POPULATION GENETICS, FORAGING ECOLOGY, AND TROPHIC RELATIONSHIPS OF GREY WOLVES IN CENTRAL SASKATCHEWAN

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ABSTRACT

Habitat fragmentation and anthropogenic development influence the level of isolation and security in and around protected habitats affecting wolf movements and the distribution and abundance of their prey. In light of recent concern about the ecology of animals in protected areas, I initiated a research project to investigate the molecular and foraging ecology of grey wolves in and around Prince Albert National Park (PANP), Saskatchewan.

Estimates of genetic diversity and population structure can be used as surrogates to detect effects of habitat degradation on wolves. Genetic diversity was high in these populations relative to other North American wolf populations. My results suggest that wolves in central Saskatchewan form a panmictic population, however there is some evidence showing partial isolation of one group of wolves within PANP. I speculate that the level of human activity such as road networks, hunting, and trapping act as dispersal impediments to this isolated group. Further, the genetic homogenization, indicating high population turnover, of wolf groups that use the periphery and adjacent areas of PANP may also contribute to the observed genetic subdivision. The partially isolated NW group, characterized by slightly lower diversity indices, low migration rates, and higher levels of allele fixation, indicated this group was a more stable social unit comprised of more related individuals.

Knowledge of wolf food habits and how they change over time is a fundamental component to understanding wolf ecology. Using scat analysis I evaluated wolf foraging ecology by calculating indices of occurrence/faeces (OF) and percent prey biomass contribution: white tailed deer contributed 43% and 33% respectively to wolf diet; elk (33%, 50%), moose (7%, 14%), beaver (5%, 2%), and snowshoe hare (2%, <1%). I found no evidence of livestock depredation nor did wolves prey on bison or caribou. There were no differences in OF indices between years. Prey selectivity was apparent in both years with wolves selecting elk and avoiding beaver. A diversity of ungulate prey are readily available to wolves in this system; however, scat analysis and tests for prey selection indicate a preference for elk. I presume this is a choice made to balance risk with profitability of food items in concordance with optimal foraging theory.

I examined trophic relationships between the grey wolf and 18 mammalian species from the boreal forest of central Saskatchewan, Canada, using $\delta^{13}C$ and $\delta^{15}N$ stable isotope values measured in hair samples. Variance in isotope values for wolves and other carnivores was investigated as a proxy for dietary variation. IsoSource, an isotopic source partitioning model, quantified the relative proportions of 5 most likely prey items in the diets of wolves. I compared these results with investigations of faecal contents using percent biomass contributions of prey items in wolf diet. I found no difference between percent biomass measures and mean percent contributions derived from IsoSource. Despite social foraging, my results indicate highly variable diets among individual wolves and I discuss this in terms of boreal wolf ecology.

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DEDICATION

This thesis is dedicated to the memory of Dr. Malcolm A. Ramsay. His curious mind, philosophical nature, and devotion to ecology ignited in me a passion for investigating the natural world.

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1.0 GENERAL INTRODUCTION

1.1 Wolf ecology in fragmented habitats

Few areas of the globe remain unaffected by pervasive human activity. Mosaics of agriculture, urbanization, logged forest, and degraded land dominate much of the landscape (Saunders et al. 1991). Alterations in the landscape have ultimately resulted in shifts of geographic distributions of numerous species, particularly those that require large tracts of contiguous habitat to survive. Changes in the distributions of large carnivores, in particular, chronicle the repercussions of such landscape alterations (Wilcox and Murphy 1985, Woodruffe 2001, With 2004). Key factors influencing the viability of large carnivore populations are habitat continuity, an adequate prey base, and freedom from persecution (Mech 1995).

Human activity, large-scale industrial development, and human population expansion have led to the extirpation of grey wolves (*Canis lupus*) throughout most of the United States (Mech 1995, Clark et al. 1996). Through direct persecution, North American wolf ranges have contracted to encompass about half of their historic range (Mech and Boitaini 2003). However, current factors threatening the long-term survival of extant wolf populations and other large carnivore populations are human disturbance and habitat exploitation and alteration (Hummel and Pettigrew 1991, Paquet and Hackman 1995, Clark et al. 1996).

A threshold or critical scale likely exists at which landscapes become fragmented enough to restrict an individual’s movements or become unsuitable to occupy. When habitat patches become, in effect, islands, such as many North American national parks, restriction of movement of large carnivores such as wolves is of special concern (Newmark 1995, Woodruffe and Ginsberg 1998). Human pressures such as roads, hunting, and trapping that surround many protected areas likely influence the dispersal ability of wolves.

1.2 Dispersal and inter-population movement

The ability of animals to move across the landscape often influences gene flow in populations. At the population level, dispersal may be regarded as inter-population gene flow or gene migration (Slatkin 1987). Population structure (Chessier et al. 1993), genetic diversity (Hedrick 1995, Paetkau et al. 1998), and inter population source-sink dynamics (Dias 1996) are all dispersal-mediated. Community structure and function across a landscape are also products of dispersal at the ecosystem level (Mouquet et al. 2001).

Ultimate benefits for dispersal are inbreeding avoidance and lower competition for mates and resources. Wolves live in socially structured family groups generally consisting of a mated pair, their offspring, and older adult offspring helpers from previous generations (Mech and Boitani 2003). Unrelated individuals from neighboring packs also make up a portion of a given pack (Lehman et al.1992). Therefore, most of the individuals in the group are related to some degree.

Average territory sizes for wolf packs occupying forested habitats range from 185 km^2 where prey densities are high, and up to 568 km^2 where prey densities are low (Paquet and Carbyn 2003). Therefore, wolves require vast spaces for foraging (Paquet and Carbyn 2003). Also, wolves are highly vagile animals capable of dispersing ~ 1000 km (Gese and Mech 1991, Wydeven et al. 1995, Wabakken et al. 2001). During long-range dispersal movements and regular home-range use, wolves in many regions of North America encounter human induced landscape changes such as agriculture, clear cuts, and roads, particularly when venturing outside of protected areas (Mladendoff et al. 1995, Woodruffe et al. 1998).

Roads and cleared landscapes allow increased access to remote areas by humans, thereby increasing hunting, trapping, and direct vehicle mortality of wolves. In southeast Alaska, wolf harvest was significantly and positively correlated with the linear km and density of roads (Person et al. 1996), where 44% of wolves were killed directly from the road system. Several other studies have shown a strong relationship between road density and wolf survival (Thiel 1985, Jensen et al. 1986, Mech et al. 1988, Fuller 1989). Wolves recolonizing Wisconsin selected areas with low road density (<0.45 km/km2) (Mladendoff et al. 1995). Wolves generally do not persist in areas with average road densities greater that 0.6 km/km^2 (Thiel 1985, Jensen et al. 1986, Mech et al. 1988, Fuller 1989).

Dispersal constraints, such as habitat fragmentation and its associated effects, can affect the pattern of relatedness among wolves within an area. In Minnesota, wolf packs are restricted to areas of protected woodland and dispersal is inhibited by habitat discontinuities caused by agriculture and development (Lehman et al. 1992). Juvenile wolves may be unable to disperse as easily across fragmented habitats and remain within their natal pack system rather than joining an established neighbouring pack (Wayne et al. 1995). Also, wolf dispersal occurs predominantly during winter months (Fuller 1989, Gese and Mech 1991), coinciding with periods when they are vulnerable to trapping pressure. Gese and Mech (1991) documented wolves leaving their natal territories as pups or yearlings, suggesting they may be naïve to the dangers of roads or anthropogenic pressures such as hunting and trapping. Furthermore, dispersal often follows pre-dispersal forays (Messier 1985, Gese and Mech 1991) where wolves may experience the negative effects of roads and hunting pressure, thereby discouraging dispersal events and limiting gene flow. Accordingly, wolves that do disperse are likely at an increased risk of mortality (Mech 1995). Differences among areas in the number of close relatives shared between packs reflected habitat constraints on dispersal (Lehman et al. 1992). Therefore, human influences affect the genetic variability of partially isolated populations in disturbed areas (Wayne et al. 1995).

1.3 Influence of foraging ecology on wolf population structure

Carnivore densities and distribution often reflect resource abundance, which generally relates to prey resources (Fuller and Sievert 2001, Carbone and Gittleman 2002). Short- and long-term changes in prey abundance and availability can act as major factors influencing population viability of large carnivores (Fuller and Sievert 2001). Natal-habitat dispersal or habitat imprinting, which may include the influence of prey, has been demonstrated in coyotes (*Canis latrans;* Sacks et al. 2004), cuckoos (*Cuculis sp*; Vogl et al. 2002), butterflies (*Parnassius smintheus)* (Keghobadi et al. 1999) and recently in wolves (Carmichael et al. 2001, Geffen et al. 2004). When prey availability changes within a habitat, indirect changes in behaviour also occur that contribute to observed changes in carnivore densities (Fuller and Sievert 2001). For example, wolf pack territory size was negatively correlated with white-tailed deer (*Oidelcoileus virginianus*) density in Wisconsin (Wydeven et al. 1995). As wolf movements change with dynamic prey populations, the genetic structure of wolf population may be influenced as result of prey specialization (Carmichael et al. 2001).

1.4 Study objectives

The paucity of information about population genetics and foraging ecology of wolves in central Saskatchewan prompted the need to collect baseline information on genetic variation and population structure in this region, as well as diet composition. My objectives were to evaluate the dietary and genetic patterns observed in protected versus unprotected wolf populations in Saskatchewan. To accomplish this, I employed non-invasive research techniques such as scat and hair collection for genetic and stable isotopic analyses.

In chapter 2, I examined genetic variation and population structure of wolves in and around PANP. I used 11 microsatellite loci to identify population structure and diversity and make inferences about wolf dispersal capabilities at a broad scale. A highly structured population might indicate limited movement among putative subpopulations.

I further investigate foraging ecology and prey selection by wolves in PANP using scat analysis in Chapter 3. I evaluated the diet composition of wolves, assessed patterns in prey selection, and discussed the results in the context of foraging theory and risk-avoidance behaviour.

Lastly, In chapter 4, I examined wolf diets using stable isotope analysis to observe individual and population variation in diet on a larger temporal scale and explore wolf trophic relationships in the boreal forest.

1.5 Conservation need

Estimates of genetic population diversity and structure are crucial in conservation biology where it is often necessary to understand whether populations are genetically isolated from each other, and if so, to what extent (Johnson et al. 2001). Preserving genetic diversity is important because of the long-term evolutionary potential it provides populations and species. Rare alleles, or combinations of alleles, may confer no immediate advantage but could be well suited to future environmental conditions (Madsen et al. 1996, Saccheri et al. 1998, Higgins and Lynch 2001, Balloux and Lugon-Moulin 2002). Furthermore, knowledge of population genetic structure provides valuable guidelines for conservation strategies and management (Eizerik et al. 2000, Johnson et al. 2001). Links between habitat characteristics, including prey availability and use, provide important baseline information giving context for any apparent structuring of populations. Diet composition and trophic relationships provide insight into community interactions and contribute to the knowledge from which conservation and management decisions are drawn (Clark et al. 2001).

1.6 References

Balloux, F. and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology 11: 155-165.

Bouzat, J. L., H.H. Cheng, H. A. Lewin, R. L. Westemeier, J. D. Brawn and K. N Paige. 1998. Genetic evaluation of a demographic bottleneck in the Greater prairie chicken. Conservation Biology 12: 836-843.

Carbone, C. and J. L. Gittleman. 2002. A common rule for the scaling of carnivore density. Science 295: 2273-2276.

Carmichael, L. E., J. A. Nagy, N. C. Larter and C. Strobeck. 2001. Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. Molecular Ecology 10: 2787-2798.

Chessier, R. K., O. E. J. Rhodes, D. W. Sugg, and A. Schnabel. 1993. Effective sizes for subdivided populations. Genetics 135: 1221-1232.

Clark, T. W., D. J. Mattson, R. P. Reading, and B. J. Miller. 2001. Interdisciplinary problem solving in carnivore conservation: and introduction. Pages 223-240 *in* Gittleman, J. L., Funk, S. M., MacDonald, D. W., and Wayne, R. K., editors. Carnivore Conservation. Cambridge University Press, Cambridge.

Clark, T. W., P. C. Paquet, and A .P. Curlee. 1996. Large carnivore conservation in the Rocky Mountains of the United States and Canada. Conservation Biology 10: 936-939.

Dias, P. C. 1996. Sources and sinks in population biology. Trends in Ecology and Evolution 11: 326-330.

Eizerik, E., C. B. Indrusiak, W. E. Johnson. 2000. Jaguar population viability analyses: evaluation of parameters and a case study of a remnant population in a South American subtropical rain forest. *In* T. A. Medellin, C. Chetkiewicz, A. Rabinowitz, K. H. Redford, J. G. Robinson, E. Sanderson, and A. Taber, editors. Jaguars in the New Millennium: A Status Assessment, Priority Detection and Recommendation for the Conservation of Jaguars in the Americas. Universidad Nacional Autonoma de Mexico/Wildlife Conservation Society.

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

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

Fuller, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monogographs 105. 41pp.

Fuller, T. K. and P. R. Sievert. 2001. Carnivore demography and the consequences of changes in prey availability. Pages 163-178 *in* J. L Gittleman, S. M. Funk, D. Macdonald and R. K. Wayne, editors. Carnivore Conservation. Cambridge University Press, Cambridge, UK.

Geffen, E., M .J. Anderson and R. K. Wayne. 2004. Climate and habitat barriers to dispersal in the highly mobile gray wolf. Molecular Ecology *in press*.

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

Gotelli, D., C. Sillero-Zubiri, G. D. Applebaum, M. S. Roy, D. J. Girman, J. Garcia Moreno, E. A. Ostrander, and R. K. Wayne. 1994. Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. Molecular Ecology 3: 301-312.

Hedrick, P. W. 1995. Gene flow and restoration: the Florida panther as a case study. Conservation Biology 9: 996-1007.

Hedrick, P. W. and S. T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics 31: 139-162.

Higgins, S. I. and M. Lynch. 2001. Metapopulation extinction caused by mutation accumulation. Proceedings of the National Academy of Sciences USA 98: 2928-2933.

Hummel, M. and S. Pettigrew. 1991. Wild hunters: predators in peril. Key Porter Books Ltd., Toronto.

Jensen, W. F., T. K. Fuller, and W. L. Robinson. 1986. Wolf (*Canis lupus*) distribution on the Ontario to Michigan border near Sault Ste. Marie. Canadian Field. Naturalist 100: 363-366.

Johnson, W. E., E. Eizirik, M. Roelke-Parker, and S. J. O'Brien. 2001. Applications of genetic concepts and molecular methods to carnivore conservation, Pages 334-358 *in* J. L. Gittleman, S. M. Funk, D. Macdonald and R. K. Wayne, editors. Carnivore Conservation. Cambridge University Press, Cambridge.

Keyghobadi, N., J. Roland, C. Strobeck. 1999. Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). Molecular Ecology 8: 1481-1495.

Kohn, M. H. and R. K. Wayne. 1997. Facts from Feces revisited. Trends in Ecology and

Evolution 12: 223-227.

Laikre, L. and N. Ryman. 1991. Inbreeding depression in a captive wolf (*Canis lupus*) population. Conservation Biology 5: 33-40.

Lehman, N., P. Clarkson, L. D. Mech, T. J. Meier, and R. K. Wayne. 1992. A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. Behavioral Ecology and Sociobiology 30: 83-94.

Mace, G. M., T. B. Smith, M. W. Bruford, and R. K. Wayne. 1996. Molecular genetic approaches in conservation-an overview of the issues. Pages 1-12 *in* T. B. Smith and R. K. Wayne, editors. Molecular Genetic Approaches in Conservation. Oxford University Press, Oxford.

Madsen, T., B. Stille, and R. Shine. 1996. Inbreeding depression in an isolated population of adders, *Vipera berus*. Biological Conservation 75: 113-118.

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

Mech, L. D., S. H. Fritts, G. L. Radde, and W. J. Paul. 1988. Wolf distribution and road density in Minnesota. Wildlife Society Bulletin 16: 85-87.

Meier, T. J., J. W. Burch, L. D. Mech and L. G. Adams. 1995. Pack structure and genetic relatedness among wolf populations in a naturally-regulated population. Pages 293-302 *in* L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Edmonton, AB, Canada.

Messier, F. 1985. Solitary living and extraterritorial movements of wolves in relation to social status and prey abundance. Canadian Journal of Zoology 63: 239-245.

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

Mouquet, G. S., E. E. Mulder, V. A. A. Jansen, and M. Loreau. 2001. The properties of competitive communities with coupled local and regional dynamics. Pages 311-328 *in* J. Clobert, E. Danchin, A. A. Dhondt and J. D. Nichols, editors. Dispersal. Oxford University Press, Oxford, UK.

Newmark, W. D. 1995. A land-bridge perspective on mammalian extinctions in western North American national parks. Conservation Biology 9: 512-526.

Paetkau, D. H., G. F. Shields and C. Strobeck. 1998. Gene flow between insular, coastal and interior populations of brown bears in Alaska. Molecular Ecology 7: 1283-

1292.

Paquet, P. C. and A. Hackman. 1995. Large carnivore conservation in the Rocky Mountains: a long-term strategy for maintaining free-ranging and self-sustaining populations of carnivores. World Wildlife Fund (Canada), Toronto, ON.

Paquet, P. C. and L. N. Carbyn. 2003. Wolf, *Canis lupus*. Wild mammals of North America: Biology, Management, and Conservation. *In* G. A. Feldhammer, B. C. Thompson and J. A. Chapman. Johns Hopkins University Press. Baltimore, MD, USA.

Person, D. K., M. Kirchoff, V. Van Ballenberghe, G. V. Iverson, and E. Grossman. 1996. The Alexander Archipelago wolf: a conservation assessment. United States Department of Agriculture-Forest Service. General Technical Report. PNWGTR-384. Portland, Oregon, USA.

Randi, E., V. Lucchini, and F. Francisci. 1993. Allozyme variability in the Italian wolf (*Canis lupus*) population. Heredity 71: 516-522.

Reed, J. Z., D. J. Tollit, P. M. Thompson, and W. Amos. 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. Molecular Ecology 6: 225-234.

Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392: 491-494.

Sacks, B. N., S. K. Brown, and H. B. Ernest. 2004. Population structure of California coyotes corresponds to habitat specific breaks and illuminates species history. Molecular Ecology 13: 1265-1275.

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

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

Theil, R. P. 1985. The relationship between road densities and wolf habitat suitability in Wisconsin. American Midland Naturalist 113: 404-407.

Vogl, W., M. Taborsky, B. Taborsky, Y. Teuschl, and M. Honza. 2002. Cuckoo females preferentially use specific habitats when searching for host nests. Animal Behavior 64: 843-850.

Wabakken, P., H. Sand, O. Liberg, A. Bjarvall. 2001. The recovery, distribution, and population dynamics of wolves on the Scandinavian peninsula, 1978-1998.

Canadian Journal of Zoology 79: 710-725.

Wayne, R. K. 1992. Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. Conservation Biology 6: 559-569.

Wayne, R. K. and C. Vila. 2003. Molecular genetic studies of wolves. Pages 317-343 *in* L. D. Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, USA.

Wayne, R. K., N. Lehman, and T. K. Fuller. 1995. Conservation genetics of the Gray wolf. Pages 399-408 *in* L. N. Carbyn, S. H. Fritts, and D R. Seip, editors. Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Edmonton, AB, Canada.

Wayne, R. K., N. Lehman, D. Girman, P. J. P. Gogan, D. A. Gilbert, K. Hansen, R. O. Peterson, U. S. Seal, A. Eisenhawer, L. D. Mech and R. J. Krumenaker. 1991. Conservation genetics of the endangered Isle Royale Gray wolf. Conservation Biology 5: 41-51.

Wiens, J. A. 2001. The landscape context of dispersal. Pages 96-109 *in* J. Clobert, E. Danchin, A. A. Dhondt, and J. D. Nichols, editors. Dispersal. Oxford University Press, Oxford, UK.

Wilcox, B. A. and D. D. Murphy. 1985. Conservation strategy: the effects of fragmentation on extinction. American Naturalist 125: 879-887.

With, K. A. 2004. Metapopulation dynamics: perspectives from landscape ecology. Pages 23-44 *in* I. Hanksi and O. Gaggiotti, editors. Ecology, Genetics and Evolution of Metapopulations. Elsevier Academic Press, Burlington, MA, USA.

Woodruffe, R. 2001. Strategies for carnivore conservation: lessons from contemporary extinctions. Pages 61-92 *in* J. L. Gittleman, S. M. Funk, D. MacDonald and R. K. Wayne. Carnivore Conservation. Cambridge University Press, Cambridge, UK.

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

Wydeven, A. P., R. N. Schultz, and R. P. Thiel. 1995. Monitoring of a gray wolf population in Wisconsin. 1979-1991. Pages 147-156 *in* L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, AB, Canada.

2.0 GENETIC DIVERSITY AND DIFFERENTIATION OF GREY WOLF POPULATIONS IN CENTRAL SASKATCHEWAN

2.1 Introduction

Central to evolutionary ecology and conservation biology is the investigation of animal movement and dispersal in rapidly changing landscapes (Young and Clarke 2000). Habitat fragmentation, anthropogenic development, and prey distribution influence animal movements and genetic structure of animal populations, including the propensity for inbreeding (Wayne et al. 1992, Young and Clarke 2000). Of special concern are wide-ranging, long-lived carnivores that require vast spaces of intact habitat (Gittleman et al. 2001).

