DISPERSAL IN A PLAIN LANDSCAPE:
WOLVES IN SOUTHWESTERN MANITOBA, CANADA

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“You must not know too much or be too precise or scientific about birds and trees and flowers and watercraft; a certain free margin, and even vagueness…ignorance, credulity… helps your enjoyment of these things”

Walt Whitman

“With their parallel lives, animals offer man a companionship which is different from any offered by human exchange… a companionship offered to the loneliness of man as a species”

John Berger

Abstract

Much of our understanding of the effects of fragmentation derives from landscapes with obvious barriers to movement. The effects of human-caused fragmentation require further study in physically-simple landscapes: areas where barriers and ecological discontinuities (e.g. climate, prey distribution) can, as far as possible, be eliminated as causes for population genetic structure. I test the prediction that human-caused fragmentation reduces dispersal and creates genetic population structure even across short distances and physically-simple landscapes. Considerable fragmentation from agricultural development has occurred in the Riding Mountain National Park (RMNP) region in southwestern Manitoba, Canada, since the 1950s. I examine microsatellite and mitochondrial DNA from gray wolves (*Canis lupus*). Microsatellite genetic structure is evident between RMNP and the Duck Mountains 30 km further north (F_{ST} = 0.074, 95% CI [0.048-0.104]) and consistent with fragmentation. Neither mutation nor natural selection is expected to have contributed significantly. Hence, human-caused fragmentation of a physically-simple landscape can cause cryptic genetic structure in vagile organisms on fine spatiotemporal scales. Mitochondrial DNA haplotypes show additional support for cryptic genetic structure. Both gray wolves and eastern wolves (proposed as species *C. lycaon*) occur in the Duck Mountains, but eastern haplotypes seem absent or rare in RMNP. In territorial organisms, tolerance toward relatives can promote kin clusters, which could further encourage within-patch dispersal in a fragmented landscape. However, inbreeding can reduce disease resistance. I tested for correlations between parasite burden and 1) individual parental relatedness and 2) homozygosity, and spatial overlap and allele sharing between radio-collared wolves from RMNP. Eight of 18 blood samples indicated exposure to Canine Distempervirus and one adult wolf died from distemper. All 18 showed Canine Parvovirus exposure. Individual parental relatedness or homozygosity was not significantly correlated with parasite burden. Allele sharing was not significantly correlated with overall spatial overlap. No successful RMNP wolf dispersal has been documented, and the inbreeding coefficient $F_{IS} = 0.085$ (95% CI [0.052-0.118] suggests high kinship. The Great Plains is now an intensely human-managed landscape, and similar results could be expected for other vagile and low-density species where detection of cryptic genetic structure can function as an important indicator in conservation management.

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List of Common Abbreviations and Acronyms

AMOVA	Analysis of Molecular Variance
Bovine TB	Bovine Tuberculosis
CDV	Canine Distempervirus
CI	Confidence Interval
CWD	Chronic Wasting Disease
FCA	Factorial Correspondence Analysis
FDR	False Discovery Rate
CPV	Canine Parvovirus
GIS	Geographic Information Systems
IR	Internal relatedness
MCP	Minimum Convex Polygon
MtDNA	Mitochondrial DNA
N_m	Number of migrants per generation
PC	Principal Component
RMNP	Riding Mountain National Park
SAMOVA	Spatial analysis of molecular variance
SEPM	Spatially Explicit Population Model
TNTC	Too Numerous to Count
UTM (NAD 83)	Universal Transverse Mercator (North American Datum 83)

Chapter 1

General Introduction

Dispersal: Individual decision and population adhesive

Dispersal often results in gene flow, the movement and integration of genes from one population to another (Ross 2001). Animals disperse for a variety of reasons, including social aggression, population regulation, mating opportunities, and avoidance of resource competition and inbreeding (Lidicker 1975, Greenwood 1980, Bekoff et al. 1984, Messier 1985, Ross 2001). Classical population genetic theory predicts that mutation, drift and selection lead to genetic differentiation of local populations, whereas gene flow contradicts these processes and determines the extent to which evolution within local populations occurs independently (Slatkin 1987). Gene flow is due to movement of individuals, gametes, extranuclear material such as mitochondria, and, at times, the extinction and recolonization of entire populations (Slatkin 1987).

Physical barriers such as mountain ranges have been found to limit dispersal and gene flow in plants, amphibians and mammals (reviewed in Taberlet et al. 1998), and can reduce dispersal in vagile species such as the Canadian lynx (*Lynx canadensis*) (Rueness et al. 2003). Long-distance gene flow occurs sufficiently often in many species to cause genetic homogeneity over a wide geographic range (Slatkin 1985). Nonetheless, sharp gradients can exist between environments favouring different characters (Hewitt 1988, Doebell and Dieckmann 2003). Across taxa, ecological and behavioural factors (Wilcox 1980, Wilson et al. 2000, Dearborn et al. 2003, Davis and Stamps 2004) contribute to genetic structure within species. Importantly, the presence of cryptic population genetic structure[1] can increasingly be detected with improved genetic and statistical techniques. Such population structure has been attributed to diet specialization, natal habitat-biased dispersal (preference for dispersal into familiar habitat) and climate in vagile taxa represented by cetaceans (Hoelzel et al. 1998), felids (Ernest et al. 2003, Rueness et al. 2003) and canids (Carmichael et al. 2001, Sacks et al. 2004). Ernest et al. (2003) found moderate genetic differentiation ($F_{ST} = 0.07$) between mountain lions (*Puma concolor*) separated by a geographic distance of 50 km by the crest of the Sierra Nevada range in California. They note that geographical barriers do not inhibit mountain lion gene flow in this area, but discuss the possible effects of ecological, geographic and human-caused factors.

Increasing human-caused landscape fragmentation and habitat loss is forcing a growing number of species to conform to a metapopulation structure (Hanski 2001). ‘Fragmentation’ has several interpretations (Lindenmayer and Fisher 2006) but fragmentation due to human activity generally results in a landscape with remnant areas of native vegetation surrounded by a matrix[2] of agricultural or other developed land (Saunders et al. 1991). The classic metapopulation is a group of partially isolated populations that undergo local extinctions and recolonizations (Frankham et al. 2002). Lack of spatially explicit population definition (Holderegger and Wagner 2006) and the colonization – extinction requirement (Noss et al. 1996) limit application of metapopulation theory to many real populations. However, landscape genetics, combining landscape ecology and population genetics, is increasingly used to examine

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[1] Cryptic population structure: discrete genetic subdivisions corresponding to borders between spatial units with no gaps in a species’ distribution and no physical barriers to movement (Sacks et al. 2005).

[2] Matrix: areas surrounding reserves (and other wilderness areas) and altered to various extents by human use (Franklin 1993).

spatial genetic patterns in relation to landscape variables (see review by Manel et al. 2003). Whereas human-caused landscape fragmentation can increase selection for long-distance dispersal (Wiens 2001) it could also facilitate selection for adaptation to local conditions (Ashley et al. 2003).

Genetic variation depends on mutation rate, selection intensity and effective population size (Hedrick 2001). Hence, gene flow can act as a conservative force by preventing independent adaptation and evolution in different populations, and as a creative force through the spread of novel genes (Slatkin 1987). Processes disproportionally affecting persistence and maintenance of evolutionary potential in small populations are increasingly recognised (see review in Frankham 2005) and a major concern for small populations is genetic drift overriding natural selection (Frankham et al. 2002). Deleterious alleles of small effect (negative fitness consequence of low magnitude) become effectively neutral in small populations and can become fixed, although selection seems capable of retaining vital alleles despite strong and enduring drift (Munguia-Vega et al. 2007).

There is emerging evidence of human-caused evolutionary change across taxonomic groups (see review in Ashley et al. 2003). Further research is needed on the influence of human-caused landscape fragmentation on dispersal and gene flow (Frankham 2005), and the minimum difference in scale at which human disturbance is incorporated into the next higher levels of biological organization (Urban et al. 1987). Landscape fragmentation can affect individual dispersal decisions and, therefore, gene flow and genetic population structure (Van Vuren 1998). Fragmentation is rarely open to experimental manipulation (Kareiva et al. 1996), and physical landscape barriers will likely have additional but unknown influence on dispersal and gene flow. For instance, human-caused barriers represented by freeways have reduced gene flow in coyotes (*Canis latrans*) and bobcats (*Lynx rufus*) (Riley et al. 2006). New findings also suggest that a combination of landscape features with low permeability can influence fine-scale genetic structure in the absence of dispersal barriers. Importantly, cryptic population structure has been identified in species such as wolverines (*Gulo gulo*) (Cegelski et al. 2003, Guillot et al. 2005) and roe deer (*Capreolus capreolus*) (Coulon et al. 2006) in landscapes fragmented by humans. Though highly mobile, wolverines are also very sensitive to human disturbance (Guillot et al. 2005). In contrast, roe deer occupy relatively small home ranges and data for n = 9 deer suggested a mean dispersal distance of about three km (Coulon et al. 2006).

Whereas the effects of physical movement barriers on dispersal and gene flow is relatively well understood, the influence of human-caused fragmentation requires further study in physically simple landscapes: areas where barriers to movement and natural ecological discontinuities (e.g. climate, mountain-lowland transitions) can, as far as possible, be eliminated as causes for population genetic structure. This should be examined in organisms where high gene flow is expected to limit independent evolution within local population units, so that fine-scale spatiotemporal effects can be quantified and incorporated into both theory and conservation practice.

Dispersal response to human-caused landscape fragmentation

Although factors such as size and location will determine the history of human influence, reserves and natural areas are important benchmarks for biodiversity due to their emphasis on ecological integrity (Noss 1995). The landscape matrix, therefore, plays a critical role in connectivity (Lord and Norton 1990, Franklin 1993, Kramer-

Schadt et al. 2004) and further attention is needed toward managing regional landscapes with reserves and their surrounding matrix as a whole (Noss et al. 1996). Evolutionary consequences of human-caused landscape fragmentation are often overlooked (Ashley et al. 2003) and could include loss of ecological effectiveness (Soulé et al. 2003) and evolutionary potential (Crandall et al. 2000). Propagules in certain isolated plant populations have shown morphological adaptations toward short-distance dispersal (Cody and Overton 1996). Similar adaptive changes can be difficult to assess for organisms lacking life history stages dedicated to dispersal. However, a measure of (presumed) neutral gene flow between neighbouring areas can help establish the extent to which selective factors are likely to operate independently within populations.

Inbreeding redistributes genetic variation and increases homozygosity (Frankham et al. 2002), and individuals with high heterozygosity may better be able to resist infection and mount immune responses slowing disease progression once infected (Acevedo-Whitehouse et al. 2005). Small, isolated or inbred populations may be unable to respond to a novel infection (Dhondt 1996, Keller and Waller 2002) and be increasingly susceptible to epidemic outbreaks of pathogens acquired from more common species (Dobson and Grenfell 1995). Dispersers may also act as disease vectors between populations (Robertson et al. 2006). Hence, the combination of naïve immune systems and low genetic variation in many small and isolated populations can exacerbate mortality from introduced disease, further reducing genetic diversity (O'Brien et al. 1985, May 1988, Hess 1996). Fluctuating population sizes in many wild species could also result in the need for > 10 immigrants per generation to maintain genetic variation (Vucetich and Waite 2000).

In mammals, early dispersers can maximize reproductive output by taking immediate advantage of reproductive potential, but higher mortality risk at this age may act as an opposing selective force balancing their reproductive output with that of biders (individuals waiting for a breeding opportunity to arise, Bekoff 1977). Biding can also remove the mortality risks of dispersal (Bekoff et al. 1984), which are a particular concern for many wide-ranging species in small reserves (Soulé and Simberloff 1986, Van Vuren 1998). In territorial species, movements may also be restricted by risk of conflict with adjacent territory holders, and degree of relatedness between neighbours may influence tolerance (Lambin and Yoccoz 1998). These authors suggest that competition for space could be reduced between kin, allowing for increased density and survival. Whereas philopatry (lack of dispersal) could help preserve locally adaptive gene complexes, especially in species with low fecundity (Shields 1983), inbreeding reduces fitness in small and isolated populations (Keller and Waller 2002). I define a patch as a fragment of wilderness (natural area not significantly modified by humans) isolated from other such areas by a matrix, and within-patch dispersal as dispersal limited to the confines of the natal patch. Individuals in isolated patches must balance potential fitness reductions (e.g. reduced offspring survival) from inbreeding with mortality risks associated with dispersal. Tolerant neighbouring territory holders could therefore potentially influence individual decisions on whether and when to disperse.

Research questions

Human-caused landscape fragmentation could have wide-ranging and subtle consequences on dispersal and gene flow, and ability to act across narrower spatial and temporal scales than previously believed. Common applications of population genetics to conservation tend to use neutral genetic markers and avoid genes under selection, so that selection does not ‘interfere’ with the study of mutation, drift and gene flow (Ashley at al. 2003). Degree of population difference in neutral molecular markers may therefore not reflect degree of adaptive difference, which has important implications for conservation management (Hedrick 1999, Crandall et al. 2000). However, dispersal response to human-caused fragmentation can be investigated by testing predictions on the flow of (assumed) selectively neutral genes within a historically well-connected landscape using organisms with high potential for gene flow.

The cohesion species-concept (Templeton 1989) highlights the importance of both genetic exchangeability (gene flow between organisms) and ecological exchangeability (shared ecological niche). Isolated landscape patches seem to have less genetic variation (Frankham et al. 2002), and the role of human-induced ecological changes in contemporary evolution is increasingly recognised (Reznick and Ghalambor 2001, Ashley et al. 2003). Loss of environmental heterogeneity can reduce divergent selection and promote hybridization and reversal of speciation (Seehausen et al. 2008). The evolutionary consequences of reduced environmental heterogeneity within human-dominated landscapes for closely related taxa thus require further study.

If pathogen – host relationships are affected by increasing isolation, we also need more research on how disease interacts with genetic factors when wide-ranging organisms take on a metapopulation configuration. This highlights the need to test for inbreeding and pathogen correlations in historically contiguous populations now increasingly limited to smaller patches within a landscape matrix. Higher relatedness within a patch could be associated with neighbouring territory holders displaying increased tolerance and spatial overlap, but simultaneously with elevated parasite burdens due to high levels of homozygosity.

Finally, the long-term effects of factors believed to influence dispersal across landscapes fragmented by human activity can be difficult to determine. However, features believed to influence movement can be examined in a spatially explicit population model (SEPM) to predict their relative importance using current knowledge of patches and the surrounding matrix. This permits exploration of how elusive but potentially critical factors such as infectious disease epidemics may affect dispersal. A spatially explicit model of movement also permits evaluation of how changes in mortality risk associated with human features could influence disperser success.

Thesis objectives

My primary objective is to determine whether a physically simple landscape fragmented by humans reduces dispersal, resulting in genetic drift and subsequent genetic population structure. Most temperate landscapes are subject to human alterations (Franklin 1993), and the Great Plains of North America is now an intensely human-managed landscape (Guertin et al. 1997). I investigate the effects of this relatively uniform landscape matrix on dispersal through detailed analyses of spatial genetic population structure in a wide-ranging organism. I then examine whether the landscape matrix is associated with evolutionary change through hybridizarion in two closely related taxa.

My secondary objective is to determine potential consequences (costs and benefits) of philopatry and inbreeding within isolated patches. I then examine long-term dispersal across the matrix through modelling. Here, I test the effects of varying mortality rate from factors predicted to be associated with emigration rate (disease) and landscape resistance to dispersal (human-caused mortality).

My final objective is to summarise my results in view of how dispersal and fragmentation might interact in influencing long-term ecological niche. My overall goal with this study is to contribute to our understanding of dispersal in landscapes fragmented by humans, and its consequences for conservation of ecological processes and evolutionary potential. I also aim to further non-invasive research methods in investigating genetic consequences of human-caused landscape fragmentation, including the potential effects of fragmentation on wildlife disease dynamics.

Hypotheses and Predictions

I first hypothesize that human-caused landscape fragmentation, even across short distances and physically simple landscapes, influences animal dispersal. Importantly, fragmentation impacts at fine scales are uncertain and species-specific and it is plausible that the null hypothesis of panmixus (or low genetic differentiation) can not be rejected. Testing predictions on gene flow using a highly mobile animal thus allows me to examine a minimum difference in scale at which human change affects biological organisation (Urban et al. 1987) instead of a null hypothesis known to be false (Johnson 1999, Burnham and Anderson 2002). Subsequently, I propose that the landscape matrix influences evolutionary processes. Finally, I hypothesize that dispersal within the confines of a landscape patch, and subsequent high relatedness, has fitness-related costs and benefits. Expected costs are elevated parasite burdens in individuals with higher parental relatedness and levels of homozygosity. Expected benefits are increasing range overlap (and thus area available for movement and feeding) for territorial organisms surrounded by related neighbours. I make the following predictions:

1) *Human-caused fragmentation reduces dispersal and creates genetic population structure even across short distances and physically-simple landscapes.*

2) *The landscape matrix reduces environmental heterogeneity and niche divergence, promoting hybridization in closely related taxa.*

3A) *In normally outbred organisms with high dispersal capability, individual parental relatedness values are positively correlated with disease and parasite burdens.*

3B) *In territorial organisms, degree of range overlap between neighbours is correlated with degree of relatedness.*

Study area and organism

Reserve size and survival ability in the landscape matrix determines species persistence in many parks (Wilcove and May 1986, Newmark 1995). Because of an emphasis on balancing biodiversity protection and human use, national parks and adjoining biosphere reserves (UNESCO 2007) are valuable settings in which to examine cross-boundary conflicts between conservation and local development (Schonewald-Cox et al. 1992). Considerable human-caused landscape fragmentation has occurred in the region surrounding Riding Mountain National Park (RMNP) in southwestern Manitoba, Canada. Agricultural development has removed forest cover to the Park edge (McNamee 1993). Several mammalian species, such as beaver *(Castor canadensis)*, fisher *(Martes pennanti)* and and American marten *(M. americana)* were extirpated from the area and RMNP is considered a wilderness “island” within an agricultural region (Carbyn 1980, Noss 1995).

