bunkhouse in Blairmore. Paul Frasca, of Tembec, allowed the crew to stay at their bunkhouse with a wonderful view in Parsons. I am thankful to Paul Ronellonfitch for finding space for us at the Suncor/Shell drilling camp near the Panther River where we thoroughly enjoyed coming home to a well cooked meal everyday and got to learn a bit more about drilling an oil well. In the Blaeberry valley we spent some luxurious nights at Leo and Karen Downey's Sanctuary Resort and Rocky Mountain Buffalo Ranch. Thanks again to everyone that opened up their doors to us and made the field work possible. It would have been some long cold nights otherwise.

Prior to starting my thesis I was practically illiterate when it came to the world of genetics, but I was extremely fortunate to be shown the ropes by some of the most respected and competent people in genetics today. My first foray into the laboratory component of genetics occurred in the most agreeable location of Los Angeles, and could not have occurred in better hands. John Pollinger, from Robert Wayne's lab at UCLA, gave me my initiation and laid the foundation for the work to come. I also received support and advice from Deborah Randall and Bridget vonHoldt at UCLA. My true mentor when it came to all aspects of genetics was Corey Davis, who spent innumerable hours explaining, and re-explaining concepts, techniques, and analyses to me. Without Corey (and his robotic side-kick) I could not have attempted all of the lab work that lay ahead of me. Lab space and unadulterated advice were provided by Curtis Strobeck. I also want to thank the rest of my adopted lab on the 6th floor that were always available to point me in the right direction when I invariably strayed from the right path, or to share in a beverage when the path was lost

completely. Greg Wilson, in particular, provided me with advice on analyses, and Lindsay Carmichael was a ready supply of knowledge of wolf genetics. Thanks to Greg Wilson and Stephanie Nakada for taking on the onerous task of editing early versions of my thesis. This work is definitely lacking in glamour but every red pen mark (and there were lots of them) was appreciated. I am indebted to Jenn Davis, Justine Moe, John Brzustowski, and Dion Pasichnyk for their assistance with sample preparation and DNA extractions.

Kristopher Sabourin helped walk me through the maze that is ACCESS and I came out on the other side far better off.

I was very fortunate to land in the Derocher lab considering I worked on such a 'small' carnivore. My lab mates Seth Cherry, Mark Edwards, Sarah Medill, John Nagy, Emily Parks, and Lindsay Towns were always nearby and ready to help with any problem that arose, or at least attempt it. We were able to mix discussions of science with any other topic and it was this group of people that made my graduate experience something I will never forget. I know that the relationships I forged with them will continue on into each of our professional careers.

I would like to thank my academic supervisory committee, Dave Coltman, Evelyn Merrill, Paul Paquet, and Cliff White for their support and advice. They helped me to understand science as well as the entire process of being a graduate student. Their encouragement throughout the process was appreciated and I was honoured to have their input into my study. Heading my academic supervision was Andy Derocher, and I reserve the most thanks for him. His door was always open, and he would never hesitate to guide me through the most menial or daunting of tasks. Andy is a master of putting science and life into context and I am grateful for his support as I, at times, muddled my way through this thesis.

Finally, I would like to thank my family for their unending support and encouragement. I have realized in the past few years how fortunate I am to have the family that I do.

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CHAPTER 1 - General Introduction

1.1 Introduction

Conservation and management at the proper spatial scale require an understanding of the size and extent of populations being studied. While there are many factors that influence population structure the role of dispersal is key (Howard 1960; Hastings 1993; Olivieri *et al.* 1995; Newman & Tallmon 2001; Amarasekare 2004; Cadotte 2006). Increasingly, genetic analysis is being used to measure dispersal and to provide novel insights into population structure of wildlife (e.g. Paetkau *et al.* 1995; Cegelski *et al.* 2003; Sacks *et al.* 2004; Eriksson *et al.* 2004; Worley *et al.* 2004).

An often cited definition of dispersal is "the permanent movement an individual makes from its birth site to the place where it reproduces or would have reproduced if it had survived and found a mate" (Howard 1960). Gene flow, or effective dispersal, occurs when successful reproduction occurs following either natal or breeding dispersal (Greenwood 1980). When gene flow occurs the fitness of individuals, genetic structure of populations, and probability of population persistence is altered (Rasa 1987; Newman & Tallmon 2001; Callaghan 2002; De Villiers *et al.* 2003). Direct measures of dispersal are difficult to obtain and are often skewed towards shorter dispersal distances due to finite study areas (Koenig *et al.* 1996; Baker *et al.* 1998; Forero *et al.* 2002). Genetic measures of dispersal help address the problem of spatial scale by allowing more individuals to be sampled over a larger area (Koenig *et al.* 1996). Genetic methods have been used to measure dispersal in a variety of species such as river otters, *Luntra canadensis* (Blundell *et al.* 2002), lions, *Panthera leo* (Spong *et al.* 2002), black-billed magpies, *Pica pica* (Hyung Eo *et al.* 2002), dolphins, *Tursiops aduncus* (Möller & Beheregaray 2004), and many others.

When rates of dispersal vary between habitats or populations they can contribute to source – sink dynamics (Kawecki & Holt 2002). Traditional determinations of source and sink populations require information on immigration, emigration, births, and deaths. In source-sink systems, source populations have growth rates, $\lambda > 1$, sink populations have $\lambda < 1$, and surplus individuals move from areas of high quality to areas of low quality (Pulliam 1988; Runge *et al.* 2006). Due to the difficulty in collecting the required demographic information there have been few studies that have conclusively shown the existence of source-sink dynamics (Diffendorfer 1998). While not fulfilling the strict definition, asymmetric rates of dispersal between populations can suggest source-sink dynamics in the absence of demographic data (Kawecki & Holt 2002).

In the last decade molecular genetic techniques have improved to allow the use of non-invasively collected samples from a wide range of

sources such as scat (Höss *et al.* 1992), hair (Woods *et al.* 1999), feathers (Segelbacher 2002), blood (Scandura 2005), saliva (Constable *et al.* 2001), and urine (Valière & Taberlet 2000) for individual identification and population genetics (Taberlet & Luikart 1999). Genetic samples collected non-invasively may be error prone (Gerloff *et al.* 1995; Gagneux *et al.* 1997; Bradley & Vigilant 2002; Creel *et al.* 2003), but methods such as the multiple-tubes approach during PCR (Navidi *et al.* 1992; Taberlet & Luikart 1999), quantitative PCR of DNA products (Morin *et al.* 2001), and quantification of errors following genotyping (McKelvey & Schwartz 2004) can help reduce and identify errors making the use of such samples feasible. Often, many more individuals may be sampled over a wider area by collecting non-invasive samples than from traditional means of live-capture or harvest. As well, many species are difficult to capture, making collection of non-invasive samples an attractive alternative.

Wolves (*Canis lupus*) are large, highly mobile carnivores that are capable of dispersing far distances (Ballard *et al.* 1983; Gese & Mech 1991; Mech *et al.* 1995; Boyd & Pletscher 1999; Wabakken *et al.* 2001) making it difficult to delineate populations and to follow the fate of dispersing individuals. Traditionally, wolf dispersal studies have utilized telemetry from radio or satellite collars, but these studies required extensive amounts of labour, time, and expense. Both Gese & Mech (1991) and Boyd & Pletscher (1999) required more than 15 years of capturing, collaring and monitoring wolves to gather sufficient data to characterize dispersal in the populations they studied. The alternative is indirect and direct genetic methods for determining population structure and dispersal (Slatkin 1987). Gene flow and relatedness in wolf packs has been examined using blood and tissue samples collected during radio-collaring and from hunter and trapper harvested individuals (Lehman *et al.* 1992; Forbes & Boyd 1997; Carmichael *et al.* 2001), however, non-invasive DNA-based population sampling for wolves is a relatively new development (Lucchini *et al.* 2002; Valière *et al.* 2003; Creel *et al.* 2003; Scandura 2005). Collaring large numbers of adult and juvenile wolves from the majority of packs within a study area to determine the extent of exchange of individuals between subpopulations is logistically problematic, and can result in injuries to captured wolves (Kuehn *et al.* 1986; Valerio *et al.* 2005). By substituting lifelong observations of individuals with a short, focused study of genetic relationships it is possible to examine population structure and large-scale movement patterns that have occurred in the recent past.

I studied a wolf population in the Canadian Rocky Mountains where varying public perception and persecution through time has resulted in cycles of extirpation and recolonization (McTaggart Cowan 1947; Gunson 1983; Hayes & Gunson 1995; Musiani & Paquet 2004). There is a long history of wolf research in the national parks of western Canada (McTaggart Cowan 1947; Huggard 1993; Paquet 1993; Hebblewhite 2000; Callaghan 2002; Hebblewhite *et al.* 2005), but less research has focussed

in areas where legal harvest occurs (Schmidt & Gunson 1985; Kuzyk 2002). Management of wolves in this area mirrors the focus of research, as federal and provincial jurisdictions have different priorities for wolves. A large scale examination of how wolves in exploited and protected populations interact, demographically and genetically, has never been undertaken.

The primary objectives of this study were to: 1) examine feasibility of using non-invasive samples for genetic analysis of wolves; 2) examine wolves within the Canadian Rocky Mountains for genetic structure at individual, pack, and population levels; 3) identify migrants and the patterns of migration between subpopulations; 4) examine patterns of asymmetric dispersal which may be indicative of source-sink dynamics; 5) compare male and female dispersal patterns; and 6) quantify the influence of human and natural features on the landscape that may act as barriers to wolf dispersal. For genetic analyses I used 13 polymorphic microsatellite loci and 1 Y-chromosome microsatellite for sex determination.

In my second chapter I examined wolf population structure at an individual, pack and population level from samples collected primarily non-invasively, but also from live-captured and harvested wolves. Starting at the individual level I calculated pairwise relatedness measures between all wolves. Relatedness was regressed against geographic distance between pairs of wolves to look for isolation by distance. At the pack level I again employed pairwise relatedness statistics, but this time to examine the differences in relatedness between individuals within packs and between packs. I compared levels of relatedness for all individuals in a pack, within sexes, and between sexes. Genetic differentiation between packs was measured using F_{st} and D_s. Mean relatedness of individuals within packs was compared between packs that occurred in areas where legal harvest of wolves occurred and where harvest is not allowed. At the population level I used a Bayesian clustering analysis to determine if subpopulations of genetically distinct groups of wolves exist within the study area.

In chapter 3 I examined dispersal between the subpopulations identified in the clustering analysis of the previous chapter. Dispersers and their natal subpopulations were identified using 3 different genetic assignment tests. The number of dispersing individuals between subpopulations was compared to see if asymmetric flow had occurred. Asymmetry in dispersal rates for males and females was also examined. I selected a series of potential barriers to test their degree of influence on wolf dispersal. Three potential barriers of anthropogenic origin and 2 of natural origin were tested using a partial Mantel test.

Finally, in chapter 4 I conclude the thesis with a synthesis of the results from the 2 data chapters. I discuss the management and conservation implications of this research for wolves in the Canadian Rocky Mountains.

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CHAPTER 2 - Genetic structure of wolves (*Canis lupus*) at the individual, pack, and subpopulation level in the Canadian Rocky Mountains

2.1 Introduction

Practical management of wide-ranging wildlife species requires an understanding of the spatial extent and structure of the population in question. Populations have commonly been delineated along socio-political boundaries, such as international or state borders; geographic features, such as mountain ranges or rivers; and even sampling areas, all of which may have little or no relation to the underlying biological structure. Many mammal, and especially carnivore, species have continuous distributions across the landscape and can make large individual movements within their range. Population structure can be difficult to discern in such circumstances, but genetic methods have proven a valuable tool for doing so (e.g. gray wolves, *Canis lupus* (Carmichael *et al.* 2001), coyotes, *Canis latrans* (Sacks *et al.* 2005), polar bears, *Ursus maritimus* (Paetkau *et al.* 1995), wolverine, *Gulo gulo* (Kyle & Strobeck 2001; Cegelski *et al.* 2003)). These genetic analyses can reveal cryptic population structure which may have gone unnoticed with other methods (Sacks *et al.* 2005).

Genetic structuring of populations occurs through the interaction of isolation and gene flow within and between local populations (Slatkin 1987). Genetic differentiation may occur across a range of scales from local (e.g. Coltman *et al.* 2003; Brouat *et al.* 2003; Bouzat & Johnson 2004) to regional (e.g. Cegelski *et al.* 2003; Eriksson *et al.* 2004) to a continental level (e.g. Kyle & Strobeck 2001; De Barro 2005; Geffen *et al.* 2004). Differentiation may arise from isolation by distance (Wright 1943; Pfenninger *et al.* 1996), however other factors such as kin-related social structure in solitary (Støen *et al.* 2005) or social species (Lehman *et al.* 1992; Pope 1992; Girman *et al.* 1997), habitat affinities during dispersal (Sacks *et al.* 2004), naturally occurring physical barriers (Keyghobadi *et al.* 1999; Carmichael *et al.* 2001; Hyung Eo *et al.* 2002; Walker *et al.* 2003; Worley *et al.* 2004), human created barriers (Epps *et al.* 2005; Proctor *et al.* 2005), prey specialization (Carmichael *et al.* 2001), or large scale climatic differences (Geffen *et al.* 2004) may be the underlying processes resulting in genetic structure.

Wolves are social animals living in family groups (Mech 1970; Lehman *et al.* 1992) and are large, highly mobile carnivores capable of dispersing long distances (Ballard *et al.* 1983; Gese & Mech 1991;

Wabakken *et al.* 2001). The historical range of gray wolves is the largest of terrestrial mammals, but their persistence and management has been a constant biological and political issue (e.g. Forbes and Theberge 1996, Haight *et al.* 1998, Wabakken *et al.* 2001, Carroll *et al.* 2003, Theuerkauf *et al.* 2003). Within North America wolves have experienced significant range contractions following European settlement and more recently expansion to previously occupied areas following reintroductions (Breitenmoser *et al.* 2001) and natural recolonization. By 1930 human persecution had reduced wolves south of Jasper National Park in the Canadian Rocky Mountains to occasional travelling individuals (McTaggart Cowan 1947). However, through natural recolonization wolves returned to the majority of this portion of the Canadian Rocky Mountains, only to be extirpated by humans once again in the 1950s (Gunson 1983). Since then, wolves from contiguous populations in northern Alberta and British Columbia have recolonized portions of the Canadian Rocky Mountains from which they were extirpated, and currently occupy a near continuous distribution through the region (Hayes & Gunson 1995). Wolves in Canada, including the Canadian Rocky Mountains, are subject to legal hunting, trapping, and management actions that may produce pack level or localized extirpations throughout the range. Although, within this range zones of protection for wolves in national and provincial parks exist, creating a mosaic landscape of safe and risky habitats.

Recent advances in molecular genetics have provided new insight into social behaviour of animals (Ross 2001) and a further improvement in molecular techniques allows the use of non-invasively collected samples such as scat (Höss *et al.* 1992), hair (Woods *et al.* 1999), and urine (Valière & Taberlet 2000) for individual identification and population genetic studies of wildlife species (Taberlet & Luikart 1999). Genetic samples collected non-invasively may be error prone (Gerloff *et al.* 1995; Gagneux *et al.* 1997; Bradley & Vigilant 2002; Creel *et al.* 2003). However, methods such as the multiple-tubes approach during PCR (Navidi *et al.* 1992; Taberlet *et al.* 1996), quantitative PCR of deoxyribose nucleic acid (DNA) products (Morin *et al.* 2001), minimization of error generating procedures (Paetkau 2002) and quantification of errors following genotyping (McKelvey & Schwartz 2004; Broquet & Petit 2004; Bonin *et al.* 2004) can all be utilized to make non-invasive samples viable sources of DNA. Collection of non-invasive samples from some species allows for a larger geographic area to be sampled in a shorter period than traditional methods of capturing individuals and collecting samples. Reduction of injuries and mortality that may occur to study animals during live capture (Kuehn *et al.* 1986; Boyle *et al.* 2006) and immobilization (Valerio *et al.* 2005) is a major benefit of using non-invasive samples.

Non-invasive collection of wolf genetic material was used to effectively sample a large number of wolves over a short period in Europe (Lucchini *et al.* 2002; Valière *et al.* 2003) and the United States (Creel *et al.* 2003). However, environmental conditions vary across the range of

global wolf distribution, and may influence the rate and degree of DNA degradation of non-invasively collected samples. The Canadian Rocky Mountains in winter tend to be cold and dry, which are excellent conditions for preserving DNA. Studies of other species have shown tissue and blood samples to be higher quality sources of DNA than non-invasively collected hair or scat (Lathuilliére *et al.* 2001; Hedmark *et al.* 2004), yet this remains to be tested on wolves in the Canadian Rocky Mountains. Non-invasive samples allow population genetic questions to be addressed over a large geographic scale, such as that in the Canadian Rocky Mountains.

One such question that can be addressed is sex ratios in wolves. There is little evidence to suggest that sex ratios for adult wolves are skewed towards one sex (Mech 1970; Meier *et al.* 1995; Fuller *et al.* 2003). However, most reports of wolf sex ratios are based on capture events which may be biased towards one or the other sex. Large scale non-invasive sampling of wolf packs provides an opportunity to test the hypothesis of equal representation of sexes.

Given the ability of wolves to disperse long distances (Fritts 1983; Boyd & Pletscher 1999), populations would not be expected to have extensive genetic structure. On a continental scale in North America conflicting results exist for isolation by distance, from no pattern of isolation for wolves (Roy *et al.* 1994), to changes in genetic distances over space related to climate (Geffen *et al.* 2004). Smaller scale studies have shown that genetic differentiation increased with distance at regional levels (Forbes & Boyd 1997; Vilà *et al.* 1999; Carmichael *et al.* 2001). Where continuous distributions across a landscape occur it is unlikely that distinct genetic subunits, or subpopulations, will occur unless barriers to gene flow exist, and this is especially true for highly mobile species such as wolves. Population sub-division has been found in wolves inhabiting islands within the Alaskan archipelago (Weckworth *et al.* 2005). In the island system oceanic barriers reduce gene flow resulting in population differentiation. This pattern also occurs in Europe where small, disjunct populations occur in islands of suitable habitat surrounded by human dominated landscapes (Vilà *et al.* 1999). From the continuously distributed population of wolves through the Canadian Rocky Mountains I would expect to see weak population structure following a pattern of isolation by distance.

Wolf packs that experience intense harvest may have reduced structure at the pack level (Jêdrzejewski *et al.* 2005). High turnover of individuals within wolf packs can occur due to harvest or other sources of anthropogenic mortality. While cases of incest have been reported for wolves, immigrants are more likely to take on reproductive roles when a pack has been reduced in size through harvest (Smith *et al.* 1997; Jêdrzejewski *et al.* 2005). Immigration of non-family members would reduce the mean relatedness, and therefore structure of packs. Within the Canadian Rocky Mountains wolves are exposed to legal harvest in some

areas, and afforded protection from harvest elsewhere, providing a system to test the effect of harvest on pack structure.

The objectives of this study were to examine wolves within the Canadian Rocky Mountains for genetic structure at individual, pack, and population levels. I collected genetic material using invasive and non-invasive methods from wolves across the region to address these issues. A suite of 13 microsatellite markers were examined using a combination of isolation by distance measures and a model based clustering procedure.

2.2 Methods

2.2.1 Study Area

The study area covered approximately 145,000 km^2 (Figure 2.1) and straddled the continental divide along the Canadian Rocky Mountains in British Columbia and Alberta. The western boundary was the height of land west of the Rocky Mountain Trench and the eastern limit was the Alberta Highway 22. The southern limit was the Canada/United States border and the northern boundary was the Kakwa River, Alberta. This area includes Banff, Jasper, Yoho, and Kootenay National Parks, as well as a range of provincial protected areas in both Alberta and British Columbia.

The region is dominated by rugged mountain ranges and wide, flat valley bottoms aligned south-southeast to north-northwest. The Columbia River Trench follows a similar alignment, however it extends from the northern portion of the study area southward into the United States, creating continuous valley bottom habitat for the length of the study area. Elevations range from 357 to 3937 m asl with three major east – west passes bisecting the mountain ranges.

Three major east-west highways and associated rail lines cross the study area and follow naturally occurring passes: Highway 16 follows the Yellowhead Pass in the north; the TransCanada Highway bisects the center of the study through the Kicking Horse and Rogers Pass; and to the south of the study area Highway 3 crosses through the Crowsnest Pass.

Ungulates available as prey for wolves include elk (*Cervus elaphus*), moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), bighorn sheep (*Ovis canadensis*), mountain goat (*Oreamnos americanus*), and caribou (*Rangifer tarandus*). Livestock, mainly cattle, occur primarily in the southern end of the study area and are occasionally depredated.

2.2.2 Sample Collection and Preservation

Scat, hair, blood, and tissue were collected in three ways (1) from non-invasive samples (hair, scat, and blood in snow); (2) from handling of live animals (hair and blood); and (3) from wolf carcasses (tissue).

Non-invasive samples of wolf DNA were collected in winter while snow tracking uncollared and collared wolf packs Winter tracking was used because DNA amplification is more successful when samples are collected in winter as opposed to summer, and tracking in snow-free conditions would be logistically difficult (Lucchini *et al.* 2002). Tracks from uncollared wolf packs were located while travelling on foot, by snowmobile, or truck through wolf habitat. Once tracks were located, wolves were backtracked (followed in the opposite direction wolves were travelling) unless tracks were > 24 hours old when forward and backtracking were used to increase the total distance followed. When possible, consecutive days were spent following a single set of tracks to increase the probability of collecting samples from all members of a pack. I attempted to collect three times as many samples as individuals estimated to be in a pack based on track counts. Several wolf packs had radio-collared individuals and telemetry was used to locate these packs for tracking and sample collection by colleagues. The number of wolves in each pack estimated from track counts was compared to the number of wolves typed genetically.

Scat samples were stored in sealed plastic bags at -20°C from collection until DNA extraction which occurred within 10 months. Scat was treated at -80°C for > 48 hours to render eggs of *Echinococcus multilocularis* and *E. granulosus* parasites inactive (Veit *et al.* 1995; Hildreth *et al.* 2004), thereby reducing the risk of human infection when handling the samples. Following DNA extraction sub-samples were collected and stored in 100% ethanol for long-term storage and possible re-extraction.

Hair was collected from bed sites, natural snags on vegetation, and wherever wolf hair was detected during tracking. Winter wolf bed sites tend to be discrete depressions in the snow with each bed site containing hair, and therefore DNA of one individual. As many follicle-bearing hairs as possible were collected from each bed site (range 1- >40). Tufts of small under hair found in association with bed sites or grooming areas were also collected. Roots were not visible to the unaided eye on these hairs, however it was presumed that roots or possibly saliva were present and could be used as a DNA source. Hair samples were stored in paper envelopes at room temperature in low humidity conditions before DNA extraction.

Samples from live captured wolves were collected from 1990-2005 by a variety of organizations and individuals. Hair was plucked or blood drawn from wolves at capture. Blood was stored in EDTA tubes at -20°C until DNA was extracted. Plucked hair samples were stored similar to hair

collected non-invasively. Information on age, sex, and reproductive status of wolves was often collected during capture events. Tissue samples from legally harvested wolves and other sources of human-caused mortality were provided by trappers and government agencies, and stored at -20°C in sealed plastic bags until DNA extraction.

Blood, tissue, hair, and DNA samples were sub-sampled and stored at the Parks Canada DNA Repository at the University of Alberta.