Ranging throughout most of the Northern Hemisphere, grey wolves are considered one of the most vagile predators (Mech 1970, Fritts 1983, Merrill and Mech 2000, Paquet and Carbyn 2003). Wolves occur in diverse ecosystems from desert to dense forest and arctic islands (Mech 1970, Nowak 1999). They disperse over great distances and topographic barriers to find mates and territories (Mech and Boitani 2003). However, at some geographic scale, distance prevents the exchange of individuals between populations and is conceptually an important impediment to dispersal. Patterns of isolation with distance are not expected for highly mobile and territorial canids like grey wolves (Chepko-Sade1987). Previous studies support this expectation by demonstrating a lack of correlation between genetic differentiation and distance in grey wolves (Wayne et al. 1992, Roy et al. 1994, Vila et al. 1999). However, Forbes and Boyd (1997) elucidated such a correlation at a finer geographic scale. In addition to isolation by distance, population structuring can also result over climatic clines (Geffen et al. 2004) and in areas where the prey base dramatically changes and wolves specialize on those prey (Carmichael et al 2001, Musiani 2003). Wolf populations can also be adapted to local conditions and show specializations concerning den-site use, foraging habitats, and physical environment (Carmichael et al. 2001, Musiani 2003, Geffen et al. 2004).

North American wolf populations are generally considered genetically diverse (Roy et al 1994). However, recent genetic work has eluded that historical (pre-extermination) wolves show more than twice the diversity of their modern conspecifics (Leonard et al. 2004). A two-thirds loss of unique genetic haplotypes resulted due to the large scale extermination of wolves in the coterminous United States (Leonard et al. 2004). Further, loss of genetic variation in small and isolated wolf populations has been shown and is of special concern (Wayne et al 1992, Randi et al. 1993, Gotelli et al. 1994, Fritts and Carbyn 1995, Wayne and Vila 2003).

Gene flow and population structure are largely determined by natal dispersal (Taylor and Taylor 1977, Slatkin 1987). Short-distance (1-100 km) dispersal is common in wolf populations (Gese & Mech 1991, Messier 1985a) and there have been documentations of wolves dispersing upwards of ~ 1000 km (Van Camp & Gluckie 1979, Fritts 1983, Boyd et al 1995, Wabakken 2002). Long distance dispersal events may be more common than we think due to the low probability of detecting dispersers.

Originally distributed province-wide, current wolf range in Saskatchewan, Canada, scarcely extends past the southern limit of the diminishing parkland and boreal ecosystems. As forests rapidly recede (Hobson et al. 2002, Fitzsimmons 2002), habitat for wolves continues to diminish, increasing the risk of wolf-livestock conflict, hunting access, and road mortality. Rates of large-scale forest clearing for agricultural purposes and logging are high in the fringe of the boreal forest (Hobson et al 2002, Fitzsimmons 2002). For many species of large carnivore, even within protected areas like parks, human conflicts are the most important cause of mortality (Newmark 1995, Woodruffe & Ginsberg 1998, Callaghan 2002). When wolves attempt to move beyond protected area boundaries, such as would be the case during extraterritorial forays that precede dispersal and dispersal itself (Messier 1985), risk of mortality is presumably high (Mech 1995). Prince Albert National Park (PANP), the only protected wolf habitat in Saskatchewan, is at increasing risk of isolation due to the above factors.

My objectives were to: 1) evaluate levels of genetic variation found in wolf populations inhabiting central Saskatchewan, and 2) elucidate the genetic population structure of wolves across a potentially fragmented landscape. I predicted high levels of natural genetic variation, relative to extant North American wolf populations, within these populations because the relatively contiguous nature of the landscape would allow wolves to mix freely. I also predicted that wolves in central Saskatchewan would retain low levels of genetic subdivision among populations given their mobility, home range size, and dispersal capability.

2.2 Methods

2.2.1 Study area

My study area was in central Saskatchewan (Figure 2.1). PANP, the core study area, harbours a wolf population (~2 wolves/100 km^2 - Paquet & Carbyn 2003) dependent on a multi-prey system. Predominantly boreal forest, PANP is 3,875 km^2 and the largest protected wilderness in Saskatchewan. Cultivated land, grazing areas, forest harvesting, road construction, and fur-trapping lines are common on adjacent provincial lands. For a detailed description of the study area see Chapter 3.

2.2.2 Non-invasive research

Approaches to population genetics have recently expanded to include non-invasive research techniques (Palsbøll 1999, Kohn and Wayne 1999, Luccini et al. 2002). The ability to isolate and analyse DNA from opportunistically collected hair and faeces has allowed scientists to produce genetic information as detailed as identities for individuals in a population (Kohn et al. 1999, Ernest et al. 2002, Luccini 2002), facilitating investigation into population genetic structure, genetic history, and population size estimation (Kohn et al. 1999, Wayne and Vila 2003).

The use of microsatellite markers has advanced genetic investigation of animal populations (Balloux and Lugon-Moulin 2002). Microsatellites are single-locus genetic markers consisting of simple sequence motifs (Kohn & Wayne 1997). They are neutral, co-dominantly inherited, highly polymorphic, and scattered throughout the genome of all living organisms. Using polymerase chain reaction (PCR), these markers can be

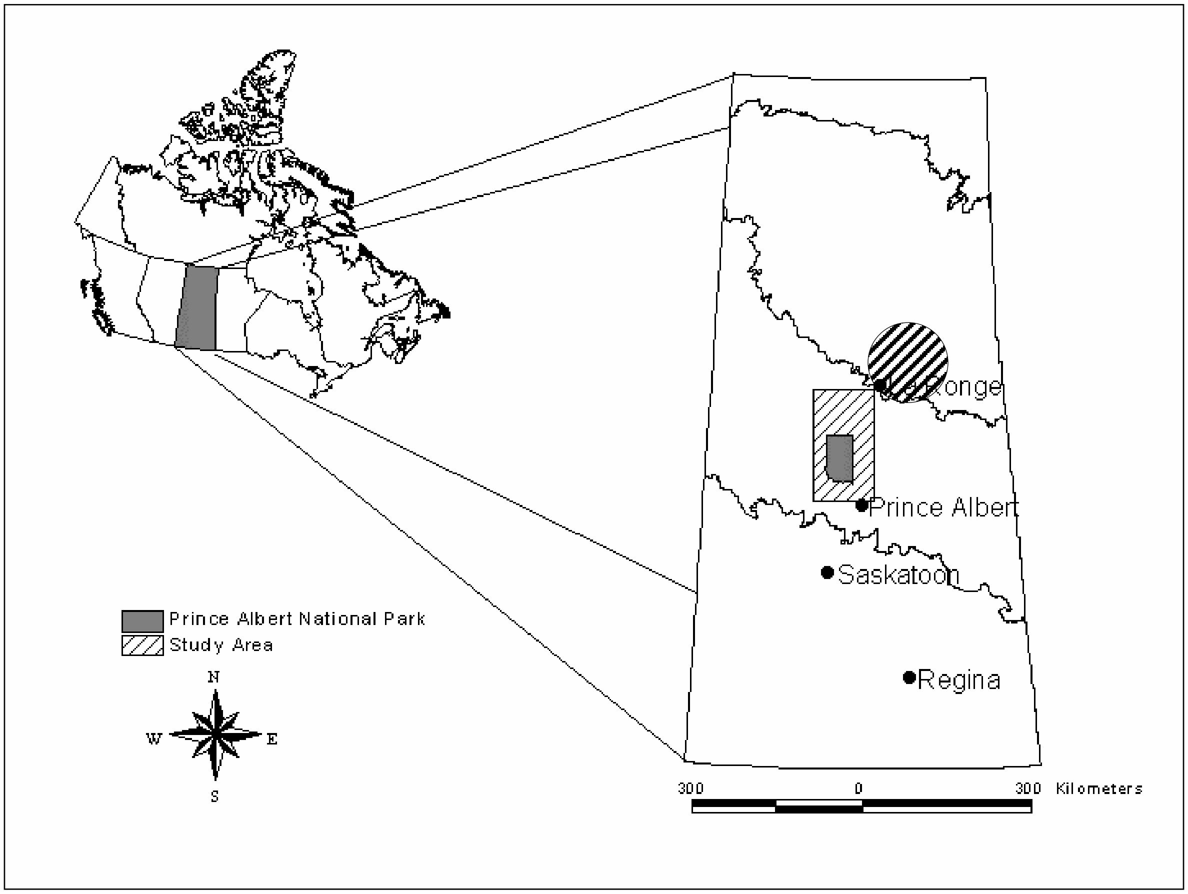

Figure 2.1. Study area in the boreal forest of central Saskatchewan, Canada.

amplified from small and degraded sources of DNA and are ideal for studying genetic variation, population structure, and gene flow in many free ranging animal populations (Kohn & Wayne 1997, Kohn et al 1999). DNA microsatellites cloned from the domestic dog (*Canis familiaris*) genome offer the ability to obtain new insights into grey wolf population genetics (Ostrander et al.1993, Roy et al. 1994, Forbes and Boyd 1996). Using these techniques, capture and handling of animals is eliminated, making it effective for investigating endangered and difficult to capture species.

2.2.3 Genetic samples

I sampled wolf faeces (scats) throughout PANP and surrounding areas in 2003. Wolf scats were collected wherever encountered on trails, lakeshores, other linear features, and by back-tracking wolves. GPS location (UTM) and date were recorded and samples were stored frozen in plastic bags. Scats were differentiated from those of sympatric coyotes based on associated signs and tracks, scat size (>30 mm in diameter for wolves), and appearance. I collected only intact and relatively fresh scats in summer. I also collected tissues from wolf mortalities that occurred within and outside PANP. One hundred and 50 scats and 16 hide samples were available for analyses. Putative populations were defined by sampling location (Figure 2.2). I schematically divided PANP into 4 quadrants; I assigned samples collected in the northeast quadrant to the NE group, the northwest samples to the NW group, the southeast samples to the SE group, the southwest samples to the SW group, and the La Ronge samples to the LR group.

2.2.4 DNA extraction

I extracted and isolated DNA from scats using a Qiagen® DNA mini stool kit, and from tissue samples using a Qiagen® DNeasy Tissue kit following manufacturer protocols. I quantified DNA in sample using fluorometry. Test results of poorly amplifying scat DNA indicated a lack of wolf specific target DNA present. I used negative controls throughout.

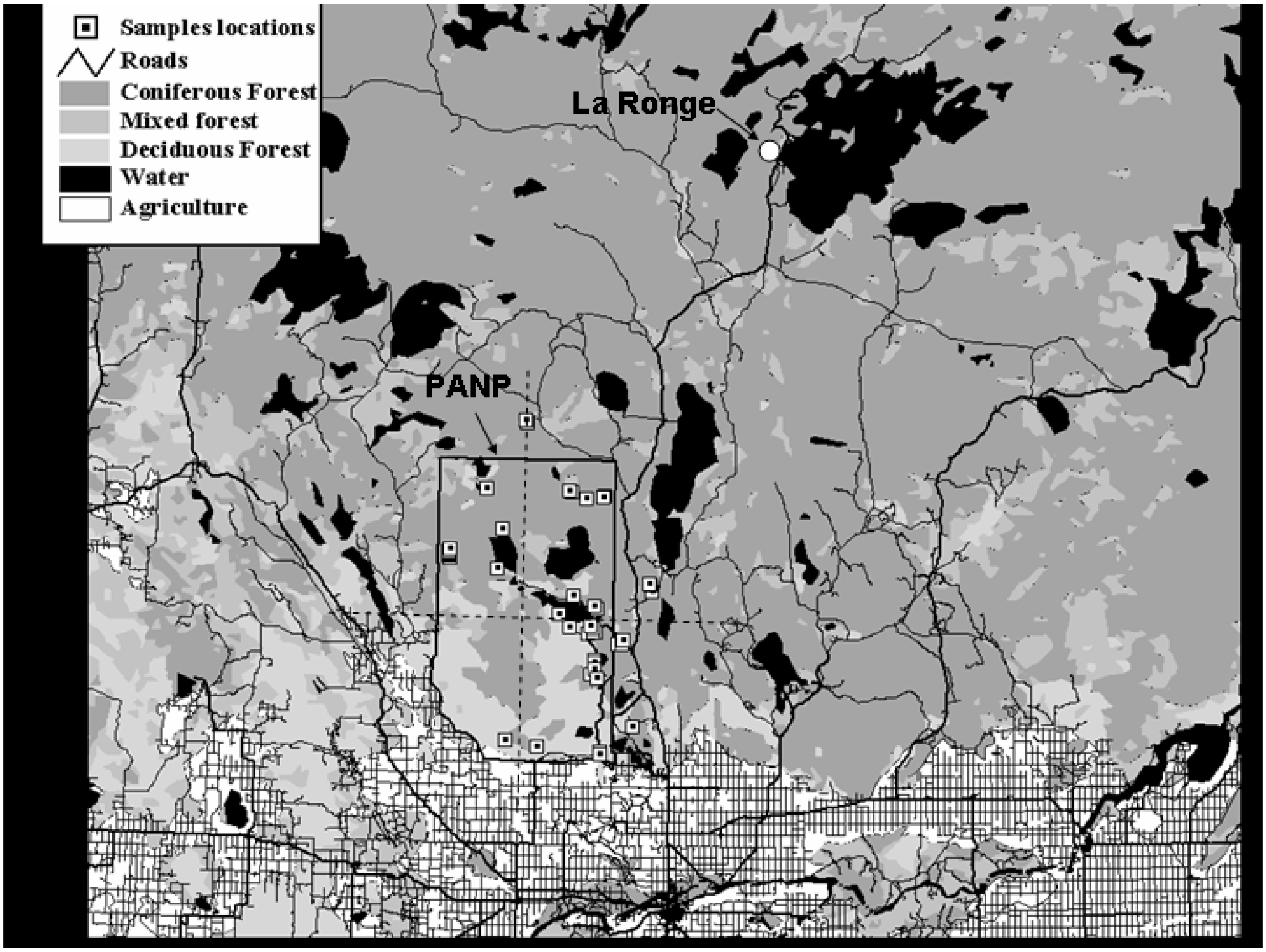

Figure 2.2. Wolf scat sample locations in central Saskatchewan (not all samples are shown).

2.2.5 Identification of microsatellite alleles

DNA markers used in the screen are shown in Table 2.1. A positive control plus 2 samples representing sample type (tissue and scat) were tested against 13 fluorescently labeled microsatellite markers using PCR. Original PCR conditions optimized for all 13 markers were Mg^{++} concentration, annealing temperature, and primer concentration. Additional conditions optimized to attain scat DNA amplification were DNA concentration, *Taq* concentration, and the number of PCR program cycles and length of extension step. Stringent conditions used to genotype “good” samples were: 1.5 to 2.0 mM $MgCl_{2,}$ 55° annealing temperature, 35 cycles, 1 U *Taq* per rxn; Liberal conditions used to genotype “poor” samples were: 1.5 to 2.0 mM $MgCl_{2,}$ 55° annealing temperature, 45 cycles, 2 U *Taq* per rxn. DNA fragments were resolved using the LICOR™ 4200 DNA Analyzer system, and scored using GeneImagIR (LICOR™). PCR artefacts such as allelic dropout and generation of false alleles may obscure true patterns of diversity due to dilution and/or degradation of faecal DNA. PCR on duplicate sample of faecal material clarified such inconsistencies in genotyping. Low volumes of some samples prevented complete testing with all markers or supplemental extractions of DNA.

2.2.6 Microsatellite diversity, population relationships and gene flow

Genetic polymorphism of each population was measured as the mean number of alleles per locus (A), the observed heterozygosity (H_O), and the expected heterozygosity from Hardy-Weinburg (H-W) assumptions (H_E; Nei 1978, 1987). I used H_E because it is a less biased index of genetic variability and is highly correlated with H_O (Nei and Roychoudhury 1974). Heterozygosity statistics were calculated by GENETIX (Belkhir et al. 2000) with a test based on Black and Krafsur (1985).

Spatial structure in microsatellites was examined by conventional F- statistics using the estimator theta (θ) (Weir and Cockerham 1984). Theta measures the proportion of total variation due to differences between subpopulations. I used GENETIX (Belkhir et al. 2000) to perform these calculations. Significances of θ were calculated after 1000 permutations. Matrices of pairwise θ-values between groups were calculated for the data and the level of significance for the values was estimated through a randomization test (as implemented by the program GENETIX). A matrix of pair-wise Euclidean distances

Table 2.1. Sequences of the 13 microsatellite DNA markers used in genotyping wolf samples.

Name	Forward Primer	Reverse Primer
PEZ01	ggctgtcacttttccctttc	caccacaatctctctcataaatac
c09.250	ttagttaacccagctcccca	tcaccctgttagctgctcaa
FH2054	gccttattcattgcagttaggg	atgctgagttttgaactttccc
VWF.x	ctccccttctctacctccacctctaa	cagaggtcagcaagggtactattgtg
c20.253	aatggcaggattttcttttgc	atctttggacgaatggataagg
FH2001	tcctcctcttctttccattgg	tgaacagagttaaggatagacacg
FH2096	ccgtctaagagcctcccag	gacaaggtttcctggtccca
FH2010	aaatggaacagttgagcatgc	ccccttacagcttcattttcc
FH2017	agcctctataatcacgtgagcc	cccagtaccaccttcaggaa
*FH2006	tgggggcgttaagagtaatg	ctaggcctaaacccctgagc
PEZ08	tatcgactttatcactgtgg	atggagcctcatgtctcatc
*MS34b	agccattcctggccgagtcc	ggtccccttttgccatagtgt
MS41b**	tcctctaattttcccctctc	ctgctcgaccctcttctctg

* these markers were dropped from the analysis

** y-chromosome marker (sex-linked)

between the central locations (UTM) for each wolf group was also calculated. All matrices were tested for correlation using Mantel's test (Liedloff 1999). Genetic relationships among groups were calculated for microsatellite DNA using Nei's genetic distance measure, D_s (Nei 1978). The number of migrants per generation, *Nm,* was estimated from θ-values by the expression $\theta = 1/(1 + 4\,Nm)$. A neighbor-joining tree was inferred from the D_s values using PHYLIP 3.57 (Felsenstein 1993). The pattern of 2-dimensional spatial structuring of groups was represented through Multidimensional Scaling (MDS) performed on the matrix of pairwise θ-values obtained from microsatellite data (SPSS, version 12.5).

2.3 Results

2.3.1 DNA extraction

The results showed amplification with only the controls and tissue sample for 11 of the markers. Marker MS34b did not amplify and was dropped from the analysis. Marker FH2006 was monomorphic and also dropped. Quantification of representative samples showed low DNA concentrations in samples prepared from scats. I eliminated poor quality samples and continued with relatively good samples. Thus, 75 out of 150 scat samples and 16 tissue samples were successfully genotyped using 11 microsatellite markers. Seventy-one individual wolves that I genotyped were sampled from within PANP, and 16 individuals used other areas next to the park (87 individuals total). I found 4 matching genotypes among these indicating that some individuals were sampled more than once. The grand total of individual wolves was 83; 62% were males and 38% were females. Subsequent analyses for genetic diversity and population structure were performed using 10 loci; sex-linked marker MS41B was removed to prevent a bias in heterozygosity measures.

2.3.2 Microsatellite diversity

I found consistent deviations from H-W equilibrium at locus PEZ01 and locus MS41b with deficiency in observed heterozygosity ($p < 0.001$). In addition, locus c09.250 deviates from H-W equilibrium in 4 out of 5 putative groups ($p < 0.001$). When putative populations are grouped together, all loci do not fulfill H-W equilibrium, likely due to heterozygosity deficiency, indicating that this is not a single panmictic population.

The observed levels of genetic variation are presented in Table 2.2. The mean number of alleles per locus ranged from 4.0 for the NW group to 6.0 for the SE group. Heterozygosity values (H_E) were high relative to other North American wolf populations (Forbes and Boyd 1997) and similar among groups, ranging from 0.5484 for the NW group to 0.6612 for the LR group.

2.3.3 Population structure and gene flow

Microsatellite matrices of θ-distances and D_s (Table 2.3) were not correlated with a matrix of geographic distances (km) between subgroups as indicated by Mantel tests (r = 0.30, p = 0.32; r = 0.58, p = 0.10, respectively). I used pairwise N_m-values to estimate the number of individuals that migrate between each pair of sampling localities (Table 2.4). The SE and SW groups had the highest values of N_m, whereas the NW had the lowest. I found no correlation between N_m values and geographical distance between localities (Mantel's test; r = -0.32, p = 0.73). Genetic structuring of wolf groups was also captured by an MDS plot (Figure 2.3); the NW wolf group was segregated from all other groups along dimension 1. The topology of populations illustrated by a neighbor-joining tree (Figure 2.4) was consistent with genetic structuring seen in the MDS plot.