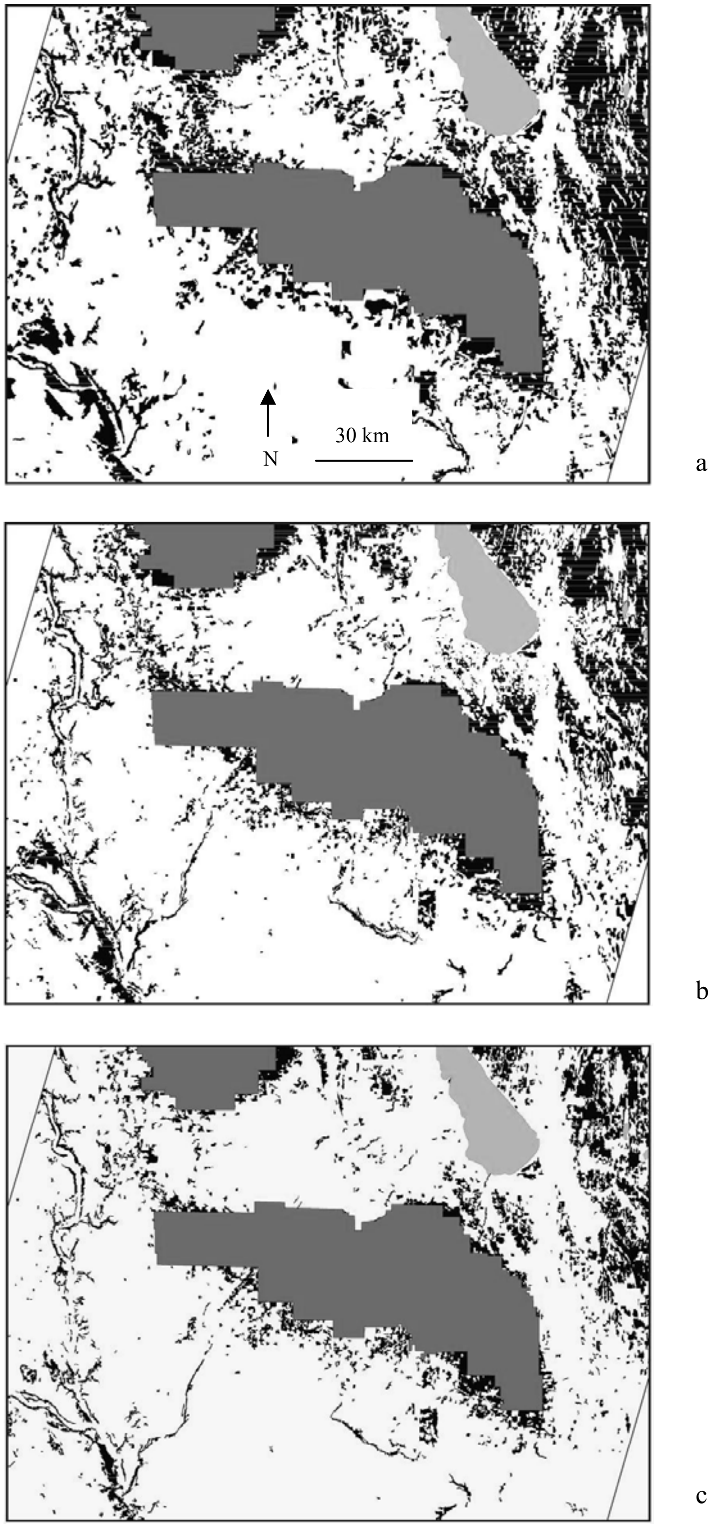

Figure 1.1. Loss of forest extent (black) between Riding Mountain National Park (lower grey reserve) and the Duck Mountains (upper grey reserve) for a) 1950s, b) 1970s, and c) 1991. White represents areas of agricultural development. Reproduced from Walker 2001 with permission.

RMNP (N 50° 46’, W 099° 59’) is located at the transition of the Prairie and Boreal Plain ecozones (Environment Canada 1993), and forest cover was almost continuous between RMNP and Duck Mountain Provincial Park and Forest (hereafter the Duck Mountains) 30 km farther north until the 1950s (Figure 1.1, Walker 2001). However, only 14% forest cover remained by 1991 and intense agricultural development in the center effectively severed RMNP from other forested areas (Walker 2001). Agriculture is now the dominant land use and occupies approximately 58% of the area around RMNP (35% cropland and 23% rangeland), whereas managed public land (including parks) comprises 16% (Parks Canada 2004). Regional road development is extensive, and exclusive of RMNP includes 30 000 km of roads at a density of 0.7 km of road per km^2 (Parks Canada 2004). The total km distance of roads within the biosphere reserve has increased by only 2% since 1948; hence most roads have existed for over 50 years (Canadian Parks and Wilderness Society 2004).

My primary study area includes the Duck Mountains (altogether 5184 km^2) and Riding Mountain Biosphere Reserve (15 000 km^2), which encompasses the core protected area RMNP (2974 km^2) and 15 surrounding rural municipalities. The area is part of the Manitoba Escarpment, a series of highlands in western Manitoba separated by broad valleys, and encompasses numerous lakes and ponds; deciduous, boreal and mixed forest; rough fescue grasslands, and extensive marshes and wetlands (Manitoba Conservation 2004, Parks Canada 2006). The climate is continental interior, with cold winters and moderate snow depths (Carbyn 1982a). The average growing season is variable but averages 72 days (Parks Canada 2004). Elk *(Cervus elaphus)*, moose *(Alces alces)*, beaver and white-tailed deer *(Odocoileus virginianus)* are abundant. Endemic mule deer (*O. odocoileus*) are now rare and have been replaced by invasive white-tailed deer over the past century. Other large mammal species include wolves *(Canis lupus)*, black bears *(Ursus americanus)*, coyote, lynx, red fox *(Vulpes vulpes)*, snowshoe hare *(Lepus americanus)* and several mustelid species.

A combination of disease and human-caused mortality and landscape fragmentation could threaten long-term survival of the RMNP wolf population (Carbyn 1982a, Fritts and Carbyn 1995, Parks Canada 2002). Though wolves have dispersed several hundred kilometers in biophysically similar landscapes (Fritts 1983, Gese and Mech 1991, Wydeven et al. 1995), they have suffered extensive human-caused mortality outside the RMNP boundary (Carbyn 1982b, Fritts and Carbyn 1995). The combined impacts of landscape change and human-induced mortality has likely reduced dispersal between the Park and surrounding areas (Fritts and Carbyn 1995, Parks Canada 2002).

Wolves are territorial mammals with low effective population size (Mech and Biotani 2003) and high behavioural plasticity in food acquisition (Weaver et al. 1996), and are considered limited primarily by food availability (Haight et al. 1998). They were historically widely distributed throughout Eurasia and North America (Kurtén and Anderson 1980) with high rates of gene flow (Vilà et al. 1999), and have been present in southwestern Manitoba for at least 5000 years (Goulet 2000). Wolves occupied the RMNP region until a combination of hunting, trapping, land clearing and poisoning appears to have caused a local extirpation around 1900 (Carbyn 1980). Reports from Park wardens and residents confirmed that wolves had returned by the 1930s, possibly via dispersal from the Duck Mountains (Fritts and Carbyn 1995). Winter snow tracking and aerial observations indicate that the RMNP population has numbered approximately 70 - 75 individuals in late winter over the past 5 years (RMNP unpub. data).

High wolf dispersal capability and annual productivity suggest resilience to modest levels of human disturbance if refugia are distributed across the landscape within distances of approximately < 196 km (see review in Weaver et al. 1996). Hence, for wolves, the RMNP region constitutes a historically well-connected landscape with high likelihood of gene flow. However, human-caused extirpation has caused significant range reduction in wolves (Kurtén and Anderson 1980, Ellegren et al. 1996, Vilà et al. 1999, Leonard et al. 2005) and their persistence in the landscape matrix is sensitive to human tolerance (Fritts and Carbyn 1995, Boyd and Pletscher 1999, Carroll et al. 2006).

Recent genetic research suggests that two putative species of wolf inhabit Manitoba, with gray wolves *C. lupus* occupying RMNP and eastern wolves *C. lycaon* inhabiting areas farther north including the Duck Mountains (Wilson et al. 2000b). They propose that eastern and red wolves *C. rufus* be considered one species, *C. lycaon*, which evolved on the North American continent independently of the gray wolf and shared an evolutionary lineage with the coyote *C. latrans* until 150 000 – 300 000 years ago. Gray wolves and coyotes are sympatric throughout RMNP and occupy different ecological niches (Carbyn 1982b, Paquet 1992), with no evidence of hybridization. Eastern wolves interbreed with both gray wolves and coyotes (Grewal 2001). Hybridization with coyotes appears to be common in agricultural landscapes of southeastern Ontario and the Great Lakes area where large ungulates have been replaced by smaller and medium-sized prey such as white-tailed deer and beaver (Schmitz and Kolenosky 1985, Schmitz and Lavigne 1987, Lehman et al. 1991, Sears et al. 2003). Coyotes are well-adapted to the matrix landscape (Gier 1975), and hybridization is a concern for the RMNP population (Carbyn 1980).

Eastern wolf range may now include Minnesota, Manitoba and northwestern Ontario, although the extent of interbreeding with other canids is uncertain, especially at the range edge (Wilson et al. 2000b, Grewal 2001, Kyle et al. 2006). Hybridization due to biophysical processes such as range expansion after glaciation would be considered natural (Kyle et al. 2006). Nonetheless, human-dominated landscapes seem to favour coyotes (Gier 1975, Lehman et al. 1991) and hybrid canids (Sears et al. 2003, Leonard and Wayne 2007). The matrix may not only limit dispersal of canids occupying the large ungulate predator niche, but also promote dispersal of canids adapted to human-altered environments. Wolves are the primary predators of elk in RMNP, and conserving predation processes is vital for the Park's ecological integrity (Parks Canada 2002). The presence of eastern wolves, a canid that appears to hybridise with coyotes in human-dominated environments, is therefore potentially significant for prey – predator relationships in and around RMNP. As humans modify ecological niches within the matrix, successful dispersal of animals well-adapted to these environments might lead to genetic swamping and altered evolutionary paths for small isolated populations such as RMNP wolves.

Thesis organisation

In Chapter two I test the first two predictions. I use highly variable co-dominant nuclear microsatellite markers, and compare the results with maternally inherited mitochondrial DNA (mtDNA) and skull morphology. This permits comparison of population genetic structure across several scales, and assessment of morphological traits expected to be associated with hybrid canids. In the third Chapter I test predictions

3A and 3B by examining potential costs (inbreeding, parasite load) and benefits (tolerant relatives in neighbouring territories) of philopatry.

In Chapter 4, I test predictions on long-term dispersal in the study area. This is a valuable means of exploring potentially important factors that are unpredictable and poorly understood, such as infectious disease epidemics. Long-term questions for RMNP (Carbyn 1980) include why, given the abundant food, there are not a) more wolves, and b) more sign of dispersal. I, therefore, explore dispersal using park habitat (RMNP, the Duck Mountains) as areas where wolves can establish territories and breed, and the matrix as an area where wolves can travel but not establish territories and breed. I use data from the study area and values from the literature on population size, pack numbers, and fecundity. I predict that dispersal will be influenced both by the number of individuals that emigrate from a patch and the degree of landscape resistance between patches. Simulated disease mortality in pups will likely reduce emigration from the patch by lowering resource competition. Landscape resistance to dispersal include roads and negative human attitudes (through road mortality and people killing wolves).

In Chapter five I summarize my findings and outline potential long-term conservation implications. Human-induced selection could have complex effects on ecosystem processes such as predator – prey relationships within the landscape matrix, which may again influence wildlife disease dynamics. I discuss the relationship between human-induced selective factors in the matrix and the ecological role of large-ungulate predators that pursue their prey over a distance (and are likely to capture ungulates in poor and diseased condition) and explore this further in Appendix H.

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Chapter 2

Dispersal in a plain landscape: Short-distance genetic differentiation and a *lupus – lycaon* cline in southwestern Manitoba wolves

Abstract

The effects of human-caused fragmentation require further study in physically-simple landscapes: areas where movement barriers and natural ecological discontinuities (e.g. climate, mountain-lowland transitions) can, as far as possible, be eliminated as causes for population genetic structure. I test the prediction that human-caused fragmentation reduces dispersal and creates genetic population structure even across short distances and physically-simple landscapes. Considerable fragmentation from agricultural development has occurred in the Riding Mountain National Park (RMNP) region in southwestern Manitoba, Canada, since the 1950s. I examine microsatellite and mitochondrial DNA from gray wolf (*Canis lupus*). Microsatellite genetic structure is evident between RMNP and the Duck Mountains 30 km further north (F_{ST} = 0.074) and consistent with fragmentation. Neither mutation nor natural selection is expected to have contributed significantly to structure at this scale. Hence, human-caused fragmentation of a physically-simple landscape can cause cryptic genetic population structure in vagile organisms on a fine spatiotemporal scale. Mitochondrial DNA haplotypes show additional support for cryptic genetic structure. Both gray wolves and eastern wolves (proposed as species *C. lycaon*) occur in the Duck Mountains. However, eastern haplotypes seem absent or rare in RMNP, where n = 19 haplotypes grouped with *C. lupus* and one haplotype was intermediate between coyotes, eastern and red wolves. Inclusion of Prince Albert National Park samples does not support extending eastern

wolf range into Saskatchewan, and the genetic cline between *C. lupus* and *C. lycaon* type wolves may represent contemporary canid evolution. Residents have reported animals intermediate between wolves and coyotes, and I examine cranial morphology for signs of smaller, and more coyote-like, features. I found no obvious differences in skull features. The Great Plains is now an intensely human-managed landscape, where detection of cryptic genetic structure can function as an important indicator in conservation management of vagile and low-density species.

Introduction

We need further understanding of the minimum difference in scale at which human disturbance is incorporated into the next higher levels of biological organization (Urban et al. 1987). For example, landscape fragmentation can affect dispersal decisions of individual animals, and, hence, gene flow and genetic population structure (Van Vuren 1998). The result is often metapopulations, where dispersal and subsequent re-establishment of populations are necessary for long-term persistence (Hanski 2001). Classical population genetic theory predicts that mutation, drift, and selection cause genetic differentiation between local populations, whereas gene flow contradicts these processes and determines the extent to which evolution within local populations occurs independently (Slatkin 1987). Physical barriers such as mountain ranges have been found to limit dispersal and gene flow in plants, amphibians and mammals (reviewed in Taberlet et al. 1998), and human-caused barriers represented by freeways have reduced gene flow in coyotes (*Canis latrans*) and bobcats (*Lynx rufus*) (Riley et al. 2006).

I here define population as “A group of organisms that interbreed and share a gene pool” (Ridley 1993, p. 638), and between-population dispersers represent only a small proportion of most populations (Rousset 2001, Waser et al. 2001). Genetic structure can persist among populations of widely distributed and mobile organisms due to movement patterns, behaviour and divergent selection pressures (see e.g. Hoelzel et al. 1998, Dearborn et al. 2003). Optimal dispersal probability and distance depend on the risk of establishing in unsuitable habitat, and local adaptation of dispersal strategies appears possible in patches above a certain size (Gros et al. 2006).

Human-caused landscape fragmentation could augment selection for increased dispersal distances (Wiens 2001) or facilitate selection for adaptation to local conditions (Cody and Overton 1996, Ashley et al. 2003). New findings also suggest that a combination of landscape features with low permeability can influence fine-scale genetic structure in the absence of dispersal barriers. Such cryptic population genetic structure has been identified in species such as wolverines (*Gulo gulo*) (Cegelski et al. 2003, Guillot et al. 2005a) and roe deer (*Capreolus capreolus*) (Coulon et al. 2006) in landscapes fragmented by humans. Importantly, cryptic population structure has also been attributed to diet specialization, climate and natal habitat-biased dispersal (preference for dispersal into familiar habitat) and in vagile taxa represented by cetaceans (Hoelzel et al. 1998), felids (Ernest et al. 2003, Rueness et al. 2003) and canids (Carmichael 2001, Geffen et al. 2004, Sacks et al. 2004, Pilot et al. 2006). Natal habitat-biased dispersal could act as a natural selection mechanism for risk aversion and create gene flow patterns corresponding with changes in habitat type (Davis and Stamps 2004). Processes disproportionally affecting small populations are increasingly recognised as important for persistence of evolutionary potential (see review in Frankham 2005). Hence, human-caused landscape fragmentation could have wide ranging and subtle consequences on dispersal and gene flow, and the ability to act across narrower spatial and temporal scales than previously believed. Further research is therefore needed to determine the spatiotemporal consequences of human-caused landscape modification on gene flow in the absence of other factors believed to cause genetic population structure. Such consequences should be examined in organisms where high gene flow is expected to limit independent evolution within local population units, so that fine-scale spatiotemporal effects can be quantified and incorporated into both theory and conservation practice.