2.2.3 DNA Extraction and Microsatellite Typing

DNA extractions were performed in a room physically separated from where amplified PCR products were stored and handled to reduce the risk of contamination of DNA samples. Negative controls were used throughout the extraction and typing process to monitor for contamination (Taberlet *et al.* 1996). Scat samples were extracted using the QIAamp DNA Stool Mini-kit (Qiagen) with slight modifications to manufacturer recommendations (i.e., incubated at 70ºC with Proteinase K for 30 minutes instead of the recommended 10 minutes). Blood, hair, and tissue samples were extracted using Qiagen Dneasy extraction kits (Qiagen) following the manufacturer's directions. The number of hairs extracted per sample varied from 1 to 30 for guard hairs and > 50 for samples of under hair.

Microsatellite loci were selected from markers developed from the dog genome. Thirteen microsatellite markers: FH2088, FH2054, FH2004, FH2001, FH2010, FH2096, FH2422 (Breen *et al.* 2001), FH3313, FH2834 (Hapke *et al.* 2001), PEZ8, PEZ9, PEZ12, and PEZ19 (Neff *et al.* 1999) (Table 2.1); and the Y-chromosome sexing marker MS34A (Sundqvist *et al.* 2001) were used to generate genotypes.

Polymerase chain reactions (PCR) and dilutions were set up using a Biomek FX automated robotic pipetting system (Beckman-Coulter) to reduce human pipetting error (Bonin *et al.* 2004). PCR amplifications were performed using a 384-well format Eppendorf Mastercycler thermocycler (Eppendorf, Hamburg). Two methods were used to optimize PCR success of different sample types. For scat, hair and blood in snow samples the microsatellite multiplex combinations (Table 2.1) were amplified in a 10 µl volume containing 5 µl of Qiagen Multiplex Mix (Qiagen), 1 µl of primer mix (100 mM concentration of fluorescently labelled forward and unlabelled reverse primers), 0.4 µl of 10mg/ml bovine serum albumin (BSA), and 3.6 µl of DNA. PCR conditions for non-invasive samples had an initial denaturation and activation of the HotStarTaq of 15 minutes at 95ºC, followed by 30 cycles consisting of 30 seconds denaturation at 94ºC, 90 seconds annealing at 59ºC, and a 60 second extension at 72ºC. A final extension step of 30 minutes at 60ºC completed the PCR sequence.

Blood and tissue samples were amplified in a 10 µl volume containing 5 µl of Qiagen Multiplex Mix, 2 µl water, 1 µl of primer mix (100 mM concentration of fluorescently labelled forward and unlabelled reverse

primers), and 2 µl of DNA. PCR conditions for blood and tissue samples were an initial denaturation and activation of the HotStarTaq of 15 minutes at 95°C, followed by 35 cycles of 30 seconds denaturation at 94°C, 90 seconds annealing at 58°C, and 90 seconds extension at 72°C. A final extension step of 10 minutes at 72°C completed the PCR sequence.

Amplification products were diluted using the automated Biomek FX system and loaded on an ABI Prism 3100 Avant capillary DNA sequencer (Perkin-Elmer). Scans were scored using the GENEMAPPER ® 3.0 software package (Applied Biosystems) and then manually checked for scoring errors.

2.2.4 Genotyping Success and Genetic Diversity

Using data from all of the provisional multi-locus genotypes generated (n = 1981) I measured success as the mean number of loci that amplified for each sample type. I examined how scat storage method and the number of hairs used in an extraction affected the amplification success of DNA.

A sub-sample of blood and tissue derived genotypes that amplified all loci and were known to be from unique individuals across the entire sampling range, were used to calculate expected (H_E) and observed (H_O) heterozygosity and null allele frequencies in Cervus 2.0 (Marshall *et al.* 1998). Allelic diversity was calculated as the number of alleles per locus. Linkage disequilibrium and exact tests for Hardy-Weinberg equilibrium were implemented in Genepop 3.4 (Rousset & Raymond 1995).

2.2.5 Individual Identification

A modified version of Taberlet *et al.*'s (1996) multiple tubes approach incorporating a matching protocol (Frantz *et al.* 2003) was used to determine genotypes for non-invasive samples (Figure 2.2). Scat samples were amplified and genotyped ≥ 7 times, hair samples five times and blood and tissue samples twice. All the genotypes for a given sample were then compared and pooled to create a single provisional consensus genotype. To determine the provisional consensus genotype for non-invasive samples, each allele of a heterozygous genotype had to be present ≥ 2 times and each allele for a homozygous genotype had to be present ≥ 3 times. When more than 2 alleles were seen at a given locus the genotype was considered to be erroneous and was not recorded. The multi-locus genotypes derived were then provisionally accepted as the consensus genotype for a sample before examining them for matches with all other genotypes (Frantz *et al.* 2003).

Similar to other non-invasive genetic studies, it was possible that multiple samples came from an individual, and therefore multiple matching

genotypes for an individual existed (Taberlet & Luikart 1999). It was unknown which wolf each non-invasive sample came from making it necessary to group matching provisional consensus genotypes together to ensure each wolf was represented by a single genotype. Additionally, it was possible that more than one wolf, and therefore sample, may share the exact same genotype with another wolf by chance. As the number of loci compared between genotypes decreases, the probability of two individual wolves sharing the same genotype increases. I used a criteria for the number of loci necessary in a genotype to ensure individual wolves had a low probability of matching with other wolves using probability of identity statistics (Paetkau & Strobeck 1994). Probability of identity is used to calculate the probability that two individuals from a population have the same multi-locus genotype, and is based on the number of loci used, the allele frequencies, and the relatedness of individuals within a population (Waits *et al.* 2001). Originally probability of identity was calculated as:

$$PID = \sum p_i^4 + \sum (p_i p_j)^2$$

where p_i and p_j are the *i*th and *j*th alleles and i ≠ j (Paetkau *et al.* 1994). I used 2 methods to estimate probability of identity that are derived from the original formula. The first considers a population to be randomly mating populations and takes into account population size:

$$PID_{unbias} = \frac{n^3(2a_2^2 - a_4) - 2n^2(a_3 + 2a_2) + n(9a_2 + 2) - 6}{(n-1)(n-2)(n-3)} \qquad \text{(eqn 1)}$$

where n is the sample size, a_i equals $\sum p_j^i$, and p_j is the frequency of the *j*th allele (Paetkau *et al.* 1998). PID_{unbias} is commonly used as a lower bound for determining an adequate number of loci to use in individual identification, however when a population is highly structured or contains many related individuals even PID_{unbias} will underestimate the true probability of identity (Taberlet & Luikart 1999). A more stringent measure commonly used as an upper bound for theoretical probability of identity can be calculated using:

$$PID_{sib} = 0.25 + (0.5\sum p_i^2) + (0.5(\sum p_i^2)^2) - (0.25\sum p_i^4) \qquad \text{(eqn 2)}$$

where p_i is the frequency of the *i*th allele (Evett *et al.* 1998). PID_{sib} assumes all individuals sampled are full siblings giving a conservative estimate of probability of identity. The observed probability of identity will fall somewhere between these upper and lower bounds (Taberlet & Luikart 1999).

A sub-sample of blood and tissue derived DNA that had amplified all 13 loci from known individuals was used to calculate theoretical probability of identity. I calculated the values of eqn 1 and 2 using Gimlet

v1.3.2 (Valière 2002) with the loci ranked from least to greatest heterozygosity to account for the possibility that some scat samples may have amplified only the least variable loci.

All samples were then put through a matching protocol to assign samples to unique individuals using the program CERVUS (Marshall *et al.* 1998). Based on the calculated PID_{sib} values (see Results) samples were required to match at 7 loci, while allowing for 1 locus of the 7 to not match. Allowing for one mismatching locus accounts for possible errors in genotyping poor quality DNA samples, while the PID_{sib} values remain low enough with the 6 matching loci to be confident that the samples originate from the same wolf. When matching samples had 1 mismatched locus the original lane traces from Genemapper were reviewed and a subjective decision of which was the true allele was made based on peak quality and strength between the different samples.

A more stringent criterion for acceptance was used for provisional genotypes that did not match with any other samples. If two genotypes had only six loci each it is possible that they originated from the same wolf, but if the loci amplified from each sample were all or partially different, they may erroneously appear to be two wolves. I required non-matching provisional genotypes to have amplified ≥ 10 loci to ensure that any single genotype could be compared with ≥ 6 loci of any other provisional genotype.

2.2.6 Sex Assignment

Individual samples were tested for sex using the Y-chromosome microsatellite marker MS34A (Sundqvist *et al.* 2001). The presence of an allele for this locus indicated the sample was derived from a male wolf and the absence of an allele indicated a female. For non-invasive samples I required the allele to be seen a minimum of three times for each sample before I assigned it as a male. PCR failure of the MS34A locus in a given sample would result in the sample being incorrectly labelled a female, even though it may have originated from a male. To avoid assigning the incorrect sex, I statistically determined the rate of amplification failure at which sex determination became unreliable. I compared sex ratios obtained from hair and combined blood and tissue samples, assuming these samples would have fewer errors, to sex ratios obtained from scat samples. A G-test for heterogeneity was used to compare different cut-off values of amplification success between the sample types.

2.2.7 Error Rate Quantification

Two main sources of error occur when genotyping; allelic dropout and false alleles (Navidi *et al.* 1992; Gerloff *et al.* 1995; Taberlet *et al.*

1996). Allelic dropout is defined as "the possibility of not detecting an allele in heterozygous individuals" (Taberlet *et al.* 1996), and false alleles are false-positive allele. False alleles may be due to contamination of samples, non-specific amplification, or slippage during PCR (Bradley *et al.* 2002). Precautions were taken to avoid contamination of samples, both in the field and laboratory, however the process of generating genotypes from non-invasive samples is error prone, and quantification of error rates is necessary. Multiple methods of error rate estimation have been devised based on theoretical models (Miller *et al.* 2002), and empirical explorations (Mowat & Paetkau 2003; Hoffman & Amos 2005), see reviews by (Bonin *et al.* 2004; Broquet & Petit 2004).

The genotyping error rates for scat and hair samples were determined by comparing provisional consensus genotypes of non-invasive samples to higher quality sources of DNA from the same wolves or independent DNA extractions from the same non-invasive sample. Scat samples were collected from captive wolves from which hair was plucked to act as reference genotypes. The captive wolves were mainly fed a diet of road-killed deer and the samples were collected in summer, and then frozen shortly after defecation to simulate the conditions occurring in the field. Additionally, DNA was extracted from several scat samples twice and the resulting provisional consensus genotypes were compared. It was impossible to collect reference samples for hair found in bed sites during snow tracking so plucked hairs from wolves captured for collaring by co-operators were compared to blood samples drawn from the same wolves during the capture event. Wolf hair samples collected non-invasively ranged from individual hairs to > 40 hairs collected from a single site so I examined error rates for a range of hairs in a single extraction (1, 2, 4, and 8 hairs).

The mean probability of allelic dropout for a given locus, *j*, was calculated in two ways:

$$ADO_j = \frac{D_j}{A_j} \qquad \text{(eqn 3)}$$

$$ADO_{hetj} = \frac{D_j}{A_{hetj}} \qquad \text{(eqn 4)}$$

where D_j is the number of amplifications missing one allele for locus *j*, A_j is the total number of positive amplifications, and A_{hetj} is the number of heterozygous positive amplifications for locus *j*. Allelic dropout cannot be detected in homozygous genotypes making eqn 3 a biased estimate of allelic dropout (Creel *et al.* 2003) and eqn 4 an unbiased estimate as it only considers heterozygous genotypes (Broquet & Petit 2004). These locus specific rates of allelic dropout can then be calculated as a ratio of allelic dropout over *L* loci to the total number of genotypes (eqn 5) or the total number of heterozygote genotypes (eqn 6):

$$P_{ADO(all)} = \frac{\sum_{j=1}^{L} ADO_j}{\sum_{j=1}^{L} A_j} \quad \text{(eqn 5)}$$

$$P_{ADO(het)} = \frac{\sum_{j=1}^{L} ADO_j}{\sum_{j=1}^{L} A_{hetj}} \quad \text{(eqn 6)}$$

False alleles can be detected in heterozygote and homozygote genotypes, hence a single unbiased equation was used to calculate per locus false alleles:

$$FA_j = \frac{F_j}{A_j} \quad \text{(eqn 7)}$$

where F_j is the number of amplifications containing a false allele for locus j, and A_j is the total number of genotypes examined with positive amplifications of locus j. The total probability of false alleles is then:

$$P_{FA} = \frac{\sum_{j=1}^{L} FA_j}{\sum_{j=1}^{L} A_j} \quad \text{(eqn 8)}$$

Given the formulas above it was possible to calculate the total probability of error in a provisional consensus genotype as:

$$P_{error} = P_{FA} + P_{ADO(all)} \quad \text{(eqn 9)}$$

The biased estimate of allelic dropout (eqn 4) was used in the calculation of P_{error} as it is impossible to sum the unbiased measure of allelic dropout (eqn 5) with the total probability of false alleles (eqn 7) (Broquet & Petit 2004), however I report the results of biased and unbiased allelic dropout.

Error rates were estimated for provisional multilocus genotypes, however the matching protocol followed as a further error reducing measure which was not considered in the error calculation. Other steps which reduced error included (1) no sample amplifying < 6 loci were used (samples that amplified < 6 loci had larger error rates); (2) any sample that did not match another sample had to have amplified ≥ 10 loci (this precaution forced all samples to overlap with ≥ 6 loci in any other sample fulfilling the requirements of the PID_{sib} rule); (3) matching samples were allowed to mismatch at only one locus to account for possible genotyping error; and (4) robotic pipetting was used for PCR and dilution set ups reducing human error.

Wolf and coyote are sympatric in the study area, and coyote scat may have been erroneously collected as wolf scat. Only experienced wolf trackers were used during field collection and extra caution in identification of scats was taken when coyote tracks were present. However, when coyote tracks were not present there was a low probability that the scats collected originated from coyotes. Presence of coyote tracks was recorded when samples were collected. To address this issue I genotyped 5 tissue samples from coyotes from the study area and found 9 unique alleles from 7 loci that did not appear in any of the 482 wolf blood- and tissue-derived genotypes. All of the coyote samples had from one to 3 of the unique alleles in a genotype. Consequently, I removed any sample from the analysis that had the unique coyote alleles.

2.2.8 Population Structure

A minimum convex polygon was created around all the sample locations for each pack, and the centroid of the polygon was chosen as the geographic coordinate to represent the pack using ARCGIS. When < 3 samples were collected from a pack a centroid could not be calculated, so I used the midpoint between 2 points, or a point location if only 1 sample was collected. Geographic distance between individuals within the same pack was assumed to be zero, therefore the pack location was used to geographically position all pack members. Euclidean distances between locations were used to create a distance matrix. I examined the effects of isolation by distance (Slatkin 1993) at the individual and pack level by measuring several genetic distance measures; an estimate of relatedness, *r* (Queller & Goodnight 1989), *F*-statistics (Weir & Cockerham 1984), and Nei's standard genetic distance, D_S, (Nei 1978), and where appropriate used permutations in a Mantel test (Mantel 1967) to examine significance as implemented in SPAGEDI 1.2 (Hardy & Vekemans 2002). Where data were non-normal I used non-parametric tests to assess the significance of the results. Results were considered significant at $P \leq 0.05$.

2.2.8.1 Individual Level

At the individual level, isolation by distance for individuals was measured using pairwise estimates of *r*, (Queller & Goodnight 1989) within and between sexes and for all individuals. The standard deviation of relatedness values were calculated by jack-knifing over all loci (Queller & Goodnight 1989). When ages were known, I removed young of the year from the analysis, because they had no possibility of being dispersers.

2.2.8.2 Pack Level

For pack level analysis, only packs where ≥ 4 individuals had been identified genetically were included in analyses (n = 36 packs). Mean estimates of relatedness (Queller & Goodnight 1989) between same sex, opposite sex, and all pairs of wolves within packs was compared. I compared mean relatedness of individuals within packs from protected to those from exploited regions. Packs were considered to be from protected areas if the centroid of the sample locations fell within the boundaries of a national park. Means and standard errors were calculated by jackknifing over all loci. When comparing estimates of mean relatedness between 2 groups it is incorrect to use standard parametric statistics due to interdependence of pairwise estimates (Danforth & Freeman-Gallant 1996). Instead, I used two-sample randomization tests with 10,000 iterations implemented in POPTOOLS 2.7.1 (Hood 2005) to determine differences between means. The number of times that the difference in means of the randomized groups was greater than the observed difference in means provided a measure of significance, reported as a *P*-value.

Using packs as sample units, I calculated *F*-statistics and D_s. While the presumed high level of relatedness of individuals within packs could bias the F_{ST} and D_s values, these measures provide a relative index of genetic distance between packs (Sacks *et al.* 2005) The same 36 packs were examined for isolation by distance using F_{ST} and D_s in relation to pairwise geographic distances.

2.2.8.3 Population Level

Individuals were assigned to subpopulations using the Bayesian clustering method of STRUCTURE 2.1 (Pritchard *et al.* 2000) which assigns individual genotypes into (K) groups independent of sampling location. The program estimates the probability of the data, $Pr(X \mid K)$, and the probability of individual membership in each cluster using a Markov chain Monte Carlo (MCMC) method and assuming populations are at Hardy-Weinberg and linkage equilibrium. The estimated number of groups is assumed to be the point where log-likelihood of K asymptotes (Pritchard *et al.* 2000), however other means of estimating K were also used (Falush *et al.* 2003; Evanno *et al.* 2005). Four independent runs of K = 1-25 were carried out with burn-in and Markov Chain Monte Carlo repetitions of 500 000 each and all other settings at default values. Individuals were assigned to groups based on the highest percentage membership (q). Genetic distances measured as F_{ST} and D_s were calculated between the groups determined using STRUCTURE.

2.3 Results

2.3.1 Genotyping Success and Genetic Diversity

Levels of genotyping success varied for the four sample types (Figure 2.3) with tissue providing a mean of 10.7 loci/extraction (SE = 0.25, *n* = 348) and blood a mean of 10.5 loci/extraction (SE = 0.27, *n* = 254). Both tissue and blood samples amplified significantly better than hair ($\bar{x}$ = 7.1 loci/extraction, SE = 0.25, *n* = 452; Mann-Whitney U: *P* < 0.001), which amplified significantly better than scat ($\bar{x}$ = 4.0, SE = 0.15, *n* = 927; Mann-Whitney U = 55 437, *P* < 0.001). However, scats stored in 100% ethanol ($\bar{x}$ =6.5 loci/extraction, SE = 1.00, *n* = 25) amplified no differently than hair (Mann-Whitney U = 5456, *P* = 0.769), but much better than scat stored at -20°C ($\bar{x}$ = 3.9, SE = 0.16, *n* = 902; Mann-Whitney U = 7573, *P* = 0.004). Temperature at the time of collection was negatively correlated to amplification success (r^2 = 0.006, *p* = 0.038, *n* = 741, range: -37 to +27°C), however, time since defecation showed no relationship to amplification success (r^2 = 0.004, *P* = 0.109, *n* = 683, range: 1 – 500 hours, with 94% of the samples < 96 hours old).

The number of hairs used in an extraction was positively related to the number of loci amplified for a given sample (r^2 = 0.015, *P* = 0.038, *n* = 284; Figure 2.4). While not included in the regression, clumps of under fur with roots that were not visible to the naked eye had a mean amplification success of 8.8 loci/sample (*n* = 79; Figure 2.4), which was greater than that of fewer hairs with visible roots.

Individual loci ranged in mean rates of amplification success from locus PEZ8, which produced data for 37% of samples, to locus FH2096 which amplified in 64% of the samples (Figure 2.5). The widest range of amplification success by marker occurred in the scat samples.

Through the data cleaning process I removed 789 samples from the analysis due to poor amplification. The remaining samples were used to form consensus genotypes for individual wolves. The process of combining similar genotypes resulted in the majority of wolves (76%) in the analyses having thirteen locus genotypes. The remaining 23% of genotypes amplified less than 13 loci, and contained a total of 3.2% missing data.

The blood and tissue-derived genotypes (*n* = 304) had expected heterozygosities per locus ranging from 0.2 to 0.89 ($\bar{x}$ = 0.72, SD = 0.052), with a range of 2 (FH2834) to 16 (PEZ8) alleles per locus ($\bar{x}$ = 9.9, SD = 5.54; Table 2.1). Nine loci deviated significantly from Hardy-Weinberg equilibrium for the entire population, however this result was

expected as packs are composed of highly related individuals. In all but one case microsatellites were chosen from different chromosomes to avoid linkage disequilibrium, however disequilibrium was detected in 66 of the 78 locus pairs over the entire sample of wolves. Similar to the deviations from Hardy – Weinberg equilibrium, the large numbers of locus pairs in linkage disequilibrium were likely attributable to the presence of highly related individuals in the sample.

2.3.2 Individual Identity

The 6 least variable loci from 304 blood and tissue samples provided a PID_{random} of 8.61×10^{-05} (i.e. 1/11,614 genotypes in a randomly breeding population may be identical) and a PID_{sib} of 1.87×10^{-02} (i.e. 1/55 siblings may share identical genotypes; Table 2.2). No samples with a $PID_{sib} > 1 \times 10^{-03}$ (ie. the possibility of 1/100 unique individuals sharing the same genotype) were included in the analysis

From 1981 samples, 540 individual wolves were identified, including 96 wolves identified solely from non-invasive samples, with the remainder of genotypes derived from blood and tissue, or a combination of non-invasive and invasive samples from the same wolf. Comparing pack size estimates from snow-tracking to genetic estimates for 23 packs resulted in over-estimation by genetic methods in 4 packs (maximum 3 more wolves than snow tracking), under estimation by genetic methods in 15 packs (maximum 4 wolves fewer than snow tracking), and congruence between the two methods in 4 packs (Figure 2.6). In 5 cases of underestimation by genetic methods there were fewer samples that provided usable DNA than there were individuals identified by tracking, making it impossible for the estimation from genetic methods to equal the snow-tracking count. The number of wolves identified genetically per pack ranged from 1 to 11 with a mean of 3.6 (SE = 0.59). The mean number of wolves estimated for the same packs from snow-tracking was 4.6 (SE = 0.58, range 2-12).

2.3.3 Sex Assignment

The female to male sex ratio derived from scat samples was significantly different from hair, blood, and tissue samples when amplification success was ≤ 30% (χ^2: $P < 0.001$; Figure 2.7). A G-test for heterogeneity revealed that sex ratios from blood and tissue samples were not significantly different regardless of the rate of amplification success, however there was significant heterogeneity (G-test: G = 187.3, df = 10, $P < 0.001$) of sex ratios for scat samples across the amplification success gradient. I used a simultaneous test procedure to find that once

amplification success was ≥ 30% the sex ratios for scat samples were a homogenous group. The female : male sex ratio for all 540 wolves identified was 255 : 285 (0.89 : 1).

Of the consensus genotypes (n = 540) derived from all of the possible amplifications (n = 1981) 76% amplified all 13 loci and no samples had amplification rates < 60% allowing for high confidence in the sex assignment of individual wolves.

2.3.4 Error Rate Quantification

Scat samples contained the most genotyping errors for both allelic dropout ($P_{ADO(all)}$ = 0.014, A_j = 250; $P_{ADO(het)}$ = 0.021, A_{hetj} = 332) and false alleles (P_{FA} = 0.014, A_j = 250), while the plucked hair samples had no erroneous alleles when compared to blood samples (A_j =299; A_{hetj} = 220) regardless of the number of hairs used in an extraction. Error rates varied by locus for scat samples from no errors detected in 5 loci to 14.3% error in locus FH2004, with a total per locus error rate of 2.8% over all loci (Table 2.3).