2.4 Discussion

Wolves in central Saskatchewan exhibit relatively low levels of genetic structure, with the exception of the NW group, which is separate from all other groups. Moreover, the observed structure is not a result of isolation by distance. I expected this because wolves are highly vagile animals. All other groups were genetically similar suggesting that individuals mix freely. Genetic variation was high in these groups as was the number of migrants when compared with the NW group and other wolf populations in North America (Forbes and Boyd 1996, Lehman et al. 1992). High N_m, low Fst, high allelic diversity, and high levels of heterozygosity (HE) suggest high genetic turnover in the SE and SW groups.

Table 2.2. Genetic variation at 10 microsatellite loci in central Saskatchewan wolves.

Population	n	Mean no. of alleles	Ho	He
NE	16	4.9	0.428	0.597
NW	20	4.0	0.476	0.548
SE	27	6.0	0.509	0.656
SW	11	5.0	0.544	0.591
LR	13	5.0	0.600	0.661

Table 2.3. Pairwise genetic distances (lower; Nei 1978) and Fst (θ) (upper) among central Saskatchewan wolves.

	NE	NW	SE	SW	LR
NE	0	0.0735	0.0119	0.0237	0.0560
NW	0.130	0	0.0515	0.0744	0.0887
SE	0.034	0.093	0	0.0221	0.0275
SW	0.057	0.128	0.056	0	0.0695
LR	0.130	0.168	0.074	0.162	0

Table 2.4. Pairwise number of migrants (N_m) among 5 grey wolf populations in central Saskatchewan.

	NE	NW	SE	SW	LR
NE	0	3.2	20.8	10.3	4
NW	0	0	4.6	3.1	2.7
SE	0	0	0	11	10
SW	0	0	0	0	3.7
LR	0	0	0	0	0

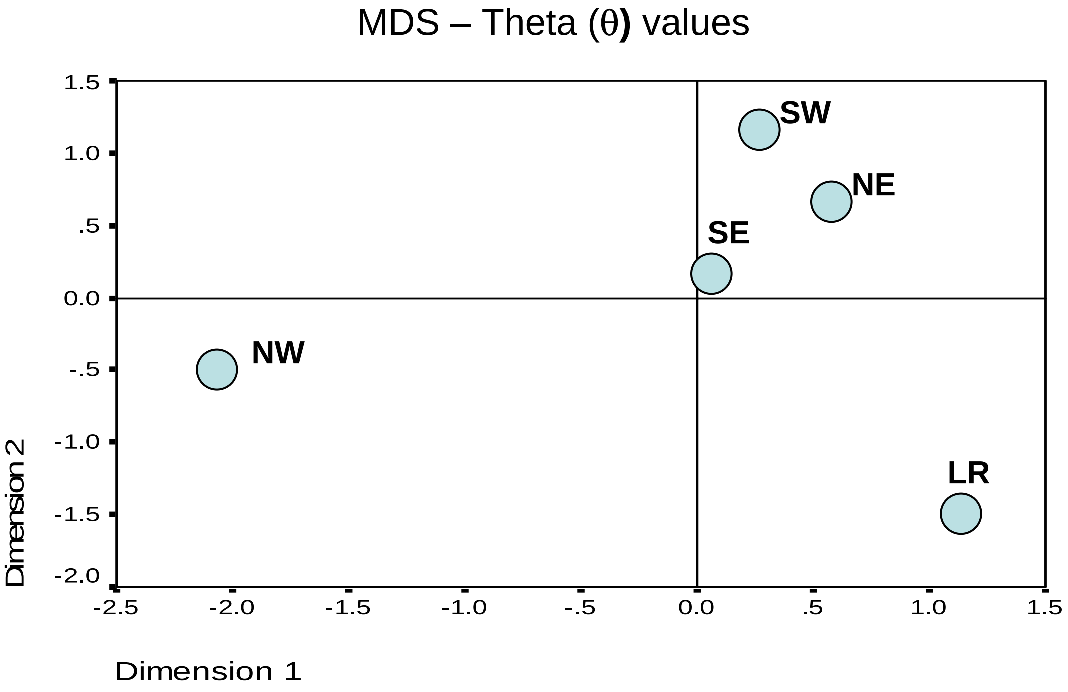

Figure 2.3. Pattern of 2-dimensional spatial structuring of wolf groups represented through Multidimensional Scaling (MDS) performed on the matrix of pairwise θ values obtained from microsatellite data.

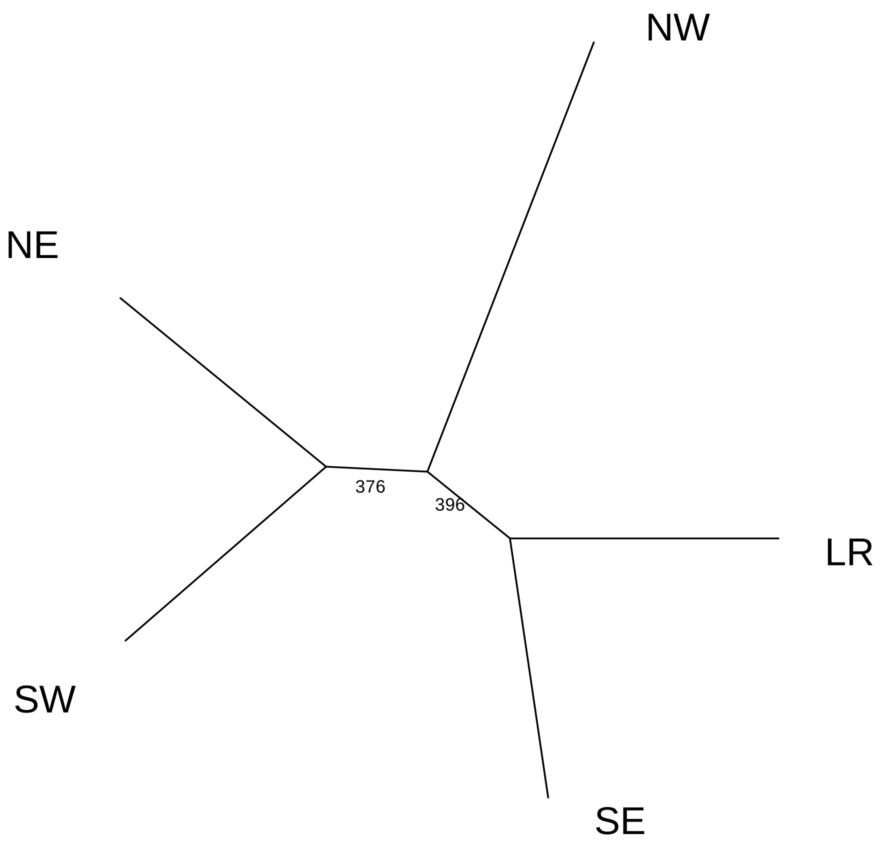

Figure 2.4. Neighbor-joining tree inferred from Nei's genetic distance measures calculated for microsatellite data.

Genetic structure of wolves from PANP and surrounding area suggest that human pressures like hunting and trapping may influence dispersal. Although there is a change in habitat type, and potentially prey distribution and abundance between PANP and Lac La Ronge, I found no significant genetic structure among these wolf groups. The partial isolation of the NW group may be explained by several ecological factors. Most wolf packs consist of a mated pair, their offspring, and adult helper offspring from previous years. Therefore, most members of a pack are closely related (Mech and Boitani 2003). These familial relationships have been confirmed using microsatellite analysis of social groups from Minnesota and Denali National Park, Alaska (Lehman et al. 1992, Meier et al. 1995). Some offspring, however, dispersed into neighboring packs or formed new packs nearby. No such inter-pack similarity was found in the Inuvik region of Canada's arctic, where wolves are heavily hunted, suggesting that higher genetic turnover may account for the absence of close family relationships (Lehman et al. 1992). In that study, one heavily exploited population had fewer kinship ties and more genetic turnover than two protected ones (Lehman et al. 1992).

Further, studies on temporal genetic variation in pack-forming coyotes during periods of locally aggressive removal indicated that exploitation perpetuated genetic homogeneity over relatively small geographic scales (Williams et al 2003). However, effects on social structure were unknown.

Wolves in the NW of PANP are less affected by hunting and trapping than other wolves in my study region because of limited access to the area by roads and trails. In addition, human activity and structures are uncommon or non-existent in this area. In comparison, all other populations are near primary (highway) and secondary roads supporting much higher levels of human activity. Accordingly, combined human pressures may be contributing to the genetic homogenization of wolf packs as follows. The pack(s) in the NW group may remain "quiet" with limited dispersal and high stability, whereas turnover in other groups may invite more dispersers causing pack instability and influencing the breeding pairs in those packs. If inter-pack kinship affects social stability and pack persistence (Wayne 1996), reducing the effects of wolf exploitation and road mortality on genetic population structure may be a conservation priority (Leader-Williams et al. 2001).

The observed structure could also be a function of sampling bias. The heavily sampled SE region of the park might be high quality habitat where a number of different packs overlap spatially but are segregated temporally. I defined groups as 1 or 2 packs using each region (quadrant). However, 3, 4, or 5 packs could be using an area at different times. Moreover, the size and shapes of territories may be structured so that all of them overlap already.

In comparison with other North American wolf populations, the observed structure in my study area was surprising. Genetic differentiation among North American wolf populations was low to moderate suggesting that exchange of a few individuals per generation is enough to prevent appreciable genetic structure by random drift (Roy et al. 1994a, 1994b, Forbes and Boyd 1996). Carmichael et al. (2001) and Musiani (2003) suggest that population differentiation occurred in Canada’s Northwest because of ecological specialization of wolves preying on different caribou herds. Similarly, the observed population subdivision of wolves in the NW region of PANP might reflect specialized use of some undocumented resource.

Genetic diversity of wolves in central Saskatchewan is high, in keeping with other North American populations (Boyd et al. 1997, Lehman et al. 1992, Roy et al. 1994, Carmichael et al. 2001). However, given increasing habitat loss and fragmentation, a future decline in genetic variation is likely (Ginsburg and Woodruffe 1997, Wayne and Vila 2003), especially considering historical genetic diversity has decreased by 50% since the historical mass persecution of wolves (Leonard et al. 2004). To prevent further declines, population sizes should be kept as large as possible. Further, intact habitat linkages should facilitate gene flow among populations. The loss of genetic variation in isolated wolf populations is of great concern. Wolves on the 450 km^2 Isle Royale, isolated by over 10 km of water, have levels of average relatedness approaching those of inbred captive populations and could suffer a decrease in fitness adversely affecting population persistence (Mace et al. 1996, Hedrick and Kalinowski 2000). Inbreeding depression in captive wolves has been documented (Laikre and Ryman 1991, Laikre et al. 1993, Fredrickson and Hedrick 2002) and implies dire consequences should this occur in wild populations. Maintaining genetic variation, particularly components that influence fitness, is vital to population persistence and the impending evolutionary response of animals to changing environmental conditions (Crandall et al. 2000).

2.5 References

Acton D. F., G. A. Padbury and C. T. Stushnoff. 1998. The eco-regions of Saskatchewan. Canadian Plains Research Centre, Saskatchewan Environment And Resource Management, Regina, Saskatchewan.

Balloux, F. and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology 11: 155-165.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, F. Bonhomme. 2000. GENETIX 4.0, logiciel sous Windows TM pour la génétique des populations. Laboratoir Génome, Populations, Interactions, CNRS UPR 9060, Université de Montpellier II, Montpellier France.

Black, W. C. and Krafsur, E. S. 1985. A FORTRAN program for the calculation and analysis of 2-locus linkage disequilibrium coefficients. Theoretical and Applied Genetics 70: 491-496.

Boyd, D. K., P. C. Paquet, S. Donelon, R. R. Ream, D. H. Pletscher, and C.C. White. 1995. Transboundary movements of a colonizing wolf population in the Rocky Mountains. Pages 135-140 *in* L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Edmonton, Alberta.

Callaghan, C. J. 2002. The ecology of gray wolf (*Canis lupus*) habitat use, survival, and persistence in the central Rocky Mountains, Canada. Ph. D. Thesis. University of Guelph, ON, Canada.

Carmichael, L. E., J. A. Nagy, N. C. Larter and C. Strobeck. 2001. Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. Molecular Ecology 10: 2787-2798.

Chepko-Sade, B. D., W. M. Shields, J. Berger, Z. T. Halpin, W. T. Jones, L. L. Rogers, J. P. Rood and A. T. Smith. 1987. The effects of dispersal and social structure on effective population size. Pages 297-321 *in* B. D. Chepko-Sade and Z. T. Halpin, editors. Mammalian dispersal patterns. University of Chicago Press, Chicago, USA.

Crandall, K. A., O. R. P. Bibida-Emonds, G. M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15: 290-295.

Ernest, H. B., E. S. Rubin, and W. M. Boyce. 2002. Fecal DNA analysis and risk assessment of mountain lion predation of bighorn sheep. Journal of Wildlife Management 66: 75-85.

Felenstein, J. 1993. PHYLIP, Phylogeny Inference Package version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.

Fitzsimmons, M. 2002. Estimated rates of deforestation in two boreal landscapes in central Saskatchewan, Canada. Canadian Journal of Forest Research 32: 843-951.

Forbes, S. H. and D. K. Boyd. 1996. Genetic variation of naturally colonizing wolves in the central Rocky mountains. Conservation Biology 10: 1082-1090.

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

Fredrickson, R. and P. Hedrick. 2002. Body size in endangered Mexican wolves: effects of inbreeding and cross-lineage matings. Animal Conservation 5: 39-43.

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

Fritts, S. H. 1983. Record dispersal of a wolf from Minnesota. Journal of Mammalogy 64: 166-167.

Geffen, E., M .J. Anderson,and R. K. Wayne. 2004. Climate and habitat barriers to dispersal in the highly mobile gray wolf. Molecular Ecology, in press.

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

Ginsberg, J. R. and R. Woodruffe. 1997. Extinction risks faced by remaining wild dog populations. Pages 75-87 *in* R. Woodruffe, J. R. Ginsberg, and D.W. MacDonald, editors. The African Wild Dog – Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

Gittleman, J. L., S. M. Funk, D. Macdonald, and R. K. Wayne, editors. 2001. Carnivore Conservation. Cambridge University Press, Cambridge, UK. 678 pages.

Gotelli, D., C. Sillero-Zubiri, G. D. Applebaum, M. S. Roy, D. J. Girman, J. Garcia Moreno, E. A. Ostrander, and R. K. Wayne. 1994. Molecular genetics of the most endangered canid: the Ethiopian wolf *Canis simensis*. Molecular Ecology 3: 301-312.

Hedrick, P. W. and S. T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics 31: 139-162.

Hobson, K. A., E. M. Bayne, and S. L. Van Wilgenburg. 2002. Large-scale conversion of forest to agriculture in the Boreal Plains of Saskatchewan. Conservation Biology 16: 1530-1541.

Kohn, M. H. and R. K. Wayne. 1997. Facts from Feces revisited. Trends in Ecology and Evolution 12: 223-227.

Kohn, M. H., E. C. York, D. A. Kamradt, G. Haught, R. M. Sauvajot, and R. K. Wayne. 1999. Estimating population size by genotyping faeces. Proceedings of the Royal Society of London B 266: 657-663.

Laikre, L. and N. Ryman. 1991. Inbreeding depression in a captive wolf (*Canis lupus*) population. Conservation Biology 5: 33-40.

Laikre, L. N. Ryman, and E. A. Thompson. 1993. Hereditary blindness in a captive wolf (*Canis lupus*) population: frequency reduction of a deleterious allele in relation to gene conservation. Conservation Biology 7: 592-601.

Leonard, J. A., C. Vila, and R. K. Wayne. 2004. Legacy lost: genetic variability and population size of historical and modern US gray wolves. Molecular Ecology. In press.

Leader-Williams, N., R. J. Smith, M. J. Walpole, K. McComb, C. Moss, and S. Durant. 2001. Elephant hunting and conservation. Science 293: 2203-2204.

Lehman, N., P. Clarkson, L. D. Mech, T. J. Meier, and R. K. Wayne. 1992. A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. Behavioral Ecology and Sociobiology 30: 83-94.

Liedloff, A. 1999. MANTEL, Version 2.0. Mantel nonparametric test calculator. School of Natural Resource Sciences. Queensland University of Technology, Brisbane, Australia.

Luccini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E. Randi. 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the Western Italian Alps. Molecular Ecology 11: 857-868.

Mace, G. M., T. B. Smith, M. W. Bruford, and R. K. Wayne. 1996. Molecular genetic approaches in conservation-and overview of the issues. Pages 1-12 *in* T. B. Smith and R. K. Wayne, editors. Molecular genetic approaches in conservation. Oxford University Press, Oxford, UK.

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

Mech, L.D. and L. Boitani. 2003. Wolf social ecology. Pp. 317-343 in L.D.Mech and L.

Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago.

Meier, T. J., J. W. Burch, L. D. Mech and L. G. Adams. 1995. Pack structure and genetic relatedness among wolf populations in a naturally-regulated population. Pages 393-302 *in* L. N. Carbyn, S. H. Fritts, and D. R. Seip, editors. Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Edmonton, Alberta.

Merrill, S. B. and L.D. Mech. 2000. Details of extensive movements by Minnesota wolves. American Midland Naturalist 144: 428-433.

Messier, F. 1985. Solitary living and extraterritorial movements of wolves in relation to social status and prey abundance. Canadian Journal of Zoology 63: 239-245.

Musiani, M. 2003. Conservation biology and management of wolves and wolf-human conflicts in western North America. Ph.D. Thesis, University of Calgary, AB, Canada.

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

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei, M. and A. K. Roychoudoury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76: 379-390.

Newmark, W. D. 1995. A land-bridge perspective on mammalian extinctions in western North American national parks. Conservation Biology 9: 512-526.

Nowak, R. M. 1999. Walker's Mammals of the World, Vol. I. Johns Hopkins University Press. Baltimore, MD, USA.

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

Palsbøll, P. J. 1999. Genetic tagging: contemporary molecular ecology. Biological Journal of the Linnean Society 68: 3-22.

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

Randi, E., V. Lucchini, and F. Francisci. 1993. Allozyme variability in the Italian wolf

(*Canis lupus*) population. Heredity 71: 516-522.

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

Roy, M. S., D. J. Girman, and R. K. Wayne. 1994. The use of museum specimens to reconstruct the genetic variability and relationships of extinct populations. Experentia 50: 551-557.

Sacks, B. N., S. K. Brown and H. B. Ernest. 2004. Population structure of California coyotes corresponds to habitat specific breaks and illuminates species history. Molecular Ecology 13: 1265-1275.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: a software for population genetics data analysis. Version 2.000. Genetics and Biometry Lab, Department of Anthropology, University of Geneva.

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

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

Taberlet, P., S. Griffin, B. Goosens, et al. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24: 3189-3194.

Taylor, L.R. and R.J. Taylor. 1977. Aggregation, migration and population mechanics. Nature 265: 415-421.

Theil, R. P. 1985. The relationship between road densities and wolf habitat suitability in Wisconsin. American Midland Naturalist 113: 404-407.

Van Camp, J. and R. Gluckie. 1979. A record long distance move by a wolf (*Canis lupus*). Journal of Mammalogy 60: 236.

Vila, C., I. R. Amorim, J. A. Leonard, D. Posada, J. Castroviejo, F. Petrucci-Fonseca, K. A. Crandall, H. Ellegren, and R. K. Wayne. 1999. Mitochondrial DNA phylogeography and population history of the grey wolf (*Canis lupus*). Molecular Ecology 8: 2089-2103.

Wayne, R. K. 1992. Mitochondrial DNA variability of the gray wolf: genetic consequences of population decline and habitat fragmentation. Conservation Biology 6: 559-569.

Wayne, R. K. 1996. Conservation genetics in the Canidae. Pages 75-118 *in* J. C.

Avise and J. L. Hamrick, editors. Conservation Genetics: case histories from nature. Cambridge University Press, Cambridge, UK.

Wayne, R. K. and C. Vila. 2003. Molecular genetic studies of wolves. Pages 317-343 *in* L. D. Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, USA.

Wayne, R. K., N. Lehman, and T. K. Fuller. 1995. Conservation genetics of the Gray wolf. Pages 399-408 *in* L. N. Carbyn, S. H. Fritts, and D R. Seip, editors. Ecology and Conservation of Wolves in a Changing World. Canadian Circumpolar Institute, Edmonton, AB, Canada.

Wayne, R.K., N. Lehman, M.W. Allard, R. L. Honeycutt. 1992. Mitochondrial DNA variability of the gray wolf – genetic consequences of population decline and habitat fragmentation. Conservation Biology 6: 559-569.

Wayne, R. K., N. Lehman, D. Girman, P. J. P. Gogan, D. A. Gilbert, K. Hansen, R. O. Peterson, U. S. Seal, A. Eisenhawer, L. D. Mech and R. J. Krumenaker. 1991. Conservation genetics of the endangered Isle Royale Gray wolf. Conservation Biology 5: 41-51.

Williams, C. L., K. Blejwas, J. J. Johnston, and M. M. Jaeger. 2003. Temporal genetic variation in a coyote (*Canis latrans*) population experiencing high turnover. Journal of Mammalogy 84: 177-184.

Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

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

Young, A. G. and G. M. Clarke, editors. 2000. Genetics, Demography, and Viability of fragmented populations. Cambridge University Press, Cambridge, UK. 438 pages.