The landscape matrix, here defined as areas surrounding reserves and altered to various extents by human use, plays a critical role in landscape connectivity (Noss et al. 1996, Franklin 1993, Kramer-Schadt et al. 2004). It is vital to understand the role of the matrix in conserving ecosystem processes and biodiversity because the matrix dominates available space (Lord and Norton 1990). Fragmentation across short distances relative to organism dispersal range thus merits further research. Wide-ranging species in small reserves are particularly at risk from conflict and mortality associated with reserve boundaries (Terborgh and Winter 1980, Soulé and Simberloff 1986, Noss et al. 1996, Woodroffe and Ginsberg 1998). The Great Plains of North America is now an intensely human-managed landscape (Guertin et al. 1997), and provides an opportunity to assess the effects of this relatively uniform landscape matrix on animal dispersal by testing predictions on fine-scale population genetic structure. Considerable landscape fragmentation has occurred around Riding Mountain National Park (RMNP) in southwestern Manitoba, Canada. Agricultural development has removed forest cover to the Park edge (McNamee 1993). Several mammalian species have been extirpated and RMNP is considered a wilderness “island” within an agricultural region (Carbyn 1980, Noss 1995). The region is located at the transition of the Prairie and Boreal Plain ecozones (Environment Canada 1993), and includes Duck Mountain Provincial Park (1424 km^2) and Forest (3760 km^2, hereafter jointly referred to as the Duck Mountains) and Riding Mountain Biosphere Reserve (15 000 km^2). The biosphere reserve encompasses the core protected area RMNP (2974 km^2) and 15 surrounding rural municipalities.

Loss of native vegetation cover does not imply habitat loss for all species (Lindenmayer and Fisher 2006). I examine genetic population structure in wolves *(Canis lupus)*, a territorial animal with low effective population size (Mech and Biotani 2003) that historically was widely distributed in Eurasia and North America (Kurtén and Anderson 1980) with high rates of gene flow (Vilà et al. 1999). Wolves show high behavioural plasticity in food acquisition (Weaver et al. 1996), and are considered primarily limited by food availability (Haight et al. 1998). Therefore, they should, theoretically, be able to live in the matrix if sufficient food is available. Wolves have dispersed several hundred kilometers in landscapes biophysically similar to my study area (Fritts 1983, Gese and Mech 1991, Wydeven et al. 1995). Nevertheless, the cumulative impacts of landscape change and human-induced mortality likely reduces dispersal between RMNP and surrounding areas (Fritts and Carbyn 1995, Parks Canada 2002). Although located 30 km south of the Duck Mountains, a combination of disease and human-caused fragmentation and mortality could threaten long-term survival of the RMNP population (Carbyn 1982a, b, Fritts and Carbyn 1995, Parks Canada 2002). Whereas fragmentation is only one of several potential factors affecting population differentiation (Kareiva et al. 1996), wolves have been present in south-western Manitoba at least 5000 years (Goulet 2000) and RMNP was well connected to the Duck Mountains until the 1950s (Walker 2001). The region therefore constitutes a historically well-connected landscape with high likelihood of gene flow. Nevertheless, RMNP wolves have been tracked for several multi-year studies since 1974 with no evidence of dispersal between the Park and surrounding areas (Carbyn 1980, Paquet 1992, G. Goulet pers. comm., RMNP unpub. data).

Molecular genetics, behaviour, and morphology should be evaluated together when examining population differentiation (Ryder 1986, O'Brien and Mayr 1991, Frankham et al. 2002). Common applications of population genetics to conservation tend to use neutral genetic markers and avoid genes under selection, so that selection does not 'interfere' with the study of mutation, drift and gene flow (Ashley at al. 2003). Degree of population difference in neutral molecular markers may therefore not reflect adaptive differences, which has important implications for conservation management (Hedrick 1999, Crandall et al. 2000). The cohesion species-concept emphasises shared ecological niche as well as genetic exchangeability (Templeton 1989). Many species or subspecies lack phylogenetically distinct mtDNA (Cronin 1993) but occupy different ecological niches (Crandall et al. 2000), which can add valuable information where genetic relationships are unclear (Philips and Henry 1992, Crandall et al. 2000).

Recent genetic research suggests that two putative species of wolf inhabit Manitoba; gray wolves *C. lupus* occupy RMNP and eastern wolves *C. lycaon* inhabit areas farther north including the Duck Mountains (Wilson et al. 2000). They propose that eastern and red wolves *C. rufus* be considered one species, *C. lycaon*, which evolved on the North American continent independently of the gray wolf and shared an evolutionary lineage with the coyote *C. latrans* until 150 000 – 300 000 years ago. Coyotes are well-adapted to the matrix landscape (Gier 1975), and eastern wolves interbreed with both gray wolves and coyotes (Grewal 2001). Hybridization with coyotes seems widespread in agricultural landscapes of southeastern Ontario and the Great Lakes area (Schmitz and Kolenosky 1985, Lehman et al. 1991, Sears et al. 2003, Leonard and Wayne 2007). The presence of a wolf type that hybridizes with coyotes in human-dominated environments could therefore be significant for gene flow between RMNP and surrounding areas. As humans modify ecological niches within the matrix, successful dispersal of animals adapted to these environments may lead to introgression and genetic swamping, thus influencing evolutionary processes in small and isolated populations such as RMNP wolves.

Some local residents from the study area have reported canids intermediate in size between gray wolves and coyotes ("brush wolves"), suggesting that hybridization might now be occurring. Coyotes and wolves are sympatric in RMNP (Paquet 1992). Interbreeding has not been documented in or near RMNP, but is a future concern (Carbyn 1980) in an agriculture-dominated landscape likely to favour coyotes or hybrid canids (Lehman et al. 1991). Hybrids may have higher fitness than either parental species in novel habitat (Arnold and Hodges 1995), and canids in certain human-dominated environments seem to be experiencing homogenizing selection toward a size intermediate between wolves and coyotes (Kolenosky and Standfield 1975, Schmitz and Lavigne 1987, Sears et al. 2003). I therefore examine available skull samples for morphology associated with wolf-coyote hybridization in Ontario (Kolenosky and Standfield 1975, Nowak 1995) to determine whether possible hybrid canids are phenotypically distinct.

I hypothesize that human-caused fragmentation, even across short distances and physically simple landscapes, influences dispersal. I also hypothesize that the landscape matrix influences evolutionary processes. I make these predictions:

Human-caused fragmentation reduces dispersal and creates genetic population structure even across short distances and physically simple landscapes.

The landscape matrix reduces environmental heterogeneity and niche divergence, promoting hybridization in closely related taxa.

Methods

Study area

The study area is part of the Manitoba Escarpment, a series of highlands in western Manitoba that are separated by broad valleys. The region encompasses numerous lakes and ponds; deciduous, boreal and mixed forest; rough fescue grasslands, and extensive marshes and forested wetlands (Manitoba Conservation 2004, Parks Canada 2006). The regional climate is continental interior, with cold winters and moderate snow depths (Carbyn 1982a). The average growing season is variable and averages 72 days (Parks Canada 2004). Elk *(Cervus elaphus)*, moose *(Alces alces)*, beaver *(Castor canadensis)* and white-tailed deer *(Odocoileus virginianus)* are abundant in the region. Endemic mule deer (*O. odocoileus*) are now rare and have been replaced by invasive white-tailed deer over the past century. Other large mammal species include wolves, black bears *(Ursus americanus)*, coyote, lynx *(Felis lynx)*, red fox *(Vulpes vulpes)*, showshoe hare *(Lepus americanus)* and several mustelid species.

Forest cover was almost continuous between RMNP and the Duck Mountains 30 km farther north until the 1950s (Walker 2001). However, only 14% remained by 1991, effectively severing RMNP from other forested areas (Walker 2001). Agriculture is now the dominant land use and occupies approximately 58% of the area around RMNP (35%

cropland and 23% rangeland), whereas managed public land (including parks) makes up 16% (Parks Canada 2004). Regional road development is extensive and, exclusive of RMNP, includes 30 000 km of roads at a density of 0.7 km of road per km^2 (Parks Canada 2004). Agricultural and road development has also occurred north of the Duck Mountains, but forest reserves connected to undeveloped areas of central Manitoba are situated approximately 10 km to the northwest. Wolves occupied the RMNP region until a combination of hunting, trapping, land clearing and poisoning probably caused a local extirpation around 1900 (Carbyn 1980). By the 1930s, reports from Park wardens and residents confirmed that wolves had returned, possibly via dispersal from the Duck Mountains (Fritts and Carbyn 1995). The RMNP population has numbered approximately 70 - 75 wolves in late winter over the past 5 years (RMNP unpub. data).

Sampling

Two field assistants and I collected faecal and hair samples during 2003 – 2005 by skiing, snowshoeing, hiking and occasionally by snowmachine over approximately 2046 km of trails, roads, and off-road fields and forest. I obtained information about wolf movements from Parks Canada and Manitoba Conservation staff, trappers, and other local residents. Wolves roam widely and I did not attempt random grid sampling of the whole study area, but focused on known wolf travel routes within RMNP and the Duck Mountains to cover as many territories as possible. Wolves are vulnerable to exploitation outside the RMNP boundary and the surrounding matrix likely constitutes marginal habitat for pack establishment (Carbyn 1980, Fritts and Carbyn 1995, RMNP unpub. data). Apart from lone wolves observed near the Park boundary the matrix is unlikely to have many resident wolf packs (Carbyn 1980, this study). Due to a combination of low tree cover and wind, tracks are quickly obscured, making snowtracking difficult (Paquet 1989). Local residents sometimes reported seeing wolves in the landscape surrounding RMNP, but I only visited areas where terrain and forest cover suggested wolf tracks could be preserved. I opportunistically collected tissue samples available from trappers, hunters and accidental mortality, including samples from outside the primary study area. I also used wolf tissue, faecal and hair samples collected in the region over the past 13 years and stored at RMNP. I collected faecal samples in freezer bags and labelled these with UTM (NAD 83), date, number of wolf tracks seen and sample condition, and stored samples at –20˚C.

I mapped all faecal sample locations using ArcView 3.3, and selected the samples that appeared to be of best quality (fresh samples with a smooth surface) from each known or assumed pack (or single wolves in some areas). Recent winter surveys suggest about 10 – 12 wolf territories in RMNP (RMNP unpub. data), and mean early winter RMNP pack size was 8.3 individuals (SD ± 3.8, range 3-16) during 1975 – 1979 (Carbyn 1980). I estimated a similar number of wolves for the Duck Mountains based on information from local residents and Manitoba Conservation staff. I selected 207 faecal samples (roughly the number of wolves thought to occur in the RMNP – Duck Mountain area) for genotyping after the first year of tracking for an overall assessment of the region. The following year I focused on areas with little representation, including where first year samples had failed to amplify, and I selected 108 samples.

Testing for dispersal, gene flow, and evolutionary change

Dispersal and gene flow between populations change over time and space (Bossart and Prowell 1998). Fine scale population processes such as individual identification and tracking parentage and relatedness are best examined by genotypic arrays in the form of multiple microsatellite loci, which are reshuffled in each generation in sexual species (Taberlet et al. 1999, Sunnucks 2000). Allele frequencies and distribution are effective markers for gene flow, population history, and population subdivision, as changes occur at larger spatial and temporal scales than genotypic arrays (Cronin 1993, Sunnucks 2000). Allele or haplotype frequencies can be changed by genetic drift, founder effect, gene flow and selection, and are estimated most accurately by assessing allele frequencies for many genes and representative sample sizes using multiple, separate single locus markers (Cronin 1993, Sunnucks 2000). Single-locus markers can be organellar (mtDNA) and nuclear (microsatellites), and the two reflect different aspects of population biology and history (Sunnucks 2000).

Microsatellites are nuclear, highly polymorphic co-dominant single copy DNA made up of tandemly repeated short sequence motifs and scattered throughout the genome (Sunnucks 2000). They are highly useful for studying paternity, kinship, genetic variation, gene flow and population genetic structure (Kohn and Wayne 1997, Sunnucks 2000). They have higher overall variability than mtDNA (Sunnucks 2000) and can conveniently be amplified in related species with the same primers (Jarne and Lagoda 1996), and in the case of wolves it is possible to use dog *(C. familiaris)* markers. MtDNA is a maternally inherited, haploid non-recombining and extra-nuclear genome (Cronin 1993). These markers have higher mutation rate than nuclear loci (Frankham et al. 2002) but lower variability and effective population size N_e (Dhondt 1996, Sunnucks 2000). Hence, they identify taxa more rapidly and are widely used for systematics (Sunnucks 2000) and illuminating relationships between populations over larger geographic distances (Dhondt 1996), although they are limited to tracking female dispersal (Frankham et al. 2002). The mtDNA control region usually shows intraspecific variation and is useful for studying genetic variability, phylogeography, and forensics (Kohn and Wayne 1997). Gene genealogies are used to examine variation in microsatellite allele length and DNA sequences created through mutation, which illustrate population processes, phyleographic events and speciation by adding a temporal (evolutionary) dimension to the relationships among alleles (Tablerlet et al. 1999, Sunnucks 2000). Nonetheless, direct estimates of individual movements among present-day populations are the only way to obtain an ecological perspective on gene flow and the impact of environmental patchiness on movement patterns (Bossart and Prowell 1998). Contemporary evolution can be diversifying and homogenizing (Carroll 2008), but canids in agricultural landscapes appear to be experiencing homogenizing selection (Kolenosky and Standfield 1975, Schmitz and Lavigne 1987, Sears et al. 2003). I examine genetic markers at two temporal scales, nuclear and mitochondrial DNA (mtDNA), to test predicted impacts of landscape fragmentation on dispersal. I then assess morphology for evidence of hybridization and evolutionary change.

Microsatellite DNA Analyses

I used QIAamp® DNA Stool Mini Kits produced by Qiagen for DNA extraction, and the method “Protocol for isolation of DNA from Stool for Human DNA Analyses” (Qiagen 2001). I kept final extracts frozen until use. Microsatellite repeat units are between two and six base pairs (Jarne and Lagoda 1996), and the risk of obtaining false alleles is reduced by using tri- or tetranucleotide markers (Tablerlet et al. 1999).

I chose the 13 autosomal (not on sex chromosomes) tetranucleotide microsatellite markers FH2001, FH2010, FH2017, FH2054, FH2088, FH2096, FH2422

(Breen et al. 2001), FH3313, FH3725 (Guyon et al. 2003), PEZ06, PEZ08, PEZ15, PEZ19 (Halverson J. in Neff et al. 1999), and the dinucleotide Y-chromosome marker MS41B (Sundquist et al. 2001) (Table 2.1). These markers have been used successfully in other wolf genetic studies (J. Pollinger pers. comm.). I set amplification of nine or more markers as the criterion for genotyping success (Lucchini et al. 2002). This gives a probability of identity [the probability of sampling identical genotypes, denoted $P_{(ID)}$] in siblings of between 0.001-0.0001 (1 in 1000 to 10 000) at a heterozygosity level of 0.08 (Waits et al. 2001). Use of 14 markers thus allowed inclusion of a large number of samples where all autosomal markers did not amplify. Microsatellite primer sequences are listed in Appendix A.

Table 2.1. Primer mixes of the 14 microsatellite markers used for multiplexing reactions on wolf DNA from southwestern Manitoba, Canada, 2003 – 2005.

Primer mixture	**Microsatellite marker combinations**
A	FH3313, FH2422, FH2001, FH2096
B	FH2017, MS41b, FH2054
C	FH2010, PEZ06
D	PEZ08, FH3725
E	PEZ15, FH2088
F	PEZ19

Polymerase Chain Reaction (PCR) conditions optimized for the markers using the Qiagen multiplexing kit were: 95˚C/15 minutes (denaturation 94˚C/30 seconds, annealing 58˚C/90 seconds, extension 72˚C/60 seconds) times 30 PCR cycles, final extension 60˚C/30 minutes, 15˚C/HOLD. The Qiagen multiplexing kit includes a master mix which contains Taq polymerase enzyme, dNTPs (Deoxyribonucleotide triphosphate), Magnesium and buffer, as well as a Q-solution for augmenting amplification of difficult templates. A 10-µl reaction contains: 1) Qiagen master mix X2 (5 µl); 2) Q-solution 5X (1 µl); 3) Primer mix 2 µM (1 µl, 0.2 µM final concentration);

4) IRD primer 1µM (0.4 µl, infrared dye, 0.04 µM final concentration), 5) DNA template (1.5 µl, concentration unknown and variable among samples) and 6) sterile H_2O (1.1 µl).