One case of allelic dropout in blood was detected during the error rate estimation. Multiple hair samples from an individual wolf provided identical heterozygote genotypes at one locus while the reference blood sample was homozygous, suggesting that putative high quality DNA sources are not always free of error.

Unique alleles identified from the 5 genotyped coyote samples allowed me to remove 1.5% of scat samples from the analysis that originated from coyotes rather than wolves (n = 12 coyote samples from 784 scats). Each coyote genotype had 1 or 2 of the coyote specific alleles.

2.3.5 Population Structure

2.3.5.1 Individual Level

Isolation by distance was plotted for mean values of relatedness at 25 km intervals, and calculated using a Mantel permutation test for all pairwise comparisons (Figure 2.8). For both methods, relatedness decreased in a logarithmic fashion with distance for males (mean relatedness: r^2 = 0.88, Figure 2.8; pairwise: Mantel r^2 = 0.022, P = 0), females (mean relatedness: r^2 = 0.74, Figure 2.8; pairwise Mantel r^2 = 0.022, P = 0), and all individuals combined (mean relatedness: r^2 = 0.90, Figure 2.9; pairwise Mantel r^2 = 0.022, P = 0). The mean distance between individuals was 215 km (SE = 0.64, range: 0 – 683 km). Females separated by ≥ 75km were no more related than random individuals on average, and males reached a mean r = 0 at 100km.

2.3.5.2 Pack Level

Mean pairwise relatedness of individuals within packs (jackknifed over loci: $\bar{x}$ = 0.316, SE = 0.013, range 0.127 - 0.702, *n* = 816 pairs) was greater than relatedness between random individuals (jackknifed: $\bar{x}$ = -0.045, SE = 0.0011, *n* = 43,735 pairs). Females within packs were significantly more related to each other (jackknifed: $\bar{x}$ = 0.351, SE = 0.019, *n* = 271 pairs) than they were to males within the same pack (jackknifed: $\bar{x}$ = 0.293, SE = 0.01, *n* = 421 pairs; permutation test, *P* = 0.0059; Figure 2.10). However, relatedness between males within packs (jackknifed: $\bar{x}$ = 0.336, SE = 0.018, *n* = 326 pairs) did not differ from relatedness of opposite sex pairs in the same pack (permutation test, *P* = 0.581).

Overall, packs were genetically differentiated from one another (F_{ST} = 0.179, *P* < 0.001; D_s, $\bar{x}$ = 0.367). However, there was no relationship between genetic and geographic distance for F_{ST} (r^2 = 0.020, *P* = 0.131; Figure 2.11b), but D_s showed significant isolation by distance (r^2 = 0.044, *P* = 0.021; Figure 2.11a). While sample sizes were small, I found no difference in relatedness of individuals from packs within protected areas ($\bar{x}$ = 0.31, *n* = 4 packs) to those from exploited packs ($\bar{x}$ = 0.34, *n* = 32; permutation, *P* = 0.699). Due to the small number of protected packs, the lack of difference in genetic structure may have been an artefact of pack size. However, there was no difference between the mean number of pack members in exploited packs ($\bar{x}$ = 8.25, SE = 0.54, *n* = 32) and protected packs ($\bar{x}$ = 8.75, SE = 1.11, *n* = 4; permutation, *P* = 0.664), and no correlation between relatedness and the pack size was found (r^2 = 0.0053, *P* = 0.674).

2.3.5.3 Population Level

The Bayesian clustering analysis of STRUCTURE did not return a definitive number of subpopulations based on the individual genotypes. Over all the iterations, log-likelihood never reached an asymptote, but rather continued a slow increase at *K* = 25. The greatest difference in log-likelihood values occurred between *K* = 1 and *K* = 2, however the alpha value peaked and mean *q* settled at *K* = 4. Based on the output from STRUCTURE and examination of geographic concordance, 4 subpopulations were chosen as the most likely number. The four subpopulations clustered into the north divide (ND; *n* = 129 individuals assigned genetically to this group), north east-slopes (NE; *n* = 157), south east-slopes (SE; *n* = 92) and the southern divide (SD; *n* = 127; Figure 2.13). Individuals were assigned into subpopulations based on the highest percentage membership (*q*) in a group. The boundaries of the subpopulations were delineated by drawing polygons around contiguous packs containing ≥50% membership of individuals from one of the four

assigned subpopulations. Within the subpopulation boundaries there are exceptions to the ≥50% membership rule where individual wolves or small packs are composed partially or entirely of individuals assigned to one of the other subpopulations. The mean q value for $K = 4$ was 0.826 (SE = 0.0069, median = 0.897, range 0.340 – 0.983).

The four subpopulations were used to calculate F_{IS} ($\bar{x}$ = 0.013, SE = 0.016) and F_{ST} ($\bar{x}$ = 0.044, SE = 0.006) which were both significantly > 0.0 ($p < 0.01$), although values were small. Mean D_s between the four populations was 0.103 (S.E. = 0.031), with the strongest differentiation between SD and NE (D_s = 0.133) and NE and SE (D_s = 0.125; Table 2.4).

2.4 Discussion

This study is the first to examine population structure in a large number of contiguous wolf packs (n = 78) across a wide geographic area (≈145,000 km^2). This was made possible by combining non-invasive sampling with traditional methods of DNA collection during capture events. Genetic sampling of wolves frequently occurs at a small scale (3-15 contiguous packs e.g. Lehman *et al.* 1992; Lucchini *et al.* 2002; Creel *et al.* 2003) where substantial data on pack size and demography exist, or at a very large scale where sample locations are considered populations without knowledge of the genetic relationships of the wolves in question (e.g. Forbes *et al.* 1997; Geffen *et al.* 2004). By sampling across a region of continuous wolf occupancy I was able to sample a large number of neighbouring wolf packs to address both small and large scale questions of genetic structure.

2.4.1 Genetic Analysis

One of the main criticisms of non-invasive sampling for population inventories is the potential for creating false individuals due to genotyping error (Taberlet *et al.* 1996; Creel *et al.* 2003). Even if error rates are low for individual microsatellite loci the probability of an error in a multilocus genotype may be quite high (Creel *et al.* 2003). A "shadow effect" (Waits & Leberg 2000; Mills *et al.* 2000) may also occur where probability of identity is too high to identify individual genotypes. The shadow effect leads to underestimation of the number of individuals sampled. In this study I was able to compare the number of wolves estimated in a pack from snow-tracking to the estimate from genotyping non-invasive samples. Errors in estimating the number of wolves based on snow-tracking may have occurred making direct comparisons between methods difficult, but the majority of cases showed an under or equal-estimation of the number of wolves using genetic methods, indicating false individuals were not created. Given the low probability of two wolves sharing the same

genotype in this study (no samples with PID_{sib} >0.01 included in analysis) it is unlikely a shadow effect was occurring.

The amplification success achieved for faecal DNA was much lower than similar canid studies. Researchers working with Italian wolves generated full 6 locus genotypes in 65% of the samples they collected in winter and stored in 95% ethanol (Lucchini *et al.* 2002), while only 32% of the samples produced genotypes at 6 or more loci in my study. However, the amplification success I achieved was similar to that of badger (*Meles meles*) scat stored frozen and extracted with kits (Frantz *et al.* 2003). My comparison of scats stored frozen compared to100% ethanol storage showed that amplification success could have been improved by storing all samples in ethanol before extraction. Increased amplification success would have allowed more wolves to be identified and included in the analyses, but may not have altered the outcome of the analyses.

The deviations from Hardy-Weinberg equilibrium I found for the entire sampling area were not surprising. Multiple individuals were sampled from each pack, with packs generally being composed of closely related family members (Mech 1970). The sampling of family members and the presence of genetically differentiated groups likely created a Wahlund effect (Lehman *et al.* 1992). The Wahlund effect occurs when genetic measures are calculated as one large population when, in fact, the population is composed of distinct groups.

2.4.2 Error Rates

While non-invasively collected samples contributed greatly to this study, significant effort was required to allay concerns in the literature about the quality of such samples (Creel *et al.* 2003; Fernando *et al.* 2003; Buchan *et al.* 2005; Hoffman *et al.* 2005). Error rates for amplification of DNA from hair samples in the literature vary depending on the species and number of hairs collected. In alpine marmots (*Marmota marmota*), samples of single hairs generated errors 14.0% of the time and samples with 10 hairs only 0.29% (Goosens *et al.* 1998), however some species, such as hairy nosed wombats (*Lasiorhinus krefftti*), have been reliably typed with single hairs (Sloane *et al.* 1997). The wolf hair collected non-invasively showed a weak relationship between amplification success and the number of hairs used, though single hairs produced on average genotypes with 50% amplification. Error rates, however, were not affected by the number of hairs used in an extraction as no errors were detected. Given the reliability of extraction and low error rates, hair collection proved to be a superior source of DNA for wolves compared to scat stored frozen. However, the availability and ease of collecting scat continue to make it an important source of non-invasive DNA, and alternative storage methods such as ethanol improve the amplification success.

2.4.3 Population Structure

Some studies report that male wolves disperse farther or more frequently (Pulliainen 1965; Peterson *et al.* 1984; Wabakken *et al.* 2001; Flagstad *et al.* 2003; Seddon *et al.* 2006), while others recorded female wolves dispersing more often or farther (Fritts 1983; Ballard *et al.* 1987). Alternatively, many studies have found no differences between male and female dispersal patterns (Gese *et al.* 1991; Boyd & Pletscher 1999; Phillips *et al.* 2003; Kojola *et al.* 2006). My research shows some evidence towards males dispersing farther than females in Canadian Rocky Mountain wolves. Females within a pack were more closely related to each other than they were to males from the same pack, while males were equally related to one another as they were to females. If immigrants into packs were predominantly male a similar pattern of higher female relatedness within packs may occur. Pairwise relatedness between females decreased to zero at 75km while males reached that level at 100km, adding evidence that females disperse shorter distances.

Wolves are socially organized into family groups (Mech 1970; Lehman *et al.* 1992), which may lead to biased estimates of allele frequencies when entire packs are sampled (Allendorf & Phelps 1981; Hansen *et al.* 1997). Within the study area local extirpations and pack removals occur, however the dispersal potential of wolves allows territories that are vacated to be quickly filled when substantial source populations exist. Therefore, the interpretation of genetic distance measures between packs must be made with caution as the allele frequencies for a pack may completely change over the course of a single generation or even a year, thereby violating many of the assumptions of genetic distance measures. Where possible I removed known young-of-the-year from isolation by distance analyses, however the nature of non-invasive sampling does not allow for age determination. My results showed that individuals within packs were significantly more related than random individuals suggesting that packs were family groups. By sampling entire packs I was, by definition, including family groups. In contrast to the violations of assumptions this causes for genetic distance measures, the bias may be beneficial when examining contemporary dispersal. Packs likely have unique allele frequencies which may allow for the assignment of individuals to their natal pack and aid in the identification of migrants.

Exploitation of wolves may reduce relatedness of individuals within packs resulting in higher genetic diversity on a local scale (Jêdrzejewski *et al.* 2005). However, only minor effects of harvest regime on relatedness were found within a coyote population (Williams *et al.* 2003). Similarly, protected and exploited packs examined in the Canadian Rocky Mountains showed no difference in mean relatedness, suggesting wolf harvest was not reducing genetic structure at the pack level. Hunted wolf packs in Poland had a much lower mean relatedness within packs ($\bar{x}$ =

0.234, SE = 0.031, n = 67; (Jêdrzejewski *et al.* 2005)) than wolves exposed to hunting and trapping from across the Canadian Rocky Mountains ($\bar{x}$ = 0.316, SE = 0.013). Packs within the Canadian Rocky Mountain national parks are legally protected from harvest, however they still experience sources of anthropogenic mortality. Highways, railways, and management actions account for wolf deaths each year and few packs have their entire territory within the boundaries of a protected area (Callaghan 2002). Wolves that roam outside the boundaries of the parks are also exposed to legal harvest, blurring the distinction between protected and exploited packs in some cases.

The Bayesian clustering method of STRUCTURE (Pritchard *et al.* 2000) has proven effective for identifying subpopulation structure in a variety of species (e.g. Caizergues *et al.* 2003; Evanno *et al.* 2005), however, inconclusive or difficult to interpret results have been found for others (e.g. Worley *et al.* 2004; McRae *et al.* 2005). Social species, such as wolves, may have cryptic hierarchical structure making the determination of the true number of subpopulations difficult. The log-likelihood of K output by STRUCTURE did not allow for unequivocal determination of the number of subpopulations for the wolves in this study. However, when used in combination with mean q, α level, and geographical distribution of genetically assigned groups, a clearer picture of the number of subpopulations was obtained. It is possible that STRUCTURE failed to unequivocally determine the number of subpopulations due to underlying genetic structure at a finer scale. This fine scale structure may have resulted from the presence of packs composed of family groups.

In species where dispersal distances are equal to or larger than the size of the area sampled, distinct subpopulations are more likely to arise due to barriers to dispersal rather than isolation by distance. The length of the study area addressed here was less than the maximum dispersal distances recorded for wolves. However, a pattern of isolation by distance between individuals was observed in my investigation of the Canadian Rocky Mountain wolves. The strength of the genetic to geographic relationship was minimal beyond 100 km indicating that other factors must be playing a role in population subdivision that I found.

If isolation by distance is playing a small role in the genetic structuring of wolf populations in the Canadian Rocky Mountains then other forces must be influencing the structure. The Rockies have rugged topography, much of which is unsuitable for, and impassable by wolves. The continental divide appears to play a role in the division of the four subpopulations identified in my analysis (Figure 2.13). While the lower portion of the SD subpopulation is bisected by the divide, the north-eastern boundary appears to be associated with the height of land that creates the divide. Both the NE and SE subpopulations have their western most boundaries abutting the divide, while to the east of SE and SD the landscape is an unforested, agricultural landscape and is

unsuitable wolf habitat. The western limits of ND and SD are unknown, because west of the study area is continuously occupied wolf habitat which was not sampled, and wolves from that area may be genetically similar to the subpopulations identified. There are no obvious natural barriers between the northern and southern boundaries of groups NE and SE respectively, however two major transportation corridors pass through each subpopulation (Highway 16 and TransCanada Highway, respectively) close to these boundaries. A dispersing GPS collared wolf from the ND population paused for two days at Highway 16 before continuing southward uninterrupted to the NE subpopulation where it was subsequently killed in a motor vehicle collision (Neufeld, in press). Similarly, in Banff National Park individual wolves frequently crossed the TransCanada Highway using wildlife crossing structures during intra-territorial movements, however some juveniles avoided crossing the highway with the rest of the pack for several months before following the rest of the pack across the highway (Parks Canada, unpublished data). Of all the subpopulation boundaries delineated in this study the division between the NE and SE subpopulations had the least obvious explanation. The genetic division may exist due to prey specialization. Wolves throughout the study area prey heavily upon elk (Schmidt & Gunson 1985; Hebblewhite 2000), and the SE subpopulation is centered on an area of historically high elk densities, while the NE subpopulation generally had lower elk densities and higher numbers of deer (M. Hebblewhite, pers. comm.). Dispersing wolves have been found to direct their movements towards, and have higher survival within, landscapes similar to their natal territories (Gese *et al.* 1991). Familiarity of habitat may include prey species availability, with wolves specializing on certain species, thereby influencing genetic subdivision as found with some Arctic wolves (Carmichael *et al.* 2001).

This study shows the value of collecting samples across the range of continuously distributed populations rather than sampling from discrete locations. Ten years previous to this study wolf genetic structure was examined in Montana and the Canadian Rocky Mountains (Boyd & Pletscher 1997; Forbes *et al.* 1997). They collected samples from four distinct geographic locations along the Rocky Mountains (three of which were covered in my study). They found significant genetic distance between all populations over a range of 4500 km. On a larger scale, no effect of distance on genetic differentiation was found for wolves from across North America (Roy *et al.* 1994). Both of these studies used samples that were collected from restricted geographic areas, which may have resulted in the sampling of primarily family groups. If the 'populations' they sampled were comprised of highly related individuals then genetic differentiation between the populations would have appeared erroneously high. By sampling contiguous packs across a large geographic distance I was able to examine the effects of isolation by distance at a much finer scale that included within pack relationships. The

wolves I analysed appear to disperse from their natal packs to adjacent or close (< 100 km) territories after which the signal of isolation by distance greatly diminishes. The mean dispersal distance of radio-collared wolves in Montana was a similar distance of 96 km (Boyd & Pletscher 1999).

The population structure found in Canadian Rocky Mountain wolves does not meet the requirements outlined by the definition of evolutionary significant units (Moritz 1994), as migration occurs between the subpopulations, and there is no history of genetic isolation. However, there are significant differences in allele frequencies between the subpopulations I identified, suggesting they may fulfill the definition of management units (Moritz 1994). I also found significant genetic differentiation between packs of wolves, making them possible candidates for management units. Much broader geographical groupings based on mitochondrial DNA phylogeography of the global distribution of wolves has also been suggested as a possible level for management units (Vilà *et al.* 1999). The vastly different geographic scales, both within my study, and contrasted with that of Vilà *et al.* (1999), highlight the difficulty in standardizing the genetic requirements for management units. In fact, it is likely impossible to apply the designation of management units practically, given the current definition (Paetkau 1999). As Vilà *et al.* (1999) suggested, current genetic structure identified in wolves is a snapshot of dynamic historical processes, such as glaciation, that have always included some level of isolation and admixture between groups. On a contemporary scale, however, the presence or absence of wolves in an area may be of significant conservation concern. If populations are increasingly fragmented, then natural recolonization of extirpated regions may take longer, or not occur at all. My findings suggest that within the near continuous distribution of wolves across the Canadian Rocky Mountains, genetically differentiated groups exist. Conservation efforts should focus on maintaining connections between these groups to avoid isolating subpopulations similar to what has occurred in Europe (Wayne & Vilà 2003).

2.5 Conclusions

The use of non-invasively collected DNA allowed a much larger geographic area to be sampled and almost 96 wolves to be identified that otherwise would have been excluded from the analyses. Improvements continue to be made with genotyping faecal and other non-invasive samples, and the value of these methods should be considered when initiating studies of cryptic or wide ranging species.

All four subpopulations identified encompass landscapes that offer both protection from, and exposure to, exploitation. Further, all of the subpopulations span some type of political boundary, including provincial and federal (ie. national parks) boundaries, and managers should account

for wolf management practices in adjacent jurisdictions. Knowledge and maintenance of wolf movements between the subpopulations is also necessary to ensure genetic diversity and recolonization of regions where wolves may become extirpated in the future.

2.6 Figures & Tables

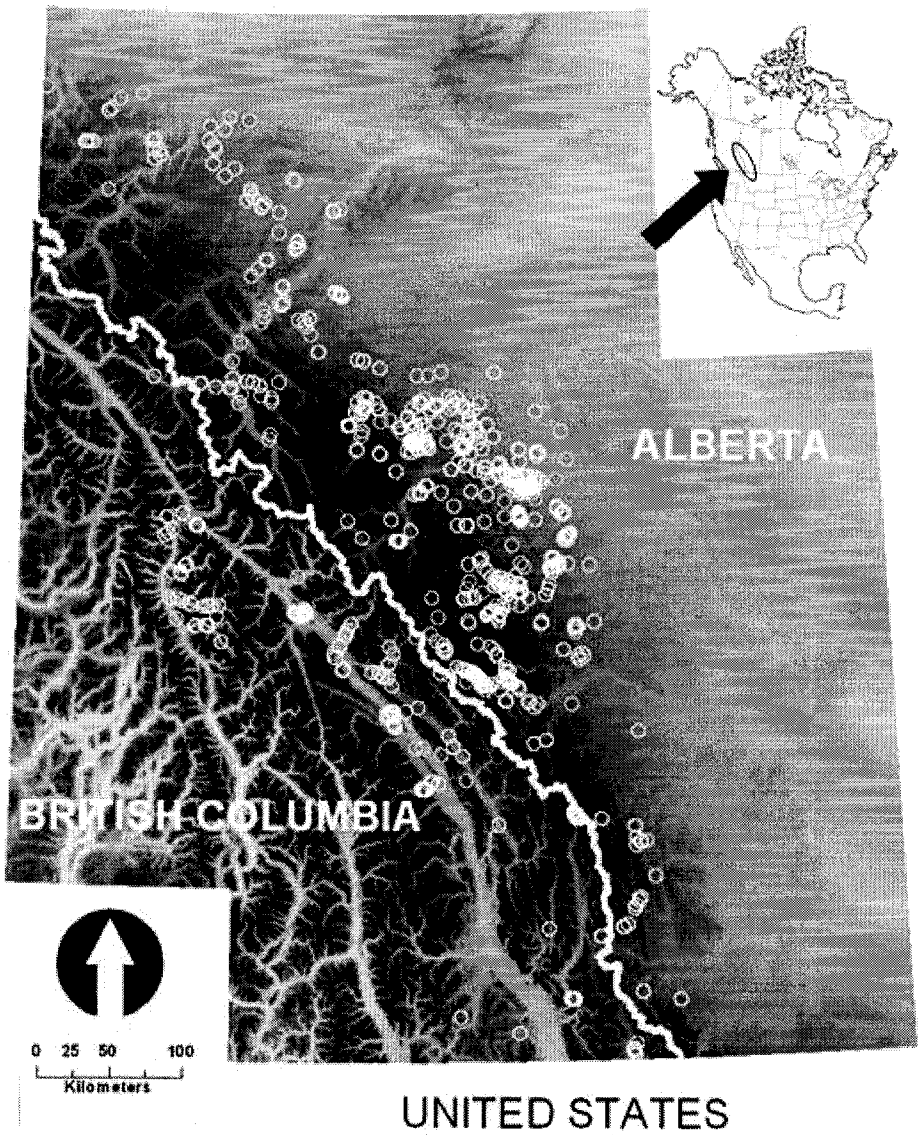


Figure 2. 1 Study area in the Canadian Rocky Mountains with wolf DNA sample locations (white circles). Elevation gradient shown from low (light) to high (dark) elevation.