3.0 FORAGING ECOLOGY AND PREY SELECTION BY GREY WOLVES

3.1 Introduction

Foraging decisions comprise one of the major determinants of various life-history strategies in predators, including spacing pattern, movement, habitat selection, social structure, reproduction, and geographical distribution (Krebs 1978, Bekoff et al. 1984). Predation profoundly influences ecosystem processes, influences natural selection, and structures ecological communities (Kotler et al. 1994, Kie 1999, Terborgh et al. 2001). The non-random nature of predation is fundamental in shaping prey community structure and plays a key role in perpetuating plant and animal diversity (Sih et al.1993, Terborgh et al. 1999, 2001). Predators often focus on infirm and naïve prey (FitzGibbon 1990) and some prefer one or more prey species to all species available (Huggard 1993, Karanth and Sunquist 1995). According to optimal foraging theory, predators should select predominantly for prey that are most profitable, irrespective of their densities or densities of other prey species (MacArthur and Pianka 1966, Chesson 1983, Krebs et al. 1983, Begon et al. 1996).

Variation in predation patterns are influenced by habitat characteristics, environmental conditions, prey assemblages, distribution, physical vulnerability, and defensive behaviour of prey, and risk of injury or mortality to the predator (Begon et al. 1996). Prey selection is a balance among profitability of prey items and their availability in the environment (Stephens and Krebs 1986, Forbes 1989). Forbes (1989) posited the dangerous prey hypothesis where handling behaviour is a function of the risk of injury to the predator. Hence, more dangerous prey should be handled more carefully (increasing time and energy demands) or not at all. Predators should then take dangerous prey less often because of longer handling times (Stephens and Krebs 1986: 128 - 150).

Grey wolves, as summit carnivores, feed on a wide variety of prey ranging in size from 1 kg to 1000 kg (Mech and Boitani 2003). Typically, wolves prey upon ungulates in all the ecosystems in which they occur (Poulle et al 1997, Jedrzejewski et al 2000, Mech and Boitani 2003), however they do not necessarily select for the largest ungulate available (Huggard 1993a). Wolves prey principally on moose (*Alces alces*) in Quebec, Yukon, and Isle Royale National Park (Messier 1985a, 1985b, Messier and Crete 1985 Peterson and Page 1988); deer in Algonquin Provincial Park, Canada, and Minnesota (Mech 1966, Fritts and Mech 1981, Fuller 1989), and muskox (*Ovibos moshatus*) and caribou (*Rangifer tarandus*) on Arctic islands (Miller 1995). Wolves in Wood Buffalo National Park (WBNP), Canada, primarily subsist on wood bison (*Bison bison*) (Carbyn et al. 1993). In Riding Mountain (Carbyn 1983) and Banff National Parks (Huggard 1993a, b), wolves preferentially prey on elk, even though many other ungulate species are available to them. Prey can vary spatially and seasonally, presumably as a function of their presence and availability. Pacific coastal wolves prey predominantly on sitka black-tailed deer (*Odocoileus hemonius*); however, they selectively capitalize on runs of fall salmon (*Onchorynchus* spp.) (Darimont and Reimchen 2002, Darimont et al. 2003). Wolves in Ontario and Manitoba substantially increase the amount of beaver eaten during summer months (Voigt et al 1976, Carbyn and Kingsley, 1979Meleshko 1986).

In western Canada, where multi-prey systems exist, wolves can choose from combinations of elk, moose, mule deer (*Oidocoileus hemonius*), white-tailed deer (*Oidecoileus virginianus*), bighorn sheep (*Ovis Canadensis*), mountain goat (*Oreamnos americanus*), bison, and caribou. In these systems, elk usually prevail in the winter diet of wolves (Carbyn 1983, Fuller 1989, Paquet 1992, Huggard 1993 a). A gap of knowledge remains in the central boreal forest of Saskatchewan where wolf foraging habits are unknown.

I collected information on wolves and their prey to i) identify major winter prey items for wolves, ii) test for prey selectivity between years (2002, 2003) under the null hypothesis of non-selective predation, and iii) examine these results in the context of foraging theory and risk-avoidance behaviour. Based on optimal diet theory, I predicted that wolves would mainly prey on elk and deer but would select elk (Carbyn 1983, Meleshko 1986, Paquet 1992, Huggard 1993 a).

In addition to ecological information, this study addresses important conservation questions. Predators such as wolves are important in ecosystem stabilization (Fryxell and

Lundburg 1994, Post et al. 2000). Describing the ecology of predator-prey systems in fragmented and unique habitats is important for understanding the behavior of wolves in human dominated systems. Such information is essential for conservationists interested in managing wolves that range outside of protected areas.

3.2 Methods

3.2.1 Study area

My study area was in central Saskatchewan (Figure 3.1). Prince Albert National Park (PANP), the core study area, harbours a wolf population (density of ~2/100km^2 - Paquet and Carbyn 2003, Urton et al. unpublished data) reliant on a multi-prey system. Predominantly boreal forest, PANP is ~3,875 km^2 and is the largest protected wilderness in Saskatchewan. Thirty percent of the area consists of lakes, streams, marshes, and bogs. The landscape is formed of steeply sloping eroded escarpments, glacial till plains, and level plateaus intermixed with sparsely treed peat-lands (Acton et al. 1998). Dominant vegetation include trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*), black spruce (*Picea mariana*), jack pine (*Pinus banksiana*) and balsam poplar (*Populus balsamifera*), as well as a variety of shrub (*Alnus* spp., *Betula* spp., *Salix* spp.) and under-story species. Potential mammalian prey species for wolves include elk, white-tailed deer, bison, moose, caribou, beaver (*Castor canadensis*), snowshoe hare (*Lepus americanus*), and smaller rodents (*Microtus* spp., *Peromyscus* spp.)(Arseneault 2003). Cultivated land, grazing areas, forest harvesting, road construction, and fur-trapping lines surround PANP and are common in adjacent provincial lands.

3.2.2 Field and laboratory procedures

I sampled wolf faeces (scats) throughout PANP and surrounding areas (Figure 3.2) during two winters (November to March of 2002 and 2003). I used scat analysis (n = 378 scats), a non-destructive and cost/time effective method (Ackerman et al. 1984, Ciucci et al. 1996), to estimate the proportion of different prey species consumed by wolves. Wolf scats were collected wherever encountered on trails, lakeshores, other linear features, and by backtracking wolves. GPS location and date were recorded and samples were stored frozen in plastic bags. These scats were differentiated from those of

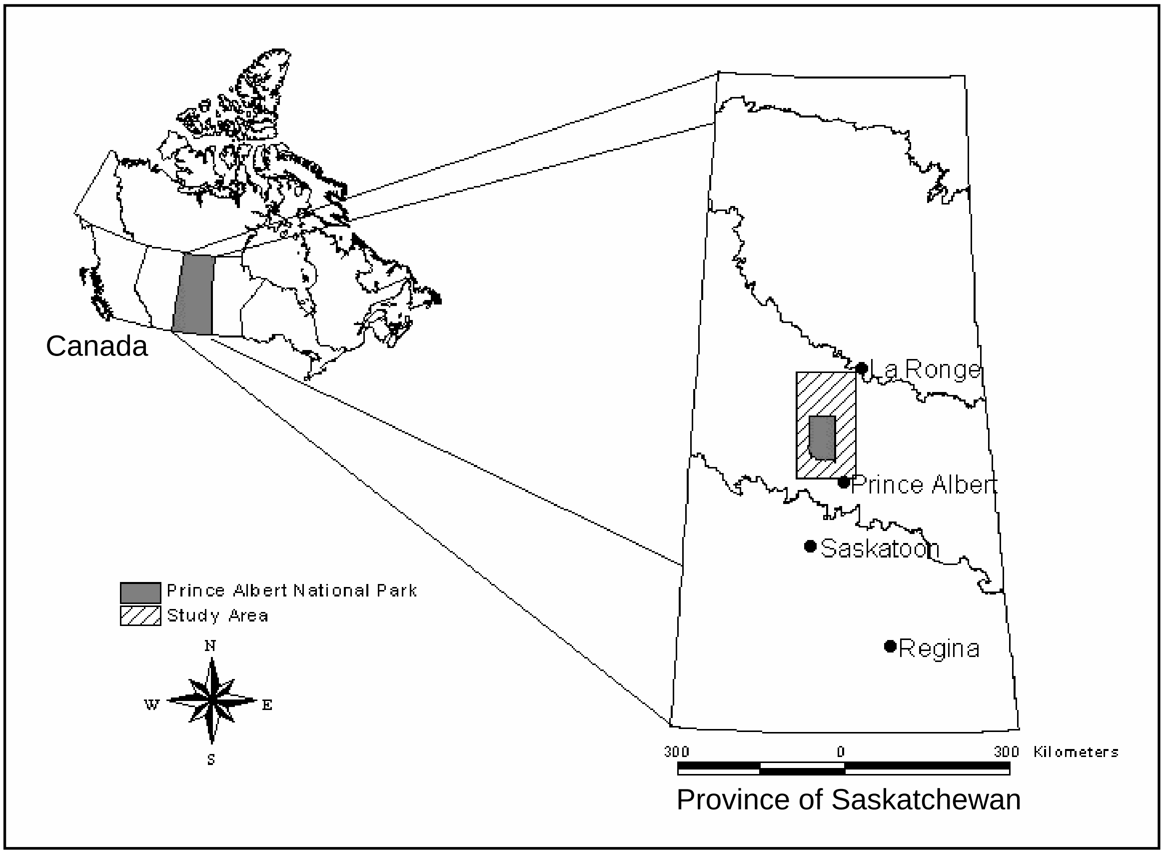

Figure 3.1: Study area in central Saskatchewan and Prince Albert National Park

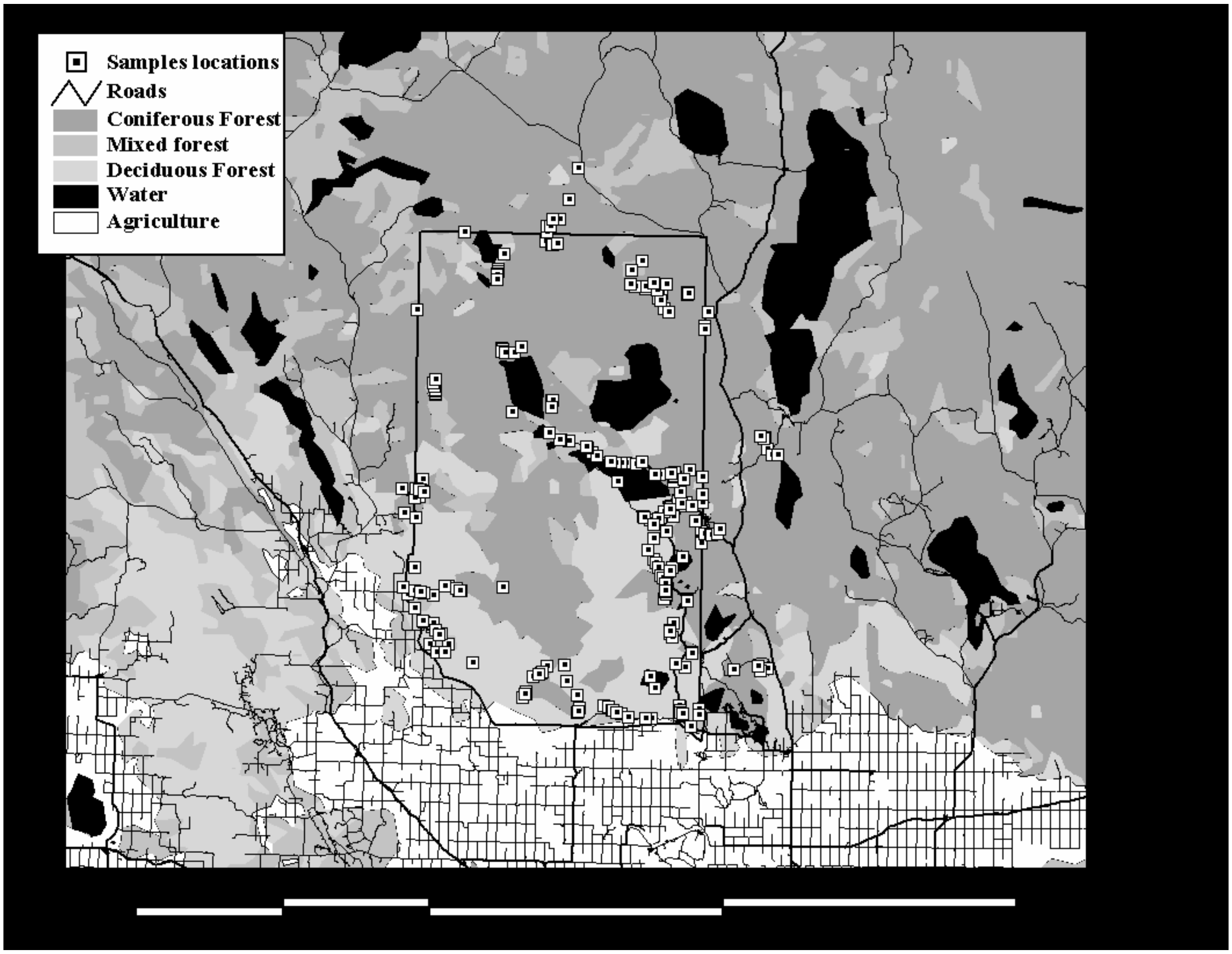

Figure 3.2. Wolf scat sample locations in central Saskatchewan and PANP (not all samples are shown).

coyote based on associated signs and tracks, scat size (>30 mm in diameter for wolves), and appearance. Scats that could not be positively identified as wolf scats were excluded from the analysis.

Laboratory procedures followed Meleshko (1986), Cuicci et al. (1996) and Kohira and Rexstad (1999). Scats were transferred to aluminum trays and oven-dried for 24 hours at 90°C as a precaution to kill hydatid tapeworms (*Echinococcus granulosus, E. multilocularis*) that may be present in scats. Latex gloves and dust masks were worn during all steps to decrease the risk of pathogen exposure. Sterilized scats were soaked for 48 hours and washed thoroughly in a fine grid sieve (1 mm) to separate macro- and microscopic materials. The remaining contents were spread in plastic trays and visually inspected for prey remains. Remains were identified by comparison with reference hair samples and with descriptions of hair characteristics. I also examined hairs using a dissection microscope and compared them with sample photos from Moore et al. (1974) and Kennedy and Carbyn (1981). I did not attempt to identify plant material.

3.3 Data analysis

3.3.1 Food habits

Prey remains in scats are biased representations of foods consumed by wolves because of different digestibility and detectability of prey items within scats (Weaver 1993, Kohira and Rextad 1999). I used two indices to describe wolf diets: occurrence/faeces (OF); the frequency that an item occurs in all scat samples, and occurrence/item (OI); the item's frequency among all items identified in all scats combined. An error rate (15%) was established by blindly re-sampling 10% of the scat collection for re-identification (Carbyn and Kingsley 1979, Fritts and Mech 1981, Ciucci et al. 1996). I used ANOVA to test for differences in OF indices between years (Zar 1999).

3.3.2 Biomass consumption

Smaller prey species, having more hair per unit body weight, produce more scats per unit prey weight consumed, leading to an over-estimation of small prey species in carnivore diets (Weaver 1993, Ciucci et al.1996). Because of biases inherent in OF and

OI indices, frequency of identification of prey remains do not give a representative picture of the consumed proportion of different prey species when the prey types vary in size (Floyd et al. 1978, Weaver 1993). A correction factor, developed by Weaver (1993) from feeding trials with captive wolves, was used to estimate the relative proportion of biomass of different prey species consumed by wolves in the study area. The equation used is as follows:

$$Y = 0.439 + 0.008x \tag{3.1}$$

Where Y = kg of prey consumed per field collectible scat and x = average weight of an individual of a particular prey type. Average prey weights were taken from Nowak (1999). Solving for Y gave an estimation of biomass consumed per collectible scat for each prey type. Multiplying Y by the number of scats found to contain a particular prey species gave the relative weights of each prey type consumed. These values were used to estimate the percent biomass contribution of different prey species to wolf diets and represent a more biologically meaningful evaluation of diet (Ciucci et al. 1996, Biswas and Sankar 2002). Biomass estimates excluded non-mammalian prey.

3.3.3 Estimation of prey selectivity

I used the program SCATMAN (Hines and Link 1993; Link and Karanth 1994) to arrive at the expected proportions of prey species in scat. The observed proportion of prey species in scats was compared with the expected proportions to determine if predation by wolves was selective. The expected proportion of scats containing a prey species was calculated using point estimates of auxiliary variables: scat rate production per prey species (λ_i) and prey density (d_i) (Link & Karanth 1994). The multinomial likelihood estimator used to compute the expected proportion of scats from a kill of a particular prey species is:

$$\Pi_i = \frac{d_i \lambda_i}{\Sigma_i \, d i \lambda_i} \tag{3.2}$$

where λ is the number of scats produced from a single kill of species i. Estimates of λ were derived using data from Floyd et al (1978) and Weaver (1993). Individual prey density estimates (No. per km^2 ± SD) were taken from Arseneault (2003); Elk: 0.19 ± 0.25, white-tailed deer: 0.94 ± .18, moose: 0.19 ± 0.22, beaver: 1.0 ± 0.5. Although density estimates have not been confirmed independently, bootstrapping procedures within the model should compensate for imprecision.

I compared observed and expected proportions of prey species using the χ^2 goodness of fit test (Zar 1999). Because density estimates are inexact and highly variable, correction factors accounting for over-dispersion in tests of goodness of fit were applied by using SCATMAN. Variability in the density estimates of each prey species and number of scats produced from a kill of any prey species is a potential source of inflation of Type I error (Link and Karantth 1994). The program SCATMAN incorporates the effect of such variability (Link and Karanth 1994) and reduces the inflation of Type I error to produce an unbiased probability value. I implemented the parametric bootstrap procedure of the program for 1000 times to alleviate the above problem. Patterns in overall prey selection were inspected for use of each species as calculated by SCATMAN. I chose a level of significance of $\alpha < 0.01$. A sensitivity analysis adjusting the coefficient of variation (CV) for scat rate production from 10% to 40% is automatically performed in each run of the model. To be conservative, I chose to examine statistics generated at CV = 40% (Karanth & Sunquist 1995). I performed this analysis on data from both years.

3.4 Results

3.4.1 Wolf food habits

Of 426 food items identified in 378 scats, the most common item was white-tailed deer in both OF and OI indices for combined years (43% and 39%, respectively), followed by elk (33%, 30%), moose (7%,6%), beaver (5%,4%), snowshoe hare (2%,1%), avian (2%, 1%), vegetation (4.5%, 4%), and other (5%, 5%); (see Table 3.1). I found no differences in OF indices between years; see Table 3.2, Figure 3.3 (ANOVA, F = 0.0684, df = 1, p = 0.804).

3.4.2 Biomass contribution

Estimation of the relative biomass contribution of different prey species to wolf diet gave a clearer assessment of prey use than results obtained from frequency of occurrence (Weaver 1993). In terms of OF, white-tailed deer contributed more than elk. However, using relative biomass, elk contributed nearly 50% of available biomass to winter wolf diet whereas deer contributed only 33%. Moose contributed 14% of biomass, and smaller mammals such as beaver and snowshoe hare showed a cumulative biomass contribution of 1.5 % (Table 3.1).

3.4.3 Prey selectivity

A comparison of observed and expected proportions of prey species in wolf scats based on individual densities rejected the hypothesis of non-selective predation in both study years (2002: $\chi^2 = 39.6$, p = 0.99, df = 3; 2003: $\chi^2 = 145.0$, p = 0.99, df = 3). Because there was evidence of selective predation in the overall pattern of prey use by wolves in each year, the results of the analyses were examined to infer selectivity for each species. In 2002, elk (p = 0.0001) and deer (p = 0.002) were found to be consumed by wolves disproportionate to their availability; i.e. wolves selectively consumed both species. I found moose and beaver consumed in proportion to their availability (p = 0.46, p = 0.49 respectively). In 2003, I found elk (p = 0.0001) and beaver (p = 0.0001) were consumed disproportionately to their availability. Wolves selectively preyed upon elk but avoided beaver. I found deer and moose to be consumed in proportion to their availability (p = 0.95, p = 0.04 respectively).