Genotyping was done with a LICOR® 4200 DNA Analyzer System, and genotypes scored using LICOR® program GeneImagIR. Two tissue control samples with known genotypes were used as reference. A number of faecal and tissue samples were too degraded or had insufficient DNA for genotyping. Twenty faecal DNA samples were re-tested to assess error rate because of generally low quality and quantity of DNA from faecal material when compared to blood and tissue. I performed DNA extractions in RMNP; all subsequent analyses were done by GenServe Laboratories at the Saskatchewan Research Council, Saskatoon.

MtDNA Analyses

I had access to 56 mtDNA sequences from Mantoba and Saskatchewan from a previous study (P. Paquet unpub. data), and 10 new samples (eight wolves, two coyotes) from the study area were also sequenced. MtDNA from the wolf control region (D-loop) was amplified by PCR and purified by GenServe Laboratories. We used primers THR-L 5'-GAATTCCCCGGTCTTGTAAACC-3' and H16498 5'-CCTGAAGTAGGA-ACCAGATG-3'. The Plant Biotechnology Institute at the National Research Council in Saskatoon sequenced all samples. Aliquots of 2 to 6 microliters were taken of each sample and mixed with primer and a premix containing fluorescently labeled nucleotides and Taq DNA Polymerase (BigDye Terminator V3.1 Kit) for a total volume of 10 microliters, which was used for PCR. For each sample, two reactions were prepared, one for the forward and one for the reverse primer. Following an initial denaturing step at 96˚C for 5 minutes, twenty-five cycles of amplification were performed for each reaction. Each cycle consisted of denaturation at 96˚ for 10 seconds, annealing at 50˚ for 5 seconds, and an extension at 60˚ for 4 minutes. This was followed by a hold at 4˚, with varying lengths of time. Ethanol precipitation was then performed twice, to remove any remains of the fluorescent nucleotides, and the samples were spun upside down for 1 minute to dry the pellets. Ten microlitres of the DNA denaturing agent Formamide was then added to dissolve the DNA. Sequencing was performed using a 3730XL DNA Analyser, supplied by Applied Biosystems. All wolf mtDNA samples were also used for microsatellite analyses and amplified successfully.

Morphological measurements

I made 13 morphological measurements on 32 wolf skulls available at the Manitoba Museum in Winnipeg, to compare genetic information from the study area with physical features (Table 2.2). Nowak (1995) suggests that these characters express major adaptive features of the wolf skull regarding overall size, cranial protection, grasping power and capacity for cutting and crushing. The measure of skull length required a larger caliper and I measured this distance to the nearest millimeter; all other measures were to the nearest 0.05 millimeter.

Table 2.2. Morphological measures on wolf skulls from the Manitoba Museum in Winnipeg, Manitoba, Canada, 2005. All skulls were considered fully-grown, based on 1) full eruption of canine teeth and 2) closing of skull sutures. Measures were recorded to the nearest 0.05 millimeter.

Number	**Measure**	**Description****
1*	length	Greatest length of skull
2	zyg width	Greatest distance across zygomata
3	P1 – M2	Alveolar length of maxillary tooth row (P^1 – M^2)
4	cheek	Maximum width across upper cheek teeth (carnassials, P^4)
5	p width	Palatal width at first premolars (P^1)
6	fr. shield	Width of frontal shield
7	M1 - orbit	Height from tooth row to orbit
8	jugal	Depth of jugal
9	carnas	Crown length of upper carnassial (P^4)
10	M2	Crown width of second upper molar (M^2)
11	a-a	Braincase width at widest point
12	b-b	Length from second upper molar (M^2) to depression in front of auditory bullae
13	g-g	Least width across frontals at constriction behind postorbital processes

*****Larger caliper required; measured to nearest millimeter.
****** Measures 1–10 described with images in Nowak (1995); 11–13 in Goulet (1993).

Statistical analyses

Below is a list of my research questions, followed by the approach and test(s) I used to answer each query:

Microsatellites

1) How many individuals does my data collection represent?

Several hair and faecal samples may originate from the same individual. Hair and tissue could also be duplicates of tissue samples from wolves later trapped or found dead.

Approach and test used: I assessed the presence of matching samples using Excel Microsatellite Toolkit (Park 2001). Allelic dropout and false alleles may affect older or poor quality samples, and all samples matching > 75% were considered to come from the same wolf. This threshold is halfway between the similarity expected of siblings and parent-offspring (50%) and a complete match (100%). The Y chromosome marker

MS41b was used to assign sex. This marker was excluded from genetic diversity analyses to avoid sex bias in heterozygosity measures, as females would always be missing the Y chromosome alleles.

2) Are the samples affected by biases common in non-invasive sampling?

Non-invasive faecal and hair samples are generally of lower quality than tissue or blood samples. Older tissue samples may also have deteriorated during storage.

Approach and tests used:

I used MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004) to assess possibilities of null alleles (alleles not amplified due to mutation at primer sites, leading to false homozygotes) large allele dropout (larger alleles may be harder to amplify and more difficult to document), and scoring errors due to stutter peaks (slippage during PCR amplification resulting in DNA fragment copies one repeat larger or smaller than the true fragment, making it difficult to distinguish homozygotes and heterozygotes).

3) Do the microsatellite alleles show isolation by distance within the study area?

If dispersal over longer distances is rare there may be geographic distances that represent thresholds to gene flow, particularly if combined with landscape fragmentation and discontinuous distribution. Conversely, animals living in family groups are expected to show high relatedness and therefore autocorrelation at shorter geographic distances.

Approach and tests used:

I used GenAlEx (Genetic Analyses in Excel) version 6 (Peakall and Smouse 2006) to examine spatial autocorrelation across all loci with a test of 999 permutations and 1000 bootstrap replicates.

4) Is the population in the study area panmictic?

As noted above, wolves disperse widely in biophysically similar landscapes and may disperse frequently and effectively (with reproduction in the new location) within the study area. Panmixus (or near-panmixus) is therefore a reasonable null hypothesis for my study area and organism.

Approach and test used: I tested for nonrandom associations of alleles at different loci (linkage disequilibrium) and difference between observed and expected ratio of genotypes (Hardy-Weinberg disequilibrium). Panmictic populations are expected to show approximate Hardy-Weinberg and linkage equilibrium (Frankham et al. 2002). I did these standard genetic diversity analyses using GENEPOP 3.4 (Raymond and Rousset 1995) and GENETIX 4.05.2 (Belkhir et al. 2004) and using the Hardy-Weinberg exact test (Guo and Thompson 1992) as implemented in GENEPOP 3.4 using the Markov chain method. I used parameter values from Coulon et al. (2006) for a population with expected low genetic differentiation (global test demerization number = 10 000, number of batches = 400, and number of iterations of batches = 3000. For tests per locus I used number of iterations per batch = 7000). To account for the testing of multiple hypotheses I followed Coulon et al. (2006) and adjusted p-values for Hardy-Weinberg and linkage equilibrium tests using false discovery rate (FDR) control outlined in Verhoeven et al. (2005). This method attempts to correct for both Type I and II errors. For m tests (the number of loci or loci pairs tested), I ranked p-values in ascending order $P_{(1)} \leq \ldots P_{(m)}$, and designated $H_{(i)}$ as the null hypothesis corresponding to $P_{(i)}$. Subsequently, k is the largest i where $p_{(i)} \leq \alpha/m \times i$, and I set $\alpha = 0.05$ to perform analyses comparative to other populations with expected low genetic differentiation (Coulon et al. 2006). Estimates for F_{IS} per locus (proportion of inbreeding within a population due to inbreeding within subpopulations) are calculated according to Weir and Cockerham (1984) and Robertson and Hill (1984).

5) Is there genetic population structure within my study area?

If the null hypothesis of panmixus is rejected, the next step is to establish the number and distribution of genetic population clusters, and determine if these correspond with observed landscape fragmentation (i.e. whether clusters are separated by matrix).

Approach and test used: Chance plays an important role in Mendelian inheritance, and the offspring of a RMNP immigrant would, at any locus, have a 50% chance of inheriting a rare allele from an immigrant parent versus a common allele from resident parent. One offspring of such a pair may thus be classified as resident whereas a full sibing could be labelled as an immigrant to the Park. This is a potential source of error, and I only examined 14 loci. However, where 'true' source populations for individuals are unknown, different methods with various underlying models can provide a range of gene flow estimates and thus relative measures of immigration and connectivity (Cegelski et al. 2003). As noted above it was difficult to collect samples in the matrix and no dispersal has been documented between RMNP and the Duck Mountains. Wolves can nonetheless cover over 70 km/day (Mech and Boitani 2003). Whereas differentiated populations are likely to be structured in spatially distinct areas (Guillot et al. 2005a, Coulon et al. 2006), I am testing for continuous distribution using discontinuous sampling. Hence, it is useful to compare gene flow estimates between approaches emphasising my null hypothesis (wolves easily move within the study area) and alternative hypothesis (no wolf dispersal documented). I therefore examined estimates between spatially explicit and non-explicit approaches, and programs assuming *a priori* population designations and not.

I examined genetic structure by comparing results from a clustering analyses based on Bayesian models; GENELAND 0.3 (Guillot et al. 2005 a, b) and one approach based on a maximum likelihood method, the Assignment Test (Paetkau et al. 1995, 1997, Waser and Strobeck 1998) using ARLEQUIN 2.00 (Schneider et al. 2000). GENELAND is spatially explicit with no *a priori* population assumptions, and considers both genetic and location data in assigning individuals to clusters (Guillot et al. 2005b). The program assumes linkage equilibrium between loci and expects that individuals are randomly located within clusters at Hardy – Weinberg equilibrium (Guillot et al. 2005b). I ran GENELAND six times to determine the number of clusters K (50 000 Markov chain Monte Carlo (MCMC) iterations, rate.max = 221 [number of samples], minimum K = 1 and maximum K = 10, nb.nuclei.max = ranging from 663 – 1500, and the Dirichlet frequency model). I considered DNA sample locations, which may have an error up to a few kilometers for hunted or trapped tissue samples, accurate for this scale of population analyses.

The Assignment Test requires *a priori* definition of populations and then attempts to assign individuals to these populations. Genotypes are not georeferenced, and whereas this represents loss of information, this test examines genotype information without the potential bias of my discontinuous sampling. Though GENELAND can be run without spatial locations, it requires sufficient discontinuity in genotypes in order to delineate clusters, and then assigns individuals to these based on posterior probabilities (Guillot et al. 2005b). Moreover, departure from model assumptions may at times result in identification of clusters that do not exist (Guillot et al. 2005a, Pilot et al. 2006). The Assignment Test in ARLEQUIN allows a test of the alternative hypothesis by defining patches separated by matrix as population clusters, and then to examine how many individuals are 'misassigned' and thus appear to be dispersers or their descendants. If many individuals appear to originate from a patch different than their sampling location, dispersal is probably common. I also examined another clustering approach based on Bayesian models; STRUCTURE 2.1 (Pritchard et al. 2000, see Appendix B).

6) Which clustering approach is best?

Which clustering result places more variation between the inferred clusters relative to within clusters?

Approach and test used: I examined analysis of molecular variance (AMOVA, Excoffier et al. 1992) to determine the distribution of genetic variation within and between genetic clusters using ARLEQUIN 2.00. I did a Factorial Correspondence Analysis (FCA) with the preferred clusters using GENETIX as outlined in Grewal (2001) to produce a visual representation. This exploratory descriptive FCA approach uses multilocus profiles to project all individuals in a three-dimensional space, using each allele as an independent variable (Roques et al. 2001). Degree of allelic diversity and rate of genetic drift may vary between different genetic markers, and the FCA approach is an attempt at grouping genetic variation according to clusters of individuals with similar genetic makeup across all nuclear markers. Data points (individuals) are coloured according to their genetic assignment (if provided in the input file), and the FCA therefore provides a visual display of the variation within and between clusters. The first axis normally explains most of the variation, followed by the second and the third axis (Belkhir et al. 2004).

7) Are the inferred genetic clusters in equilibrium (indicating stability in the frequency of alleles and genotypes)?

Do the inferred clusters show nonrandom mating or inbreeding?

Approach and test used: I examined inferred clusters for nonrandom mating and inbreeding by testing for linkage disequilibrium and departures from Hardy-Weinberg equilibrium, and estimated F_{IS} per locus in GENETIX (parameter values from Coulon et al. (2006) with global test demerization number = 10 000, number of batches = 300, and number of iterations of batches = 5000). I examined spatial autocorrelation within clusters with a test of 999 permutations and 1000 bootstrap replicates, and assessed presence of null alleles, large allele dropout, and scoring errors due to stutter peaks.

8) How much gene flow occurs between the inferred clusters?

Are inferred clusters highly differentiated, and is there evidence of sex-biased dispersal?

Approach and test used: I calculated pairwise population differentiation (F_{ST}) by Theta (Weir and Cockerham 1984) between genetic clusters using GENETIX with a test of 1000 permutations. I then calculated the number of migrants between clusters as $N_m = (1 - F_{ST})/4F_{ST}$ (Wright 1969). Next, I repeated the F_{ST} and N_m calculations for males and females separately.

MtDNA

1) Are mtDNA sequences highly divergent within the study area?

How much divergence is there between mtDNA sequences from different areas, and are any wolf sequences closely related to coyotes?

Approach and test used: I examined sequence divergence for 239 basepairs of the wolf mtDNA control region and compared recent wolf and coyote samples from my study area with previously identified haplotypes from Manitoba and Saskatchewan (P. Paquet unpub. data), eastern North America (Wilson et al. 2000) obtained from NCBI GenBank (Y. Plante pers. comm.) and Europe (Ellegren et al. 1996). I aligned haplotypes using CLC Free Workbench 3.2 (Knudsen et al. 2005) and then made manual adjustments, and created a neighbour-joining tree of haplotypes using 1000 bootstrap replicates.

Subsequently, I used the program TCS (Clement et al. 2000) to estimate gene genealogies with a method that incorporates the possibility of recombination and is well suited to analyses at the population level (Clement et al. 2000). I performed analyses including and excluding gaps in the haplotypes.

2) How is mtDNA divergence distributed spatially?

Are haplotypes spatially clustered, and do clusters correspond with landscape fragmentation?

Approach and test used: I divided the available samples into 20 groups based on their geographic origin for haplotype frequency analyses. To determine differentiation between groups I used NEIGHBOUR and DRAWTREE in the program PHYLIP 3.65 (Felsenstein 1993) to create a neighbour joining tree based on pairwise population differentiation (F_{ST}) values (Weir and Cockerham 1984) between groups calculated in ARLEQUIN 3.1 (Excoffier et al. 2006). I used Spatial Analysis of Molecular Variance (SAMOVA, Dupanloup et al. 2002) to determine geographically homogeneous local population groups maximally differentiated from each other using $k = 2 – 17$. This method establishes the variation within and between groups, and which population(s) appears most divergent. Both approaches are sensitive to sampling (Pilot et al. 2006) and I have few samples from some locations. Nonetheless, the analyses provide an initial identification of breaks in gene flow and add context to the divergence seen in the study area by inclusion of Ontario and Saskatchewan haplotypes.

Morphology

1) Is there evidence of canids with skull features intermediate between wolf and coyote?

Do wolf skulls from the Duck Mountains, where eastern wolves have been found, show sign of more coyote-like morphology, such as a slender nose and palate?

Approach and test used:

Nowak (1995) examined differences in skull size and shape between coyotes, gray wolves and eastern wolves using a canonical discriminant analysis. This approach requires prior existence of groups with known means and variances, which I do not have for my study area. To explore whether wolves from the Duck Mountain area have features consistent with a more coyote-like canid than wolves from RMNP, I examined skull variation by Principal Component Analysis (PCA) as outlined in Goulet (1993) using SPSS 10.0.1. (SPSS Inc. 1999). The objective of a PCA is to identify the variables (factors) that explain correlation patterns within a set of observed variables and extract the factors that explain most of the variance observed. I did Varimax rotation to simplify relationships between factors and variables and thus clarify interpretation. Rotation started from the original axes and converged in 10 iterations. Wolf skulls are grouped by sex and latitude zone. Starting from the south, zone 1 represents the Riding Mountains (only gray wolves documented), zone 2 the Duck Mountains (gray and eastern wolves documented) and zone 3 the Porcupine Mountains (no prior sampling).

Results

Nuclear DNA extraction, genotyping, and individual identification

1) How many individuals does my data collection represent?

More than 800 wolf faecal, hair and tissue samples were collected and 420 samples were selected for microsatellite DNA analyses based on apparent quality and collection location. I calculated allelic diversity for each marker, % error rate (based on a subset of 20 samples typed twice) and % amplification success (Table 2.3).

Table 2.3. Microsatellite marker amplification details for wolves in southwestern Manitoba, Canada, 2003 – 2005*.

Marker (locus)	**Number of alleles***	**% Error rate****	**% Amplification*****	**Repeat******	**Overall result**
FH2096	3	13	95	tetra	Moderate
FH2001	9	25	99	tetra	Good
FH2422	14	8	97	tetra	Very good
FH3313	22	48	90	tetra	Moderate
FH2054	12	33	97	tetra	Moderate
MS41b	6	0	44% show males	di	Only in males
FH2017	4	20	82	tetra	Moderate
FH2088	7	28	98	tetra	Moderate
PEZ06	14	70	99	tetra	Poor
PEZ08	13	15	39	tetra	Poor
FH3725	20	25	97	tetra	Moderate
FH2010	5	13	98	tetra	Moderate
PEZ19	10	20	93	tetra	Moderate
PEZ15	22	10	80	tetra	Good

* includes samples from Red Deer Lake (n=2) and Sherridon (n=1) in northern Manitoba, Hecla Island (n=1) and Lac du Bonnet (n=1) in central Manitoba, and the Turtle Mountains (n=1) in southern Manitoba, and inclusion of samples collected 1990 – 2003.