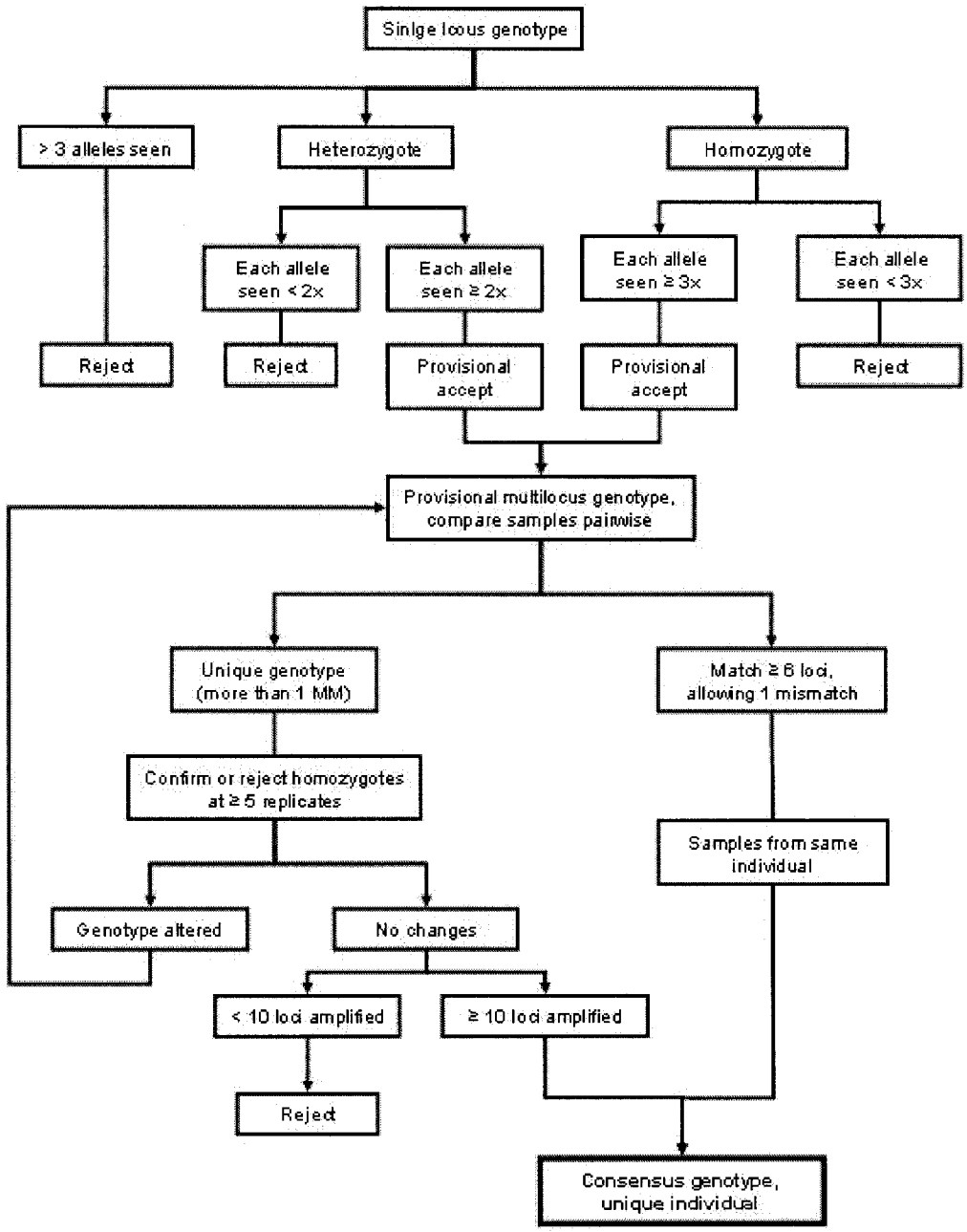


Figure 2. 2 Flow chart of provisional and consensus genotype determination for non-invasive samples collected from wolves in the Canadian Rocky Mountains. (MM = mismatch)

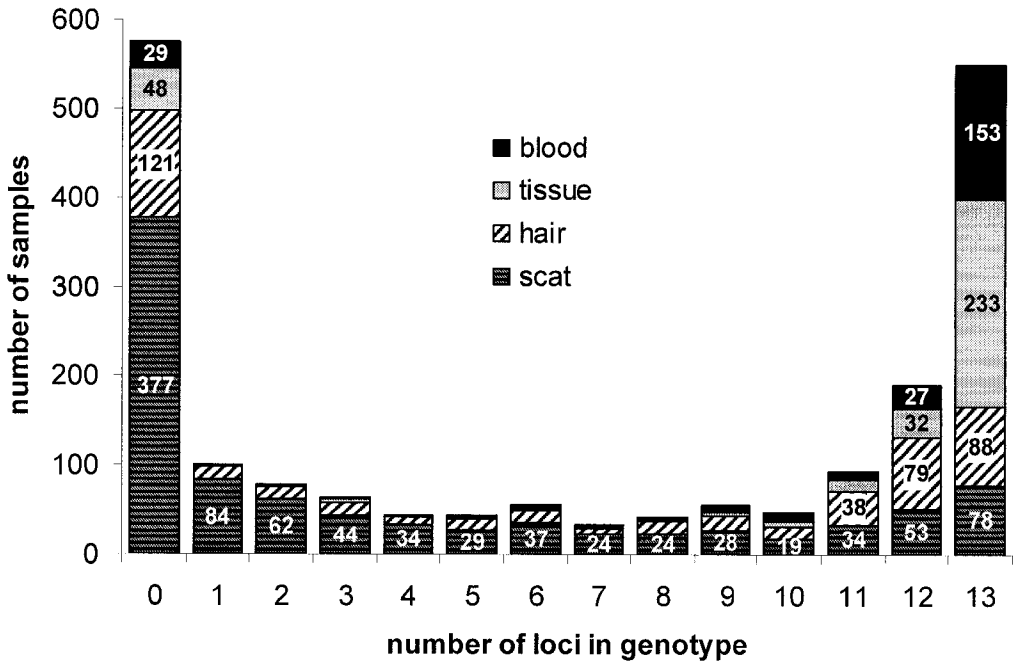


Figure 2. 3 DNA amplification success of wolf blood, tissue, scat and hair collected in the Canadian Rocky Mountains. Success ranged from complete failure (0 loci amplifying) to complete multi-locus genotypes for a given sample (13 loci amplifying). Sample size shown inside some bars.

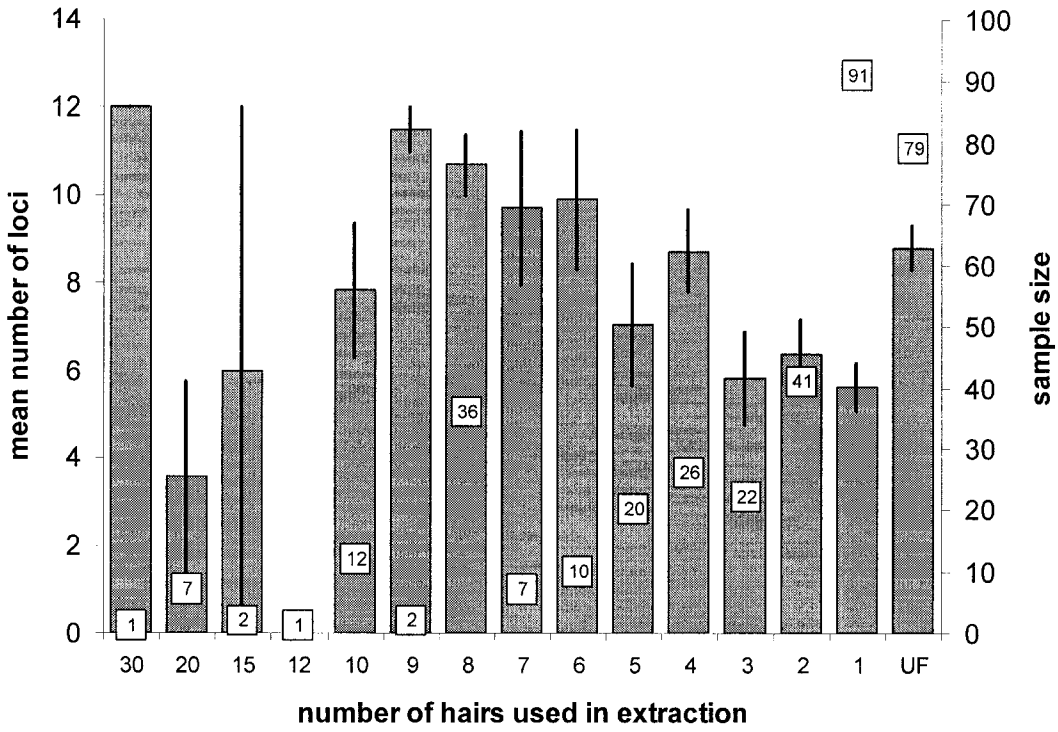


Figure 2. 4 Mean amplification success (gray bars) and number of samples genotyped (inside boxes) by number of wolf hairs used in a single DNA extraction. Error bars are 1 standard error. UF = under fur; >40 fine hairs with no visible roots.

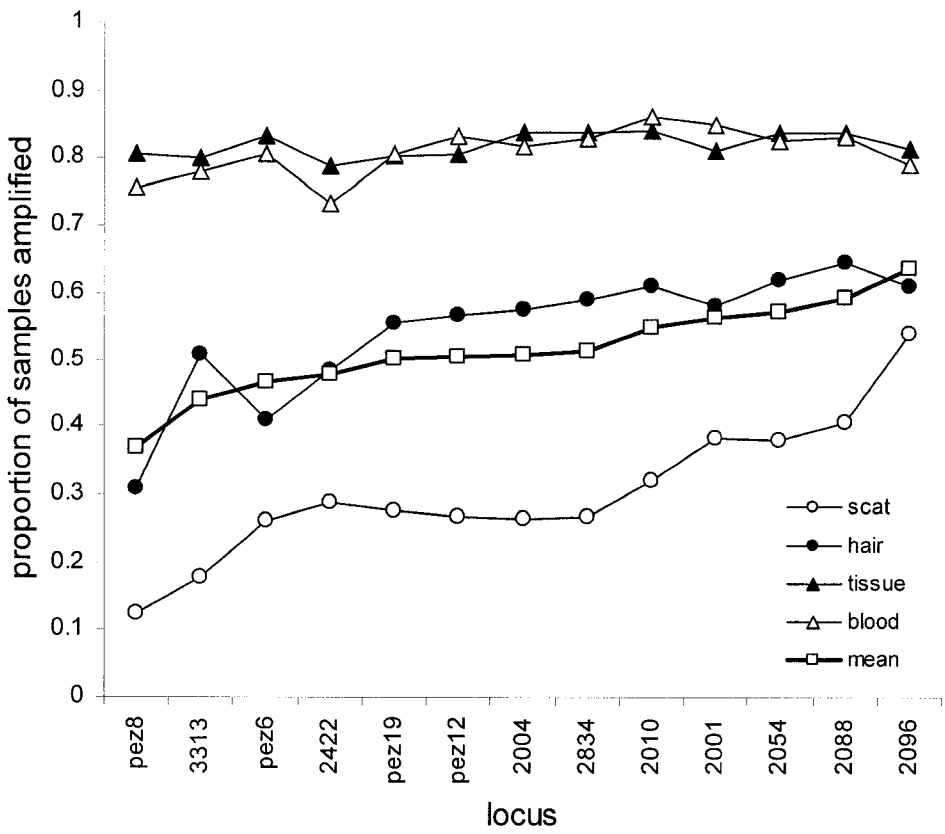


Figure 2. 5 Per locus amplification success for wolf scat, hair, tissue, blood, and the weighted mean from samples collected in the Canadian Rocky Mountains. Loci are sorted left to right from lowest to highest mean amplification success. Non-invasive samples are denoted by circles, invasive samples by triangles, and the mean for all sample types is the dark line with squares.

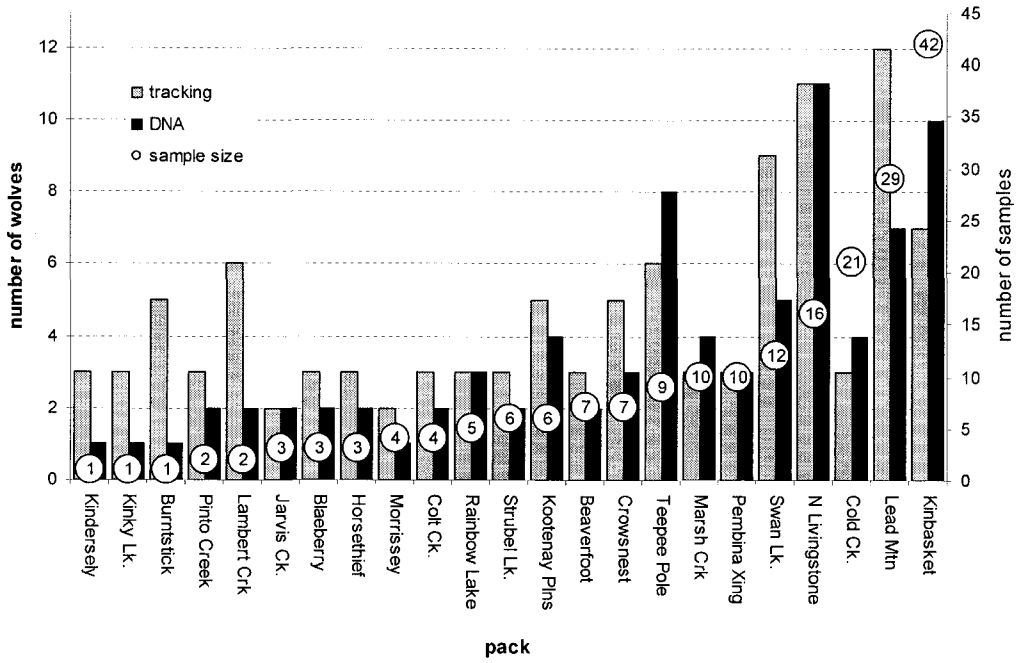


Figure 2. 6 Number of wolves identified per pack in the Canadian Rockies using snow track counts and genetic methods for packs where only non-invasive genetic samples were collected. Values in circles represent the number of samples that yielded DNA for use in determining individuals from each pack (secondary y-axis).

Table 2. 1 Individual locus diversity for the 13 microsatellite loci based on a sub-sample of 304 blood and tissue samples collected from wolves in the Canadian Rocky Mountains. Includes observed (H_O) and expected (H_E) heterozygosities, and Weir & Cockerham's F_{IS} (Weir & Cockerham 1984). Loci are organized by multiplex combinations (FH2422 was multiplexed with the sexing marker MS34, not shown). * Loci that deviate significantly from Hardy-Weinberg equilibrium ($P < 0.05$) when all individuals are pooled.

Locus	Alleles	H_O	H_E	F_{IS}	Null Allele Frequency	Allele Size Range (bp)
FH2088	7	0.68	0.68	-0.02	-0.0123	89-128
FH2054*	13	0.85	0.85	0.016	0.0045	139-172
FH2004*	15	0.78	0.84	0.073	0.0338	232-323
FH3313*	20	0.71	0.89	0.196	0.1114	343-410
PEZ19*	5	0.56	0.63	0.103	0.051	184-204
FH2001	7	0.79	0.8	0.026	0.0097	125-150
PEZ12*	11	0.75	0.78	0.041	0.0233	256-300
FH2010*	5	0.7	0.67	-0.06	-0.0297	219-235
FH2834	2	0.19	0.2	0.012	0.0038	263-265
PEZ6*	11	0.79	0.85	0.082	0.0339	167-197
PEZ8*	16	0.78	0.84	0.068	0.036	206-245
FH2096	3	0.48	0.53	0.08	0.052	96-104
FH2422*	14	0.79	0.8	0.016	0.0022	176-250
Mean	**9.9**	**0.68**	**0.72**	**0.033**	**0.0246**	

Table 2. 2 Individual and multi-locus probability of identity statistics for wolves in the Canadian Rocky Mountains over a range of microsatellite loci. Individual loci are listed from least to most informative and multi-locus probability of identity is computed with the step-wise addition of loci from least to most informative. Cumulative probability of identity is an estimate of the probability that 2 wolves will share the same genotype (e.g. for 13 loci using PID_{SIB} there is a 1 in 99,130 chance that 2 wolves will share the same genotype by chance).

single locus PID			cumulative 1/PID	
locus	unbias	sib	unbiased	sib
FH2834	0.663	0.818	2	1
FH2096	0.320	0.566	5	2
PEZ19	0.209	0.493	23	4
FH2010	0.175	0.463	129	9
FH2088	0.147	0.451	875	21
PEZ12	0.075	0.379	11,614	55
FH2001	0.066	0.366	174,833	152
FH2422	0.064	0.371	2,716,489	408
FH2004	0.043	0.341	63,573,350	1,196
PEZ8	0.042	0.343	1,520,893,539	3,486
PEZ6	0.041	0.338	37,497,375,219	10,304
FH2054	0.036	0.332	1,038,133,311,715	30,998
FH3313	0.021	0.313	49,317,496,993,582	99,130

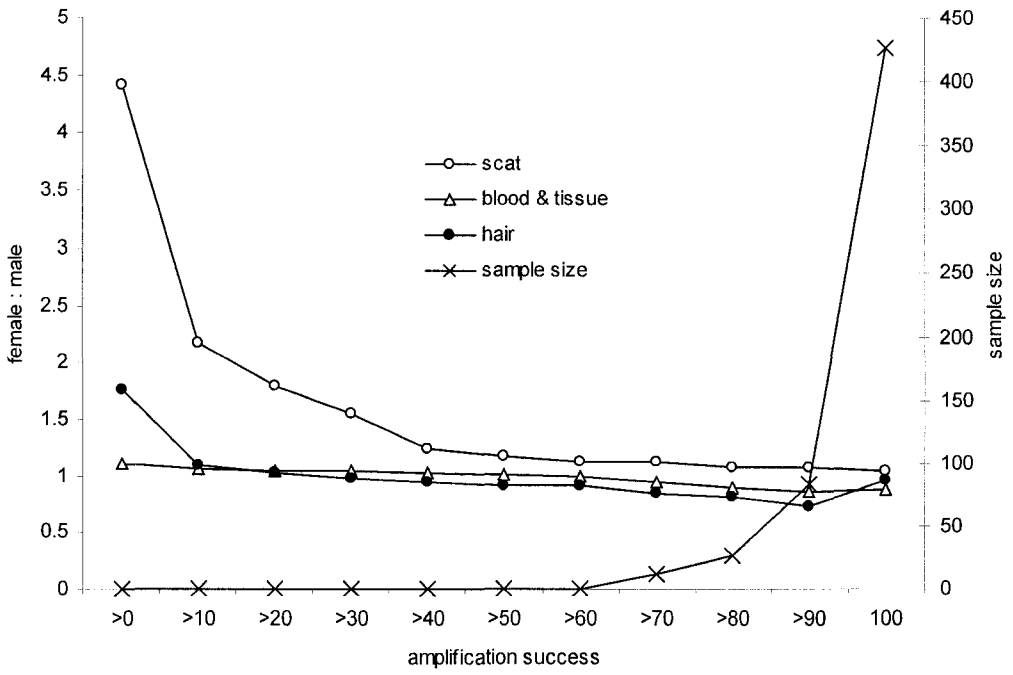


Figure 2. 7 Wolf sex ratios (female:male) obtained from all scat, hair, and blood and tissue amplifications (n = 1981), taking into account rates of PCR amplification success. Amplification success is the percentage of loci (maximum 13) that amplified for a given sample (e.g. ">50" includes samples that amplified 50-100% of the loci). The sample size line corresponds to the secondary y-axis and is the number of consensus wolf genotypes used in the final analysis that amplified at the given rate.

Table 2. 3 Genotyping error rates by locus for wolf scat samples collected in the Canadian Rocky Mountains. ADO_j is the allelic dropout rate when all loci were compared, ADO_{hetj} is for comparisons between heterozygote genotypes only, FA_j is the rate of false alleles for all loci, P_{error} is the sum of FA_j and ADO_j. A_j is the number of homo- and heterozygote loci compared, while A_{hetj} is the number of heterozygous loci compared.

Locus	ADO_j	FA_j	A_j	ADO_{hetj}	A_{hetj}	P_{error}
FH2001	0	0	25	0	21	0
FH2834	0	0	18	0	1	0
PEZ19	0	0	13	0	9	0
PEZ6	0	0	20	0	16	0
PEZ8	0	0	8	0	4	0
FH2054	0.040	0	25	0.048	21	0.040
FH2422	0.053	0	19	0.077	13	0.053
PEZ12	0	0.056	18	0	12	0.056
FH2088	0.045	0.045	22	0.059	17	0.091
FH2010	0.091	0	22	0.154	13	0.091
FH2096	0.026	0.079	38	0.050	20	0.105
FH3313	0.125	0	8	0.143	7	0.125
FH2004	0	0.143	14	0	12	0.143
Total	**0.014**	**0.014**	**250**	**0.021**	**166**	**0.028**

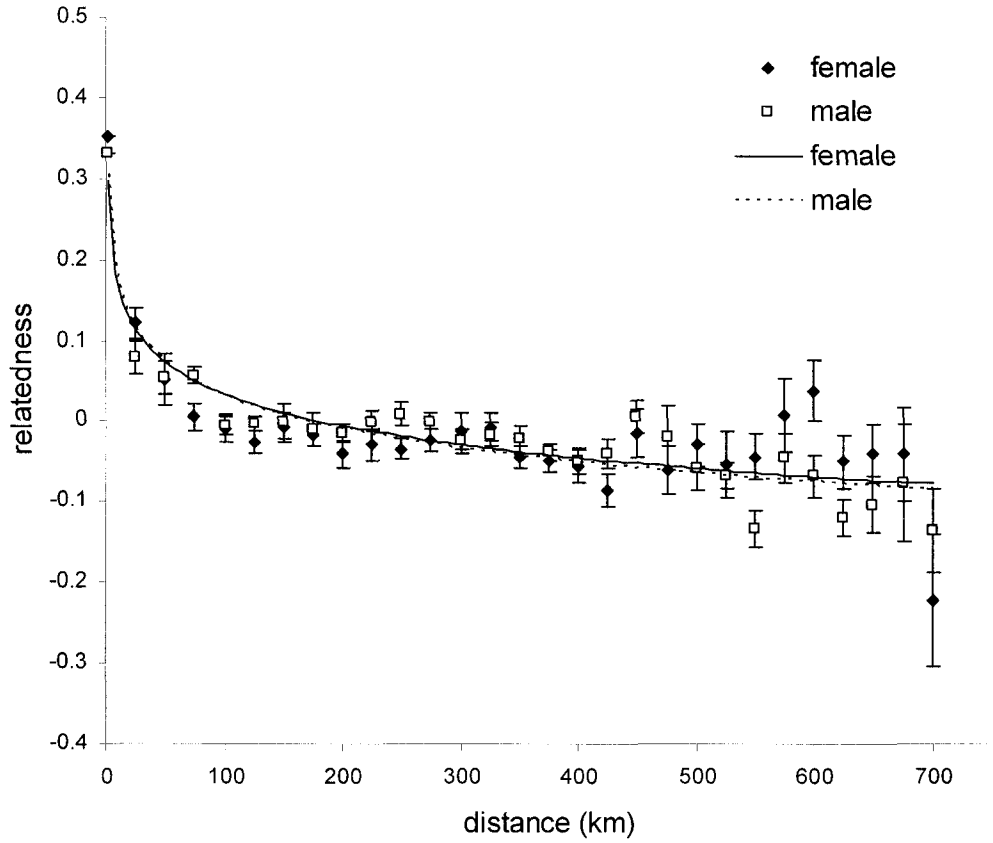


Figure 2. 8 Mean pairwise relatedness estimates (Queller & Goodnight 1989) over distance for male and female wolves in the Canadian Rocky Mountains. Logarithmic regression lines are shown for both sexes. Error bars represent one standard error.