3.5 Discussion

3.5.1 Wolf diet composition

Preference for cervids by wolves was clearly demonstrated by this study. I found elk to be the most selected prey with the largest winter contribution of biomass in the wolf diet. Despite the paucity of ecological data in this area, the patterns demonstrated by this analysis generally agree with findings from studies of wolf food habits in Riding Mountain National Park, an area similar in habitat features (Carbyn 1983, Meleshko 1986, Paquet 1992). I found that wolves selected elk in the study area in both years. Elk

Table 3.1: Composition of wolf scats and relative biomass contribution of different prey species to winter wolf diet in Prince Albert National Park (2002, 2003)

Species	No. of scats	%OF	%OI	Avg. weight (kg)	% Biomass contribution
Elk	125	33	30	286	50.48
White-tailed deer	163	43	39	120	33.77
Moose	27	7	6	390	14.23
Beaver	18	5	4	23	1.46
Snowshoe hare	1	0	0	1.5	0.06
Avian	6	2	1	n/a	n/a
Vegetation	17	4.5	4	n/a	n/a
Other	20	5	5	n/a	n/a

No. of feces = 378

No. of items = 426

Table 3.2. Composition of wolf scats in each sampling year in Prince Albert National Park

	2002			2003		
Species	No. of scats	%OF	%OI	No. of scats	%OF	%OI
Elk	28	36	34	97	32	29
White-tailed deer	21	27	26	142	47	42
Moose	16	21	20	11	4	3
Beaver	9	12	11	9	3	3
Snowshoe hare	0	0	0	1	0	0
Avian	0	0	0	6	2	2
Vegetation	1	1	1	16	5	5
Other	3	4	4	17	6	5
TOTAL	78	101	96	300	99	89
total items	82			339		

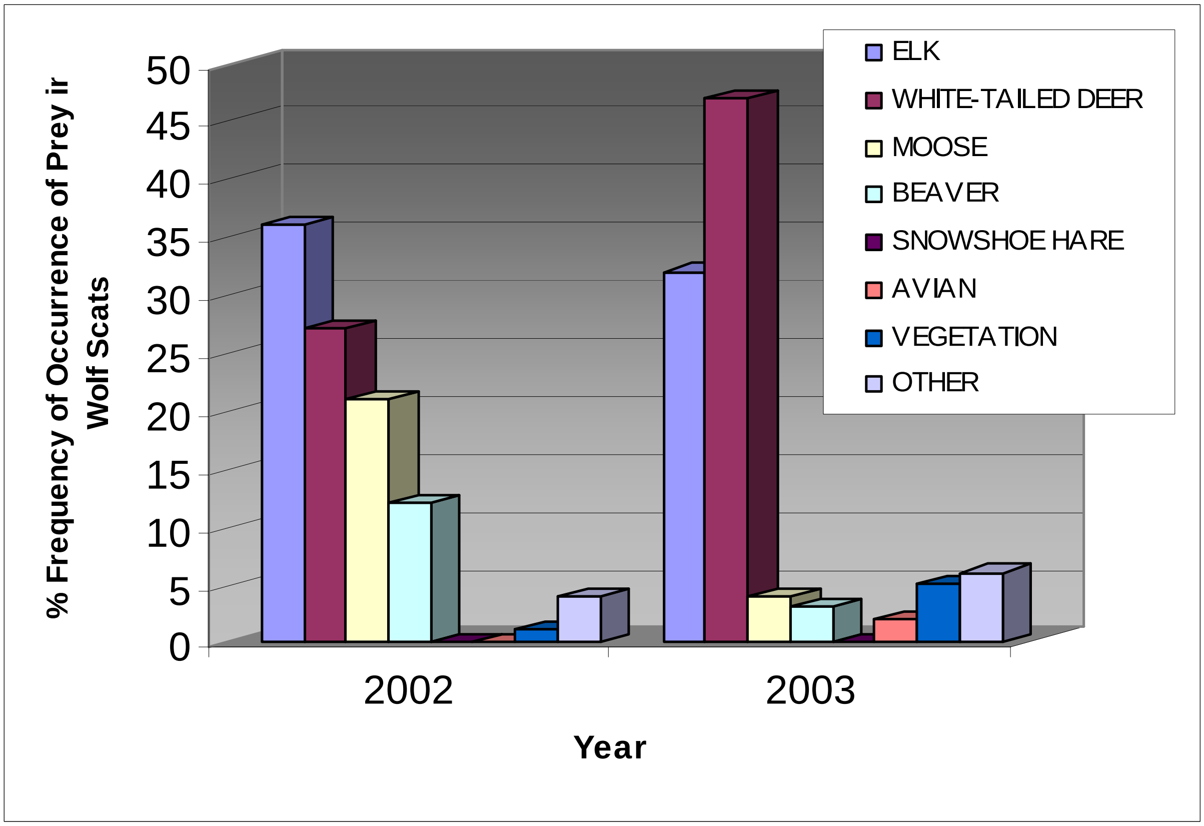

Figure 3.3: Proportions of prey species in winter wolf diets in 2002 and 2003 in PANP, central Saskatchewan.

occur in relatively high densities, contributing to increased encounter rates with predators. However, their gregarious nature may also be a factor that increases their chance of depredation (Nelson and Mech 1981, Messier et al. 1988, Huggard 1993a, 1993b, Hebblewhite and Pletscher 2002).

Deer are far more abundant in terms of density than other ungulates in this system (Arseneault 2003), explaining their standing as the most frequently occurring food item in wolf scats. However, the relative biomass contributed to wolf diet was nearly half that of elk. Moose were also found to be an important dietary item for wolves. Although this ungulate species is available to wolves, behavioural constraints may operate to relax predation on moose relative to elk and deer. Moose are generally solitary species and aggressively defend themselves and their young (Mech 1966). Killing the largest bodied ungulate in this system, with the latter behavioural characteristics, presents high risk for wolves.

Beaver were a secondary prey item for wolves and were consumed in proportion to their abundance in 2002 but avoided in 2003. Beavers are inactive above water in winter months and are not easily available to wolves. Samples collected in spring (March and April) in 2002 were over-represented leading to the conclusion of non-selection. Samples collected in 2003 underrepresented spring and therefore describe a strictly winter diet where wolves would seemingly “avoid” beaver.

The appearance of snowshoe hare and avian prey at low frequencies demonstrates the wolf’s plasticity to hunt opportunistically. Although these species are abundant and available, they appear infrequently in wolf diets. Expending energy on such small prey all the time likely does not pay.

Livestock, primarily cattle (*Bovis* spp.), occur within and near my study area. Livestock depredation by wolves is well documented throughout the world (Pullianien 1965, Fritts and Mech 1981, Gunson 1983, Tompa 1983, Vos 2000). No evidence of hair from cattle was found in 378 wolf scats. Livestock depredation either occurs so infrequently that this type of analysis could not detect it, or other ungulate species provide an adequate natural prey base facilitating a disinterest in livestock (Vos 2000). Moreover, venturing on to agricultural land may present too great a risk as wolves often have negative encounters with humans (Mazzioli et al 2002).

A herd of approximately 300 plains bison also occupies the south-west quadrant of PANP. I found no evidence of bison in wolf scats in winter. These large bodied and highly defensive ungulates may present too large a challenge and too great a cost to wolves. Although wolves that were tracked near bison ranges within the study area were observed investigating bison bedding and feeding sites, no wolf predation on bison was documented (L. O'Brodovich personal communication). A summer diet investigation my indicate that wolves prey on calves. Other natural prey may exist in sufficient abundance that switching to bison is not required. In areas such as WBNP, wolves predominantly prey on bison. However, alternate cervid prey are not abundantly or readily available (Carbyn et al. 1993, Joly and Messier 2000).

3.5.2 Risk-avoidance and optimal foraging

Animals select for a particular food type when the proportion of that type in the animal's diet is higher than that in the environment (MacArthur and Pianka 1966, Stephens and Krebs 1986). To obtain food, predators must expend time and energy, first in searching for prey and then handling it. Generalist predators such as wolves pursue both more and less profitable types of prey (Jedrzejewski et al. 2000, Huggard 1993 a, Mech and Boitani 2003). Optimally foraging predators should balance risk and profitability to maximize their overall rate of energy intake.

Wolves risk injury (Rausch 1967, Pasitschniak-Arts et al. 1988) and mortality in attempting to kill large-bodied prey (Mech 1970). Healthy, vigorous prey often escape wolf predation by fighting or fleeing (Mech 1984, Nelson and Mech 1993, Stephenson and Van Ballenberghe 1995). Weaver (1992) noted that about 25% of 1,450 wolves killed by humans in control programs in Alaska showed traumatic skull injuries, presumably inflicted by moose and other large prey. On occasion, moose, bison, elk, and deer can kill attacking wolves (Stanwell-Fletcher 1942, Frijlink 1977, Nelson and Mech 1985, Mech and Nelson 1990, Weaver 1992), with risk increasing with size of prey (Weaver et al. 1992). Several studies have shown that predators often search for less risky opportunities rather than attack such dangerous prey (Stephens and Krebs 1986: 128 -150, Forbes 1989). Although moose are available in density equal to that of elk, wolves may avoid preying on them to avoid risk. When prey that flee to avoid predation, such as elk and deer, are present in high densities and portray much lower risk relative to gains, wolves may prefer to search for and attack them. Bison are not taken at all in this system presumably because of the energy demand incurred, risk involved in capture and handling, and the availability of less risky prey. Risk involved in killing cattle may be viewed as a reduced conflict with humans (Mazzioli et al. 2002).

Further, wolves require a mean rate of 1.7 kg of prey/wolf/day for daily maintenance requirements and 3.2 kg of prey/wolf/day for successful reproduction (Mech 1970). Moose provide roughly 4.4-6.3 kg/wolf/day (Mech 1966), white-tailed deer roughly 3.8-2.9 kg/wolf/day (Paquet and Carbyn 2003), and elk falling somewhere between those ranges based on body mass. Therefore it may not be necessary to risk injury when adequate prey are available that meet a wolf's daily metabolic requirements.

My results lend support to the notion that wolves choose to balance risk in foraging strategies in accordance with the dangerous prey hypothesis (Forbes 1989). We must note, however, that predation on a prey species is likely to be determined by the density of groups rather than individuals, which influences encounter rates between the predator and its prey in a forested ecosystem (Huggard 1993 a, Karanth and Sunquist 1995, Biswas and Sankar 2002). Although density of groups affects encounter rates, group size would also be an important factor influencing the probability of sighting prey by the predator. I have data only on individual densities and so must be cautious when interpreting results. In any case, large profitable prey are readily available in this environment, yet wolves select for medium sized ungulate prey. Adapted for chase, wolves expend more energy in the pursuit of these prey, however this cost may be offset by a considerable decrease in risk of injury or death in the process.

I noted wolves capitalizing on a wide variety of prey types in this system, indicative of a generalist predator. However, prey selection is evident and is in concordance with optimal foraging theory where wolves should balance risk and energy demands with profitability. Social effects on diet, where young wolves observe experienced conspecifics while hunting and learn to recognize the same search images, may facilitate this pattern (Shettleworth et al. 1993).

Predators need considerable behavioural plasticity to use effectively prey assemblages in different ecosystems. Large body size and flexible predatory behaviour release wolves from the constraint of prey specificity. Human activity has modified most habitats available to wolves leading to changes and perhaps reductions in ungulate distribution and abundance. Given the rate of habitat loss in the boreal forest (Hobson et al. 2002, Fitzsimmons 2002), PANP remains a vital area for examining wolf food habits and the subsequent effects of human-induced change on ecosystem diversity.

3.6 References

Ackerman, B. B., F. G. Lindzey, and T. P. Hemker. 1984. Cougar food habits in southern Utah. Journal of Wildlife Management 48: 147-155.

Acton D. F., G. A. Padbury and C. T. Stushnoff. 1998. The eco-regions of Saskatchewan. Canadian Plains Research Centre, Saskatchewan Environment and Resources Management, Regina, Saskatchewan.

Arseneault A. A. 2003. Status and Management of Wildlife in Saskatchewan, 1999-2001. Saskatchewan Environment Fish and Wildlife Technical Report.

Begon, M, J. L. Harper and C. R. Townsend. 1996. Ecology: individuals, populations and communities. Blackwell Science Ltd. Oxford.

Bekoff, M., T. J. Daniels and J. L. Gittleman. 1984. Life history patterns and comparative social ecology of carnivores. Annual Review of Ecology and Systematics 15: 191-232.

Biswas, S. and K. Sankar. 2002. Prey abundance and food habits of tigers (*Panthera tigris tigris*) in Pench National Park, Madhya Pradesh, India. Journal of Zoology London 256: 411-420.

Carbyn, L. N. 1983. Wolf predation on elk in Riding Mountain National Park, Manitoba. Journal of Wildlife Management 47: 963-976.

Carbyn, L. N. and M. C. Kingsley. 1979. Summer food habits of wolves with the emphasis on moose in Riding Mountain National Park. Proceedings of the. North American Moose Conference Workshop 15: 349-361.

Carbyn, L. N., S. M. Oosenbrug and D.W. Anions. 1993. Wolves, bison and the dynamics related to the Peace Athabaska Delta in Canada’s Wood Buffalo National Park. Circumpolar Research Series, no. 4. Canadian Circumpolar Institute, University of Alberta, Edmonton, AB, Canada.

Chesson, J. 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology 64: 1297-1304.

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

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

Darimont, C. T., T. E. Reimchen and P. C. Paquet. 2003. Foraging behaviour by gray wolves on salmon streams in coastal British Columbia. Canadian Journal of Zoology 81: 349-353.

Fitzsimmons, M. 2002. Estimated rates of deforestation in two boreal landscapes in central Saskatchewan, Canada. Canadian Journal of Forest Research 32: 843-951.

Floyd, T. J., L. D. Mech and P. J. Jordan. 1978. Relation wolf scat contents to prey consumed. Journal of Wildlife Management 42:528-532.

Forbes, L. S. 1989. Prey defences and predator handling behavior: the dangerous prey hypothesis. Oikos 55: 155-158.

Frijlink, J. H. 1977. Patterns of wolf pack movements prior to kills as read from tracks in Algonquin Park, Ontario, Canada. Bijdragen Tot De Dierkunde 47:131-137.

Fritts, S. H. and L. D. Mech. 1981. Dynamics, movements and feeding ecology of a newly protected wolf population in northwestern Minnesota. Wildlife Monographs 80: 1-79.

Fryxell, J. M. and P. Lundberg. 1994. Diet choice and predator-prey dynamics. Evolutionary Ecology 8: 407-421.

Fuller, T. K., and L. B. Keith. 1980. Wolf population dynamics and prey relationships in northeastern Alberta. Journal of Wildlife Management 44:583-602.

Fuller, T. K. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs No. 105.

Gunson, J. R. 1983. Wolf predation of livestock in western Canada. Pages 102-105 *in* L. N. Carbyn, editor. Wolves in Canada and Alaska. Canadian Wildlife Service Report Number 45.

Hebblewhite, M. and D. H. Pletscher. 2002. Effects of elk group size on predation by wolves. Canadian Journal of Zoology 80: 800-809.

Hobson, K. A., E. M. Bayne and S. L. Van Wilgenburg. 2002. Large-scale conversion of forest to agriculture in the Boreal Plains of Saskatchewan. Conservation Biology 16: 1530-1541.

Huggard, D. J. 1993a. Prey selectivity in Banff National Park. I. Prey species. Canadian Journal of Zoology 71: 130-139.

Huggard, D. J. 1993b. Prey selectivity of wolves in Banff National Park, II. Age, sex, and condition of elk. Canadian Journal of Zoology 71:140-147.

Jedrzejewski, W., K. Schmidt, J. Theuerkauf, B. Jedrzejewska, and H. Okarma. 2000. Prey selection and predation by wolves in Biolwieza Primeval Forest, Poland. Journal of Mammalogy 81: 197-212.

Joly, D. O. and F. Messier. 2000. A numerical response of wolves to bison abundance in Wood Buffalo National Park, Canada. Canadian Journal of Zoology 78: 1101-1104.

Karanth, K. U. and M. E. Sunquist. 1995. Prey selection by Tiger, leopard and dhole in tropical forests. Journal of Animal Ecology 64:439-450.

Kennedy, A. J. and L. N. Carbyn. 1981. Identification of wolf prey using hair and feather remains with special reference to western Canadian National Parks. Unpublished Canadian Wildlife Service Report.

Kie, J. G. 1999. Optimal foraging and risk of predation: effects on behaviour and social structure in ungulates. Journal of Mammalogy 80: 1114-1129.

Kohira, M. and E. A. Rextad. 1997. Diets of wolves, *Canis lupus*, in logged and unlogged forests of southeastern Alaska. Canadian Field Naturalist. 111: 429-435.

Kotler, B. P., J. S. Brown and W. A. Mitchell. 1994. The role of predation in shaping the behaviour and community organization of desert rodents. Australian Journal of Zoology 42: 449-466.

Krebs, J. R. 1978. Optimal foraging: decision rules for predators. Pages 23-63 *in*. J. R Krebs and N. B. Davies, editors. Behavioural Ecology. Sinauer, Sunderland, MA.

Krebs, J.R., D.W. Stephens and W.J. Sutherland. 1983. Perspective in optimal foraging. Pages 165-216 *in* A. H. Brush and G. A. Clark, Jr., editors. Cambridge University Press, New York, USA.

Link, W. A. and K. U. Karanth. 1994. Correcting for overdispersion in tests of prey selectivity. Ecology 75: 2456-2459.

MacArthur, R. H. and E. R. Pianka. 1966. On optimal use of a patchy environment. American Naturalist 100: 603-609.

Maziolli, M., M. E. Graipel and N. Dunstone. 2002. Mountain lion depredation in southern Brazil. Biological Conservation 105: 43-51.

Mech, L. D. 1970. The wolf: the ecology and behaviour of an endangered species. Doubleday/Natural History, Garden City, NY, USA.

Mech, L .D. 1966. The Wolves of Isle Royale. U.S. National Park Service Fauna Series, no. 7. Unite States Government Printing Office. 210 pages.

Mech, L.D. 1984. Predators and predation. Pp.189-200 *in* L.K. Halls, editor. White tailed deer ecology and management. Stackpole, Harrisburg, PA.

Mech, L. D. and L. Boitani. 2003. Wolves: Behavior, Ecology and Conservation. University of Chicago Press. Chicago and London.

Mech, L. D., and M. E. Nelson. 1990. Evidence of prey-caused mortality in three wolves. American Midland Naturalist 123:207-208.

Meleshko, D. W. 1986. Feeding habits of sympatric canids in an area of moderate ungulate density. M.Sc. Thesis. University of Alberta, Edmonton, AB.

Messier, F. 1985a. Social organization, special distribution, and population density of wolves in relation to moose density. Canadian Journal of Zoology 63: 239-245.

Messier, F. 1985b. Solitary living and extraterritorial movements of wolves in relation to social status and prey abundance. Canadian Journal of Zoology 63:239-245.

Messier, F and M. Crete. 1985. Moose-wolf dynamics and the natural regulation of moose populations. Oecologia 65: 503-512.

Messier, F. J. Huot, D. Le Hanaff, and S. Luttich. 1988. Demography of the George River Caribou Herd: Evidence of population regulation by forage exploitation and range expansion. Arctic 41: 279-287.

Miller, F.L. 1995. Status of wolves in the Canadian Arctic islands. Pages. 35-42 *in* L. N. carbyn, S. H. Fritts and D. R. Seip, editors. Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, AB.

Moore, T. D., L. E. Spence and C. E. Dugnolle. 1974. Identification of the dorsal guard hairs of some mammals of Wyoming. Wyoming Game and Fish Deptartment, Cheyenne, WY. 177 pages.

Nelson, M. E., and L. D. Mech. 1981. Deer social organization and wolf predation in northeastern Minnesota. Wildlife Monograph 77.

Nelson, M. E., and L. D. Mech. 1985. Observations of a wolf killed by a deer. Journal of Mammalogy 66:187-188.

Nelson, M. E. and L.D. Mech. 1993. Prey escaping wolves, *Canis lupus*, despite close proximity. Canadian Field Naturalist 107: 245-246.

Nowak, R. M. 1999. Walker's Mammals of the World, Vol. I. Johns Hopkins University Press, Baltimore, USA.

Paquet P. C. 1992. Prey use strategies of sympatric wolves and coyotes in Riding Mountain National Park, Manitoba. Journal of Mammalogy 73: 337-343.

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

Pasitschniak-Arts, M., M. E. Taylor, and L. D. Mech. 1988. Skeletal injuries in an adult arctic wolf. Arctic Alpine Research 20:360-365.

Peterson, R. O. and R. E. Page. 1988. The rise and fall of the Isle Royale Wolves. Journal of Mammalogy 69:89-99.

Post, D.M., M.E. Conners, and D.S. Goldberg. 2000. Prey preference by a top predator and the stability of linked food chains. Ecology 81:8-14.

Poulle, M. L., L. Carles, B. Lequette. 1997. Significance of ungulates in the diet of recently settled wolves in the Mercantour Mountains (Southeastern France). Review of Ecology (Terr. Vie) 52: 357-368.

Pullianinen, E. 1965. Studies on the wolf (Canis lupus) in Finland. Annals of Zoologica Fenniscandia 2: 215-259.

Rausch, R. A. 1967. Some aspects of the population ecology of wolves, Alaska. American Zoologist 7: 253-265.

Shettleworth, S. J., P. Reid and M. S. Plowright. 1993. The psychology of diet selection. Pages 56-77 *in* R.N. Hughes, editor. Diet Selection. Blackwell Scientific Publications, Oxford, UK.

Sih, A. 1993. Effects of ecological interactions on forager diets: competition, predation risk, parasitism and prey behaviour. Pages 182-211 *in* R.N. Hughes, editor. Diet Selection. Blackwell Scientific Publications, Oxford, UK.

Stanwell-Fletcher, J. F. 1942. Three years in the wolves' wilderness. Natural History 49: 1-7.

Stephens, D. W., and J. R. Krebs. 1986. Foraging theory. Princeton University Press, Princeton, New Jersey, USA.

Stephenson, T. R. and V. Van Ballenberghe. 1995. Defense of one twin calf against wolves, *Canis lupus*, by a female moose, *Alces alces*. Canadian Field Naturalist 109: 251-253.