** twenty samples were genotyped twice to assess error rate: the number of alleles that differered in first and second amplification/40 (20 samples x 2 alleles per loci).

*** amplification was defined as successful when 9 or more markers gave results

**** tetranucleotides are repeated segments of four nucleotides (such as GGAT), while dinucleotides are repeated segments of two nucleotides (such as GA)

Of the 420 faecal, hair and tissue samples, 310 were successful in amplifying at least 9 of 14 markers. After adjusting for matching samples, I identified a total of 221 individual wolves. For two of the radio-collared wolves, samples were collected during capture and again when the animals were found dead. These samples match with the exception of one allele each (>90%) which supports the threshold criterion (>75%). I identified male wolves based on presence of Y-chromosome alleles.

2) Are the samples affected by biases common in non-invasive sampling?

There was no sign of large allele dropout. However, 11 of 14 loci (all except FH2010, FH2054 and FH2096) were identified as having possible null alleles. Loci FH2017, PEZ08, PEZ19, and MS41b showed possible stutter peaks.

3) Do the microsatellite alleles show isolation by distance within the study area?

Spatial autocorrelation across loci varied with distance throughout the study area (Figure 2.1) but was generally within confidence intervals. Microsatellite alleles were positively correlated with distance up to 60 km (indicating kinship between individuals at this spatial scale), then negatively correlated at 60 – 330 km, and subsequent values vary around zero. Overall, spatial autocorrelation within the study area does not seem to be significant.

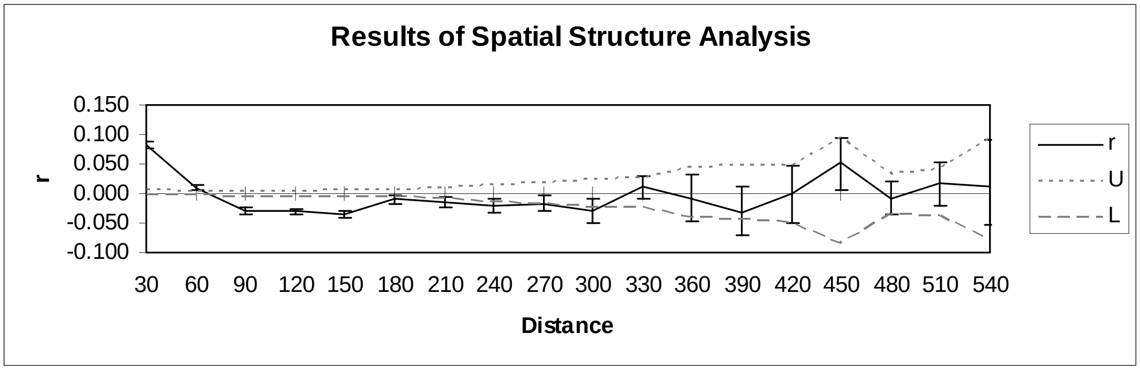

Figure 2.1. Spatial autocorrelation in microsatellite alleles across all wolves sampled in southwestern Manitoba, Canada, 2003 - 2005. The autocorrelation (kinship) coefficient is denoted by r, and distance in kilometers. U and L are upper and lower limits for the 95% confidence interval around the null hypothesis of no spatial structure as determined by 999 permutations, whereas the upper and lower error bars show the 95% confidence interval about r as determined by 1000 bootstrap replicates.

Gene flow and population genetic structure inferred from nuclear DNA

4) Is the population in the study area panmictic?

Forty-four percent of wolves scored as males, and I found altogether six alleles at the Y-chromosome locus MS41b (211, 213, 215, 217, 219 and 223). Overall, 13 of14 loci showed Hardy-Weinberg disequilibrium with levels of heterozygosity lower than expected, and 26 of 78 loci pairs showed significant linkage disequilibrium. This suggests that genetic population structure is present (Table 2.4).

Table 2.4. Genetic diversity in 221 wolves from southwestern Manitoba, Canada, 2003 – 2005*. Values significant at the 0.05 level (after FDR correction) marked in bold.

Locus	**Number of alleles**	**H_o**	**H_e n.b.****	**P-value plus S.E.*****	**F_{IS} W&C/R&H**	**Allele size range**
FH2001	9	0.6727	0.7385	**0.0014 (±0.0003)**	0.089/0.088	127 – 152
FH2010	5	0.6019	0.6542	**0.0010 (±0.0002)**	0.080/0.108	220 – 236
FH2017	4	0.1780	0.2366	**0.0000 (±0.0000)**	0.248/0.188	260 – 272
FH2054	12	0.8111	0.8420	**0.0324 (±0.0026)**	0.037/0.020	134 – 174
FH2088	7	0.5395	0.6034	**0.0002 (±0.0001)**	0.106/0.069	92 – 132
FH2096	3	0.6226	0.6458	0.5009 (±0.0027)	0.036/0.029	95 – 103
FH2422	14	0.6147	0.6976	**0.0000 (± 0.0000)**	0.119/0.259	174 – 242
FH3313	22	0.7512	0.8613	**0.0000 (± 0.0000)**	0.128/0.099	337 – 425
FH3725	20	0.7814	0.8489	**0.0000 (± 0.0000)**	0.080/0.042	130 – 194
PEZ06	14	0.6881	0.8487	**0.0000 (± 0.0000)**	0.190/0.206	164 – 198
PEZ08	13	0.6514	0.7670	**0.0281 (±0.0035)**	0.151/0.067	213 – 253
PEZ15	22	0.6776	0.8395	**0.0000 (± 0.0000)**	0.193/0.127	200 – 284
PEZ19	10	0.5707	0.6926	**0.0000 (± 0.0000)**	0.176/0.138	182 – 214
Mean	11.92	0.6278	0.7147	**0.0000**	0.12052	

*Includes samples collected between 1990–2003.

** H_e n.b. denotes that H_e values are calculated with correction for sample size bias (Nei 1978).

*** Significance values for H_e excess or deficiency calculated using the Hardy-Weinberg exact test (Guo and Thompson 1992), and adjusted for false discovery rate (FDR) control (Verhoeven et al. 2005).

5) Is there genetic population structure within my study area?

The genetic structure analyses outlined below generally assume Hardy-Weinberg and linkage equilibrium within each cluster. This may be difficult to fulfill for wolves due to their social structure where populations are composed of family groups (Thiessen 2007), and violations of these assumptions are observed in large wolf sample sets (J. Pollinger, pers. comm.). However, these are the best available reference values for establishing the extent of panmixus and comparison with other populations.

Testing for dispersal between *a priori* population clusters:

The spatially non-explicit Assignment Test

For the assignment test I entered three *a priori* populations (K = 3; The Riding Mountains, the Duck Mountains, and the Porcupine Mountains – Kettle Hills). With a few exceptions, wolves were assigned to the predicted population (Figure 2.2). Log likelihood plots of individual assignment between pairs of *a priori* populations (Figures 2.3 – 2.5) show that whereas assignment between populations was relatively equal (close to the diagonal line) for some wolves, possibly indicating mixed genetic background, most were placed into relatively well defined clusters. Some genotypes appear to fit better with a population other than their *a priori* designation. These apparently "misassigned" individuals likely represent immigrants or their descendants.

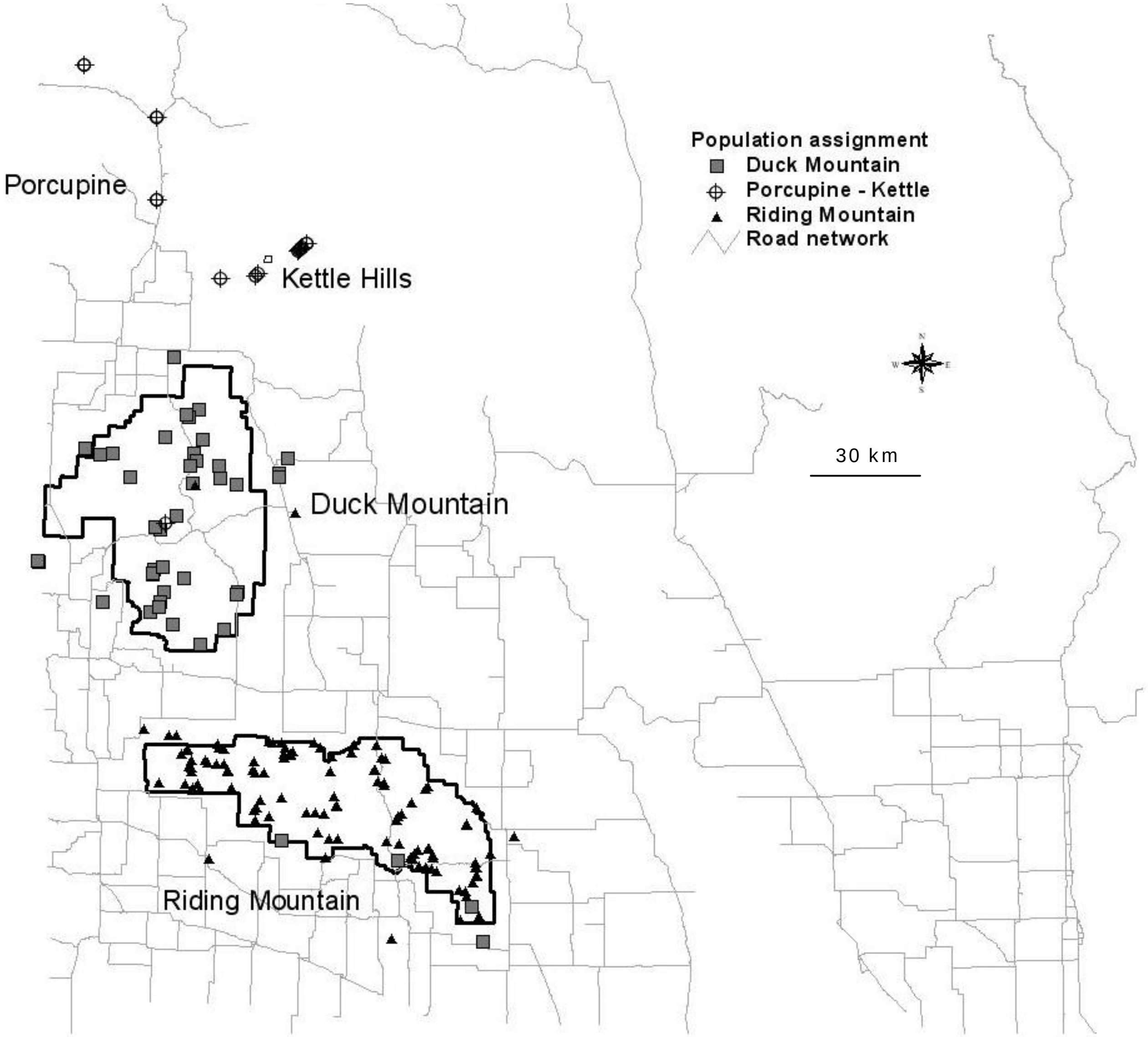

Figure 2.2. Wolf genotype assignments in southwestern Manitoba, 2003 – 2005 based on the Assignment Test (Paetkau et al. 1995, 1997; Waser and Strobeck 1998).

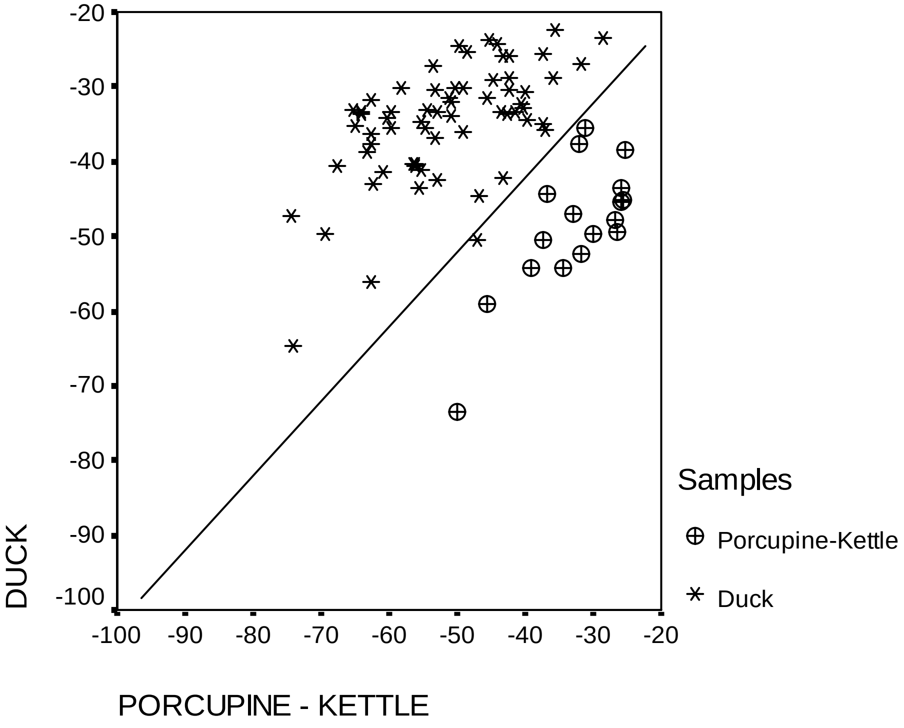

Figure 2.3. Log likelihood values for Duck Mountain versus Porcupine-Kettle wolves in southwestern Manitoba, 2003 - 2005. Sample label shows collection location.

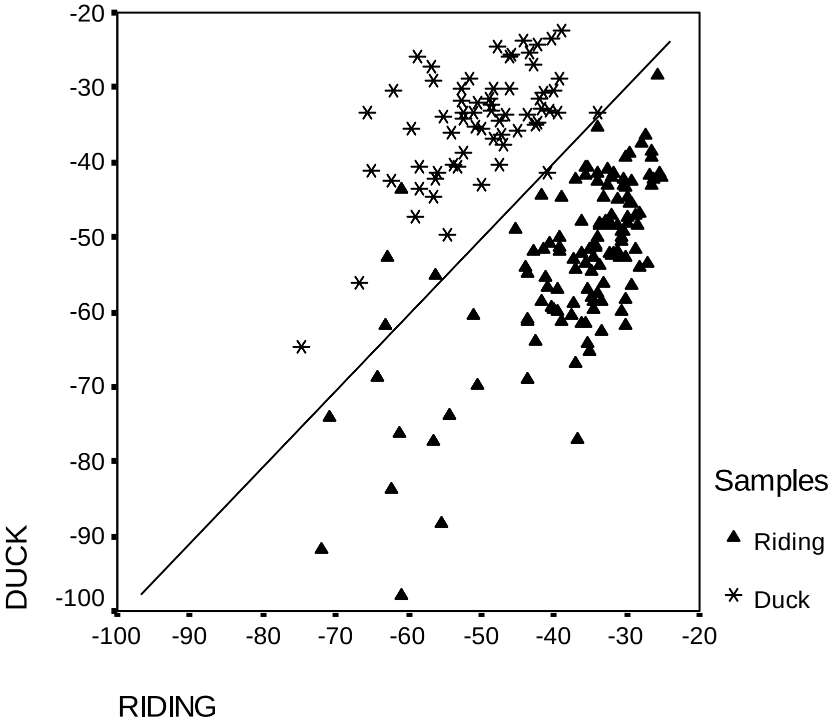

Figure 2.4. Log likelihood values for Duck Mountain versus Riding Mountain wolves in southwestern Manitoba, 2003 - 2005. Sample label shows collection location.

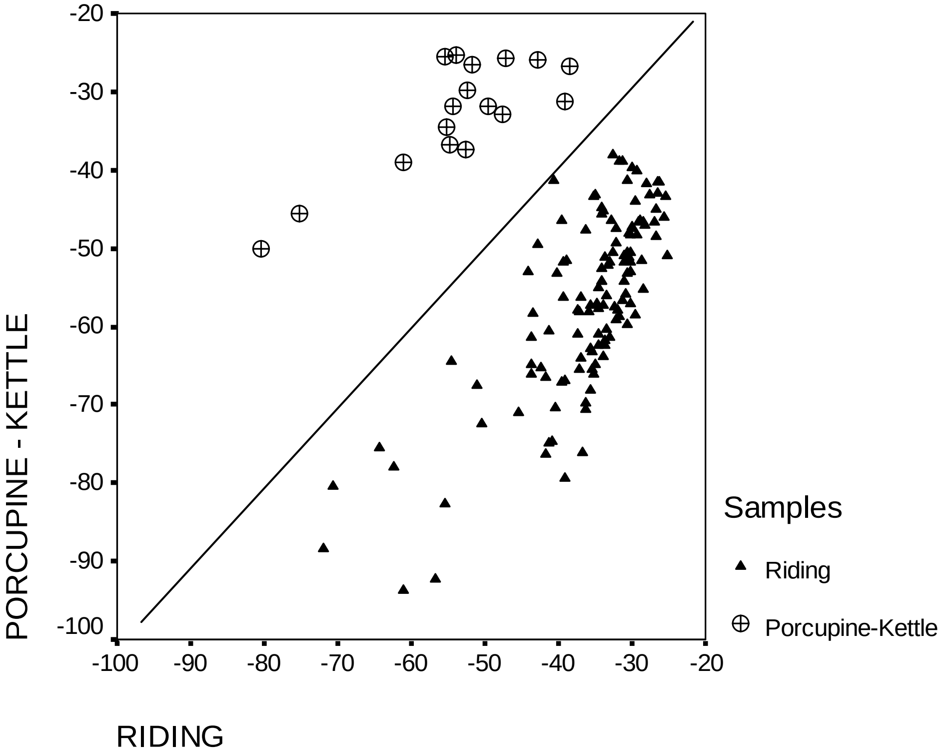

Figure 2.5. Log likelihood values for Porcupine - Kettle versus Riding Mountain wolves in southwestern Manitoba, 2003 - 2005. Sample label shows collection location.