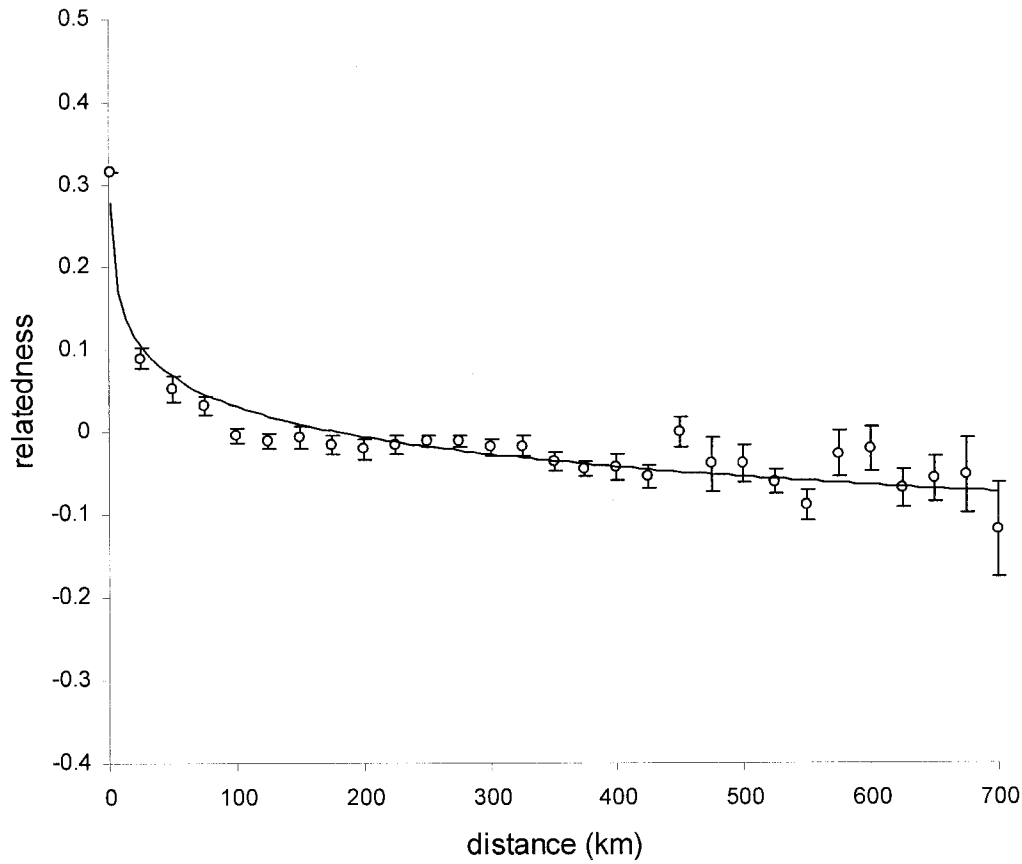


Figure 2. 9 Mean pairwise relatedness estimates (Queller & Goodnight 1989) over distance for both sexes of wolves combined. Relatedness decreased logarithmically as shown by the regression line. Error bars represent one standard error.

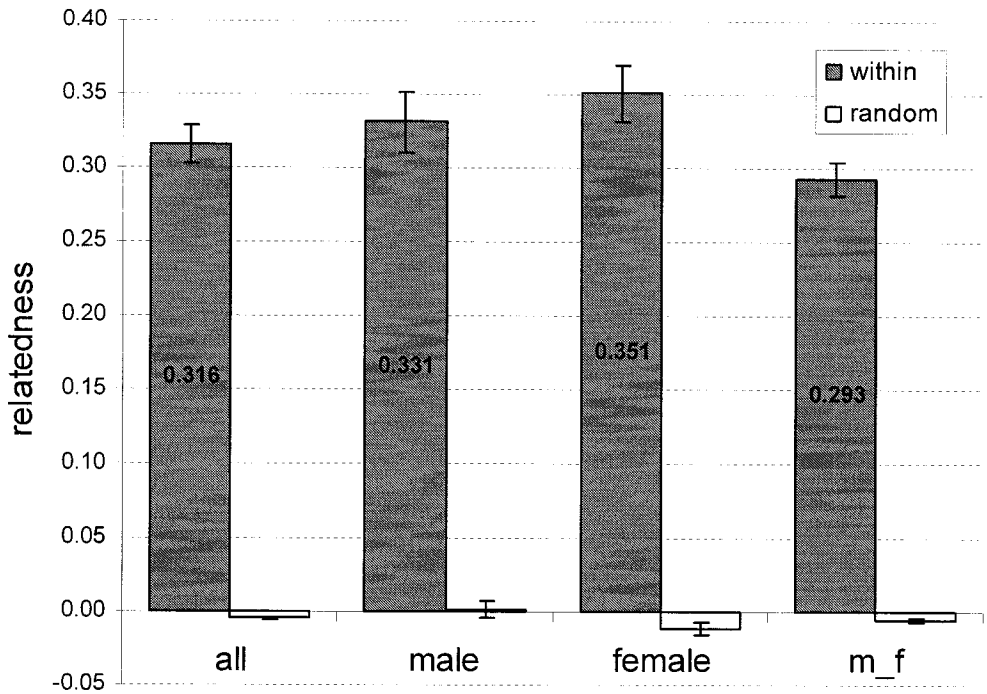
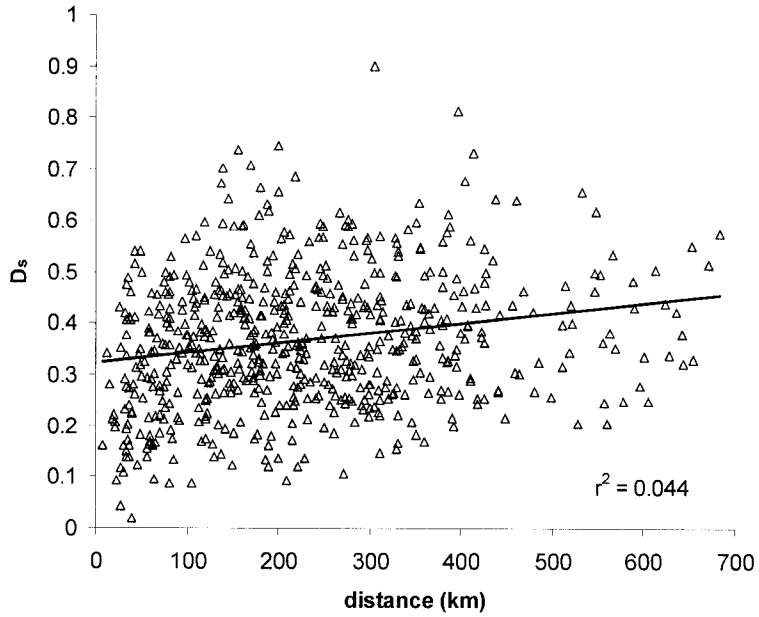


Figure 2. 10 Average pairwise relatedness, *r*, of individual wolves within packs and among random individuals across the Canadian Rocky Mountains. Comparisons are made for all members of a pack, individual sexes and between sex comparison. Error bars indicate one standard error.

a)



b)

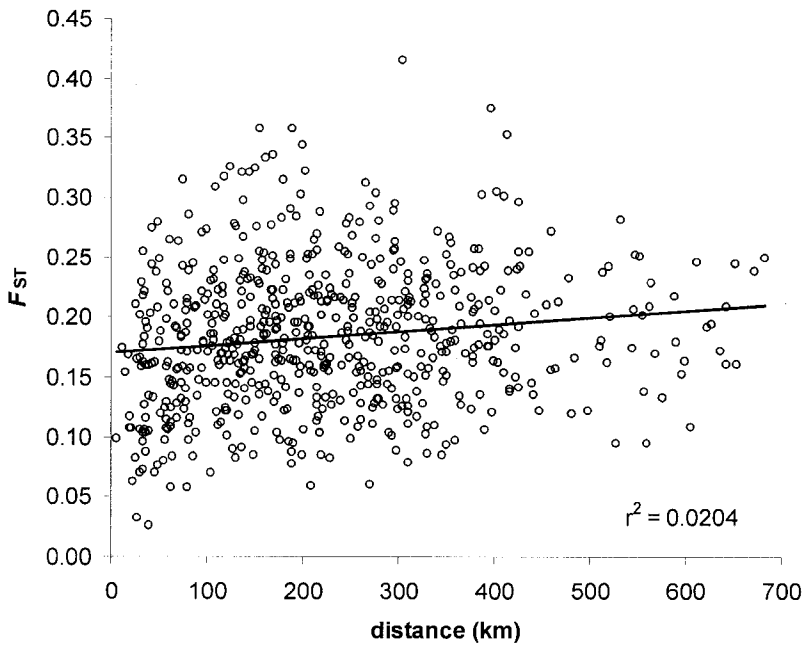


Figure 2. 11 Isolation by distance correlations for pairs of wolf packs in the Canadian Rocky Mountains. Genetic distances used are D_s (a) and F_{ST} (b). Regression line and value shown on each graph.

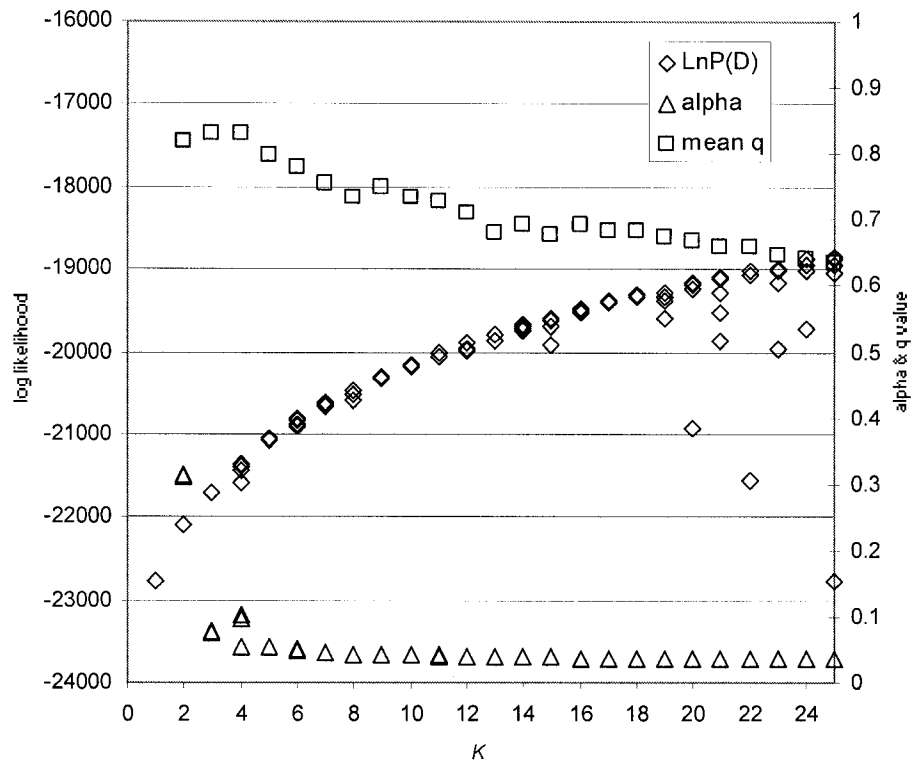


Figure 2. 12 Output from Bayesian clustering analysis of STRUCTURE 2.1 for all wolves identified genetically in the Canadian Rocky Mountains. Four iterations of the program were run for each *K*, without any prior information on population origin used. Potential groupings from 1-25 were examined. Mean *q* was calculated for each value of *K* with the data from the run with the highest LnP(D) value, and represents the mean level of assignment of an individual to a cluster. Alpha is another measure of admixture between populations for individuals.

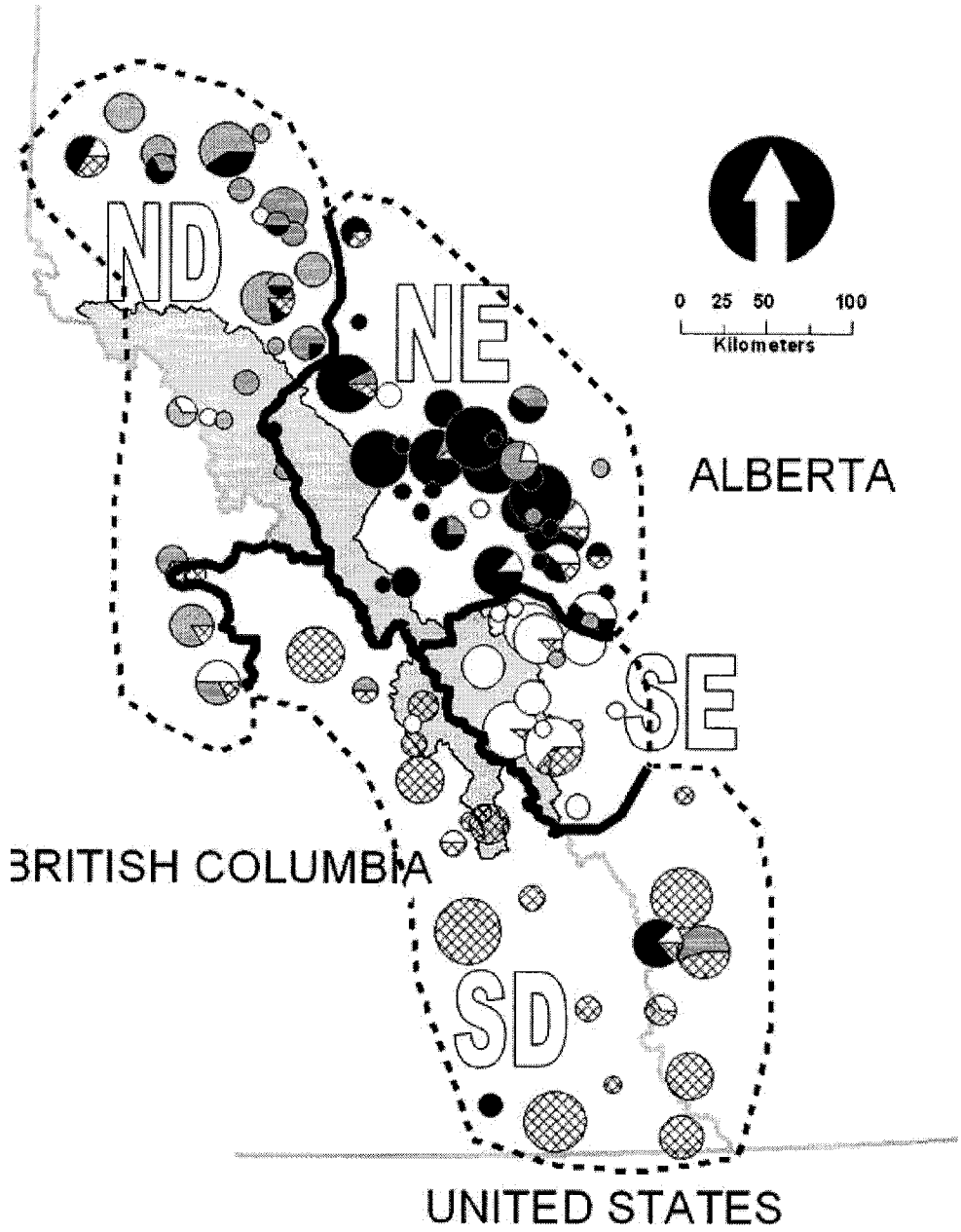


Figure 2. 13 Subpopulations of wolves in the Canadian Rockies as determined using output from the Bayesian clustering analysis of STRUCTURE. Each pack is represented by a pie chart with individual pack members shaded one of 4 colors relative to the subpopulation of their assignment. The size of the circle indicates the number of individuals genotyped from a pack (range: 1 – 14 wolves, not all wolves from every pack were successfully genotyped). Subpopulation abbreviations: ND = northern divide (black), NE = north eastslope (black), SE = south eastslope (white), SD = southern divide (dark gray). Gray polygons are national parks.

Table 2. 4 Genetic distance matrix between subpopulations of wolves in the Canadian Rocky Mountains. F_{ST} above diagonal and D_s below. Subpopulation abbreviations: ND = northern divide, NE = north eastslope, SE = south eastslope, SD = southern divide.

	SD	ND	NE	SE
SD		0.0447	0.0552	0.0477
ND	0.1041		0.0306	0.0354
NE	0.1334	0.0839		0.0537
SE	0.0974	0.0764	0.1251	

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CHAPTER 3 - Genetic analysis of contemporary wolf (*Canis lupus*) dispersal in the Canadian Rocky Mountains: barriers and asymmetric dispersal between subpopulations

3.1 Introduction

Dispersal of organisms has many short- and long-term influences on community structure and diversity (Cadotte 2006), population persistence (Newman & Tallmon 2001; Amarasekare 2004) and dynamics (Hastings 1993), individual fitness (Newman & Tallmon 2001), rates of spread, and colonization (Olivieri *et al.* 1995; Trakhtenbrot *et al.* 2005). However, the full dispersal potential for a species can be one of the most difficult population parameters to measure (Nathan *et al.* 2003).

Dispersal has been defined many ways in the literature. Dispersal can be divided into two categories: 1) natal dispersal, which is the movement of an individual from its place of birth to where it reproduces or would have reproduced if it had survived and found a mate (Howard 1960; Greenwood *et al.* 1979); and 2) breeding dispersal, which is the movement of an adult between consecutive breeding sites or groups (Greenwood & Harvey 1982). Neither of these definitions strictly imply gene flow, which is the movement of gametes, individuals, or groups of individuals that result in changes in gene frequencies in a population (Slatkin 1987).

Scientists have been measuring dispersal and gene flow using genetic methods for over 70 years. Early methods were based on indirect genetic measures of population differentiation, such as Wright's F_{ST} (Wright 1951), and the associated *Nm*, which estimates the effective number of migrants present in a population (Wright 1931). These indirect measures are based on simplistic assumptions of an island model of dispersal with equal population sizes and likely produce results correct to within only a few orders of magnitude (Whitlock & McCauley 1999), making them unsuitable for practical management purposes. More recently genetic assignment methods have been employed to identify contemporary dispersal between populations (Paetkau *et al.* 1995; Rannala & Mountain 1997; Cornuet *et al.* 1999; Pritchard *et al.* 2000; Wilson & Rannala 2003). Assignment tests compare individual genotypes to allele frequencies from predefined populations using frequentist statistics (Paetkau *et al.* 1995) or Bayesian algorithms (Rannala & Mountain 1997; Pritchard *et al.* 2000; Wilson & Rannala 2003). Results of assignment tests have been used to address a range of ecological questions including determining populations of origin for individuals (Paetkau *et al.* 1995), rates of dispersal (Waser & Strobeck 1998; Paetkau *et al.* 2004), presence of sex-biased dispersal (Favre *et al.* 1997;

Prugnolle & de Meeus 2002), and identifying barriers to dispersal (Proctor *et al.* 2005).

Demographics can vary amongst groups within a population across a landscape. When natality consistently exceeds mortality in some areas, and mortality is greater than natality in others, there may be source-sink dynamics at work (Pulliam 1988). In systems with varying demographics between groups, rates of dispersal can be asymmetric (Dias 1996). The source population, where natality exceeds mortality, will provide more migrants to the sink population, than vice versa. Sources and sinks may arise from variation in habitat quality either temporally (Virgil & Messier 2000), or spatially (Donahue *et al.* 2003). Three habitat types have been defined in heterogeneous landscapes: 1) sources, which act as net exporters of individuals; 2) true sinks, which act as net importers, and are susceptible to extinction in the absence of immigrants; and 3) pseudo-sinks, which are net importers that over time may sustain populations in the absence of immigration and eventually become sources (Boughton 1999).

Despite being studied more than almost any other wild carnivore, many questions regarding wolf (*Canis lupus*) biology remain unanswered (Fuller *et al.* 2003). Wolves have the widest naturally occurring distribution of any terrestrial mammal in the world, and are capable of some of the farthest dispersals seen in terrestrial mammals. Several studies have examined wolf dispersal (Gese & Mech 1991; Boyd & Pletscher 1999; Seddon *et al.* 2006; Kojola *et al.* 2006), yet this remains one of the least understood, and difficult aspects of wolf biology to measure.

Dispersal behaviour has important consequences for population persistence of social carnivores, such as wolves (Callaghan 2002). It is estimated that a wolf population will be composed, on average, of 10-15% dispersing wolves during the winter season (Fuller *et al.* 2003). Traditionally, radio or satellite telemetry collars have been used to quantify wolf dispersal (Gese *et al.* 1991; Boyd & Pletscher 1999). However, the inherent difficulties of capturing wolves and other species, have led to a wide range of direct and indirect genetic methods for quantifying movement of individuals (Koenig *et al.* 1996). Gene flow and relatedness in wolf packs has been examined using blood and tissue samples collected during radio-collaring and from hunter and trapper harvested individuals (Lehman *et al.* 1992; Meier *et al.* 1995; Boyd & Pletscher 1999; Carmichael *et al.* 2001). As well, non-invasive, DNA-based population sampling for wolves has been used to measure dispersal and a variety of population parameters (Lucchini *et al.* 2002; Adams *et al.* 2003; Valière *et al.* 2003; Creel *et al.* 2003; Lucchini *et al.* 2004). Recently, much work on wolf dispersal has focussed on expanding populations in Europe (Lucchini *et al.* 2002; Valière *et al.* 2003; Linnell *et al.* 2005; Kojola *et al.* 2006), yet few studies have examined dispersal in established wolf populations.

Mammals generally exhibit a pattern of male-biased dispersal (Dobson 1982), though exceptions exist (Favre *et al.* 1997; Hammond *et*

al. 2005), and dispersing females may have a larger impact on population structure than males (Tiedmann *et al.* 2000). African wild dogs (*Lycaon pictus*) are an example of a cooperatively breeding canid, similar to wolves, that follow the mammalian pattern of male-biased dispersal (McNutt 1996). While there is evidence for male-biased dispersal in some populations of wolves (Pulliainen 1965; Flagstad *et al.* 2003; Seddon *et al.* 2006), most studies have shown no propensity for one sex to disperse more than the other (Peterson *et al.* 1984; Fuller 1989; Gese *et al.* 1991; Boyd & Pletscher 1999; Phillips *et al.* 2003; Kojola *et al.* 2006).

Cryptic barriers to dispersal may exist where adjacent suitable habitats that are occupied by a species have low rates of dispersal between them (Sacks *et al.* 2004). Coyotes occupying continuous distributions in California have shown reduced dispersal between habitat types even in the absence of other barriers (Sacks *et al.* 2004). Wolves travel long distances through diverse habitats, both within their territories, and during dispersal, however evidence of barriers to dispersal for wolves is scant. Recently, genetic differentiation of wolves on a continental scale was identified, and related to differences in climate and topography (Geffen *et al.* 2004). On a smaller scale prey specialization and a major river were cited as barriers to gene flow for Arctic wolves (Carmichael *et al.* 2001), and ocean surrounding islands influenced genetic differentiation of wolves inhabiting the Alaskan archipelago (Weckworth *et al.* 2005). Wolf density may also play a role in reducing dispersal potential. Territoriality combined with high population densities may create landscapes devoid of vacant territories, and expose migrants to increased negative intraspecific interactions (Wolff 1997; Kojola *et al.* 2006). Conversely, where wolves experience high mortality from humans, turnover within packs is high and migrants may find accepting packs or vacant territories more easily (Novaro *et al.* 2005; Jêdrzejewski *et al.* 2005).

Dispersal of most species evolved with little anthropogenic influence, however human modification of landscapes and direct mortality currently influence dispersal patterns in a wide range of taxa (Epps *et al.* 2005; Wofford *et al.* 2005; Proctor *et al.* 2005; Riley *et al.* 2006). Wolves inhabit an increasingly fragmented and human dominated landscape where barriers that impede or preclude dispersal may exist. Roads and railways can be sources of mortality for wolves within their territories (Callaghan 2002; Whittington *et al.* 2005), and during dispersal (Jêdrzejewski *et al.* 2004; Neufeld, in press). As road traffic increases, the permeability of these features has been shown to decrease for wolves and other carnivores (Alexander *et al.* 2005). Accordingly, wolves often avoid high use roads within their territories (Thurber *et al.* 1994; Whittington *et al.* 2005). On the other hand, many studies have documented radio-collared wolves crossing human dominated landscapes, including high traffic roads, during periods of dispersal (Mech *et al.* 1995; Merrill & Mech

2000; Valière *et al.* 2003; Jêdrzejewski *et al.* 2004; Blanco *et al.* 2005). While roads and human dominated landscapes are often detrimental to wolves through direct mortality and alteration of behaviour within territories, to date no evidence that roads are barriers to wolf dispersal exist.