Terborgh, J., J. Estes, P. Paquet, K. Ralls, D. Boyd-Heger, B. Miller, and R. Noss. 1999. The role of top carnivores in regulating terrestrial ecosystems. Wild Earth 9: 42-56.

Terborgh, J., L. Lopez, V. P. Nunez, M. Rao, G. Shahabuddin, G. Orihuela, M. Riveros, R. Ascanio, G. H. Adler, T. D. Lamber, and L. Balbas. 2001. Ecological meltdown in predator-free forest fragments. Science 294: 1923-1926.

Tompa, F. S. 1983. Problem of wolf management in British Columbia: conflict and program evaluation. Pages 112-119 *in* L. N. Carbyn, editor. Wolves in Canada and Alaska. Canadian Wildlife Service Report Number 45.

Voigt, D. R., G. B. Kolenosky and D. H. Pimlott. 1976. Changes in summer foods of wolves in central Ontario. Journal of Wildlife Management 40: 663-668.

Vos. J. 2000. Food habits and livestock depredation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. Journal of Zoology London 251: 457-462.

Weaver, J. L. 1992. Two wolves, *Canis lupus*, killed by a moose, *Alces alces*, in Jasper National Park, Alberta. Canadian Field Naturalist 106:126-127.

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

Zar, J.H. 1999. Biostatistical Analyis. 4th edition. Prentice Hall, Upper Saddle River, NJ, USA.

Vos. J. 2000. Food habits and livestock depredation of two Iberian wolf packs (*Canis lupus signatus*) in the north of Portugal. Journal of Zoology London 251: 457-462.

4. 0 GREY WOLF TROPHIC RELATIONSHIPS IN THE BOREAL FOREST: INSIGHTS FROM STABLE ISOTOPES

4.1 Introduction

Describing and understanding patterns and functional relationships between predator communities and their prey are fundamental objectives for ecologists (Hall and Raffaelli 1997, Post 2002). Insights into trophic relationships among organisms can help define species' roles in communities and track energy flow (Post 2002). Understanding trophic interactions, community composition, and dynamics of energy flow is also vital for investigating species' resource requirements and determining how communities, and ultimately ecosystems, are regulated (Litvaitis 2001).

The Boreal forest ecosystem harbours a diversity of mammalian species. The grey wolf, a summit carnivore, limits and often regulates its prey on the landscape (Messier 1994, Terborgh et al. 1999). Carnivores such as wolves exert a strong top-down role stabilizing the trophic structure of ecosystems (Terborgh et al. 1999, Post et al. 2000, Wilmers et al. 2003). Wolf kills are important sources of food for avian and mammalian scavengers (Wilmers et al. 2003).

Within species groups, variance in trophic position or diet among individuals has several ecological, evolutionary, and conservation implications (Bolnick et al. 2003). Since natural selection acts on individuals, differences among individuals in diet can contribute to differences in individual fitness. To date, the tendency has been to describe diets of animals at the population level with little consideration of individual differences (Carbyn 1983, Meleshko 1985, Paquet 1992, Durell 2000, Bolnick et al. 2003). Conservation strategies, in particular, are traditionally based on identifying average resource requirements for a population (Durell 2000, Bolnick et al. 2003). Differential foraging strategies, such as those occurring between the sexes, may confer an advantage in fitness to each group (Casaux et al. 2001, Durell et al. 1993, Beier 1988). However, individual variability in foraging strategies may also be detrimental to some individuals in the population, especially those with niches divergent from those that are more common. In carnivores, such as wolves, kin selected traits related to social grouping may act to counter differential access of individuals to available prey (Creel and Creel 1995, Creel 1997).

Previously, our understanding of animal trophic relationships has been hampered by limitations in methodology. Several studies have described the advantages to using the stable isotope approach to augment more traditional dietary methods (Hobson et al. 1994; Hobson et al. 2000, Litvaitis 2001). Stable isotope assays provide a continuous measure of trophic position and can potentially identify relatively complex trophic strategies such as omnivory (Peterson and Fry 1987, Cabana and Rasmussen 1996). Moreover, they offer discrete information about individuals. Stable nitrogen isotope ratios show a stepwise enrichment of about 3-5 ‰ in $\delta^{15}N$ with increasing trophic level (DeNiro and Epstien 1978, 1981; Tieszen et al. 1983, Bocherens and Drucker 2003) whereas $\delta^{13}C$ values change little as carbon moves through food webs (Peterson and Fry 1987, France and Peters 1997, Post 2002). However, when $\delta^{13}C$ values of sources differ, they can be used to evaluate the ultimate sources of carbon for a consumer (Tieszen et al. 1983). Coupled $\delta^{15}N$ and $\delta^{13}C$ values provide both source and trophic information (Schell and Ziemann 1989, Hobson and Clark 1993, Hobson et al. 1994). Isotopic measurements of metabolically active tissues like blood plasma, whole blood, and muscle represent integrations of diet over days to several weeks, while bone collagen can reflect diet over a lifetime (Hobson et al. 1993). Metabolically inactive tissue, such as hair, reflects diet during the growth phase and so represents diet up to many months (Tieszen et al. 1983, Darimont and Reimchen 2002).

Trophic relationships and feeding ecology among seabirds (Hobson et al. 1994), black bears (*Ursus americanus*), grizzly bears (*Ursus arctos*) (Hilderbrand et al. 1996, Jacoby et al. 1999, Hobson et al. 2000), and more recently, grey wolves (Szepanski et al. 1999, Darimont & Reimchen 2002, Bocherens & Drucker 2003) have been studied with good success by measuring stable isotope ratios in feathers and hair. However, these studies primarily trace marine dietary inputs, which are easily differentiated isotopically from terrestrial inputs (Chisholm et al. 1982, Hobson and Sealy 1991, Ben-David et al. 1997a, 1997b). Past applications of stable isotopes used distinctive $\delta^{13}C$ and $\delta^{15}N$ values of various food sources to determine their relative contribution to an animal's diet

(Hobson et al. 1994; Ben-David et al. 1997a, 1997b,). This method relies on mass balance equations and distinct isotope signatures of various sources to determine their relative contributions to a mixed signature and is limited to solving for n+1 sources when n isotopes are used. However, recently developed models (Phillips and Gregg 2003) have since allowed the examination of ranges of possible dietary inputs in complex systems in which the number of isotopically different sources exceeds the number of isotopes used.

In this study, I applied conventional (faecal analysis) and stable isotope techniques to elucidate trophic relationships involving wolves in the boreal forest of central Saskatchewan, Canada. I was particularly interested in examining variance in isotopic signatures among individual wolves. I predicted that wolves would show among the highest δ^{15}N values for all mammals (i.e. corresponding to their assumed top predatory role) and that isotopic variance among individuals would be relatively low for these social carnivores versus other more solitary species.

4.2 Methods

4.2.1 Study area

Wolves inhabit nearly two thirds of the boreal forest of Saskatchewan from Duck Lake, (52°81 N, 106°22 W) in the south to the North West Territories in the north. This region, the Boreal Plain Eco-zone, is a forested matrix with interspersed wetlands, lakes and rivers (Acton et al. 1998). Nearly 30% of this area is covered with water. The landscape consists of steeply sloping eroded escarpments, glacial till plains, and level plateaus intermixed with sparsely treed peat-lands (Acton et al. 1998). My study area, in the central region of Saskatchewan (Figure 2.2 in Chapter 2), encompasses the Aspen Parkland and Boreal Forest Transition eco-regions and has a complex mixture of communities (Acton et al. 1998). Dominant vegetation includes trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*), black spruce (*Picea mariana*), jack pine (*Pinus banksiana*), and balsam poplar (*Populus balsamifera*), as well as a variety of shrub (*Alnus* spp., *Betula* spp., *Salix* spp.) and under-story species. Potential mammalian prey species of wolves include elk (*Cervus elaphus*), white-tailed deer (*Oidecoileus* *virginianus*), bison (*Bison bison*), moose (*Alces alces*), caribou (*Rangifer tarandus*), beaver (*Castor canadensis*), snowshoe hare (*Lepus americanus*) and small rodents (*Microtus*, *Peromyscus*, etc.) (Arsenseault 2003). Cultivated land, grazing areas, forest harvesting, road construction, and fur-trapping lines are prominent in this area. This region accounts for the bulk of the province's merchantable timber. Prince Albert National Park (PANP) comprised the core of my study area.

4.2.2 Sample collection

During the winters (Nov-Mar) of 2001/2002 and 2002/2003, I opportunistically collected hair samples from 18 mammalian species [wolf, coyote (*Canis latrans*), red fox (*Vulpes vulpes*), black bear, lynx (*Lynx canadensis*), wolverine (*Gulo gulo*), fisher (*Martes pennanti*), marten (*Martes americana*) raccoon (*Procyon lotor*), elk, white-tailed deer, bison, moose, caribou, beaver, snowshoe hare, northern flying squirrel (*Glaucomys sabrinus*), and muskrat (*Ondatra zibethica*)] from trappers. Some hair samples were also obtained from road kills.

Each sample consisted of guard hairs. All species analysed were killed in winter, therefore, I assumed hairs taken were probably grown from mid-summer to late-fall or early-winter; roughly 4 to 5 months. Sex, age, and body condition were unknown for most samples.

4.2.3 Isotopic analyses

Hair samples were cleaned with a 2:1 chloroform:methanol solution and dried for 24 hours under a fume hood. One mg sub-samples were loaded into 4x6 mm tin cups (Isomass Scientific) and combusted at 1,800 °C in a Robo-Prep elemental analyzer. Further analyses of resultant CO_2 and N_2 gases were performed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS). Isotope signatures are expressed in delta notation as ratios relative to PeeDee Belemnite (PDB) (carbon) and atmospheric N_2 (Air)(nitrogen) standards. Analytical error was estimated to be ±0.1‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$ values based on thousands of measurements of an albumen lab standard.

4.2.4 Isotopic calculations

I used Kruskal-Wallis tests to evaluate differences in isotopic values among individual wolves in three locations within and outside PANP for carbon and nitrogen (Zar 1999). I used Dunn's tests to determine which locations were different and Levene's test for homogeneity to examine equality of group variances. Finally, I used Z-tests on independent proportions with an applied Yates correction to test differences between measures of diet (Zar 1999). All tests were performed using SigmaStat (2.0).

4.2.5 IsoSource

Dietary mixing models provide an indication of relative prey consumption. Consumer diet-hair discrimination values, although not established for wolves, were based on captive experiments using red foxes (Roth and Hobson 2000), the closest relative to the wolf for which values have been measured. These values were ±3.4‰ and ±2.6‰ for δ^{15}N and δ^{13}C values, respectively, and similar to those found for other mammalian species (DeNiro and Epstien 1981, Kelly 2000). To calculate dietary endpoints of prey items, I used the mean isotopic signatures of hair corrected for dietary fractionation by applying the appropriate fractionation values for each isotope.

Measurement error and sample variability of both sources and mixtures combine to create uncertainty in source contribution estimates. These sources of uncertainty can be implicitly incorporated by the choice of tolerance values for isotopic mass balance (Phillips & Gregg 2003). I adjusted mass balance tolerance and source increment values in IsoSource to 0.1‰ and 1%, respectively.

4.3. Results

4.3.1 Mammalian trophic relationships

As expected, δ^{13}C values for wolves in the central boreal forest of Saskatchewan reflected a terrestrial C_3 dominated ecosystem (Figure 4.2). Table 4.1 summarizes mean isotopic signatures and their variances for all 18 species examined. The ranges in isotope values for wolves were broad; 5.4‰ to 11.2‰ for δ^{15}N and -19.7‰ to -24.3‰ for δ^{13}C suggesting a highly varied diet and dietary differences among individuals during the period of hair growth. I plotted mean δ^{15} N and δ^{13}C values for three groups of wolves (Figure 4.2, Table 4.2) to illustrate this variability: those from within a protected area, Prince Albert National Park (PANP), those outside PANP but at roughly the same latitude, and those 150 km north of PANP, near Lac La Ronge, SK. I found significant differences in isotope values for wolves among sites (δ^{15}N: $\chi^2 = 11.6$, df = 2, p = 0.003; δ^{13}C: $\chi^2 = 10.8$, df = 2, p = 0.005). La Ronge and Outside wolves were significantly different from PANP wolves in δ^{15}N values (Dunn's test, Q = 3.25, $p < 0.05$; Q = 2.5, $p < 0.05$) and PANP and Outside wolves differed from La Ronge wolves in δ^{13}C values (Dunn's test, Q = 3.0, $p < 0.05$; Q = 2.6, $p < 0.05$). Variances among the 3 groups were not equal for either isotope group. Wolves from La Ronge and outside PANP were more variable than those sampled inside (Levene's test, $F_{2,44} = 4$, p = 0.025 for δ^{15}N, $F_{2,44} = 7.9$, p = 0.001 for δ^{13}C)(Figure 4.2).

Range and standard deviation of the mean stable isotope values for all wolves were similar to those for coyotes, a solitary generalist carnivore (Table 4.1). Variances were also similar to those for black bears, an omnivorous species known to have a highly varied diet (Nowak 1999).

4.3.2 IsoSource

Using *a priori* knowledge of wolf diets and knowing the prey composition in my study area based on literature, I selected 5 most likely prey species to include in the IsoSource model. These were: elk, deer, moose, beaver, and snowshoe hare. I limited my consumer value to the mean calculated for wolves in and around PANP (n = 26, mean = 7.3 ‰). The ranges of feasible solutions of the distribution of relative contributions of each prey item to wolf diet produced by IsoSource are reported as 25th-75th percentile ranges. These are less influenced by outliers on the tails of the distributions than minimum and maximum values.

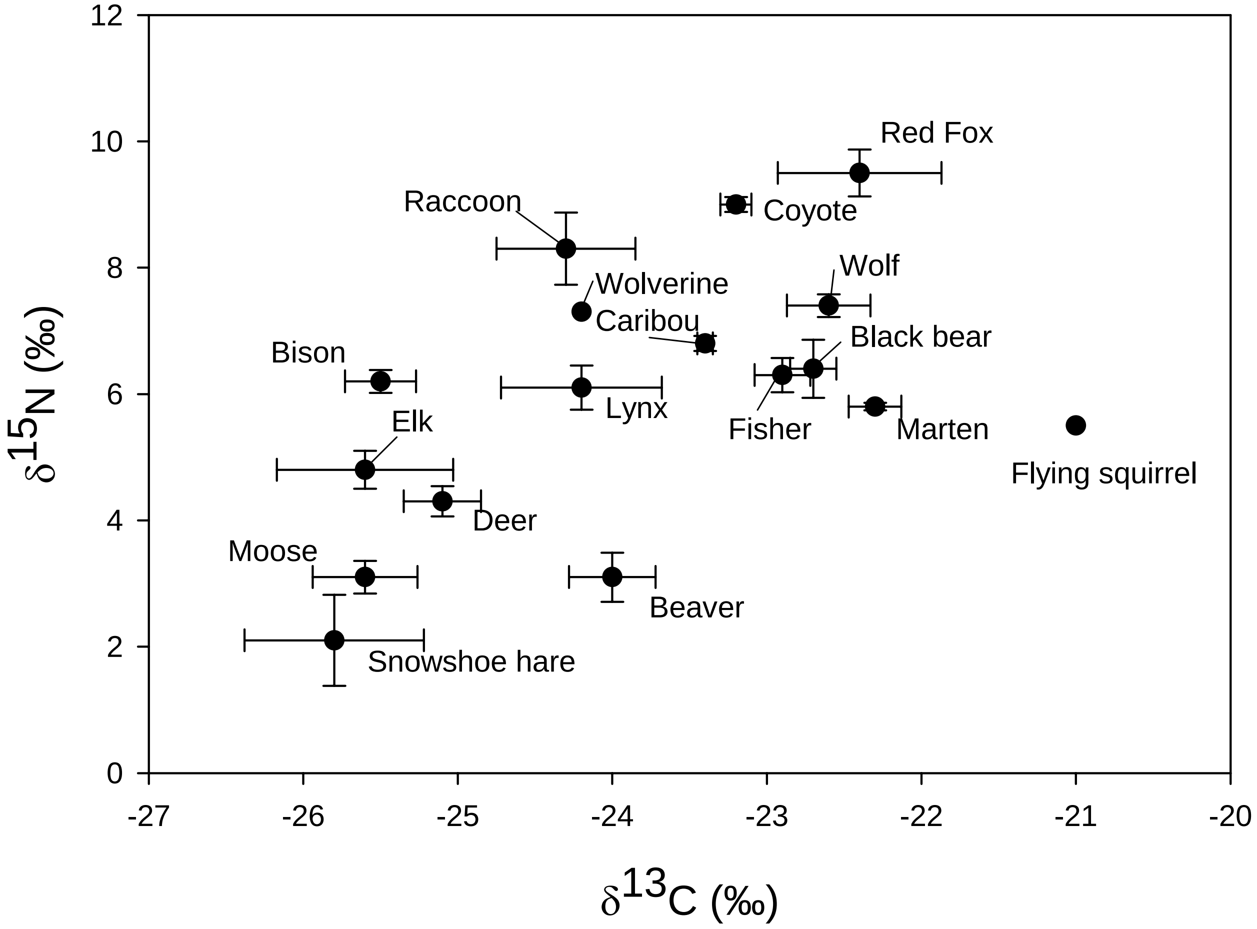

Figure 4.1. Distribution of mean δ^{13}C and δ^{15}N ± SE values (‰) for 18 boreal forest mammals sampled in central Saskatchewan sampled over two consecutive winters (2002, 2003). Values for muskrat are not included in this plot.

Table 4.1. Summary of means, standard deviations (SD) and ranges (min to max) for δ^{15}N and δ^{13}C values in hair for 18 mammalian species sampled from the central boreal forest of Saskatchewan.

Species	n	δ^{15}N (‰)	SD	range (‰)	δ^{13}C (‰)	SD	range (‰)
Wolf	47	7.4	1.22	5.4 to 11.2	-22.4	1.1	-24.3 to -19.7
Coyote	42	9	1.04	7.1 to 12.2	-23.2	0.7	-24.4 to -21.7
Red fox	9	9.5	1.3	7.3 to 11	-22.4	1.3	-23.6 to -18.7
Black bear	8	6.4	1.9	4.6 to 10.9	-22.7	0.53	-23.7 to -22.0
Marten	3	5.8	0.18	5.6 to 5.9	-22.3	0.3	-22.7 to -22.0
Raccoon	2	8.3	…	…	-24.3	…	…
Fisher	5	6.3	0.75	5.2 to 7.1	-22.9	0.5	-23.4 to -22.3
Wolverine	1	7.3	…	…	-24.2	…	…
Lynx	3	6.1	0.93	5.2 to 7.1	-24.2	1.2	-25.6 to -23 .5
Beaver	8	3.1	1.5	0.5 to 4.9	-24	1.3	-26.3 to -21.8
Moose	7	3.1	0.83	2.0 to 4.4	-25.6	0.5	-26.1 to -23.5
Bison	9	6.2	0.78	5.1 to 7.8	-25.5	1	-26.6 to -23.5
White tailed deer	10	4.3	1.1	2.7 to 6.5	-25.1	1.2	-26.3 to -22.1
Caribou	26	6.8	0.77	5.6 to 8.4	-23.4	0.3	-24.0 to -22.6
Flying squirrel	1	5.5	…	…	-21	…	…
Elk	9	4.8	1.3	2.2 to 6.8	-25.6	1.1	-27.1 to -24.2
Snowshoe hare	5	2.1	2	0.0 to 4.6	-25.8	0.9	-27.3 to -25.1
Muskrat	1	4.3	…	…	-12.1	…	…

Table 4.2. Summary of means, standard deviations (SD) and ranges (min to max) for $\delta^{15}N$ and $\delta^{13}C$ values for 3 groups of wolves sampled at different locations throughout the central boreal forest of Saskatchewan.