Testing for dispersal with no *a priori* populations:

The spatially explicit GENELAND approach

The results for number of clusters (K) were 7, 8, 9, 10, 7, and 7. Guillot et al. (2005a) note that departures from model assumptions of Hardy-Weinberg and linkage equilibrium can cause the program to overestimate the number of clusters. For this reason, and because 3 of 6 runs gave K = 7, this value appeared the best and most parsimonious choice (Figure 2.6). As suggested by Guillot et al. (2000a) I then fixed the population to K = 7 population clusters (variation = 0) and did the analyses again to estimate group membership for all individuals. The program assigned individuals to six different clusters, and two individuals were assigned equally to clusters 1 and 7 (Figure

2.7). No individuals were assigned to cluster 2. This appeared to be a “ghost” population, possibly due to departure from model assumptions (Coulon et al. 2006). Only two individuals were assigned to cluster 7 and one individual to 1.

I also did the analysis again with initial parameters settings but without reference to spatial coordinates (Guillot et al. 2000a), which allows me to compare the GENELAND results with the non-spatial Assignment Test results. Analysing the data with and without spatial coordinates also allows me to determine whether discontinous sampling appears to influence individual assignment to clusters, as I have very few DNA samples from the landscape matrix. I conducted six runs without spatial coordinates that resulted in K = 10, 9, 10, 9, 8, and 8. I chose K = 9 as the best non-spatial result, and did the analysis again with K fixed to 9 to estimate individual population membership. Non-spatial analysis resulted in identification of several clusters within RMNP, and the locations of two clusters generally correspond with the territories of two known family groups (radio-collared packs). Only two individuals were assigned to cluster 7 and one individual to cluster 5.

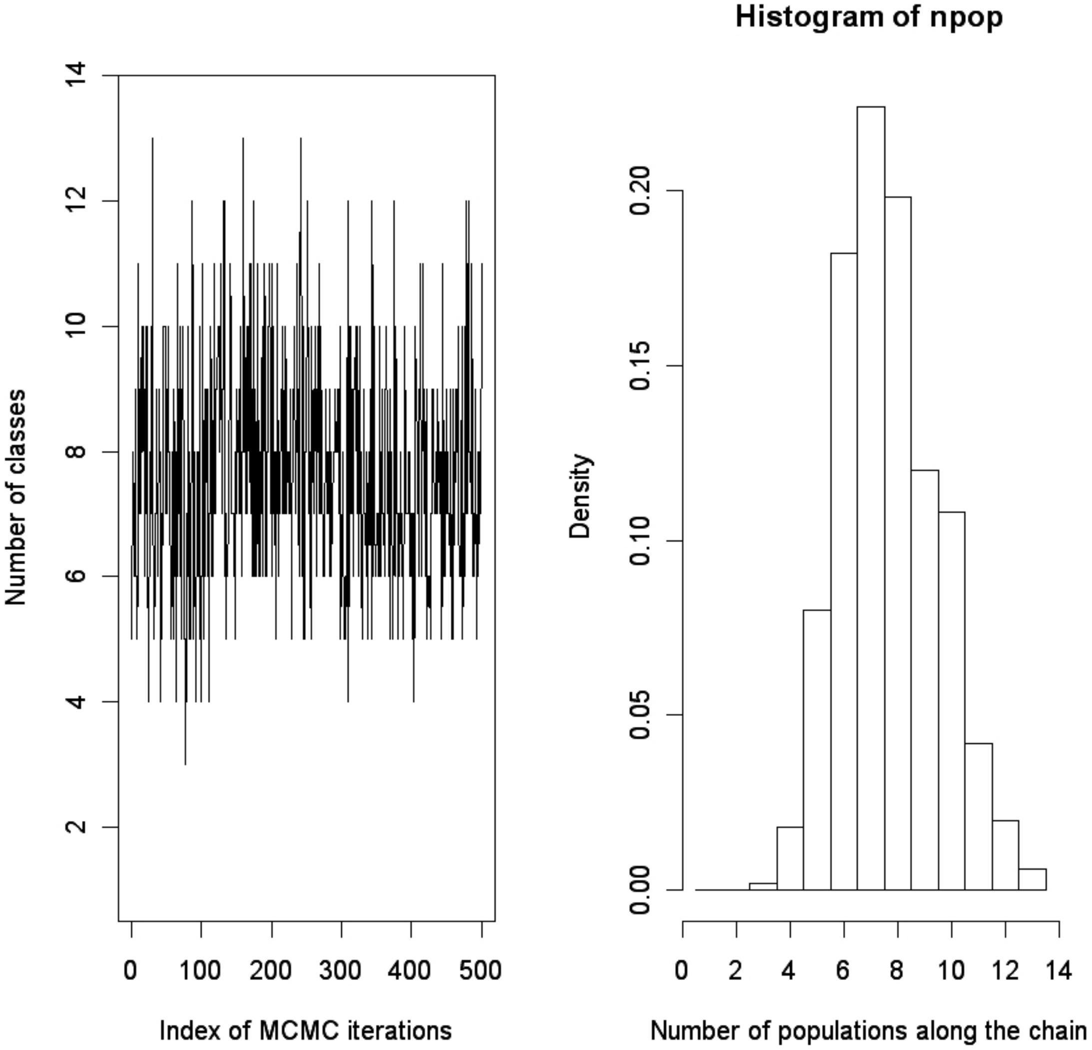

Figure 2.6. Histogram of the Markov chain Monte Carlo (MCMC) iterations and likelihood of number of populations for wolf genotype assignments in southwestern Manitoba, 2003 – 2005 based on GENELAND (Guillot et al. 2000a).

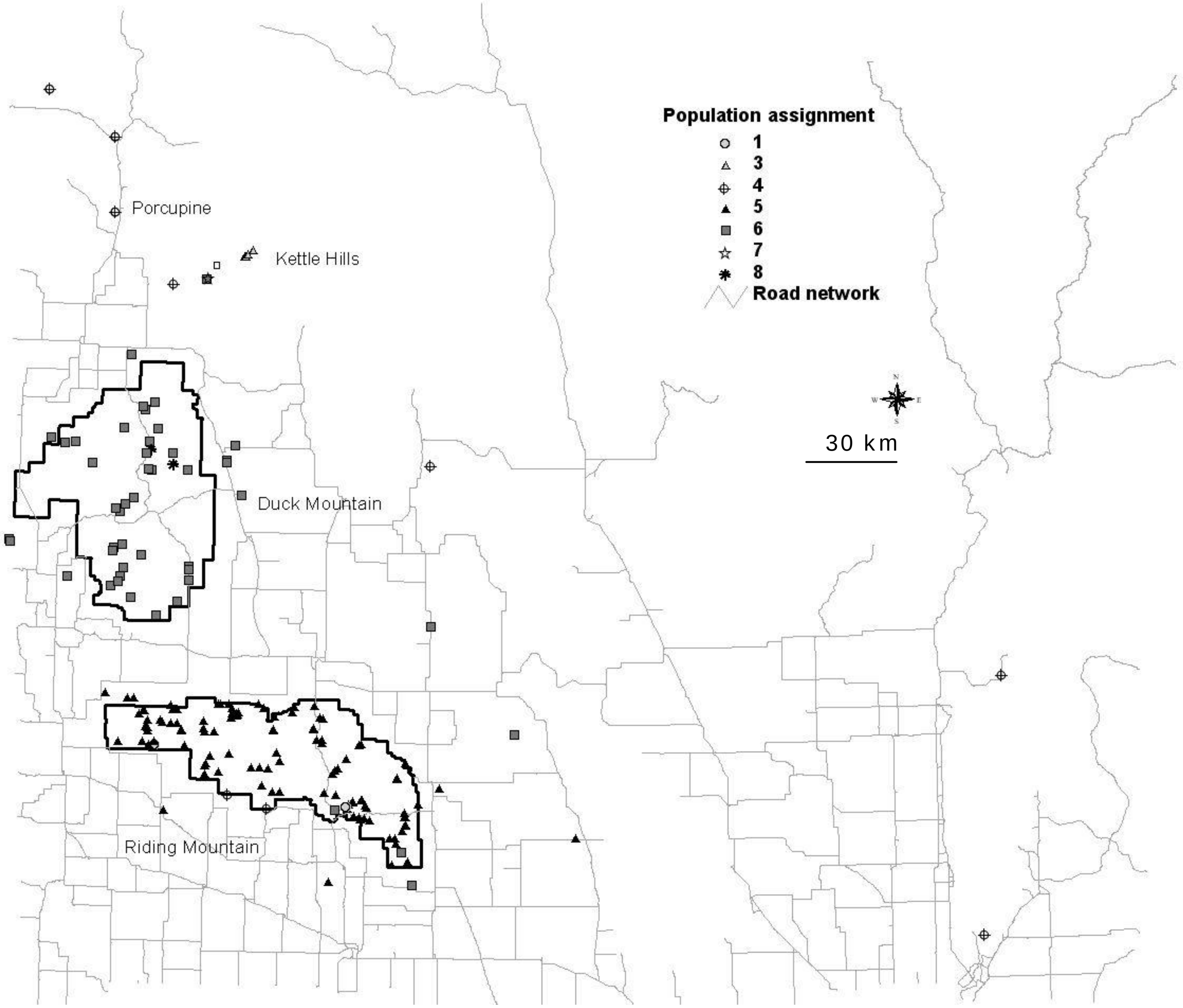

Figure 2.7. Wolf genotype assignments in southwestern Manitoba, Canada, 2003 – 2005 based on GENELAND (Guillot et al. 2000a) with K = 7 genetic clusters. Two additional wolves (collected 400 km north and 200 km south of the Park) were also assigned to cluster 4. Cluster 8 represents two Duck Mountain individuals equally assigned to clusters 1 and 7.

6) Which clustering approach is best?

In order to examine the inferred population clusters and determine degree of dispersal between clusters, I first selected the result that appeared best to maximize variation in nuclear allele frequencies between clusters. Analyses of molecular variance (AMOVA) within and between proposed clusters (Table 2.5) suggest that GENELAND results account for more microsatellite allele variation between clusters than within clusters relative to the Assignment Test.

Table 2.5. Analyses of molecular variance (AMOVA) between and within suggested microsatellite allele population clusters in southwestern Manitoba wolves, 2003 – 2005. Genetic clusters are based on the Assignment Test (Paetkau et al. 1995, 1997; Waser and Strobeck 1998) and GENELAND (Guillot et al. 2000a).

Genetic clusters	**% variation among clusters**	**% variation within clusters**	**Fixation index F_{ST} (p value)**
ASSIGNMENT TEST k=3	7.12	92.88	0.07123 (0.0000)
GENELAND k=7	8.65	91.35	0.08648 (0.0000)

I chose the spatial results suggested from GENELAND with K = 7 clusters as the most likely genetic structure for the following reasons:

- Previous analyses suggest that inclusion of spatial locations in GENELAND permits detection of cryptic population structure (Coulon et al. 2006). GENELAND and Assignment Test results show similar number and distribution of genetic clusters, but GENELAND results suggest additional population structure.
- Although the difference is small, analyses of molecular variance (AMOVA) within and between proposed clusters suggest that GENELAND results account for more variation between groups than the Assignment Test results.

Results from GENELAND and the Assignment Test agreed on overall structure of genetic clusters and, with a few exceptions, identified the same immigrants. Where the Assignment Test did not assign immigrants to the same source population as in GENELAND, the second-best assignment (the Assignment Test considered three *a priori* clusters) was consistent with GENELAND results. I therefore did not repeat the Assignment Test with GENELAND clusters. Genotyping of the Y marker MS41b may have errors, and presence of Y-chromosome alleles did not correspond to the reported sex for some animals although this could be due to erroneous reporting. Furthermore, locus PEZ06 had a 70% error rate and FH3313 had 48% error rate (other loci varied from 0 – 33%). To examine these possible sources of error before continuing analyses on the inferred clusters, I did an additional GENELAND analysis with these three loci excluded to examine their effect on genetic structure. The results remained consistent and I therefore retained all loci for further analyses of Hardy-Weinberg and linkage equilibrium within genetic clusters.

7) Are the inferred genetic clusters in equilibrium (indicating stability in the frequency of alleles and genotypes)?

Standard Genetic Analyses on the Inferred Populations

I continued standard genetic analyses for clusters 3 (Kettle Hills), 4 (Central Manitoba), 5 (Riding Mountain), and 6 (Duck Mountain, Table 2.6). The Kettle Hills and Central Manitoba clusters showed no linkage disequilibrium when adjusted for multiple tests (m = 78 loci pairs), whereas in the Riding Mountain and Duck Mountain clusters 22 and 10 pairs, respectively, differed significantly from equilibrium. The loci pairs affected varied between populations, and because none differed significantly in > 2 clusters, I retained all loci. The Central Manitoba cluster had no sign of null alleles, and the Kettle Hills cluster did not have enough data for analyses. The Riding Mountain cluster had five loci which may have null alleles (PEZ06, PEZ15, PEZ19, FH2422 and FH3313), whereas the Duck Mountain cluster had three (PEZ06, PEZ08 and FH2017). There was no sign of large allele dropout, and only one locus in one population (FH2017 in the Duck Mountain cluster) showed possible stutter. Allele frequencies for each of the four clusters are shown in Appendix C.

Table 2.6. Expected and observed heterozygosity, allelic diversity, and degree of inbreeding (F_{IS}) for four microsatellite allele population clusters in southwestern Manitoba wolves, 2003 – 2005*. Heterozygosity values are calculated with correction for sample size bias**. Values significant at the 0.05 level (after FDR correction***) marked in bold.

	Pop 3 *Kettle Hills* (n = 8, allelic div. = 2.69)				Pop 4 *Central Manitoba* (n = 14, allelic div. = 7.07)				Pop 5 *Riding Mountain* (n = 123, allelic div. 8.00)				Pop 6 *Duck Mountain* (n = 71, allelic div. = 8.92)			
Locus	Ho	He n.b.	P-value	F_{IS}	Ho	He n.b.	P-value	F_{IS}	Ho	He n.b.	P-value	F_{IS}	Ho	He n.b.	P-value	F_{IS}
FH2054	1.0000	0.7083	0.4400	-0.4550	0.7857	0.8280	0.8276	0.0530	0.7479	0.7963	0.3076	0.0610	0.9155	0.8402	0.0813	-0.0900
FH2001	0.6250	0.7083	**0.0009**	0.1250	0.4286	0.5291	0.3159	0.1960	0.6721	0.7196	0.2035	0.0660	0.7183	0.7446	0.0356	0.0360
FH2096	0.5714	0.5275	1.0000	-0.0910	0.6429	0.6111	1.0000	-0.0540	0.6723	0.6523	0.4572	-0.0310	0.5143	0.6203	0.2076	0.1720
FH2010	0.5000	0.5333	1.0000	0.0670	0.7143	0.7963	0.0070	0.1070	0.6780	0.6429	0.8345	-0.0550	0.4648	0.4764	0.5859	0.0240
FH2017	0.5000	0.4091	1.0000	-0.2500	0.1538	0.2185	0.1201	0.3040	0.2019	0.2270	0.3706	0.1110	0.0909	0.1972	**0.0001**	0.5410
PEZ08	1.0000	1.0000	-		0.8000	0.6947	0.8767	-0.1610	0.5789	0.6542	0.0664	0.1160	0.7250	0.8538	0.0292	0.1520
FH2088	0.7500	0.5000	0.4408	-0.5560	0.5000	0.5926	0.0910	0.1610	0.6167	0.6566	**0.0002**	0.0610	0.4000	0.4291	0.3051	0.0680
FH2422	0.6250	0.5250	1.0000	-0.2070	0.8571	0.8677	0.3072	0.0130	0.5124	0.6131	**0.0001**	0.1650	0.7571	0.7312	0.8212	-0.0360
FH3313	1.0000	0.7879	0.3192	-0.3040	0.7857	0.9259	0.1016	0.1560	0.7455	0.8271	0.2190	0.0990	0.7313	0.8204	0.0833	0.1090
PEZ06	1.0000	0.7833	0.3812	-0.3020	0.8571	0.8942	0.925	0.0430	0.7083	0.8372	**0.0000**	0.1540	0.6056	0.7119	**0.0000**	0.1500
PEZ19	0.0000	0.0000	-		0.5714	0.7354	0.1527	0.2300	0.5766	0.6927	**0.0008**	0.1680	0.6029	0.6930	**0.0054**	0.1310
PEZ15	0.8750	0.7750	0.4113	-0.1400	0.7857	0.8175	0.7649	0.0400	0.6068	0.7338	**0.0001**	0.1740	0.7324	0.8097	0.0672	0.0960
FH3725	1.0000	0.6917	0.0931	-0.4930	0.7692	0.8338	0.2865	0.0800	0.7815	0.8044	**0.0059**	0.0290	0.7429	0.7951	0.3468	0.0660
Total	0.7266	0.6115	0.1721	-0.2353	0.6655	0.7188	0.0959	0.0814	0.6230	0.6813	0.0000	0.0842	0.6155	0.6710	0.0000	0.0793

*Includes samples collected between 1990 – 2003.