When segments of a population are exposed to hunting and others protected from harvest, source-sink dynamics may arise. Canids are frequently subject to legal and illegal harvest, the results of which have created source-sink dynamics in several species. For example, hunting of culpeo foxes (*Pseudalopex culpaeus*) in one part of their range created a demographic sink, while nearby areas without hunting acted as a source (Novaro *et al.* 2005). Protected area boundaries are often drawn along political lines, and animals often cannot recognize the significance of such invisible boundaries. However, a group of Alaskan wolves preferentially used areas within a refuge, as opposed to outside, though this tactic was not used by all wolves within the refuge (Thurber *et al.* 1994). A number of carnivores, especially wide ranging species, were shown to have population sinks associated with the boundaries of protected areas (Woodroffe & Ginsberg 1998). At these boundaries individuals that spend the majority of their time within the protected area, and are possibly naïve to the effects of human harvest, are susceptible to harvest when they travel beyond the boundary of the protected area. Also, contrary to the belief that protected areas act as sources, black bears (*Ursus americanus*) in Banff National Park experienced equal mortality from anthropogenic sources inside the park, where they were protected from harvest, as outside where legal harvest occurred (Hebblewhite *et al.* 2003). Callaghan (2002) hypothesised that the Canadian Rocky Mountain national parks are a demographic sink for wolves, despite wolves receiving protection from harvest within.

Historically, wolves were extirpated from the southern portion of the Canadian Rockies at least twice, and the second recolonization took many years to occur (Gunson 1983; Hayes & Gunson 1995). On a smaller, more contemporary scale wolves throughout Canada are exposed to legal hunting, trapping, and management actions that may remove individuals, or packs. Within the Rocky Mountains, zones of protection occur for wolves in national and provincial parks, though these protected areas have seen periods of wolf persecution in the past. The differing management regimes create a mosaic landscape of safe and risky habitats for wolves. It is important to understand how migration occurs generally for wolves, and specifically in this region, to reduce the possibility of population isolation or extirpation.

I examined patterns of dispersal for wolves inhabiting the Canadian Rocky Mountains using population genetic analyses. The goals of this study were to: 1) identify migrants and the patterns of migration between subpopulations; 2) examine patterns of asymmetric dispersal which may be indicative of source-sink dynamics; 3) compare male and female

dispersal patterns; and 4) quantify the influence of human and natural features on the landscape that may act as barriers to wolf dispersal.

3.2 Methods

3.2.1 Study Area

The study area encompassed approximately 145,000 km^2 of mountains and foothills that straddled the Continental Divide along the Canadian Rocky Mountains in British Columbia and Alberta (Figure 3.1). The topography is dominated by rugged mountain ranges and wide, flat valley bottoms aligned south-southeast to north-northwest. The Columbia River Trench follows a similar alignment, however it extends from the northern portion of the study area southward into the United States, creating continuous valley bottom habitat for the length of the study area. Elevations range from 357 to 3937 m above sea level with 4 major east – west passes bisecting the mountain ranges. Associated with 3 of the mountain passes are transportation corridors consisting of highways and rail lines. The Highway 16 follows the Yellowhead Pass in the north; the TransCanada Highway bisects the center of the study through the Vermillion and Rogers Pass; and Highway 3 crosses through the Crowsnest Pass in the southern portion of the study area. Howse Pass is the fourth low elevation route across the Continental Divide, and currently remains in a relatively natural state.

Wolves currently occupy a near continuous geographic distribution within the Rocky Mountains and surrounding foothills and boreal forest. The south-eastern portion of the study area abuts against the prairies, where currently no wolves reside. Four contiguous protected areas (Banff, Jasper, Kootenay and Yoho National Parks) totalling 20,238 km^2 exist in the Canadian Rockies, where hunting and trapping of wolves is illegal. Through the rest of the study area wolves were legally hunted and trapped. The population of wolves in this region is considered to be healthy and increasing (Hayes *et al.* 1995; Boitani 2003).

3.2.2 Sample Collection and Genetic Analysis

Tissue samples (n = 299) were collected from wolves that were harvested or died from other causes. Collaborators collected blood samples (n = 210) from wolves captured for collaring. Scat (n = 667) and hair (n = 370) samples were collected non-invasively during snow tracking sessions. The samples were collected between 1990 and 2005. DNA extraction, amplification, and genotyping followed methods outlined in Chapter 2.

3.2.3 Dispersal

Wolves were divided into genetically unique subpopulations using the Bayesian clustering method of STRUCTURE 2.1 (Pritchard *et al.*

2000) (for details see Chapter 2). The program identifies clusters of genetically similar individuals independent of geographic location. Four subpopulations were identified: the northern divide (ND), the north eastslopes (NE), the south eastslopes (SE), and the southern divide (SD; Figure 3.1). I examined contemporary dispersal between these subpopulations using assignment tests.

Many studies use only a single genetic assignment test to identify dispersers. However, results vary between tests and the use of more than one method allows for higher confidence in the assignment of dispersers (Cegelski *et al.* 2003). To identify first generation dispersal between the 4 subpopulations of wolves in the Canadian Rocky Mountains I used 3 different genetic assignment tests: the frequentist method of Paetkau *et. al.* (1995), and 2 Bayesian methods (Rannala & Mountain 1997; Pritchard *et al.* 2000).

The frequentist and Rannala & Mountain's (1997) Bayesian assignment tests were implemented in GENECLASS2 (Piry *et al.* 2004). For both methods I used the statistical criteria of L_home/L_max, which compares the likelihood of an individuals genotype in the population sampled from, to the likelihood that the individual belongs to another sampled population. When the individual is compared to the population from which it was sampled the "leave one out" procedure was used, which excludes the sampled genotype from the population for that computation. The frequentist method calculates the probability of individual assignment to each of the subpopulations utilizing area-specific allele frequencies. In order to distinguish between true migrant individuals and individuals erroneously assigned as migrants (i.e. type 1 error), 10,000 simulated individuals were created and resampled using a Monte Carlo method (Paetkau *et al.* 2004). The resampling method creates a reference population based on the distribution of genotype likelihoods from the actual population and then compares the likelihood of each individual being assigned to the distribution. A threshold value of $P \leq 0.05$ was chosen for population assignment.

Dispersers between subpopulations were also identified using STRUCTURE version 2.1 (Pritchard *et al.* 2000). The subpopulation each individual was sampled from was used as a prior in the Bayesian model (USEPOPINFO on) to determine the probability of origin for all individuals from all subpopulations. The program assigns each individual a posterior probability of occurrence (q value) for each of the 4 subpopulations. The program was run with a migration rate (v) of 0.05 for a burn-in and run time of 1 million iterations each. Output from STRUCTURE allowed individuals to be assigned into 3 categories: 1) dispersers; 2) residents; and 3) admixed individuals. The 3 categories were based on thresholds of assignment to a population at $q \geq 0.7$ (Spencer & Hampton 2005). Wolves were considered dispersers when they had $q \geq 0.7$ for a population different from the one they were sampled in. Conversely, residents had $q \geq 0.7$ for the population they were sampled from. Admixed

individuals were intermediate between migrants and residents, and were defined by maximum q values between 0.3 and 0.7. Depending on whether an individual had a resident probability above or below $q = 0.5$, it could be genetically more a resident or a migrant, respectively. Regardless of whether an individual was more of resident or migrant origin, it could not be assigned definitively to any subpopulation.

Sample collection occurred over a number of years making it possible to "recapture" samples from the same wolf. Probability of identity statistics for siblings (PID_{sib}; Evett *et al.* 1998) were used to determine the probability that two genotypes originated from the same wolf, rather than by chance. When a matching genotype was identified from 2 different subpopulations the wolf was assumed to have dispersed. The first sample location was considered the point of origin and the last location from which the wolf was sampled was considered the end point of the dispersal movement. These known dispersers were subjected to assignment tests along with the other wolves, allowing the accuracy of those tests to be examined.

Migration rates between subpopulations were calculated using the Bayesian procedure of BayesAss 1.3 (Wilson & Rannala 2003). The program calculates migration rates for first and second generation migrants allowing for deviations from Hardy-Weinberg equilibrium. The program also accommodates missing genotype data which occurs frequently with non-invasive samples. The program was run 4 times with 427 individuals. Data was sampled at intervals of 2000 with a burn in period of one million iterations and three million iterations of the data for each run.

Indirect estimates of gene flow were calculated using Nm ($Nm \approx [1-F_{ST}] / 4F_{ST}$) between subpopulations (Wright 1943). F_{ST} was calculated in SPAGeDi (Hardy & Vekemans 2002).

3.2.4 Sex Biases and Mortality

Results from the assignment tests were used to compare dispersal patterns of male and female wolves. Sex ratios of all dispersing individuals were compared to the ratios of the population as a whole using χ^2 tests. In the same manner, the sex ratios of migrant wolves from each subpopulation were examined.

Individuals determined to be migrants were identified from samples collected from both dead (carcasses) and living (non-invasive samples and live captures) wolves. Dispersing wolves may be at higher risk of mortality when travelling through unfamiliar landscapes. If mortality rates of dispersers is higher, I would expect the ratio of genotypes derived from dead to live wolves to be higher in the sample of migrants than from the population as a whole. The ratio of dispersing wolf genotypes collected from carcasses to live captures was compared to the mortality ratio of all

wolves used in the assignments with a χ^2 test. The influence of sex on mortality during dispersal was also investigated.

3.2.5 Barriers to Dispersal

I tested several potential barriers to wolf dispersal using partial Mantel tests (Smouse *et al.* 1986). The analysis tests the correlation between two matrices while controlling the effect of a third, thereby removing spurious correlations. The three matrices I used were pair-wise relatedness between individuals (Queller & Goodnight 1989), pair-wise Euclidean distance, and the location of compared individuals on the same, or opposite side of a potential barrier. Individuals on the same side of a barrier were coded in the matrix as 1s and individuals on opposite sides of a barrier were coded as 0s (Carmichael *et al.* 2001). Five features were tested for their effect as barriers: the Highway 16, the TransCanada Highway, Highway 3, the Continental Divide, and finally the Columbia Basin Trench (Figure 3.2). The potential barriers of anthropogenic origin (Highway 16, TransCanada Highway, and Highway 3) follow low elevation valley bottoms through mountainous terrain. Similarly, the main prey species, and thus the suitable habitat for wolves, occurs in valley bottoms. Where highways follow low elevation valleys a single pack's territory will often occur on both sides of the road (Callaghan 2002; Blanco *et al.* 2005). Features that may be barriers to individuals unfamiliar with the area are less likely to restrict movement of those that travel on either side of the potential barrier. Therefore, wolf packs with territory centroids within 10 km, or those known to range on either side of the potential barrier, were excluded from the analysis. Individuals included in the analysis were determined in two ways. First, for east/west barriers (Highway 16, TransCanada Highway, Highway 3), all individuals to the north and south of the barrier up to, but not extending past, the next barrier were included (Figure 3.3). Second, for north/south oriented barriers (Continental Divide, Columbia Basin Trench) I included all individuals within 96 km of a barrier, which was the mean dispersal distance found for wolves in Montana (Boyd & Pletscher 1999). I included the Howse Pass, located between Highway 16 and TransCanada Highway, as a control valley. The Saskatchewan River valley east of Howse Pass has a low traffic volume highway much of its length, but little permanent development. Only the area to the east of the Continental Divide was included in the control analysis, as few samples were collected on the north side of the pass west of the Continental Divide. To examine possible demographic effects of barriers I analysed males and females separately (Proctor *et al.* 2005). All tests were implemented in the program ZT (Bonnet & Van de Peer 2002) with 100,000 matrix randomizations using partial residuals (Legendre 2000). Correlations were considered significant when $P \leq 0.05$, using one-tailed tests.

3.3 Results

3.3.1 Genetic Analysis

Using a combination of non-invasive and invasively collected DNA I was able to identify 540 individual wolves from at least 78 packs. For results specific to genetic analysis see Chapter 2.

3.3.2 Dispersal

The number of individual dispersers identified by the 3 assignment tests ranged from 13 to 61 (Table 3.1). Except in 2 cases, whenever a wolf was considered a migrant by any of the methods it was assigned to the same subpopulation by all tests. Two wolves (W11 and W398) were assigned to a different subpopulation by each of the assignment methods. STRUCTURE assigned the wolves to their population of capture (origin) while the other two methods determined the wolves were migrants from different subpopulations. Given the uncertain origin of these 2 wolves, I chose to be conservative and did not consider them migrants in the rest of my analyses. Using the criteria that a wolf had to be assigned as a migrant by at least 2 of the 3 assignment methods I was able to identify 39 migrants. A total of 13 wolves were identified as migrants by all 3 methods and 26 wolves were classified as migrants by 2 of the 3 methods. Twenty two wolves were classified as migrants by only one method, thereby not meeting the criteria I had set out for identifying a migrant. STRUCTURE identified the fewest number of migrants (n = 13), while the Bayesian method of Rannala & Mountain (1997) identified the most migrants (n = 59) which was 4.5 times more than STRUCTURE. Paetkau *et al.*'s (1995) frequentist method identified 41 migrants which were more similar to the Bayesian method implemented in GENECLASS2 than to STRUCTURE. The frequentist method produced 2 individuals that were not considered migrants by any other method, and Rannala & Mountain's Bayesian analysis identified 20 individual migrants at $P < 0.05$ that were considered non-migrant by the other methods. In all cases the migrants detected by STRUCTURE were similarly assigned by the other assignment tests. STRUCTURE was able to assign 96% of the wolves as residents or migrants with high certainty ($q \geq 70\%$). No migrant had $q < 0.83$, and 9 of 13 migrants had $q > 0.9$. In total, 21 individuals were considered to be admixed from the STRUCTURE analysis (range of q values: 0.438 – 0.677), 15 of which were putative migrants based on assignment by the other two methods, 4 were identified as migrants by only 1 of the GENECLASS2 assignment methods.

Additional information collected from dispersing wolves provided detail for 2 dispersal events. Wolves W200 and W207 were killed on the

same day by a hunter in the SE subpopulation. Both of these wolves were identified as migrants from the ND subpopulation using assignment tests. An estimate of relatedness, *r*, (Queller & Goodnight 1989) suggests that they were related near the level of half-siblings ($r = 0.23$). It is possible they originated and dispersed together from the same natal pack. Wolf W174, which was putatively assigned as a migrant, was lactating at the time of its death. Lactation suggests the wolf had become a breeding member of the pack it dispersed to, thereby fulfilling the requirement of gene flow.

Three wolves were directly identified as dispersers from "recaptures" of the same genotype from more than one location or pack. Wolf W306 was legally trapped in south eastern British Columbia (SD subpopulation), however 1 month before its death, 4 scats and 1 hair sample were collected from the NE subpopulation that genetically matched the tissue sample from the dead wolf. All the assignment methods placed W306 in the NE subpopulation, indicating this wolf dispersed from there to the SD subpopulation. PID_{sib} based on 13 matching loci gave a probability of 0.00001 that the 2 genotypes were identical by chance.

The second wolf (W453) was identified from a scat collected in Jasper National Park (within the ND subpopulation), and 5 months later from a tissue sample collected by a trapper in the NE subpopulation. The two genotypes matched at 10 loci ($PID_{sib} < 0.0003$). Both GENECLASS2 assignments placed the wolf in the ND subpopulation, while STRUCTURE grouped the wolf into the NE subpopulation (Table 3.1).

The third wolf directly identified as a disperser was not classified as a migrant based on my criteria for assignment tests. Two samples matching at 11 loci ($PID_{sib} < 0.0001$) were collected from a wolf (W441) 2 months apart. The first, a hair sample, was collected from the Waiprous pack in the SE subpopulation, and the second, a scat sample, was collected from a wolf in the NE subpopulation that was not associated with a pack. STRUCTURE assigned the wolf to the SE subpopulation ($q = 0.781$), though the frequentist method only weakly considered the wolf to be from the SE subpopulation. The Bayesian assignment of GENECLASS2 grouped the wolf into the NE subpopulation (Table 3.1).

Asymmetries in the numbers of migrants exchanged between subpopulations based on assignment tests were evident (Figure 3.4). The NE subpopulation received more first generation migrants than it returned to all other subpopulations, despite it having the most wolves sampled. Both the SE and ND subpopulations provided twice or nearly twice as many migrants to the NE slopes, while only one more migrant moved from the SD to the NE than *vice versa*. Migration was unidirectional from the SD to the ND, with no migrants detected moving south into the SD from the ND.

Posterior probabilities of migration rates for first and second generation migrants were below 0.05 between all pairs of subpopulations

as determined by BayesAss (Table 3.2). The 95% confidence intervals overlapped for all of the migration rates between subpopulations, except for one pair. The migration rate from the ND subpopulation to the NE was greater than to the SE subpopulation. Indirect measures of gene flow provided a similar pattern of results as the Bayesian analysis (Table 3.3). The highest migration rate (Nm = 7.92) was between the ND and NE subpopulations.

3.3.3 Sex Biases and Mortality

The sex ratio of 245 female to 266 male wolves used in the assignment tests did not differ from 1:1 (χ^2 = 0.86, P > 0.50). Similarly, the sex ratio for all wolves identified as dispersers by the assignment tests (17 F:22 M) was not different from the total population of wolves sampled (χ^2 = 0.29, P > 0.10). The ratio of females to males that emigrated from each of the subpopulations was no different from the sex ratio of the sampled population as a whole (Table 3.4).

The ratio of individuals identified from dead wolves to those from living wolves was not different between dispersers and non-dispersers (χ^2 = 0.56, P > 0.50). Of the 17 females identified as dispersers, 9 were sampled from carcasses and 8 from living wolves. For the 22 males identified as dispersers 13 were sampled living and 9 from carcasses. The ratios of dead to living dispersers when analysed by sex was not different from the population as a whole (females: χ^2 = 0.59, P > 0.50, males: χ^2 = 0.14, P > 0.90).

3.3.4 Barriers to Dispersal

There was a weak, but negative partial correlation of the Continental Divide north of the TransCanada Highway with relatedness between individuals, when distance was accounted for (partial Mantel r = -0.038, P = 0.0056), but there was no correlation south of the highway (partial Mantel r = -0.015, P = 0.29). Subsequently, I further divided the areas north of the TransCanada Highway into segments west or east of the Continental Divide, and considered areas south of the TransCanada Highway in their entirety. There was no correlation of the presence of the Highway 16 (partial Mantel r = -0.00071, P = 0.47), or Howse Pass (partial Mantel r = -0.012, P = 0.078) east of the Continental Divide, with relatedness. The presence of the TransCanada Highway east of the Continental Divide was negatively correlated with relatedness (partial Mantel r = -0.026, P = 0.014), but the same result was not found for the TransCanada Highway in British Columbia (partial Mantel r = -0.012, P = 0.292. Examination of Highway 3 showed a weak, negative correlation of that barrier with estimates of relatedness (partial Mantel r = -0.065, P = 0.0032). The presence of the Columbia Basin Trench was also negatively

correlated with relatedness of wolves on either side (partial Mantel r = -0.029, P = 0.044). However, in all cases geographic distance had a greater influence on relatedness than barriers.

3.4 Discussion

Dispersal is a difficult parameter to measure in most species. For wide ranging, difficult to capture species, such as wolves, this task can be especially time consuming and costly. Data from radio-collared wolves allowed 31 dispersers to be identified in a Montana study (Boyd & Pletscher 1999), and 75 in the eastern forests of Minnesota (Gese *et al.* 1991). The Montana and Minnesota studies took 18 and 20 years, respectively, to collect enough data to analyse dispersal patterns. Alternatively, over 3 years I was able to document 39 dispersal events using genetic methods.

3.4.1 Dispersal

Studies of wolf dispersal traditionally focus on restricted geographic areas within which a large proportion of the wolves are collared. Dispersal rates, directions, and distances are calculated based on the number of wolves that migrate from the packs they were captured in. The typical pattern that emerges is a star-shaped array of dispersal trajectories originating from a confined area. This type of study does not permit rates of dispersal into the capture area, or rates between adjacent regions to be calculated. Such problems are associated with most studies of dispersal based on a finite study area size (Koenig *et al.* 1996; Forero *et al.* 2002). By employing genetic analysis using non-invasive sampling over a large area in combination with samples from harvested and live-captured wolves, I was able to examine rates of dispersal both to, and from, subpopulations. Having both emigration and immigration data helps to understand the dynamics of a population. However, the genetic methods I used were not without drawbacks. While I was able to identify dispersers between subpopulations, I could not detect migration out of the study area. As well, migrants may have entered the study area from populations outside of my sampling area. These two problems are associated with spatial scale. The length of the study area I examined was less than that of the longest distance dispersals recorded for wolves. This would make it impossible to detect long-distance dispersers even from one end of the study area to the other, much less from the edge of the study outward. To detect all dispersal into and out of a study area it would be necessary to collect genetic samples from a distance around the main region of focus that was equal to or greater than the longest dispersal distance for the species being studied. This type of study design is impractical for wide ranging species such as wolves, however when the question of interest

involves quantifying rates of movement between specific subpopulations then genetic assignment tests are an excellent tool.

Assignment tests can accurately determine migration rates under many circumstances (Berry *et al.* 2004; Paetkau *et al.* 2004; Talle *et al.* 2005). However, accuracy of assignment is dependent upon the genetic diversity of the population, genetic distance between sites, number of loci used, number of individuals sampled and the method of assignment employed (Cornuet *et al.* 1999; Berry *et al.* 2004). Assignment of individuals is more accurate when using loci with high levels of genetic diversity (Estoup *et al.* 1998; Björnstad & Røed 2002; Berry *et al.* 2004). Average observed heterozygosity (H_o) for this study was 0.68 (Chapter 2), which was less than that of Berry *et al.* (2004; $H_o = 0.77$) who were able to assign 65-100% of individual skinks (*Oligosoma grande*), but higher than studies of other species which had ≈ 75% success assigning individuals to populations (Maudet *et al.* 2002; Manel *et al.* 2002).

Genetic distance between populations as measured by F_{st}, was positively related to the accuracy of assigning individuals to their natal population (Paetkau *et al.* 1997; Cornuet *et al.* 1999; Berry *et al.* 2004; Paetkau *et al.* 2004), however other measures of genetic distance, such as D_{LR} (Paetkau *et al.* 1997), or plotting genotype likelihoods may give a better indication of the power of assignment tests to correctly determine populations of origin (Paetkau *et al.* 2004). Berry *et al.* (2004) was able to assign 100% of skinks from populations with $F_{st} > 0.07$, and approximately 78% of individuals when $F_{st} \approx 0.04$. F_{st} between the 4 subpopulations of wolves I examined ranged from 0.031 - 0.055, which should have been sufficient power to identify the natal population of most individuals. When genotype likelihoods for individuals based on the frequentist assignment method of Paetkau (1994) were plotted the subpopulations showed varying levels of differentiation (Figure 3.5). Visual assessment of the genotype likelihood plots suggested the ND and NE subpopulations were the least differentiated, while the NE and SD subpopulations were most distinct from one another. Visual ranking of dissimilarity between the remaining pairs of subpopulations was difficult. Measures of F_{st} were concordant with the results of the genotype likelihood plots for the most and least differentiated subpopulations, and suggested that my power to distinguish migrants would be lowest between the ND and NE subpopulations.

Simulation and empirical studies have shown that adding more loci when conducting assignment tests at a given level of genetic differentiation will improve accuracy (Cornuet *et al.* 1999; Berry *et al.* 2004). However, when differentiation between populations is low, there is only a gradual rate of improvement as more loci are added (Cornuet *et al.* 1999). Cornuet *et al.* (1999) also noted that fewer loci can be balanced with more individuals sampled per population. I used 13 microsatellite loci, which in combination with a range of 87 – 164 individuals from each subpopulation, should have provided sufficient power to identify migrants.