Location	n	$\delta^{15}N$ (‰)	SD	range (‰)	$\delta^{13}C$ (‰)	SD	range (‰)
PANP	16	6.5	0.56	5.4 to 8.2	-22.9	0.32	-23.7 to -22.5
Outside PANP	14	7.4	0.98	5.4 to 9.3	-22.5	1.2	-24.3 to -20.4
La Ronge	17	7.9	1.5	5.5 to 11.2	-21.7	1.25	-23.5 to -19.7

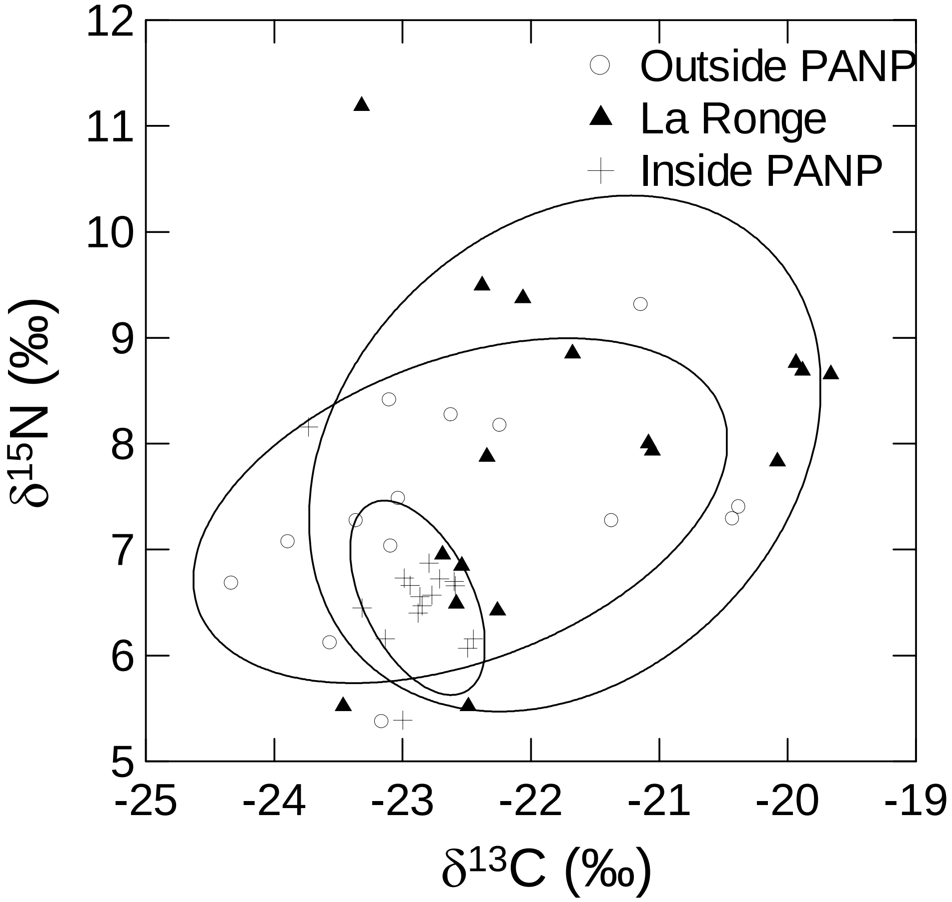

Figure 4.2. $\delta^{13}C$ and $\delta^{15}N$ values and ellipses showing one standard deviation for 3 groups of wolves sampled at different locations throughout the boreal forest of central Saskatchewan, Canada.

As solutions, Phillips and Gregg (2003) caution against using a single proportion such as the mean relative contribution to diet because it misrepresents the actual lack of uniqueness of the results of the model. I report the mean with my ranges only for comparative purposes especially when considering the results of my scat analysis. Wolves relied significantly on elk (range: 30-62%; mean: 48%), white tailed deer (11-35%; 22%), moose (7-27%; 14%), beaver (4-17%; 8%) and snowshoe hare (4-16%; 8%) (Figure 4.4).

I compared results from this isotope model with results from a faecal analysis performed blind to the isotope analyses (Urton et al., in prep.). Percent dietary biomass consumption, calculated using a regression equation specific to wolf diets (Weaver 1993), did not vary significantly from the mean estimates of relative prey contributions indicated by IsoSource (elk: $z = -0.229$, $p = 0.819$; deer: $z = 0.436$, $p = 0.663$; moose: $z = 0.220$, $p=0.826$; beaver: $z = -0.211$, $p = 0.833$; snowshoe hare: $z = -1.91$, $p = 0.056$). This measure of diet is most meaningful and reflects the relative importance of prey in diet (Ciucci et al. 1996, Weaver 1993; Scott and Shackleton 1980).

4.4 Discussion

4.4.1 Trophic relationships involving wolves

Carnivores separated into discrete isotopic groups in this boreal forest system. I initially expected wolves to be at the top of the food chain. Notably, however, red fox and coyote were more enriched in δ^{15}N and $\delta\,^{13}$C. Plant food items originating from cultivated areas may be enriched in ^{15}N relative to those from forested areas (Riga 1971, Hobson et al. 1999, Ostrom et al. 2002, Lavin et al. 2003). As scavenging species and opportunistic hunters of small rodent prey, red fox and coyote may have foraged at or near the agricultural areas interspersed in the southern boreal landscape. Although, preying predominantly on large ungulate prey, wolves also prey on smaller mammals (beaver and snowshoe hare) but tend to avoid open agricultural areas (Carbyn 1983; Meleshko 1985; Paquet 1992) therefore consuming foods more depleted in ^{15}N than coyote and red fox. Fisher and marten, opportunists and scavengers, also prey on medium-to-small sized rodents; however, they rarely venture into open agricultural areas and are restricted to dense forest cover (Nowak 1999).

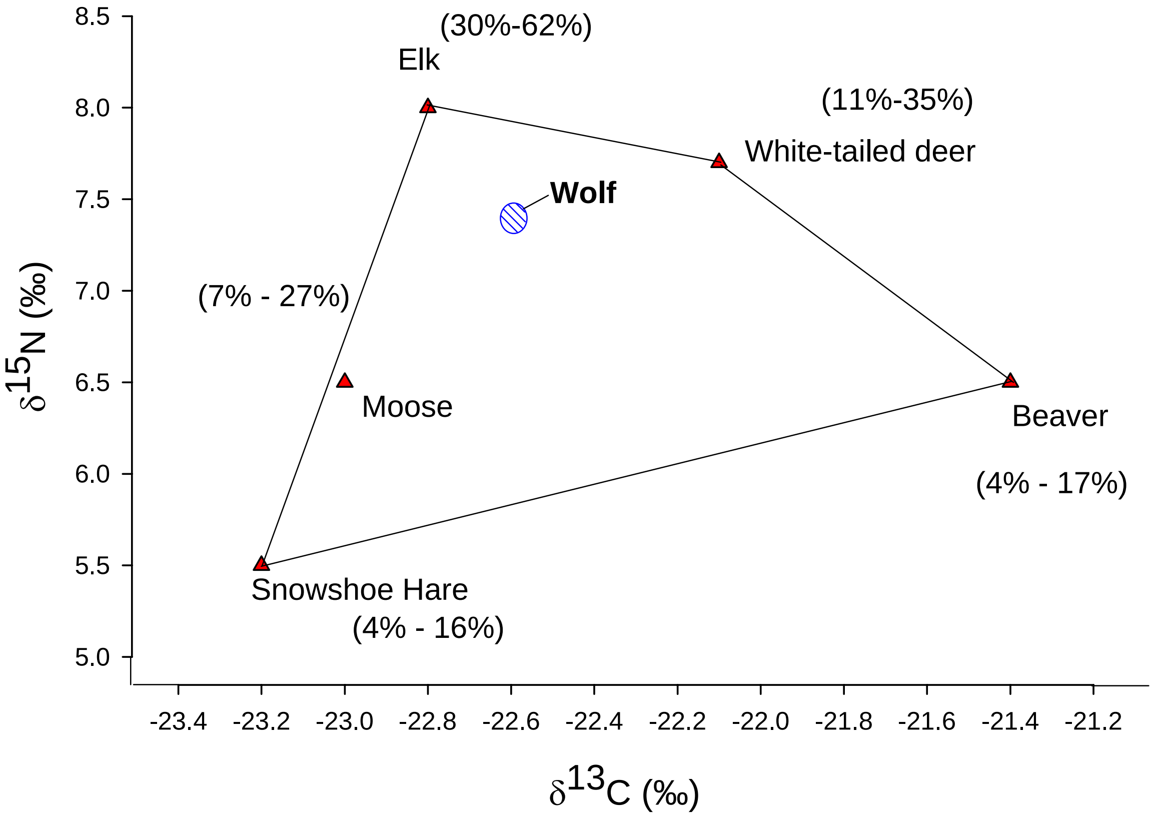

Figure 4.3. Mixing polygon for $\delta^{13}C$ and $\delta^{15}N$ values of five food sources for gray wolves in central Saskatchewan. I report the 25th to 75th percentile ranges for the calculated feasible distributions in IsoSource.

As specialists on snowshoe hare, lynx δ^{13}C and δ^{15}N values reflected their preference for consuming this prey item. They, like snowshoe hare, were the most depleted in both isotopes (excluding δ^{13}C values for marten) and clearly occupied a different niche than predatory generalists like wolves, fishers, martens, red foxes or coyotes. Black bears, an omnivorous species, showed high variation in δ^{15}N values and were positioned below other strictly carnivorous predators. High variation in δ^{15}N values indicates a highly varied diet. Black bears are known to forage extensively on forbs, berries and seeds, but also prey on insects, young of year ungulates, and frequently scavenge carcasses (Nowak 1999).

Due to dietary overlap, I could not differentiate between δ^{15}N and δ^{13}C values for elk and deer. Both species browse and graze to the same extent on similar vegetation types (Nowak 1999). Moose and beaver, although sharing identical δ^{15}N values, differed in δ^{13}C values (i.e. by 2 ‰). Both species spend much of their time foraging in aquatic habitat and wetlands, however, beaver spend considerably more time in the water and also consume the woody portions of plants like willow and aspen to a greater extent. δ^{15}N values in moose were lower than other ungulates possibly because of the high proportion of browse in their diet (Sponheimer et al. 2003, Drucker et al. 2003). Bison were enriched in ^{15}N reflecting their preference for sedges and grasses around wetlands (Nowak 1999, Stewart et al. 2003). Also, bison are foregut fermenters and digest large amounts of symbiotic microflora and this can result in ^{15}N enrichment (Sponheimer et al 2003). Caribou were even more enriched in δ^{15}N and δ^{13}C than bison and other herbivores, likely reflecting their preference for lichens (Barnett 1994; Nowak 1999; Wison and Ruff 1999, Drucker et al. 2001). Lichens, a water-stressed species, show higher δ^{13}C values than other plant forms (Barnett 1994). Similarly, I speculate that flying squirrels showed this enrichment feeding on lichens, mushrooms, and bracket fungi (Wilson and Ruff 1999); however, isotope values for fungi are unknown.

4.4.2 Individual variation

I expected similar isotopic values among individual wolves because of their social foraging behaviour (Mech 1970). Wolves hunt cooperatively and I assumed that all individuals have access to the same food resources as indicated by conventional diet studies (Scott and Shackleton 1980, Meleshko 1986, Carbyn and Kingsley 1979, Carbyn et al. 1985, Paquet 1992, Weaver 1993, Ciucci et al. 1996). However, the variability in isotopic signatures were similar to those measured for coyotes and bears, solitary generalists known to have highly varied diets.

Stable isotope values of wolves sampled outside of PANP and La Ronge were significantly different and more variable from those sampled within PANP. Differences in prey abundance and distribution may account for these differences indicating varied wolf diets. Exploited wolf populations (e.g. those occurring outside of PANP) experience higher population turnover and pack instability compared to protected wolves (Lehman et al. 1992). Increased movements of individuals among packs can also contribute to greater population genetic variation (Lehman et al. 1992, Meier et al. 1995). Enhanced movements may also be linked to isotopic variation. Unstable packs and lone individuals often have larger home ranges and are more mobile than established stable packs (Mech and Boitani 2003). A more varied diet may result as individual wolves can specialize on different prey or consume a larger variety of prey compared with animals in social groups. Our findings of higher isotopic variation within the two exploited wolf populations compared with the protected wolves of PANP are consistent with this hypothesis.

High individual isotopic variation in animals has been demonstrated to be due to consumption of a variety of marine resources and/or vegetation (Ben-David 1997, Hilderbrand et al. 1996, Szepanski 1999, Darimont and Reimchen 2002). For example, Szepanski et al. (1999) found that wolves on the coast of SE Alaska had variable isotopic signatures due to differential consumption of marine-derived salmon resources. They also found that interior Alaska wolves, in a system where individuals have limited access to salmon and abundant access to caribou, showed little isotopic variation among individuals. In contrast I found considerable isotopic variation among individuals in a completely terrestrial C3 system with no access to extremely different isotopic prey such as marine resources. High intraspecific variation in isotope ratios indicates large and consistent diet variation (Bolnick et al. 2003). Given the high prey diversity in the boreal forest, individual dietary specialization is possible but was unexpected due to the probable predominance of social foraging.

Bolnick et al. (2003) demonstrate that between-individual variation in resource use can constitute a large portion of a population's dietary niche width. Individual specialization can result from stochastic events, patchy environments, or behavioural variation among individuals (Bolnick et al. 2003). These short- and long-term processes may cause individuals to use different resources if they have different preferences or resource use efficiencies, reflecting variable morphological, behavioural, or physiological capacities to handle alternative resources (Bolnick et al. 2003). Diets of wolves are often generally examined using information based on kills or faecal remains. These techniques cannot readily differentiate lone individuals from those belonging to packs. The stable isotope data, however, give individual dietary history.

Wolves, when belonging to packs, often hunt cooperatively, but not all pack members necessarily have access to the same resources from a kill. Depending on age and social structure of a pack, each individual may not have the same access to all parts of the carcass or any access at all. Also, participation in packs may change seasonally and this may or may not overlap with the period of hair growth (Mech 1970). As obligate predators and opportunists, wolves eat small mammals, fish and birds when and where available (Darimont et al. 2004). Lower ranking individuals who cannot support their metabolic requirements on the left-overs of what their pack kills may also subsist in this manner. Lone individuals may also rely more on these types of prey items.

Wolves exhibit strong territoriality (Mech 1970, Mech and Boitani 2003). In patchy environments where soil types, vegetation types, and even prey type and distribution vary across the landscape, wolves occupying different territories may consume different prey resulting in isotope signature variation. Stable isotope signatures in terrestrial C3 food webs have been shown to vary among biomes due to differing biogeochemical processes (Cormie and Schwarcz 1994) and anthropogenic influences on the landscape (Lavin et al. 2002). Wolves foraging in multiple biomes may express different isotopic signatures relative to those wolves restricted to one biome throughout their lives.

Black bear and mustelid remains have been found in wolf scats (Kohira 1997, White et al. 2002) and the incidence of wolves preying on black bears has been documented in British Columbia, Manitoba and Minnesota (Horesji et al. 1984; Paquet and Carbyn 1986; Rogers and Mech 1988, Darimont et al. 2004). It is plausible that wolves may have access to discarded black bear carcasses from hunting outfitters and baiting operations in our study area (Paquet, Giroux, Archer, personal communications). Garbage in rural areas is also potentially accessible to wolves. Nutritional stress may further account for individual isotopic variation (Hobson et al. 1993).

4.4.3 Dietary mixing models

My derived mixing polygon in Isosource was broad with wolf mean isotopic values (the "mixture") situated nearest to elk and deer. Elk made up most of the diet followed by white-tailed deer, moose, beaver, and snowshoe hare. My results are consistent and other estimates using conventional techniques (Urton et al.in prep., Meleshko 1986, Carbyn & Kingsley 1979, Carbyn et al. 1985, Paquet 1992). Relative prey contributions as indicated by IsoSource therefore reflect a realistic picture of assimilated diet when compared with conventional faecal methods despite each method representing different time periods.

Consideration of the metabolic pathway between diet and tissue for each element of interest is important when using stable isotopes to derive consumer diets (Krueger and Sullivan 1984, Schwarcz 1991, Ambrose and Norr 1993, Tieszen and Fagre 1993). In carnivores, structural lipids and proteins are assimilated into consumer tissues; however, carbohydrates (e.g. in plants and berries) consumed by carnivores may not be readily detected since they are poor sources of nitrogen and carbon tends to be evolved as CO_2 during metabolism (Schwarcz 1991, Tieszen and Fagre 1993, Hobson and Stirling 1997). If wolves consumed plant materials directly, these would add to isotopic variability (typically by lowering $\delta^{15}N$ values) and might not readily be reflected in wolf $\delta^{13}C$ values. Plant materials such as grass and conifer needles have been noted in wolf scat, however, these materials do not break down during digestion and so their components would not be assimilated into tissues. On occasion, wolves consume berries for a short period during the year (Messier, personal communication) but these sparse foraging bouts likely do not contribute to variability over the period of hair growth.

I have shown intrapopulation variation in stable isotope values previously unrecognized in wolves. Although isotopic variation is not necessarily synonymous with dietary variation, we consider this the most parsimonious explanation for our boreal study area. Because the integration of prey resources into hair is over months, the variation I observed is likely a result of dietary differences and not basal trophic level shifts because these should average out during the integration period (Bearhop et al. 2004). I was able to compare directly individuals along a single diversity scale over a known temporal integration period fulfilling many important assumptions regarding the measurement of species niche width (Bearhop et al. 2004). Stable isotopic mixing models that focus on mean isotopic signatures have proved to be a robust measure of diet at the population level. The possibility of incorporating individual variance in isotope signatures into dietary mixing models is an important prospect for refining investigations of food web models, predator-prey interactions, and competition (DeAngelis and Gross 1992). Moreover, variance in the measurements of proxies that determine niche width among individual species is ecologically meaningful. Future studies should attempt to refine the understanding of physiological and environmental factors influencing dietary variation. Further empirical analysis of intrapopulation niche variation and individual dietary variance will greatly improve our knowledge of the evolution of complex ecological interactions involving wolves and other species.

4.5 References

Acton D. F., G. A. Padbury, and C. T. Stushnoff. 1998. The eco-regions of Saskatchewan. Canadian Plains Research Centre, Saskatchewan Environment and Resource Management, Regina, Saskatchewan.

Ambrose S. H., L. Norr. 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. Pages 1-38 *in* J.B. Lambert, G. Grupe, editors. Prehistoric human bone-archaeology at the molecular level. Springer, New York, NY, USA.

Arseneault A. A. 2003. Status and Management of Wildlife in Saskatchewan, 1999-2001. Saskatchewan Environment Fish and Wildlife Technical Report.

Barnett, B. A. 1994. Carbon and nitrogen isotope ratios of caribou tissues, vascular plants and lichens from northern Alaska. MS Thesis, University of Alaska, Fairbanks.

Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73: 1007-1012.

Beier, P. 1988. Reanalysis of data on sex differences in white-tailed deer diets. Journal of Mammalogy 69: 435.

Ben-David M., R. W. Flynn, D. M. Schell. 1997a. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111: 280-291.

Ben-David M., T. A. Hanley, D. M. Schell. 1997b. Seasonal changes in diets of coastal and riverine mink: the role of spawning pacific salmon. Canadian Journal of Zoology 75: 803-811.

Bocherens H. and D. Drucker. 2003. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. International Journal of Osteoarchaeology 13: 46-53.

Bolnick, D. I., R. Svanback, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. American Naturalist 161: 1-28.

Cabana, G. and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences (USA) 93: 10844- 10847.

Carbyn, L. N. and M. C. Kingsley. 1979. Summer food habits of wolves with the emphasis on moose in Riding Mountain National Park. Proceedings of the North American Moose Conference Workshop 15: 349-361.

Carbyn, L. N., P. C. Paquet, and D. W. Meleshko. 1985. Ecological studies of wolves and coyotes in Riding Mountain National Park, Manitoba. Unpublished Progress Report.

Casaux R., M. Favero, P. Silva, A. Baroni. 2001. Sex differences in diving depth and diet of Antarctic shags at the South Shetland Islands. Journal of Field Ornithology 72: 22-29.

Chisholm B. S., D. E. Nelson, and H. P. Schwarcz. 1982. Stable carbon ratios as a measure of marine versus terrestrial protein in ancient diet. Science 216: 1131-1132.

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

Cormie, A. B. and H. P. Schwarcz. 1994. Stable isotopes of nitrogen and carbon of North American white-tailed deer and implications for paleodietary and other food web studies. Paleogeography Paleoclimatology Paleoecology 107: 227-241.

Creel, S. 1997. Cooperative hunting and group size: assumptions and currencies. Animal Behavior 54: 1319-1324.

Creel, S. and N. M. Creel. 1995. Communal hunting and pack size in African wild dogs, *Lycaon pictus*. Animal Behavior 50: 1325-1339.

Darimont, C. T. and T. E. Reimchen. 2002. Intra-hair stable isotope analysis implies seasonal shift to salmon in Gray wolf diet. Canadian Journal of Zoology 80: 1638-1642.

Darimont, C. T., M. H. H. Price, N. N. Wichester, J. Gordon-Walker and P. C. Paquet. 2004. Predators in natural fragments: foraging ecology of wolves in British Columbia's central and north coast archipelago. Journal of Biogeography 31: 1-11.

DeAngelis, D. L. and L. J. Gross. 1992. Individual based models and approaches in Ecology. Chapman and Hall. New York, USA.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimicha Cosmochimica Acta 42: 495-506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Cosmochimica Acta 45: 341-351.

Drucker D., H. Bocherens, and D. Billiou. 2003. Evidence for shifting environmental conditions in Southwestern France from 33 000 to 15 000 years ago derived from carbon-13 and nitrogen-15 natural abundances in collagen of large herbivores. Earth and Planetary Sciences Letters 216: 163-173.

Drucker D., H. Bocherens, A. Pike-Tay, and A. Mariotti. 2001. Isotopic tracking of seasonal dietary change in dentine collagen: preliminary data from modern caribou. Earthand Planetary Sciences 333:303-309.

Durell, S. E. A. Le V dit. 2000. Individual feeding specialization in shorebirds: population consequences and conservation implications. Biological Review of the Cambridge Philosophical Society 75: 503-518

Ehleringer, J. R. and P. W. Rundel. 1988. Stable Isotopes: History, Units and and instrumentation Pages 1-15 *in* P. W. Rundel, J. R. Ehleringer and K. A. Nagy, editors. Stable Isotopes in Ecological Research eds. Springer-Verlag, Berlin.

Fitzsimmons, M. 2002. Estimated rates of deforestation in two boreal landscapes in central Saskatchewan, Canada. Canadian Journal of Forest Research 32: 843-951.