** H_e n.b. denotes that H_e values are calculated with correction for sample size bias (Nei 1978).

*** Significance values for H_e excess or deficiency calculated using the Hardy-Weinberg exact test (Guo and Thompson 1992), and adjusted for false discovery rate (FDR) control (Verhoeven et al. 2005).

I calculated pairwise F_{ST} values for population differentiation using Theta (Weir and Cockerham 1984), and number of migrants between populations (N_m, Wright 1969). Overall values are outlined in Table 2.7, and values for males and females are shown in Tables 2.8 and 2.9, respectively.

Table 2.7. Pairwise F-values (Theta, Weir and Cockerham 1984; upper) and number of migrants (N_m, Wright 1969; lower) between microsatellite allele population clusters in southwestern Manitoba wolves, 2003 - 2005. F_{ST} values in bold, F_{IS} in regular font, and 95% confidence intervals are estimated using 1000 bootstrap replicates.

	Kettle Hills	**Central Manitoba**	**Riding Mountain**	**Duck Mountain**
Kettle Hills* n = 8	--------	**0.131 [0.075-0.181]** -0.014 [-0.069-0.049]	**0.096 [0.048-0.143]** 0.072 [0.036-0.108]	**0.117 [0.075-0.152]** 0.061 [0.011-0.112]
Central Manitoba n = 14	1.66	--------	**0.056 [0.042-0.072]** 0.084 [0.050-0.117]	**0.057 [0.035-0.077]** 0.081 [0.037-0.122]
Riding Mountain n = 123	2.34	1.12	-------	**0.074 [0.048-0.104]** 0.085 [0.052-0.118]
Duck Mountain n = 71	1.88	4.17	3.13	--------

*Values based on very small sample size.

Table 2.8. Pairwise F-values (Theta, Weir and Cockerham 1984) and number of migrants (N_m, Wright 1969) for males between microsatellite allele population clusters in southwestern Manitoba wolves, 2003 – 2005. F_{ST} values in bold, F_{IS} in regular font, and 95% confidence intervals are estimated using 1000 bootstrap replicates.

	Kettle Hills	**Central Manitoba**	**Riding Mountain**	**Duck Mountain**
Kettle Hills* n = 3	--------	**0.099 [0.037-0.159]** -0.047 [-0.098-0.016]	**0.081 [0.018-0.144]** 0.054 [0.010-0.096]	**0.111 [0.048-0.172]** 0.028 [-0.042-0.105]
Central Manitoba n = 9	2.28	--------	**0.063 [0.046-0.082]** 0.049 [0.019-0.084]	**0.080 [0.050-0.117]** 0.023 [-0.026-0.079]
Riding Mountain n = 57	2.84	3.73	-------	**0.084 [0.051-0.123]** 0.054 [0.012-0.096]
Duck Mountain n = 32	2.01	2.87	2.74	---------

* Values based on very small sample size.

Table 2.9. Pairwise F-values (Theta, Weir and Cockerham 1984) and number of migrants (N_m, Wright 1969) for females between microsatellite allele population clusters in southwestern Manitoba wolves, 2003 – 2005. F_{ST} values in bold, F_{IS} in regular font, and 95% confidence intervals are estimated using 1000 bootstrap replicates.

	Kettle Hills	**Central Manitoba**	**Riding Mountain**	**Duck Mountain**
Kettle Hills* n = 5	--------	**0.190 [0.109-0.260]** 0.037 [-0.08-0.152]	**0.102 [0.052-0.144]** 0.086 [0.039-0.142]	**0.130 [0.080-0.169]** 0.086 [0.027-0.161]
Central Manitoba n = 5	1.07	--------	**0.053 [0.032-0.078]** 0.118 [0.065-0.167]	**0.063 [0.029-0.098]** 0.128 [0.063-0.199]
Riding Mountain n = 66	2.20	4.43	-------	**0.067 [0.039-0.093]** 0.112 [0.066-0.159]
Duck Mountain n = 39	1.68	3.75	3.47	---------

* Values based on very small sample size.

Figure 2.8 shows FCA represented by three-dimensional clusters of allele frequencies. The first axis accounts for most of the variation (35.98%), and segregates the RMNP and Duck Mountain clusters. Kettle Hills individuals are placed between these two and Central Manitoba individuals closer to RMNP. Some individuals are not attributed to either cluster and seem distinct from all other wolves.

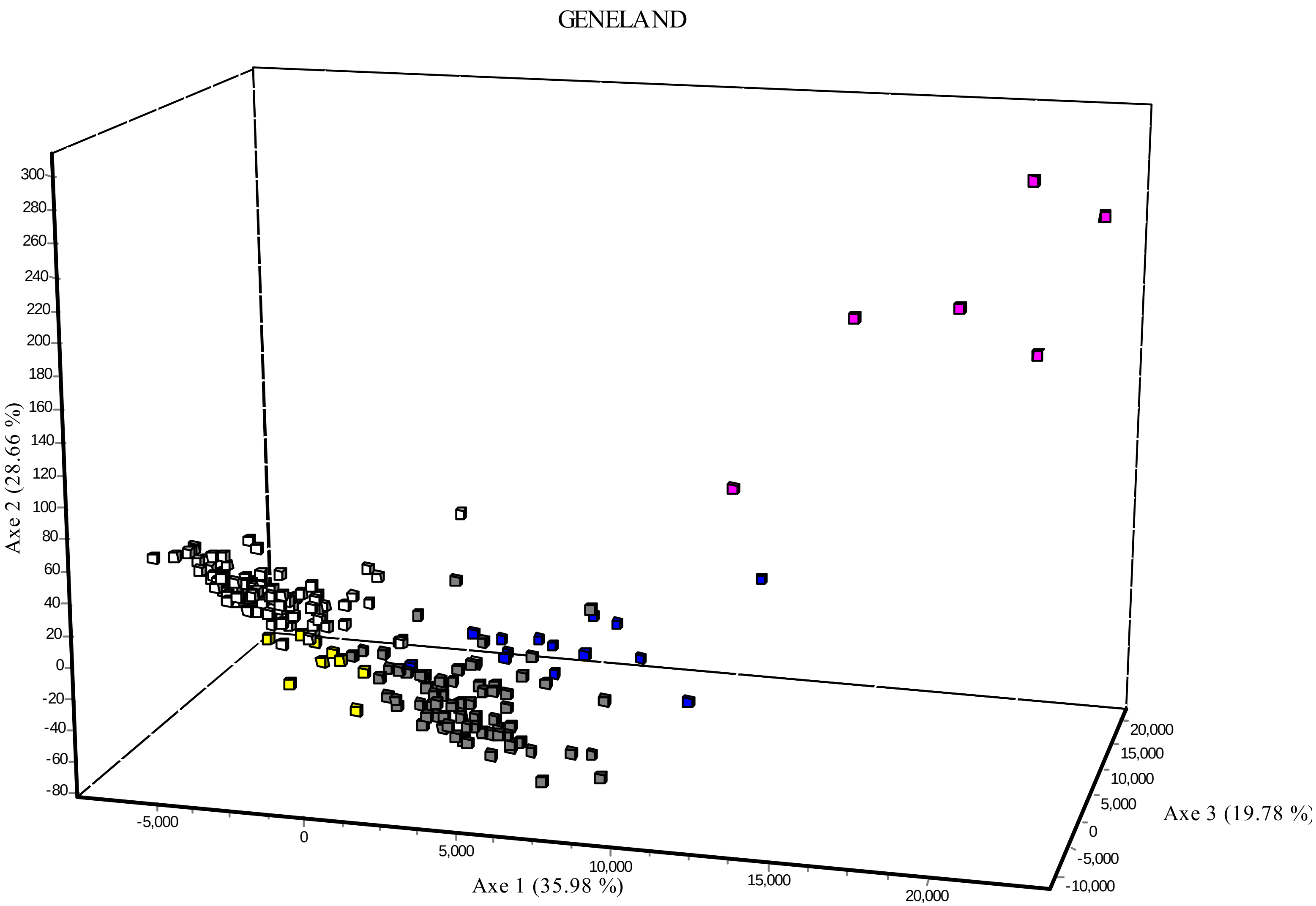

Figure 2.8. Factorial Correspondence Analysis (FCA) of three-dimensional allele frequency structure in southwestern Manitoba wolves, 2003 - 2005. White: Riding Mountain cluster; yellow: Kettle Hills; gray: Duck Mountains; blue: Central Manitoba; pink: wolves not assigned to any cluster. In each cluster, an artificial individual represents the population centre.

The Kettle Hills and Central Manitoba clusters did not have enough data to examine spatial autocorrelation. Results for Riding Mountain (Figure 2.9) and Duck Mountain (Figure 2.10) clusters show initial positive correlation between relatedness and distance, followed by negative correlation starting near 40 km. In the Riding Mountain cluster, the relationship is negative until about 150 km after which it climbs above zero and remains positive. A similar situation is seen in the Duck Mountain cluster. Overall, spatial autocorrelation within each area did not seem significant.

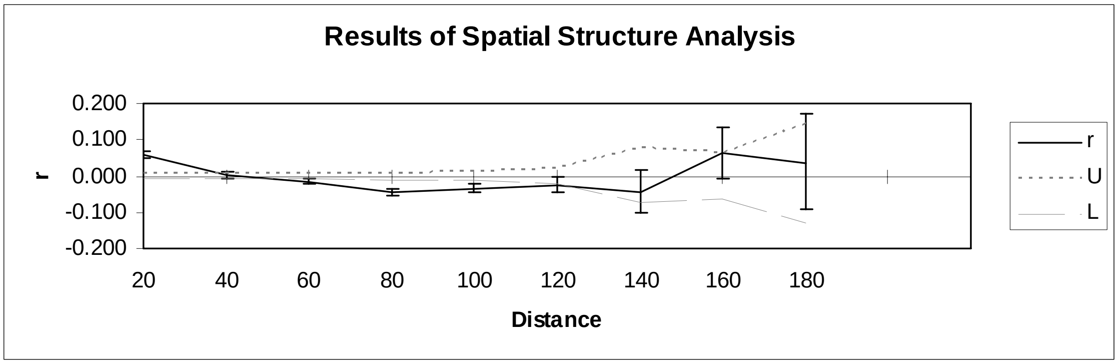

Figure 2.9. Spatial autocorrelation in wolf microsatellite alleles for the Riding Mountain cluster in southwestern Manitoba, 2003 - 2005. The autocorrelation (kinship) coefficient is denoted by r, and distance in kilometers. U and L are upper and lower limits for the 95% confidence interval around the null hypothesis of no spatial structure as determined by 999 permutations, while the upper and lower error bars show the 95% confidence interval about r as determined by 1000 bootstrap replicates.

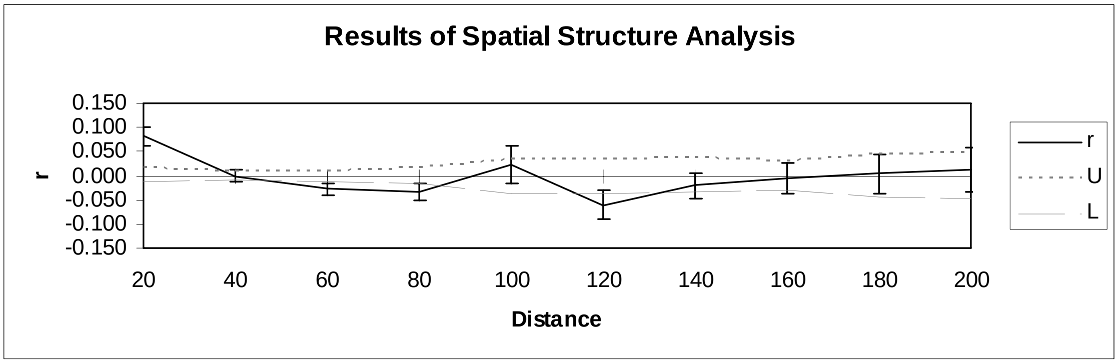

Figure 2.10. Spatial autocorrelation in wolf microsatellite alleles for the Duck Mountain cluster in southwestern Manitoba, 2003 - 2005. The autocorrelation (kinship) coefficient is denoted by r, and distance in kilometers. U and L are upper and lower limits for the 95% confidence interval around the null hypothesis of no spatial structure as determined by 999 permutations, while the upper and lower error bars show the 95% confidence interval about r as determined by 1000 bootstrap replicates.

MtDNA haplotype frequencies and sequence divergence

1) Are mDNA sequences highly divergent within the study area?

I found 13 haplotypes within my study area, and an additional seven from other regions of Manitoba and Saskatchewan (Table 2.10). Of these, four Manitoba and one Saskatchewan variants were extracted from coyotes. Haplotypes C3, C22 and C23 were previously found in Manitoba by Wilson et al. (2000a), and C22 and C23 are found across the prairies. Northern Manitoba have three haplotypes that appear location specific (13, 14, 15) whereas Prince Albert National Park (PA) have two (16, 17). Seven haplotypes (5 – 12) were found only within RMNP, and two (C3, 12) only in the Duck Mountains. Appendix D shows haplotype frequencies found in this study within regions in Manitoba and Saskatchewan. Table 2.11 outlines grouping and frequencies of North American haplotypes examined for sequence divergence.

Table 2.10. Canid mtDNA haplotypes found in Manitoba and Saskatchewan. For sample location information see Table 2.11.

Haplotype	Duck	EMan	NEMan	NWMan	PA	RMNP	Total
C3	2						2
C22			1	5	4		10
C23	2	2		2		13	19
1						1	1
2						1	1
3						1	1
4					1		1
5						1	1
6						1	1
7						1	1
8						1	1
9						1	1
10						1	1
11						1	1
12	1						1
13				1			1
14				1			1
15				1			1
16					18		18
17					2		2

Table 2.11. North American canid mtDNA haplotypes per location, with sample sizes. Samples originating from *Canis latrans* and *C. rufus* are specified, other samples include *C. lupus* and the proposed *C. lycaon.* Haplotypes with C-prefix are from Wilson et al. 2000. C22 was also identified in a *C. lupus* reference sample from Montana provided to P. Paquet, and 18 from a *C. rufus* sample contributed by J. Leonard.

Sample code	Sample description	Haplotype with number of samples	No. of samples
NWMan	Northwestern Manitoba	C22 (5), C23 (2), 13 (1), 14 (1), 15 (1)	10
NEMan	Northeastern Manitoba	C22 (1)	1
EMan	Eastern Manitoba	C23(2)	2
Duck	Duck Mountains, MB	C3 (3), C22 (1), C23 (3), 12 (1)	8
PA	Prince Albert NP, SK	C22 (4), 16 (18), 17 (2)	24
PA latrans	Prince Albert *Canis latrans*	4 (1)	1
RMNP	Riding Mountain NP, MB	C23 (13), 5 (1), 6 (1), 7 (1), 8 (1) 9 (1), 10 (1), 11 (1),	20
RM latrans	Riding Mountain *Canis latrans*	1 (1), 2 (1), 3 (1)	3
Montana	Montana, USA	C22 (1)	1
Rufus	*Canis rufus*	C2 (9), C19 (3), 18 (1)	13
Algonquin	Algonquin Provincial Park, ON	C1 (7), C9 (1), C14 (3), C17 (1), C19 (1)	13
NAlgonquin	North of Algonquin, ON	C1 (1), C16 (1), C23 (1)	3
OH latrans	Ohio, USA *Canis latrans*	C5 (1)	1
TX latrans	Texas, USA *Canis latrans*	C4 (1), C6 (2), C7 (1), C8 (1), C10 (1), C11 (1),C12 (1), C15 (1), C18 (2), C19 (12), C20 (2), C21 (2)	27
NWT	North West Territories	C23 (1)	1
N QC	Northern Quebec	C23 (1)	1
NW ON	Northwest Ontario	C13 (2), C24 (1)	3
Nbor ON	Northern boreal Ontario	C23 (1)	1
FF ON	Fort Francis, Ontario	C23 (1)	1
S ON	Southern Ontario	C1 (1), C9 (1), C14 (2), C19 (4)	8

Haplotypes from northern and eastern Manitoba, RMNP, and Prince Albert generally cluster together and close to European gray wolf haplotypes in a neighbour-joining tree (Figure 2.11). Two northern Manitoba haplotypes (14, 15) and one from RMNP (11) are close to C22, whereas 6, 7, 8, 9 and 10 from RMNP are near C23, although haplotype 6 is divergent from the rest. The two Prince Albert variants (16, 17) and 13 from northern Manitoba group nearest to the European haplotypes. Coyote variant 3 from RMNP is most similar to C9 from Algonquin Provincial Park and southern Ontario, and C8 from Texas coyotes. RMNP coyote variant 1 is most similar to C18 and C19 from Texas coyotes and red wolves, and coyote variant 2 groups with coyote haplotype 4 from

Prince Albert although the two are quite divergent. The red wolf haplotypes 18 and C2 cluster together.