Cegelski *et al.* (2003) suggest the accuracy of assignment in empirical studies where the actual population of origin is unknown can be evaluated by examining the concordance among assignment tests. This method can be especially useful where the data set may not fulfill the assumptions of some or all of the tests (Cegelski *et al.* 2003). The 3 assignment methods I used each returned a different number of migrants. The Bayesian method of Rannala & Mountain (1997) as implemented in GENECLASS2 identified 61 individuals as migrants, 20 of which were not assigned as migrants by the other 2 methods. The frequentist method implemented in GENECLASS2 identified 42 migrants, only 2 of which were not identified as migrants by the other assignment tests. All 13 individuals classified as migrants by STRUCTURE were similarly assigned by the other methods. Based on the criteria that an individual was considered a migrant only if it had been identified as such by at least 2 assignment methods I was able to identify 39 putative migrants. Over half (58%) of the 26 putative migrants identified only by the 2 GENECLASS2 methods were considered admixed by STRUCTURE, and for individuals identified as migrants only by Rannala & Mountain's method 4 of 20 were considered admixed by STRUCTURE. The frequentist method assigned 16 of the 20 individuals resident probabilities ranging from 0.05 – 0.10 which were above the threshold to be considered migrants, yet were not assigned strongly to their subpopulation of capture. The level of statistical stringency applied could influence whether or not individuals are considered migrants. I examined how a change in stringency for the 2 GENECLASS methods would affect identification of migrants by altering the cut-off value from $P < 0.05$ to $P < 0.10$. When the changes were made the frequentist method identified 73 migrants, 15 of which were not identified by the Bayesian method, and the Bayesian method identified 77 migrants, 32 of which were not identified by the frequentist method. Reducing the stringency of the method led to similar total numbers of dispersers identified by the two methods, but increased the proportion of individuals that one method declared a migrant and the other identified as resident from 17% at $P < 0.05$ to 31% at $P < 0.10$. Berry et al. (2004) found the number of migrant skinks between some populations was over-estimated by Rannala & Mountain's method, which reinforces the need to use more than one assignment test.

On average 10 – 15% of a wolf population are migrants during the winter (Fuller *et al.* 2003). The number of migrants I detected was approximately half that expected. However, I detected migration only between subpopulations, and undoubtedly migration is also occurring between packs within subpopulations. Pack to pack movements occur, but are difficult to determine using the genetic methods I employed. Collared wolves within my study area were observed dispersing between packs (M. Hebblewhite, pers. comm.; L. Neufeld, pers. comm.; N. Webb, pers. comm.). I attempted to quantify between pack dispersal by implementing similar genetic analyses as I used for subpopulations, but by

substituting packs for subpopulations. Results of assignment tests were highly variable and the confidence intervals of BayesAss were overlapping in almost all cases. The method did show some promise, but the small sample size for individuals within packs is likely a limiting factor.

Information on dispersal and population size alone do not provide sufficient information to define sources and sinks in the strictest sense, unless local populations are at equilibrium in terms of growth, or the difference between local immigration and emigration are much greater than the amount which the population's growth rate varies from 1.0 (Runge *et al.* 2006). As well, source-sink dynamics should be measured over longer periods to avoid labelling of areas as sources or sinks based on short term data, which may be skewed by stochastic processes (Dias 1996). Therefore, if one assumes a simplistic view of sources and sinks, where over one generation a source is a net exporter of individuals and a sink is a net importer (Boughton 1999), then one can speculate on these dynamics based solely on dispersal data. Asymmetric rates of dispersal were observed between several of the wolf subpopulations in the Canadian Rocky Mountains. This asymmetry was evident from the results of genetic assignment tests, and Bayesian estimation of recent migration rates. I did not have data on growth or survival rates for the subpopulations and therefore could not evaluate source – sink dynamics explicitly. However, the information on dispersal rates relative to the number of samples collected from each population reveals current patterns of migration that may be indicative of source – sink dynamics. Genetic assignment tests showed the NE subpopulation received more immigrants from every other subpopulation than it supplied emigrants. In concordance with the assignment test results, the highest numbers of migrants were detected moving from the ND to NE using the Bayesian algorithm of BayesAss, and the least differentiation between subpopulations using F_{st} was between the NE and ND. As a net importer of migrants the NE subpopulation can be ruled out as a source, and may be acting as a sink. Alternatively, assignment tests indicate more emigrants are leaving the SD subpopulation than immigrants entering. As a net exporter, the SD subpopulation may be functioning as a source. No migrants were detected in the SD subpopulation that originated in the ND, adding further evidence to SD being a source, but confusing the role of ND (which is a net exporter to the NE and SE subpopulations according to assignment tests).

Alternative hypotheses to source – sink dynamics exist to explain asymmetric dispersal between habitats varying in quality. A non-territorial bird, the citril finch (*Serinus citrinella*), exhibited a pattern of 'sources and pools', where migration occurred asymmetrically from low to high quality habitats opposite to what would be expected from source – sink theory (Senar *et al.* 2002). Survival of finches was greater in the high quality habitats, and Senar *et al.* (2002) hypothesized that high quality habitats were acting as demographic pools operating below carrying capacity due

to the high quality and quantity of resources available. Given the territorial nature of wolves, and the minimum spatial requirements of wolf packs (Fuller *et al.* 2003), the idea of pools of high density is unlikely. Source – sink theory originated from an evolutionary perspective where maladaptive choices lead to the extinction of certain phenotypes, however the theory generally does not include the effects of human-caused mortality on an ecological time scale (Delibes *et al.* 2001). Attractive sinks may occur where individuals choose to occupy territories based on the quality of the resources available, but some factor, often anthropogenic in nature, reduces survival or recruitment (Delibes *et al.* 2001). A persistent population may be maintained in an attractive sink even when harvest is occurring, as long as the total amount of sink habitat does not increase to the point that the entire population declines (Delibes *et al.* 2001). Without information on survival and growth rates it is impossible to determine if any of the wolf subpopulations in the Canadian Rockies are acting as attractive sinks. Varying rates of wolf hunting and trapping pressure throughout the region may provide the conditions necessary for attractive sinks to exist. Where wolves are heavily hunted and trapped, prey species may respond numerically, making the region more attractive to wolves. Wolves dispersing into reindeer management areas which are of higher quality habitat than their natal territories were more likely to be killed by humans than wolves dispersing to other regions in Finland (Kojola *et al.* 2006).

Historic wolf occupation in the Canadian Rockies is a good example of how source – sink dynamics can change over time. When wolves were extirpated from the southern portion of the Canadian Rockies in the 1950s, natural recolonization took over 30 years to occur. Despite large source populations existing in the northern portions of the Rockies and the boreal forests, reproducing populations were slow to establish. Wolf free areas may have been maintained as sinks through persistent human persecution (Gunson 1983), but other factors such as prey abundance may also have played a role. Currently, the majority of suitable habitat in the Canadian Rockies is occupied by wolves, and acts as a source population for natural recolonization of regions in the Rocky Mountains in the U.S.A. (Boyd & Pletscher 1999).

3.4.2 Sex Biases and Mortality

The assignment tests did not reveal any differences in the number of males and females that dispersed between subpopulations. These results are consistent with the majority of studies of wolf dispersal that have found no sex bias in rates of dispersal.

If a migrant dies during dispersal, or before it has an opportunity to reproduce in its new territory, then no gene flow has occurred. For the majority of migrants I identified it was not possible to determine if they had reproduced in their new territory. I found no difference between the number of migrant genotypes from dead wolves and the number of

resident wolves sampled from carcasses. This suggests that migrants and residents are equally susceptible to mortality, but does not allow quantification of effective gene flow.

3.4.3 Barriers to Dispersal

Wolves are highly mobile and individuals were found crossing a wide variety of landscape features. Beside anecdotal reports (e.g. Neufeld, in press), roads have not been shown to act as barriers to wolf dispersal. The results of my research show that some high traffic roads impede, but do not necessarily exclude, wolf movement across them.

The TransCanada Highway east of the Continental Divide had a negative influence on the relatedness between individuals. This reduction in relatedness suggests the highway is reducing dispersal between the regions on either side. A significant portion of this highway is fenced to restrict wildlife access onto the road, and the only means to cross in this section is through crossing structures designed for wildlife. Wolves use these crossing structures (Clevenger & Waltho 2000), however information is lacking on the success of dispersing wolves using the structures. Young-of-the-year wolves from territories that straddle the TransCanada Highway in the fenced region have been observed refusing to cross through the structures with the rest of the pack (Parks Canada, unpublished data). With time, the young wolves followed the pack through the crossing structures. It is uncertain how dispersing wolves, unfamiliar with these crossing structures, would perceive them. The majority of the unfenced portion of the highway has 4 lanes of high volume traffic. West of the Continental Divide the TransCanada Highway did not appear to be acting as a barrier. The western portion of the highway is not fenced, and also receives less vehicle traffic presumably making it more permeable for wolves.

Highway 3 to the south also appeared to be a barrier to gene flow. This highway has less vehicle traffic than the TransCanada Highway, but extensive urban development is associated with the valley through the Crowsnest Pass. The analysis I used was unable to determine the relative influences of traffic versus urban development on wolf connectivity, but was effective at identifying linear features in general that reduced permeability. No correlation between reduced relatedness and the Highway 16 or the control region of the Howse Pass was observed. The Columbia Basin Trench was correlated with reduced relatedness between wolves and therefore potentially acted as a barrier, however this result may have been confounded by the proximity of the Continental Divide. Few wolf packs were sampled between the Columbia Basin Trench and the Continental Divide, so the partial Mantel test would have included primarily individuals east of the Divide in the calculation. More samples would be required to increase confidence in the assumption of the Trench acting as a dispersal barrier to wolves.

The one natural feature that I tested reduced connectivity between some regions. The Continental Divide north of the TransCanada Highway was correlated with reduced dispersal, but the Divide south of the TransCanada Highway was not. South of the TransCanada Highway the distance between the Divide and non-mountainous region in Alberta narrows. With fewer mountains to cross, wolves may be better able to disperse over the Divide in this region. As well, there is no suitable wolf habitat east of the Rockies for wolves living south of the TransCanada Highway, thereby restricting them to dispersal north or west. Wolves living north of the TransCanada Highway can disperse in any direction to find suitable habitat, and may choose easier routes than crossing the Continental Divide. In mountainous terrain wolf territories primarily occupy valley bottom habitat that is restricted in width. Wolves dispersing through mountainous terrain have been shown to travel at low elevations and may be more likely to encounter an unfamiliar, and possibly hostile, pack than if they were travelling through a relatively flat landscape where the probability of encountering the resident pack would be lower. The influence of inaccessible mountainous terrain and conspecific aggression of resident wolf packs encountered at low elevations may combine to restrict wolf movement over the Continental Divide.

It is possible the reductions in connectivity I attributed to anthropogenic influences may have been due to historic genetic differentiation, rather than contemporary effects from roads (Epps *et al.* 2005). Given the dispersal potential of wolves this hypothesis is unlikely. All of the highways and the control area of the Howse Pass follow similar topographical features through the mountains. There is little reason to believe the TransCanada Highway and Highway 3 were associated with features that would create a barrier while none existed for the Highway 16 or the Howse Pass. Conversely, a natural barrier, the Continental Divide, has existed for millions of years, and has likely always acted as a natural barrier to wolf movement.

3.5 Conclusions

Genetic measurement of wolf dispersal patterns proved to be effective. Caution must be exercised when using assignment tests, however, as the method chosen to identify migrants can result in disparate estimates of the number of contemporary dispersers. By using 3 different assignment tests I was able to present a range of possible dispersal rates between subpopulations. To identify putative migrants I used the conservative approach of requiring congruence between at least 2 of 3 of the methods before assigning an individual migrant status.

Wolves are a resilient species as evident by their ability to repopulate areas from which they had previously been extirpated in the Canadian Rockies and conterminous United States. However, recolonization generally follows a significant reduction of organized wolf

persecution by humans, and population persistence is largely dependent on societal values towards wolves at a given time (Musiani & Paquet 2004). Increasing urban and industrial development in areas currently occupied by wolves in the Canadian Rockies could reduce the ability of wolves to disperse across anthropogenic features. The increasing influence of anthropogenic barriers, in association with previously occurring natural barriers, could act to slow recolonization if extirpations occur. Asymmetric rates of dispersal, suggestive of source – sink dynamics, currently exist between the 4 wolf subpopulations in the Canadian Rockies. Connectivity between the subpopulations should be maintained or enhanced to allow potential sinks to be rescued demographically by sources.

Future work should pair measures of population growth or survival of wolves in different habitats and/or populations with dispersal rates to allow source – sink dynamics to be explicitly quantified. A combination of genetic and traditional collaring methods would be effective in gathering the necessary demographic and dispersal data. Mandatory reporting of wolf harvest and simultaneous collection of tissue samples for genetic analysis by government agencies would help further this goal.

3.5 Figures & Tables

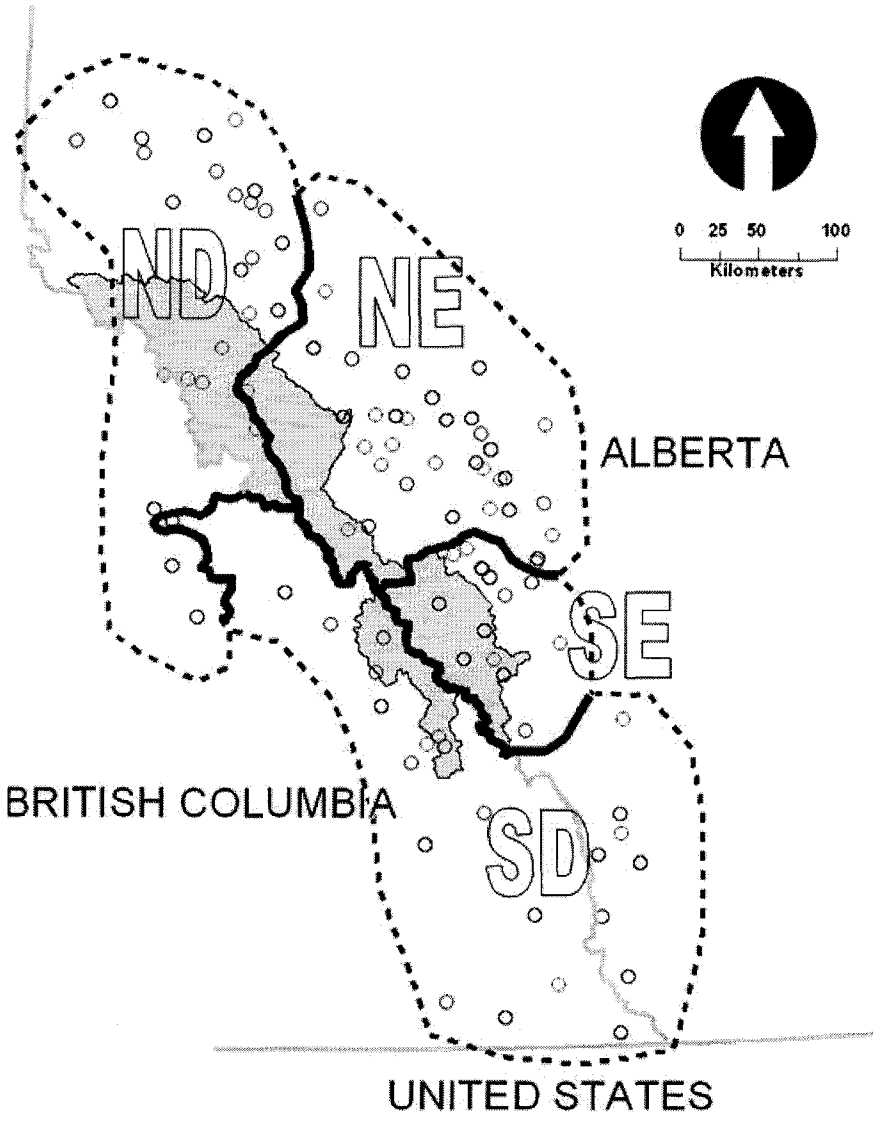


Figure 3. 1 Study area within the Canadian Rocky Mountains showing sample locations and subpopulation boundaries of wolves as determined by genetic similarity using STRUCTURE (Pritchard *et al.* 2000). Solid black line indicates division between subpopulations and dashed line indicates the boundary of the sampling region, beyond which it was not possible to determine genetic associations. Light gray line indicates provincial and international boundaries. Map also shows wolf pack locations (white circles) and national parks (gray polygons). Subpopulation abbreviations: ND = northern divide, NE = north eastslopes, SE = south eastslopes, SD = southern divide.

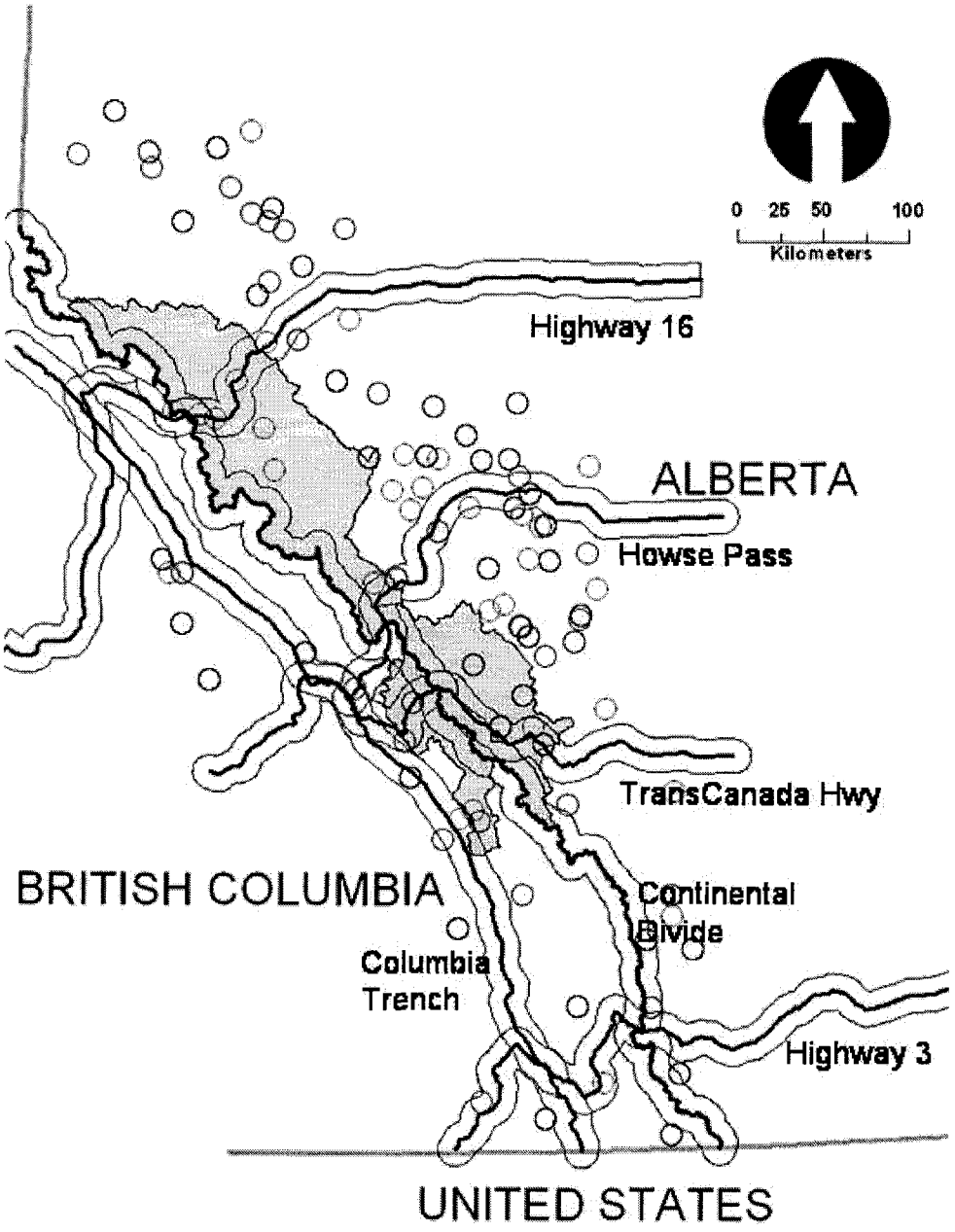


Figure 3. 2 Potential barriers to wolf dispersal examined using partial Mantel tests with relatedness, geographic distance and the presence or absence of a barrier as parameters. The 5 barriers tested were: the TransCanada Highway, Highway 16, Highway 3, Continental Divide, and Columbia Basin Trench. Samples within a 10km buffer (light black lines) around barriers were not included in analyses. Wolf pack locations are shown as black circles.

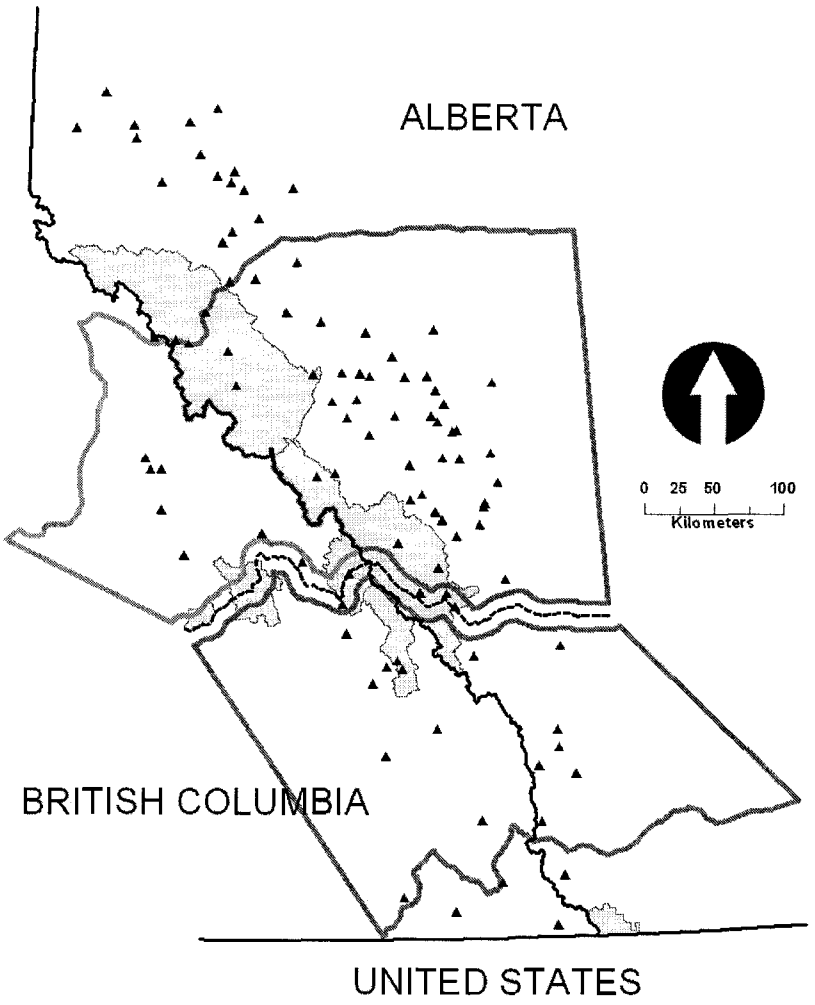


Figure 3. 3 Sampling area used to test correlation of relatedness with the presence of the TransCanada Highway as a barrier using a partial Mantel test. The TransCanada Highway is shown as a dotted line, and the 2 gray polygons to the north and south represent the regions individual wolf genotypes were drawn from. No samples were drawn from within a 10km buffer zone on either side of the potential barrier to avoid including packs with territories that overlap the barrier. The northern boundary of the north polygon was the Highway 16 and the southern boundary of the southern polygon was Highway 3. The Continental Divide, shown as a black line running through the middle of the 2 polygons was used to further subdivide the polygons into northwest, northeast, southwest, and southeast. Similar methods were employed for determining the area to draw genotypes from for the other potential barriers. Black triangles are wolf packs, generally composed of multiple wolves. The areas shaded light gray are national parks where wolf harvest is illegal.