France, R. L. 1995. Differentiation between littoral and pelagic food webs in lakes using carbon isotopes. Limnology and Oceanography 40:1310-1313.

France, R. L. and R. H. Peters. 1997. Ecosystem differences in the trophic enrichment of 13C in aquatic food webs. Canadian Journal of Fisheries and Aquatic Sciences 54: 1255-1258.

Hall, S. J. and D. G. Raffaelli. 1997. Food-web patterns: What do we really know? Pages 395-421 *in* A. C. Gange and V. K. Brown. Multitrophic Interactions in Terrestrial Systems. Blackwell Science, Oxford, UK.

Hilderbrand, G. V., Farley, S. D., Robbins, C. T., Hanley, T. A., Titus, K. and Servheen, C. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology 74: 2080-2088.

Hobson, K. A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. Marine Ecology Progress Series 95: 7-18.

Hobson, K. A. 1999. Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: implications for evaluating trophic relationships and breeding origins. The Condor 101: 799-805.

Hobson, K. A., Alisauskas, R. T. and Clark, R. G. 1993. Stable-nitrogen isotope enrichment in avian tissue due to fasting and nutritional stress: implications for isotopic analyses of diet. The Condor 95: 388-394.

Hobson, K. A., E. M. Bayne and S. L. Van Wilgenburg. 2002. Large-scale conversion of forest to agriculture in the Boreal Plains of Saskatchewan. Conservation Biology 16: 1530-1541.

Hobson K. A. and Clark R. G. 1993. Turnover of 13C in cellular and plasma fractions of blood: implications for non-destructive sampling in avian dietary studies. Auk 110: 638-641.

Hobson K. A., Stirling I. 1997. Low variation in blood $\delta^{13}C$ among Hudson Bay polar bears: implications for metabolism and tracing terrestrial foraging. Marine Mammal Science 13: 359-367

Hobson, K. A., McLellan, B. N. and Woods, J. G. 2000. Using stable carbon and nitrogen isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. Canadian Journal of Zoology 78: 1332-1339.

Hobson, K. A., J. F. Piatt and J. Pitocchelli. 1994. Using stable isotopes to determine seabird trophic relationships. Journal of Animal Ecology 63: 786-798.

Hobson, K. A. and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 84: 9-18.

Hodum, P. J. and K. A. Hobson. 2000. Trophic relationships among Antarctic fulmarine petrels: insights into dietary overlap and chick provisioning strategies inferred from stable-isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses. Marine Ecology Progress Series 198: 273-281.

Horesji B. L., G. E. Hornbeck, and R. M. Raine. 1984. Wolves, Canis lupus, kill a female black bear, *Ursus americanus*, in Alberta. Canadian Field Naturalist 98: 368-369.

Jacoby, M. E., G. V. Hilderbrand, C. Servheen, C. C. Schwartz, and S. M. Arthur. 1999. Trophic relationships of brown and black bears in several western North American ecosystems. Journal of Wildlife Management 63: 921-929.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78: 1-27.

Kirchoff, M. D. and D. N. Larsen. 1998. Dietary overlap between native Sitka black tailed deer and introduced elk in southeast Alaska. Journal of Wildlife Management 62: 236-243.

Kohira, M. 1997. Diets of wolves, *Canis lupus,* in logged and unlogged forests of southeastern Alaska. Canadian Field Naturalist 111: 429-435.

Krueger H. W. and C. H. Sullivan. 1984. Models for carbon isotope fractionation between diet and bone. Pages 205-220 *in* J. R. Turnland and P.E. Johnson, editors. Stable isotopes in nutrition ACS Symposium Series 258, American Chemical Society, Washington, DC, USA.

Litvaitis, J. A. 2000. Investigating food habits of terrestrial vertebrates. Pages 165-190 *in* L. Boitani and T.K. Fuller, editors. Research Techniques in Animal Ecology: Controversies and Consequences. Columbia University Press, NY, USA.

Mech, L. D. 1970. The wolf: the ecology and behaviour of an endangered species. Doubleday/Natural History, Garden City, NY.

Meleshko, D. W. 1986. Feeding habits of sympatric canids in an area of moderate ungulate density. Ph.D. Thesis. University of Alberta, Edmonton, AB.

Messier F. 1994. Ungulate population models with predation: a case study with the North American moose. Ecology 75: 478-488.

Minagawa, M. and E. Wada. 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochimica Cosmochimica 48: 1135-1140.

Nowak, R. M. 1999. Walker's Mammals of the World, Vol. I. Johns Hopkins University Press. Baltimore & London.

Ostrom, N. E., L. O. Hedin, J. C. Von Fisher, G. P. Roberston. 2002. Nitrogen transformation and NO3- removal at soil-stream interface: a stable isotope approach. Ecological Applications 12: 1027-1043.

Paquet, P. C. 1992. Prey use strategies of sympatric wolves and coyotes in Riding Mountain National Park, Manitoba. Journal of Mammalogy 73: 337-343.

Paquet, P.C., Carbyn L. N. 1986. Wolves, *Canis lupus*, killing denning black bears, *Ursus americanus*, in the Riding Mountain National Park area. Canadian Field Naturalist 100: 371-372.

Peterson, B. J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293-320.

Phillips, D. L. and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136: 261-269.

Phillips, D. L. and P. L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia 130: 114-125.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83: 703-718.

Riga A., H. J. Van Praag and N. Brigode. 1971. Rapport isotopique natural de l'azote dans quelques sols forestiers et agricoles de Belgique sournuis a divers vraitemments aulturneaux. Geoderma 6: 213-222.

Rogers L. L., L. D. Mech. 1981. Interactions of wolves and black bears in northeastern Minnesota. Journal of Mammalogy 62:434-436.

Roth, J. D. 2002. Temporal variability in arctic fox diet as reflected in stable-carbon isotopes: the importance of sea ice. Oecologia 133: 70-77.

Roth, J. D. and K. A. Hobson. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. Canadian Journal of Zoology 78: 848-852.

Schell, D. M., S. M. Saupe, and N. Haubenstock. 1989. Natural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat usage. Pages 261-269 *in* P.W. Rundel, J.R Ehleringer,a nd K.A. Nagy. Stable Isotopes in Ecological Research. Springer-verlag, New York.

Schell, D. M. and P.J. Ziemann. 1989. Natural Carbon Isotope tracers in Arctic aquatic food webs. Pages 231-251 *in* P.W. Rundel, J.R Ehleringer,a nd K.A. Nagy. Stable Isotopes in Ecological Research. Springer-Verlag, New York.

Schwarz, H. P. 1991. Some theoretical aspects of isotope paleodiet studies. Journal of Archaeological Science 18: 261-275.

Scott, B. M. V. and D. M. Shackleton. 1980. Food habits of two Vancouver Island wolf packs: a preliminary study. Canadian Journal of Zoology 58: 1203-1207.

Sponheimer M., T. Robinson, L. Ayliffe, B. Roeder, J. Hammer, B. Passey, A. West, T. Cerling, D. Dearing and J. Ehleringer. 2003. Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}N$ values from a controlled feeding study. International Journal of Osteoarchaeology 13: 80-87.

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

Terborgh, J., J. Estes, P. Paquet, K. Ralls, D. Boyd-Heger, B. Miller, and R. Noss. 1999. The role of top carnivores in regulating terrestrial ecosystems. Wild Earth 9:

42-56.

Theberge, J. B., S. M. Oosenburg and D. H. Pimlott. 1978. Site and seasonal variation in food of wolves, Algonquin Park, Ontario. Canadian Field Naturalist 92: 91-94.

Tieszen, L. L., J. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia 57:32-37.

Tieszen, L. L., T. Fagre. 1993. Effects of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. Pages 127-156 *in* J. B. Lambert and G. Grupe, editors. Prehistoric human bone-archaeology at the molecular level. Springer, Berlin.

Vanderklift, M. A. and S. Ponsard. 2003. Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. Oecologia 136: 169-182.

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

White, K. S., H. N. Golden, K. J. Hundertmark and G. R. Lee. 2002. Predation by wolves, *Canis lupus*, on wolverines, *Gulo gulo*, and American marten, *Martes americana*, in Alaska. Canadian Field Naturalist 116: 132-134.

Wilmers, C. C., R. L. Crabtree, D. W. Smith, K. M. Murphy, and W. M. Getz. 2003. Trophic facilitation by introduced top predators: grey wolf subsidies to scavengers in Yellowstone National Park. Journal of Animal Ecology 72: 909-916.

Wilson, D. E. and S. Ruff. 1999. The Smithsonian Book of North American Mammals. Smithsonian Institution Press. Washington and London.

Zar, J. H. 1999. Biostatistical Analyis. 4th edition. Prentice Hall. Upper Saddle River, NJ, USA.

5.0 GENERAL DISCUSSION

5.1 Synthesis

The overarching goal of this thesis is to asses how human-caused ecosystem change affects the behaviour and ecology of wolves. Ultimately, the capacity for animal populations to adjust to, or withstand rapidly changing environments is unknown. Although landscape changes are ubiquitous over time, the present rate and scale of impacts affecting ecosystems are unprecedented. The rapidity and magnitude of such change raise great concern regarding the ability of animals like wolves to adapt and survive.

In Europe, widespread environmental changes resulting primarily from anthropogenic activities resulted in wolf extirpation from all but the most remote regions of the continent (Zimen and Boitani 1975, Boitani 1992, Randi et al. 1993, 1995, 2000). However, as small remnant populations now rebuild themselves, they are showing a remarkable resilience to human-caused disturbances (i.e., Italian wolves: Boitani 1992). The natural recolonization of wolves to areas in the United States and Canada where they were also extirpated (Mech 1995, Mech and Boitani 2003) is further evidence of resilience (Weaver et al. 1996). However, nearly all recovered populations remain at risk and require direct intervention by managers to ensure survival (Mech and Boitani 2003). Moreover, the long-term effects of population declines and range contraction, from an evolutionary perspective, are unknown (Boitani 1992). In theory, decreases in heterozygosity, because of population bottlenecks and varying degrees of inbreeding, contribute to decreased survival and reproduction and lower individual and population fitness (Bouzat et al. 1998, Wayne and Vila 2003, Leonard et al. 2004). Clarifying whether genetic diversity and structure of a population have a natural basis or result from human activities and fragmentation of range is important (Wayne and Vila 2003).

Human-caused environmental change, if not slowed, presents complicated challenges for wildlife management. Carnivore populations across North America have demonstrated susceptibility to human-caused fragmentation of the landscape (Proctor

2003, Wayne et al 1992). Wolverines, for example, have increased genetic population structure at the southern edge of their North American distribution (Kyle and Strobeck 2002). Lynx (Campbell 2002) and grizzly bear (Proctor 2003) populations were found to be genetically structured across a major highway. Fragmentation may limit the ability for dispersal to affect "source to sink" processes (Proctor 2003). Fragmentation may also increase selective pressure on wolves by increasing human-caused mortality near the perimeters of populations. National parks are a primary example of this phenomenon (Newmark 1995, Woodruffe and Ginsberg 1998, Callaghan 2002).

Pack instability created by human-caused wolf mortalities, as I propose in Chapter 2, raises the possibility that, although much of the area surrounding PANP is contiguous with the park, potential habitat may not provide security due to human activity (e.g. hunting, trapping, roads) outside the park. Most North American wolf populations have been managed without consideration of harvest times, characteristics of areas where harvest is allowed, or methods used to kill wolves. In addition, few jurisdictions have monitored the effects of harvesting (Hayes and Gunson 1995).

Approximately 4-11% of wolves are trapped annually in North America (varying regionally) (Boitani 2003). Although rigorous assessments are lacking, this rate of mortality is not believed to limit wolf populations, except along the southern fringes of their distribution (Theberge 1991, Hayes and Gunson 1995, Boitani 2003). Wolf populations in my study area are at the southern limit of their range where habitat fragmentation and human activity are prominent.

My data represent a 1-year snapshot of the genetic structure and diversity of wolf populations in central Saskatchewan. Wolf populations are considered plastic given wolf vagility, and if sampled over multiple years, the NW population may show more admixture in subsequent years relative to 2003. The same population should be sampled over time in order to identify possible changes (increases or decreases) in structure.

A fundamental component of wolf habitat is the distribution and abundance of prey. Specifically, wolves and their prey are inextricably linked. Therefore, diet composition of wolves provides pertinent information about what prey are important for sustaining wolf populations. In addition, the wolf's diet forms the core of human conflict with this large carnivore (Boitani 2003). Elucidating wolf diets is important because as environmental conditions change, relationships between wolves and prey also change. In Chapter 3, I show that wolves used a wide variety of prey, indicative of a generalist predator. I show that prey selection is in concordance with optimal foraging theory where wolves balance risk and energy demands with profitability. Selection of elk as a prey preferred by wolves may contribute to understanding which habitats are important for maintaining populations of wolves and which are prime dispersal destinations. If natal habitat dispersal does occur in wolves, knowledge of prey use is of great benefit when attempting to understand this dynamic (Sacks et al. 2004).

In Chapter 4, stable isotope techniques provide a robust measure of broad diet, similar to that of faecal analysis, but with additional information about individual variation in diet. My results indicate that individual specialization may be more common in social carnivores than the literature suggests. Variance among individual species is ecologically meaningful and future studies should attempt to tease apart physiological and environmental factors influencing this variation. Information about individual diet variation has profound implications for the ecology of individuals, a facet of ecology currently neglected. Although examining average dietary patterns is useful for whole populations, natural selection operates at the individual level. Variation in individual foraging strategies and habitat preferences affects what we see at the population level and remains key when studying the evolutionary ecology of organisms. Further, community relationships elucidated by my stable isotope analyses provided a snap shot of the energy dynamics among mammals in the boreal forest, lending insight into wolf ecology not previously documented or expected.

5.2 Conservation and management implications

Conserving genetic diversity is important because of the long-term evolutionary potential it provides. Rare alleles, or combinations of alleles, may confer no immediate advantage but could be well suited to future environmental conditions. Small populations that have lost rare alleles have less potential to adapt (Bouzat et al. 1998, Randi et al. 2000). Thus, prudent biologists and managers should aim at maintaining gene flow among local populations to alleviate the potential deleterious effects of small populations and genetic isolation. Accordingly, effective management of wolf populations requires some idea of genetic population structure. It is also useful to assess dietary patterns for wolves within and outside protected areas to enhance our knowledge of predator-prey relationships that may influence wolf movements, ranges, and subsequent population structure.

My research provides critical baseline data and insight into the potential vulnerability of wolf populations that could result from genetic deterioration in national parks. In addition, it provides context for concerns over genetic viability in other fragmented wolf populations. This investigation will also significantly contribute to the development of novel and effective research methods that reduce risk imposed by invasive research. Once refined, these techniques will have broad applicability for low-impact investigation of animal populations.

5.3 Conclusion

I have elucidated interesting and potentially important patterns in wolf population ecology. Although my study was not experimental, I was able to measure variation in an existing ecological state in a purposeful and organized manner. I was also able to describe some probable proximate causes for that state.

Exploratory studies of this nature are practical, informative, and serve to stimulate and direct further research. Wolves are extremely difficult to study at any scale. Capture and handling are dangerous and stressful for study animals and handlers. Although radio telemetry has provided ecologists with insights into the ecological plasticity and variability of many species, non-invasive genetic and isotopic techniques have recently enabled new insights in wildlife research.

Additional research should be considered for wolf populations in central Saskatchewan. Aside from further investigation into wolf population genetics and foraging ecology, fine-scale habitat modeling may be required to explain wolf distribution. Whether wolves are uniformly distributed across the landscape or if dispersal by wolves is density dependent is unknown. Furthermore, very little is known about the density, distribution, and abundance of prey in this area. The findings of this study highlight the need for continued research in the central boreal ecosystem of Saskatchewan.

5.4 References

Boitani, L. 1992. Wolf research and conservation in Italy. Biological Conservation 61: 125-132.

Boitani, L. 2003. Wolf conservation and recovery. Pages 317-343 *in* L.D. Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, USA. 448 pp.

Bolnick, D. I., R. Svanback, J. A. Fordyce, L. H.Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: incidence and implications of individual specialization. American Naturalist 161: 1-28.

Bouzat, J. L., H. H. Cheng, H. A. Lewin, R. L. Westemeier, J. D. Brawn and K. N Paige. 1998. Genetic evaluation of a demographic bottleneck in the Greater prairie chicken. Conservation Biology 12: 836-843.

Callaghan, C. J. 2002. The ecology of Gray wolf (*Canis lupus*) habitat use, survival, and persistence in the central Rocky mountains, Canada. Ph. D. Thesis. University of Guelph, ON, Canada.

Campbell, V. A. 2002. Population genetic structure of Canada lynx (*Lynx canadensis*) in Alberta. M.Sc. Thesis. University of Alberta, Edmonton, AB. 74pp.

Hayes, R. D. and J. R. Gunson. 1995. Status and management of wolves in Canada. Pages 21-33 *in* L. N. Carbyn, S. H. Fritts, and D. R. Siep, editors. Ecology and Conservation of wolves in a changing world. Canadian Circumpolar Institute, Edmonton, AB.

Kyle, C. J. and C. Strobeck. 2002. Connectivity of peripheral and core populations of North American wolverines. Journal of Mammalogy 83: 1141-1150.

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

Mech, L. D. and L. Boitani. 2003. Wolf social ecology. Pages 317-343 *in* L.D.Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago. 448 pp.

Newmark, W. D. 1995. A land-bridge perspective on mammalian extinctions in western North American national parks. Conservation Biology 9: 512-526.

Proctor, M. F. 2003. Genetic analysis of movement, dispersal, and population fragmentation of grizzly bears in southwestern Canada. Ph.D. Thesis. University of Calgary, Calgary, AB. 147 pp.

Randi, E., F. Francisci, and V. Lucchini. 1995. Mitochondrial DNA restriction fragment-length polymorphism in the Italian wolf (Canis lupus). Journal of Zoology, Systematics, Evolution and Resources 3:97-100.

Randi, E., V. Lucchini, M. F. Christensen, N. Mucci, S. M. Funk, G. Dolf, and V. Loeschcke. 2000. Mitochondrial DNA variability in Italian and East European wolves: detecting the consequences of small population size and hybridization. Conservation Biology 14: 464-473.

Randi, E., V. Lucchini, and F. Francisci. 1993. Allozyme variability in the Italian wolf (*Canis lupus*) population. Heredity 71: 516-522.

Taberlet, P., L. P. Waits and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. Trends in Ecology and Evolution 14: 323-327.

Theberge, J. B. 1991. Ecological classification, status, and management of the gray wolf (*Canis lupus*) in Canada. Canadian Field Naturalist 83:122-128.

Wayne, R. K. and C. Vila. 2003. Molecular genetic studies of wolves. Pages 317-343 *in* L.D. Mech and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago. 448 pp.

Wayne, R.K., N. Lehman, M.W. Allard, R. L. Honeycutt. 1992. Mitochondrial DNA variability of the gray wolf – genetic consequences of population decline and habitat fragmentation. Conservation Biology 6: 559-569.

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

Zimen, E., and L. Boitani. 1975. Number and distribution of wolves in Italy. Z. Saugetierk. 40:102-112.

APPENDIX A.

Caveats in non-invasive genetic sampling

Faecal material yields low quantities of degraded DNA (Taberlet et al. 1996, Luccini et al. 2002). Target DNA extracted from non-invasive samples might produce contamination, allelic dropout, or scoring of false alleles (Taberlet et al. 1996, Luccini et al. 2002, Miller et al. 2002). Therefore, any large-scale project should be carefully assessed through pilot studies.

It is important to address the limitations associated with conducting non-invasive genetic sampling and analyses. When measuring genetic diversity in a population, allelic dropout (only 1 allele of a heterozygous individual is detected) can reduce the observed heterozygosity (H_O), potentially resulting in erroneous concerns about extinction risks caused by inbreeding, low N_e, or low genetic variation (Taberlet et al. 1999). When investigating genetic population structure (F_{st} and genetic distance), erroneous estimates of allele frequencies might alter F_{st} estimates, Nm, and phylogenies. Reduced Ho can also generate a false Wahlund effect wrongly suggesting that substructure exists within the sample population (Taberlet et al. 1999). However, in these cases the severity of these errors is low. An instance where one must be overly cautious in interpreting results is when using these data for paternity and parentage analyses or population assignments. (Taberlet et al. 1999)

Some major technical challenges may be alleviated by (Taberlet et al. 1999): 1) limiting the degradation of DNA before extraction by maximizing sample preservation, 2) using tissue specific extraction kits that have greatly improved extraction success over classical phenol-chloroform DNA extraction, 3) using “hot start” PCR amplificaiton allowing for more PCR cylces that detect single target molecules when conditions are optimized, 4) using a “multiple tube approach” where repeat PCR experiments with multiple aliquots of the same DNA extract are performed; this method is useful for detecting errors, and 5) avoiding contamination by conducting pre- and post- PCR experiments in separate labs, minimizing handling of PCR extracts, and using negative controls.

References

Luccini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E. Randi. 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the Western Italian Alps. Molecular Ecology 11: 857-868.

Miller, C., P. Joyce, L. P. Waits. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160: 357-366.

Taberlet, P., S. Griffin, B. Goosens, et al. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24: 3189-3194.

Taberlet, P., L. P. Waits, and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. Trends in Ecology and evolution 14: 323-327.