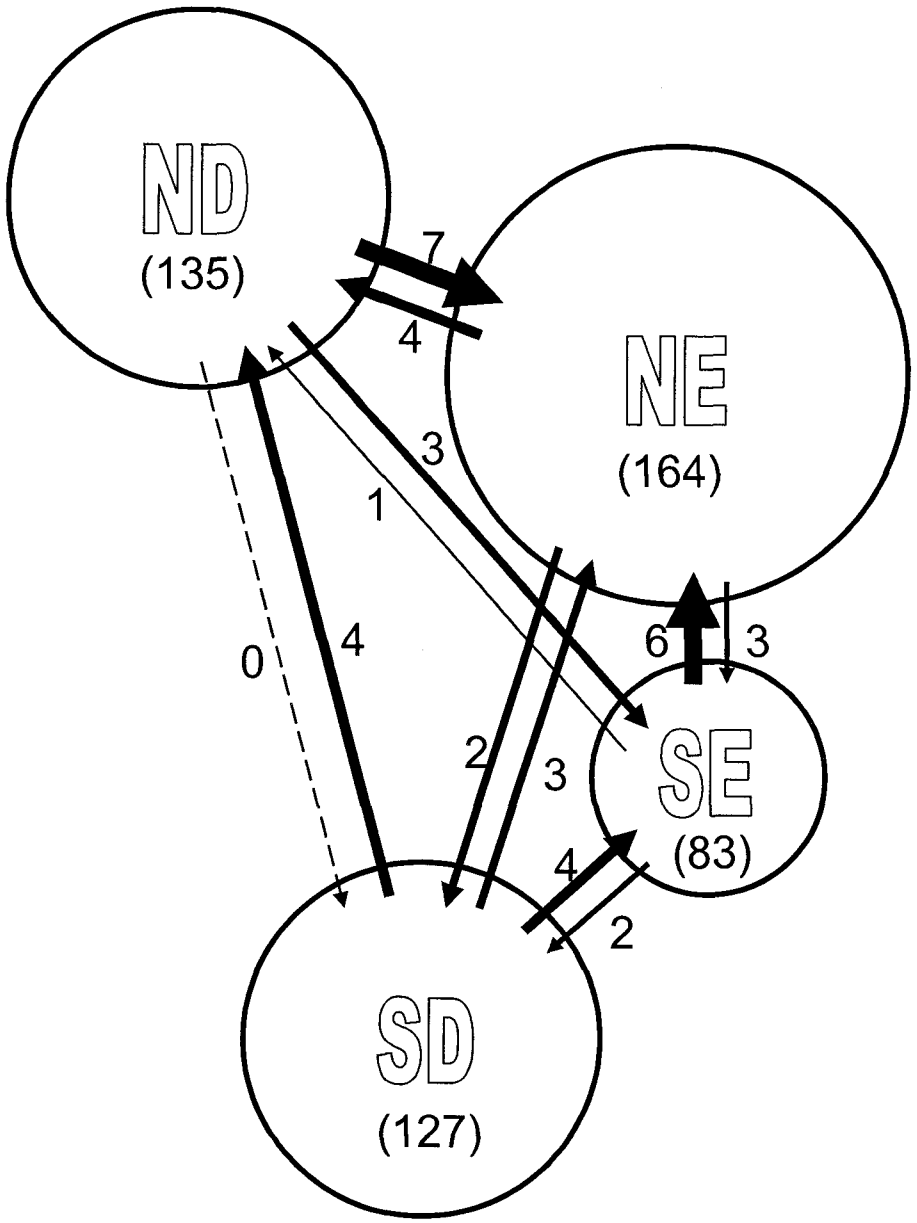
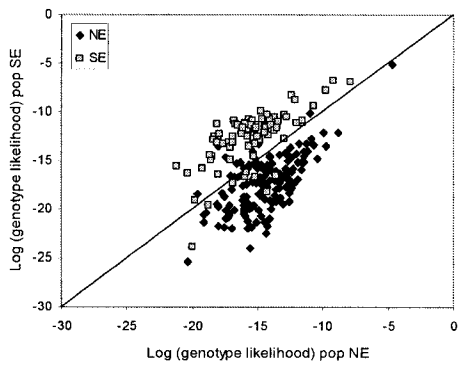


Figure 3. 4 Direction and number of wolves dispersing between subpopulations in the Canadian Rocky Mountains as determined by genetic assignment tests. Putative migrants were identified as such by at least 2/3 assignment tests. Thickness of lines indicates estimated number of migrants moving between subpopulations. Dashed line from ND to SD indicates zero migrants. Circles indicating subpopulations are scaled to sample size (as listed in brackets beneath subpopulation abbreviation).

a)



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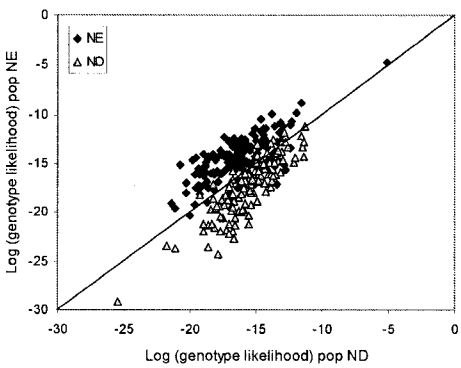

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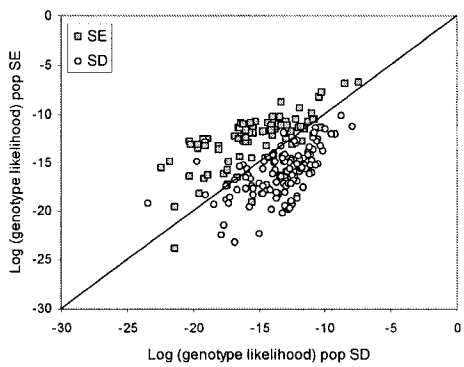

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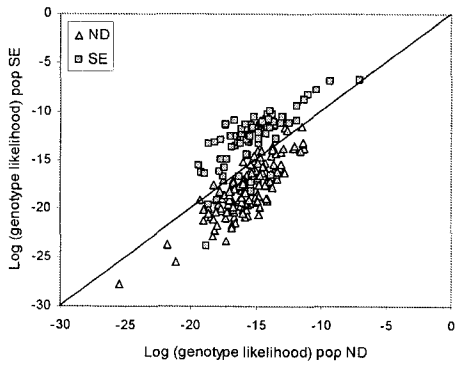

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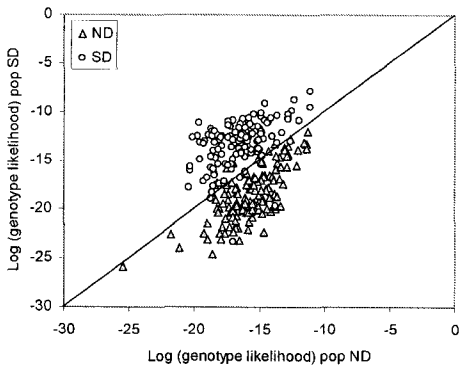

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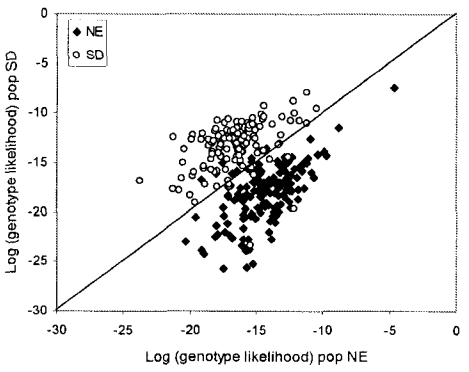

Figure 3. 5 Log likelihood ratios for individual wolf assignment to subpopulations based on the frequentist assignment method. The sloped line indicates no differentiation between individual genotype frequencies. Migrants are genetically assigned to one subpopulation, but were sampled from the other.

Table 3. 1 Individual wolves identified as migrants and their assignment to 4 subpopulations in the Canadian Rocky Mountains using 3 genetic assignment tests. Wolves above the single line through the table were considered migrants by 3/3 assignment methods, between the single and doubled line were considered migrants based on 2/3 assignment methods, and wolves below the doubled line were only identified by one assignment method and were not considered migrants. (*) indicates wolves that were identified as direct dispersals by collection of the same genotype in more than one subpopulation. AM = admixed individuals.

		Geneclass frequentist				Geneclass Bayesian				STRUCTURE			
sex	geog origin	assign pop	res prob	mig	Lh/Lmax	assign pop	res prob	mig	Lh/Lmax	assign pop	Res prob	mig	max q
F	SD	NE	0	yes	7.906	NE	0	yes	7.55	NE	0	yes	0.962
M	SD	NE	0	yes	7.618	NE	0	yes	8.56	NE	0	yes	0.995
M	SE	ND	0	yes	5.069	ND	0	yes	6.036	ND	0	yes	0.939
M	SE	SD	0	yes	3.612	SD	0	yes	7.497	SD	0	yes	0.999
F	NE	SE	0	yes	4.326	SE	0	yes	6.408	SE	0	yes	0.999
F	NE	SE	0	yes	4.431	SE	0	yes	4.411	SE	0.01	yes	0.979
F	NE	SD	0	yes	3.582	SD	0	yes	5.363	SD	0.01	yes	0.989
M	SE	NE	0	yes	3.952	NE	0	yes	3.881	NE	0.01	yes	0.904
M	SE	SD	0	yes	3.366	SD	0	yes	3.334	SD	0.04	yes	0.926
M	SE	ND	0	yes	2	ND	0	yes	4.745	ND	0.04	yes	0.862
M	ND	SD	0	yes	3.075	SD	0	yes	4.519	SD	0.11	yes	0.887
F	NE	ND	0	yes	2.835	ND	0	yes	4.6	ND	0.12	yes	0.839
F	NE	ND	0	yes	3.713	ND	0	yes	3.674	ND	0.12	yes	0.87
F	SE	NE	0	yes	2.929	NE	0	yes	2.744	NE	0.04	AM	0.517
M	NE	SD	0	yes	2.483	SD	0	yes	3.997	SD	0.18	AM	0.572
F	ND	SD	0	yes	1.682	SD	0	yes	1.848	SD	0.34	AM	0.629
F	NE	SD	0	yes	2.534	SD	0	yes	2.494	SD	0.34	AM	0.615
M	SD	SE	0	yes	1.174	SE	0	yes	1.144	SD	0.44	AM	0.438
M	NE	SE	0	yes	2.759	SE	0	yes	1.35	SE	0.45	AM	0.507
M	NE	SE	0	yes	2.078	SE	0	yes	2.073	NE	0.46	AM	0.458
F	NE	SE	0	yes	1.281	SE	0	yes	1.257	NE	0.5	AM	0.502
M	NE	SE	0	yes	1.583	SE	0	yes	1.569	NE	0.51	AM	0.514
M	NE	ND	0	yes	2.027	ND	0	yes	1.962	NE	0.52	AM	0.521
M	NE	ND	0	yes	1.275	ND	0	yes	1.224	NE	0.53	AM	0.528
M	NE	ND	0	yes	1.892	ND	0	yes	1.877	NE	0.53	AM	0.53
M	SD	NE	0	yes	1.715	NE	0	yes	1.678	SD	0.54	AM	0.537
F	SE	ND	0	yes	0.982	ND	0	yes	2.197	SE	0.56	AM	0.559
F	NE	ND	0	yes	2.261	ND	0	yes	2.221	NE	0.6	AM	0.595
F	**SD**	**NE**	**0**	**yes**	**1.04**	**ND**	**0**	**yes**	**0.13**	**SD**	**0.6**	AM	**0.6**
M	NE	ND	0	yes	1.344	ND	0	yes	1.33	NE	0.8	no	0.796
M	**NE**	**SE**	**0**	**yes**	**1.33**	**ND**	**0**	**yes**	**2.01**	**NE**	**0.8**	**no**	**0.8**
M	ND	SE	0	yes	1.151	SE	0	yes	1.123	ND	0.8	no	0.799
F	SE	SD	0	yes	1.163	SD	0	yes	1.106	SE	0.81	no	0.807
F	SE	SD	0	yes	1.087	SD	0	yes	1.065	SE	0.82	no	0.823
M	ND	NE	0	yes	1.833	NE	0	yes	1.815	ND	0.83	no	0.83
M	ND	SD	0	yes	1.689	SD	0	yes	1.663	ND	0.85	no	0.848
F	ND	SD	0	yes	1.106	SD	0	yes	1.13	ND	0.86	no	0.864
F	ND	NE	0	yes	1.188	NE	0	yes	1.19	ND	0.87	no	0.865
M	ND	NE	0	yes	0.983	NE	0	yes	0.951	ND	0.87	no	0.873
M	ND	NE	0	yes	1.051	NE	0	yes	1.05	ND	0.91	no	0.905
F	SD	SE	0	yes	0.909	SE	0	yes	0.863	SD	0.93	no	0.929
M	NE	NE	0.1	no	0.334	ND	0	yes	3.229	ND	0.32	AM	0.677
F	NE	NE	0.1	no	1.032	SE	0	yes	1.056	SE	0.32	AM	0.65
M	SE	SE	0.1	no	0.339	NE	0	yes	0.337	SE	0.53	AM	0.531
F	SE	SE	0.1	no	0.099	SD	0	yes	1.619	SE	0.64	AM	0.64
F	SD	SD	0.1	no	0.104	NE	0	yes	0.109	SD	0.74	no	0.738
M	SD	SD	0.1	no	0.634	ND	0	yes	0.22	SD	0.78	no	0.775
M	SE	SE	0.1	no	0.692	NE	0	yes	2.404	SE	0.78	no	0.781
F	NE	NE	0.6	no	0	ND	0	yes	1.079	NE	0.78	no	0.782
F	NE	NE	0.1	no	0.541	ND	0	yes	0.524	NE	0.79	no	0.788
M	NE	NE	0.1	no	0.909	ND	0	yes	0.86	NE	0.82	no	0.816
F	SD	SD	0.1	no	0.414	ND	0	yes	0.409	SD	0.82	no	0.818
F	NE	NE	0.1	no	0.822	SD	0	yes	0.818	NE	0.85	no	0.854
M	ND	NE	0	yes	1.042	ND	0.5	no	0	ND	0.86	no	0.862
M	ND	ND	0.1	no	0.934	NE	0	yes	0.933	ND	0.88	no	0.881

M	NE	NE	0.1	no	0.579	ND	0	yes	0.559	NE	0.89	no	0.885
M	NE	NE	0.1	no	0.822	SE	0	yes	0.81	NE	0.89	no	0.887
M	SE	SE	0.1	no	0.674	SD	0	yes	0.686	SE	0.91	no	0.913
F	SD	SD	0.1	no	0.41	NE	0	yes	0.398	SD	0.91	no	0.914
M	NE	NE	0.1	no	0.999	ND	0	yes	0.992	NE	0.92	no	0.915
F	ND	ND	0.1	no	0.703	SE	0	yes	0.696	ND	0.93	no	0.931
F	ND	NE	0	yes	1.142	ND	0.5	no	0	ND	0.94	no	0.938
M	SE	SE	0.1	no	0.05	SD	0	yes	0.063	SE	0.97	no	0.969
	43				**61**				**13**				

Table 3. 2 Means and 95% confidence intervals (in brackets) for posterior distributions of migration rates between wolf subpopulations determined using BayesAss. Values in bold along the diagonal are the proportion of individuals derived from the source population each generation (ie. non-migrants). All migration between subpopulations was < 10%.

	Rate from			
To	ND	SE	NE	SD
northern divide (ND)	**0.974** (0.926, 0.997)	0.005 (0, 0.023)	0.008 (0, 0.036)	0.013 (0.001, 0.047)
south eastslopes (SE)	0.003 (0, 0.015)	**0.980** (0.948, 0.997)	0.005 (0, 0.021)	0.012 (0.001, 0.037)
north eastslopes (NE)	0.041 (0.016, 0.070)	0.026 (0.010, 0.047)	**0.928** (0.893, 0.957)	0.006 (0, 0.017)
southern divide (SD)	0.008 (0, 0.028)	0.010 (0, 0.034)	0.014 (0.001, 0.036)	**0.969** (0.935, 0.993)

Table 3. 3 Pairwise F_{ST} and the derived migration rates (*Nm*) between the 4 wolf subpopulations identified in the Canadian Rocky Mountains. F_{ST} values are above the diagonal, and *Nm* below.

	ND	SE	NE	SD
ND	-	0.035	0.030	0.044
SE	6.81	-	0.053	0.047
NE	7.92	4.41	-	0.055
SD	5.34	4.99	4.28	-

Table 3. 4 Sex ratios of emigrated wolves from 4 subpopulations in the Canadian Rocky Mountains. Expected values for χ^2 tests of the 4 subpopulations was based on the ratio of females to males for all wolves used in the assignment tests. The observed ratio for total wolves used in the assignment tests was compared to the expected value of a population with an equal sex ratio.

	Female	Male	Total	Ratio	χ^2	*P*
northern divide (ND)	4	6	10	0.67	0.25	>0.75
north eastslopes (NE)	3	6	9	0.50	0.77	>0.75
southern divide (SD)	6	5	11	1.20	0.19	>0.90
south eastslopes (SE)	4	5	9	0.80	0.04	>0.975
total wolves in tests	245	266	511	0.92	0.86	>0.50

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CHAPTER 4 – Conclusion

4.1 Summary

In my thesis, I used molecular genetics to examine population structure of wolves (*Canis lupus*) in the Canadian Rocky Mountains, and factors that influenced that structure. I identified 4 subpopulations of wolves based on genetic similarity and geographic proximity of individuals. These subpopulations appeared to be divided along natural and anthropogenic boundaries, though I could not explain the presence of some of the divisions. Genetic isolation by distance was present in the population, but occurred on a local scale and was likely not a factor in subpopulation division. The rate of dispersal between subpopulations was moderate and not biased towards either sex, however females appeared to remain closer to their natal pack than males. Dispersal of first generation migrants between the subpopulations was asymmetric. More migrants moved into the north eastslopes (NE) subpopulation from the other 3 subpopulations than travelled in the opposite direction, suggesting source-sink dynamics may be operating. Partial Mantel tests (Mantel 1967; Smouse *et al.* 1986) revealed several features on the landscape that reduced dispersal rates. Both natural and anthropogenic features were identified as barriers.

The barrier analysis allowed me to speculate on the origin of subpopulation boundaries. In several cases areas of reduced dispersal were correlated to the presence of natural and anthropogenic features that occurred near the divisions between subpopulations. The Continental Divide between Highway 16 and the TransCanada Highway coincides with a division between subpopulations. As well, the TransCanada Highway east of the Continental Divide occurs close to the boundary between the SE and NE subpopulations. These results show that a combination of natural and anthropogenic factors play a role in forming genetically identifiable subpopulations. Other factors, such as prey specialization, that I was unable to measure may also play a role in the division of the population.

Without information on growth rates or survival of individuals I could not say with certainty that the asymmetric dispersal observed was evidence of source-sink dynamics. Longer term data is necessary to determine whether the observed patterns were due to intrinsic habitat differences between subpopulations or due to stochastic effects, such as increased harvest or disease.

The non-invasive methods used in this study show great promise for future genetic work on wolves in cold climates. Many non-invasive studies for other species use scented lures to draw animals to some type of hair removal device. Wolf territories cover large areas and extensive time and effort would be required to attract a pack to a specific site and

there is a high probability that once at the site, large packs would leave mixed samples on barbed wire hair collection devices. For my study, instead of attracting wolves to a point location, I used snow tracking to follow wolves to where samples occurred naturally. By combining scat collection with the novel approach of collecting hair from bed sites I was able to identify 96 wolves which would have gone undetected if only samples from captures or carcasses were used. Continuing improvements in fecal DNA extraction methods will make non-invasive sampling of wolves even more efficient, and the need to capture wolves specifically for DNA analysis will cease to exist.

4.2 Future Work

This study uses non-invasive genetics to identify subpopulation structure of wolves in the Canadian Rocky Mountains and potential sources of subpopulation boundaries, as well as providing a base of genetic information to monitor changes in subpopulation distributions and genetic diversity through time. Future research should focus on further understanding of divisions and movement of wolves within subpopulations.

Repeated sampling of the study area through time would result provide a framework for capture-mark-recapture analysis, direct identification of dispersing individuals, and changes in subpopulation boundaries. A significant effort is required to sample such a large area, however the contacts made during my research and the possibility of formalizing sample collection from hunters would reduce the time and effort required. Even sampling on a smaller scale across the range of the study area would allow changes to be monitored and changes in population structure to be quantified.

In order to understand source – sink dynamics that may be occurring better information on population demographics is required. Efforts to this end are currently under way for a portion of the east slopes of the Canadian Rocky Mountains (N. Webb, pers. comm.), and will provide a greater understanding of the role of harvest on wolf population demographics. The majority of adult wolf mortality in the Canadian Rockies is human related (Callaghan 2002; N. Webb, pers. comm.). Data on age and sex of harvested wolves in conjunction with yearly population estimates would allow estimates of population growth (λ). When calculated regionally or by subpopulation, differences in λ and asymmetric dispersal rates could be quantified and associated with the presence or absence of sources and sinks. Management of wolves on a broad scale would benefit from such data.

The advent of GPS and satellite telemetry allows the exact path and start and end points of individual collared wolves to be known, and will provide insight into route choices and habitat preferences of wolves during dispersal. To date information on individual wolf dispersal paths exist

anecdotally and no study has addressed this question directly. As GPS collars are increasingly used on wolves information on dispersal patterns will slowly accumulate. This individual level data will help to identify areas of dispersal barriers and preferable dispersal habitat which is currently lacking.

Where multiple prey species exist wolves may preferentially forage on certain ungulates which may influence subpopulation structure (Carmichael *et al.* 2001). Analysis of diet for packs from each of the subpopulations would help determine if population subdivision is being influenced by affinities toward certain prey species. Diet can be determined by physical and genetic analysis of scat already collected for non-invasive genetic study (Farrell *et al.* 2000; Deagle *et al.* 2005). Linking genetic identification of individuals with their dietary preferences is a powerful tool that can have wide ranging applications.

4.3 Conclusions

In a landscape that is increasingly influenced by anthropogenic changes, wildlife populations can be fragmented into progressively smaller and more isolated units (Crooks 2002; Proctor *et al.* 2005; Epps *et al.* 2005). Wolves are often assumed to be immune to population isolation from habitat fragmentation due to their ability to travel long distances through a variety of landscapes. This study is one of the first to show genetic subpopulation structure of wolves in a geographically continuous population and link the differentiation to anthropogenic influences. As human presence and changes to the landscape increase, subpopulations of wolves may become increasingly isolated, and barriers to dispersal may slow or eliminate recolonization of extirpated areas. Maintenance of existing, and creation of new travel corridors through human dominated landscapes is necessary to ensure wolf movement between subpopulations.

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