In the Duck Mountains, C3 and 12 group together and near C1 from the Algonquin Provincial Park area and southern Ontario. Wilson et al. (2000a) found C3 only in Manitoba, and haplotype 12 differes from C3 by 2 basepairs. The remaining RMNP haplotype (5) was found in a faecal sample from within RMNP, and is located closer to eastern wolves, red wolves, and coyotes in the neighbour-joining tree than to other RMNP variants. However, nuclear DNA from this sample identified the individual as a RMNP wolf. The TCS gene genealogies with gaps included (Figure 2.12) and excluded (Figure 2.13) further supports a distinction between gray wolf haplotypes and all others. In Figure 13 without gaps there are fewer haplotypes, as 7 is here identical to 10 and C23, 11 with C22, 16 with 17, 1 with C19 and C21, 18 with C2, and C12 with C11. Although gap removal changes the genealogy, both clearly distinguish gray wolf haplotypes from all others, and indicate that Duck Mountain (C3, 12) and Algonquin area (C1) haplotypes are closest to gray wolves. In Figure 12, haplotype 5 from RMNP is closest to 1 from a RMNP coyote and C13 from northwest Ontario wolves. With gaps removed, haplotype 5 is closest to 18 from red wolves and again to C13 (Figure 2.13).

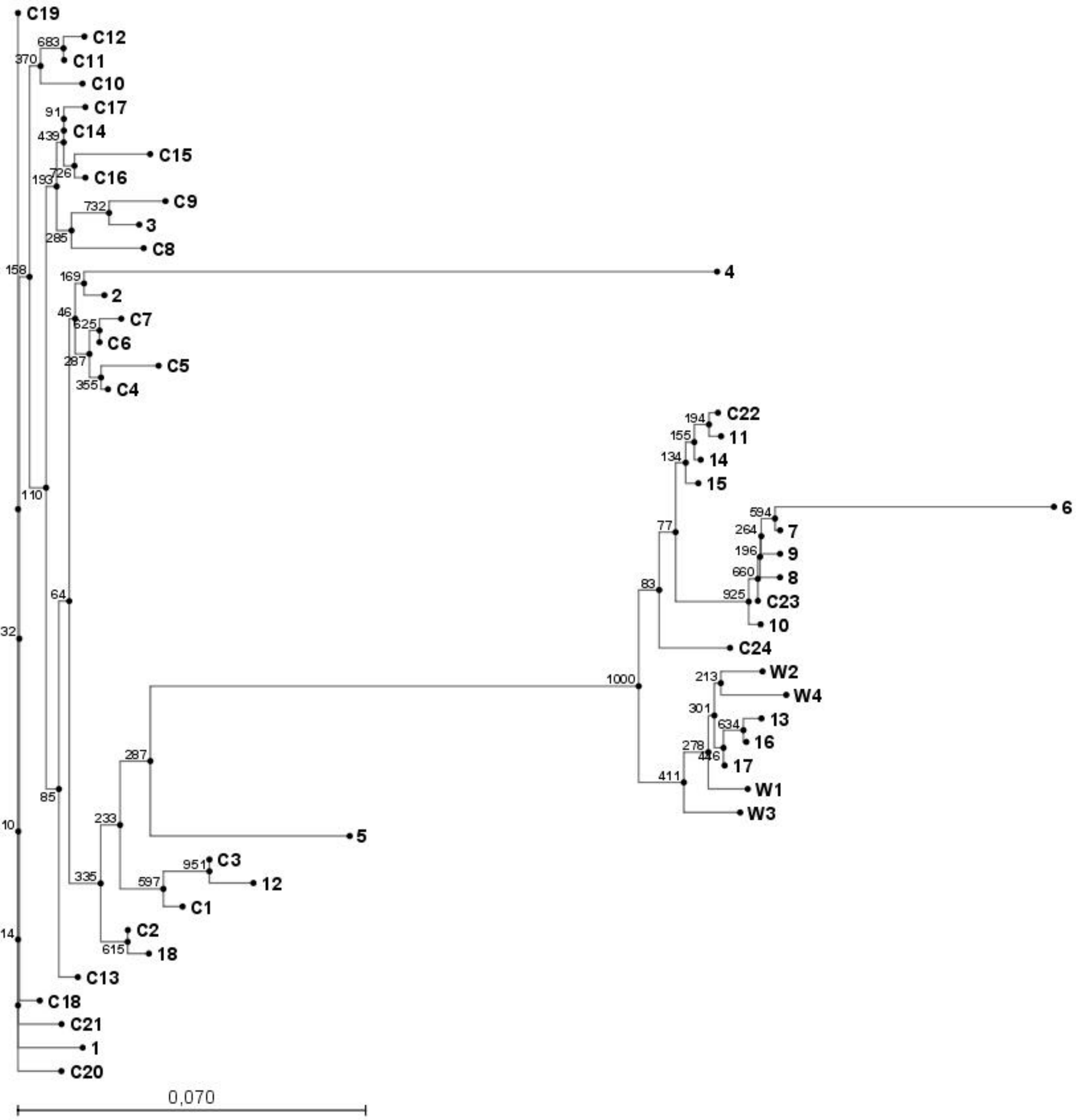

Figure 2.11. Neigbour-joining tree of sequence divergence for 239bp of the mtDNA control region for *Canis latrans, C. lupus, C. rufus* and the proposed *C. lycaon*. Bootstrap values show support for nodes based on 1000 bootstrap trees (replicates). Haplotype details are listed in Table 2.11. European *C. lupus* haplotypes W1 – W4 from Ellegren et al. (1996). Starting from the top, haplotypes C19 – C4 represents *C. latrans*, the branch with haplotypes C22 – W3 represents *C. lupus*, and haplotypes 5- C20 represents *C. latrans, C. rufus* and the proposed *C. lycaon*.

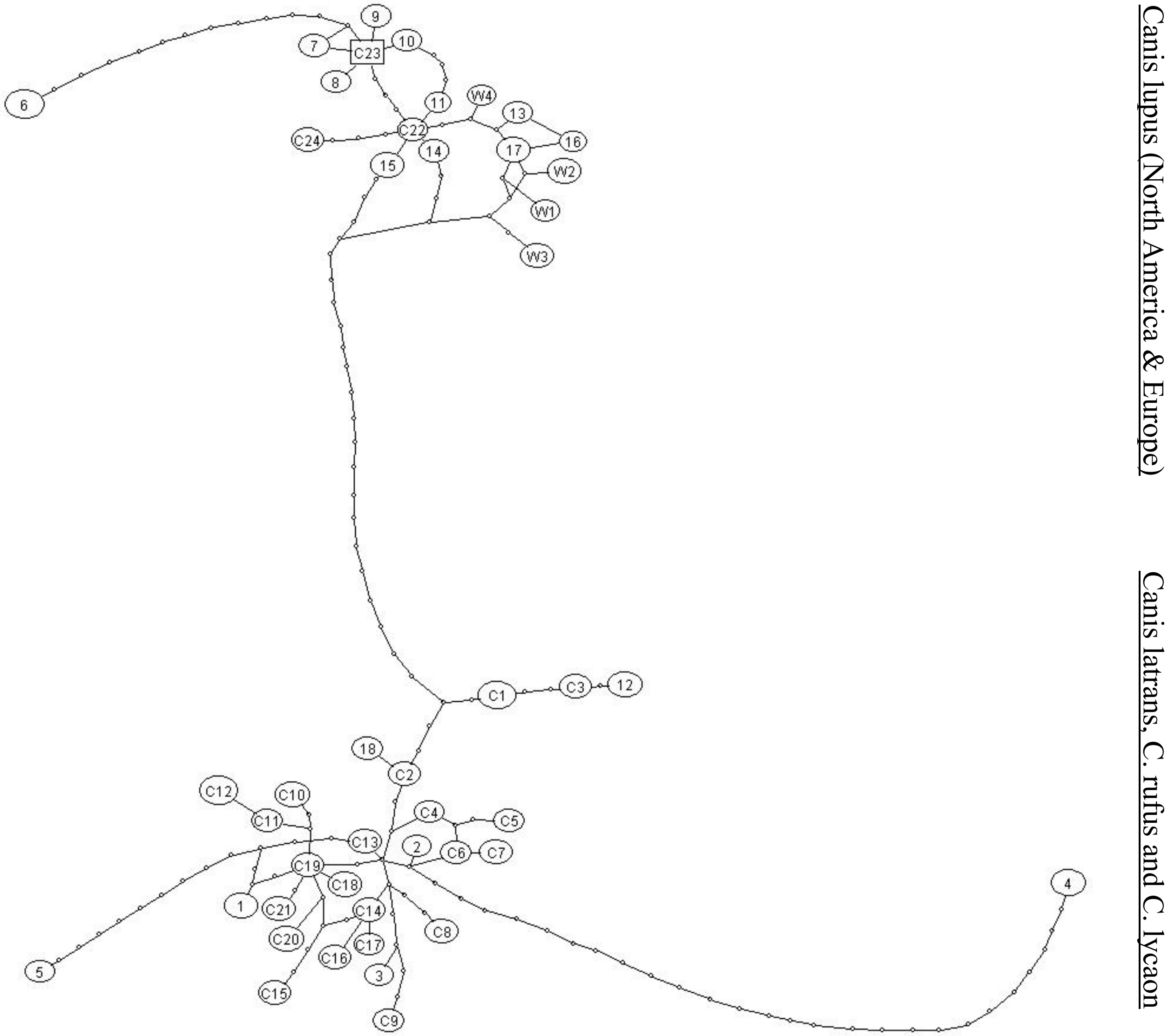

Figure 2.12. Gene genealogy including haplotype gaps for 239bp of the mtDNA control region for *Canis lupus* (top half of figure) and *C. latrans*, *C. rufus* and the proposed *C. lycaon* (bottom half of figure). Haplotype details are listed in Table 2.11. European *C. lupus* haplotypes W1 – W4 from Ellegren et al. (1996).

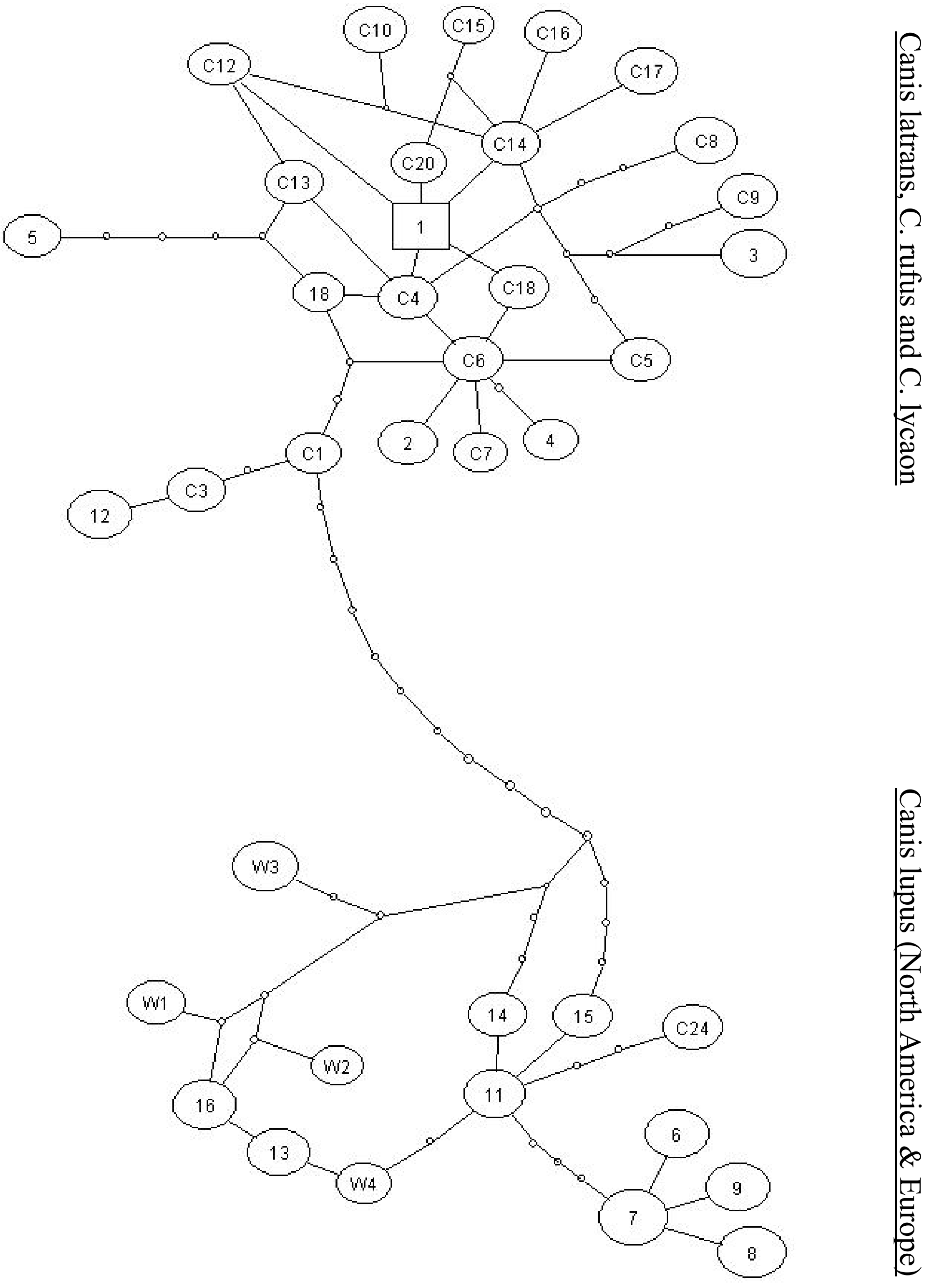

Figure 2.13. Gene genealogy with haplotype gaps removed for 239bp of the mtDNA control region for *Canis latrans, C. rufus* and the proposed *C. lycaon* (top half of figure) and *C. lupus* (bottom half of figure). Haplotype details are listed in Table 2.11. European *C. lupus* haplotypes W1 – W4 from Ellegren et al. (1996).

2) How is mtDNA divergence distributed spatially?

Gene flow and population genetic structure inferred from mtDNA could be strongly affected by uneven distribution of haplotypes in my available samples. However, the results indicate that higher-order groupings may to some extent be based on geographical proximity (Appendix E). SAMOVA results suggest similar groups (Appendix F).

Wolf Skull Morphology

1) Is there evidence of canids with skull features intermediate between wolf and coyote?

Male wolves are generally larger in linear skull dimensions (Jolicoeur 1959, Kolenosky and Stanfield 1975, Table 2.12) and because of the differences in size I did separate factor analyses for males (n = 19) and females (n = 13).

Table 2.12. Mean values and standard deviations for wolf skull measures in southwestern Manitoba. All measures are in millimeters. Measurement details are provided in the Methods.

Measure	**Duck males (n = 7)**	**RMNP males (n = 10)**	**Mean males with st.dev. (n = 19)***	**Duck females (n = 8**	**RMNP females (n = 4)**	**Mean females with st.dev. (n = 13)***
1.length	255.43	261.20	259.8 (12.2)	255.50	243.50	251.3 (11.5)
2. zyg. width	137.43	138.70	139.2 (8.6)	138.37	134.55	136.7 (4.5)
3. P1 - M2	85.35	88.02	87.1 (3.3)	84.94	81.44	83.5 (4.1)
4. cheek	79.66	82.65	81.6 (3.7)	80.18	79.74	79.7 (3.4)
5. p. width	32.22	32.36	32.6 (2.4)	32.52	31.43	32.1 (1.8)
6. fr shield	63.14	63.04	63.4 (7.1)	62.16	63.89	62.5 (3.8)
7. M1-orbit	39.02	40.56	40.2 (2.2)	39.61	37.09	38.7 (2.2)
8. jugal	19.36	19.16	19.5 (1.7)	19.33	18.06	18.8 (1.3)
9. carnas.	25.14	26.17	25.9 (1.2)	24.99	23.99	24.7 (1.3)
10. M2	13.64	14.01	14.0 (0.9)	13.76	13.21	13.6 (0.5)
11. a-a	66.45	65.39	66.1 (2.9)	66.84	63.24	65.8 (2.3)
12. b-b	68.36	68.09	68.3 (4.1)	67.27	63.50	65.9 (4.1)
13. g-g	41.63	39.40	40.7 (3.2)	40.65	39.40	40.6 (3.7)

*The Porcupine Mountain female and two males are included in the study area mean values.

<u>Male wolves</u>

Factor loadings for each variable (Table 2.13) show partial correlation after rotation between each male skull measure and the three factors extracted as principal components. The results indicate that the first principal factor (PC1) was associated with skull size in males, and accounted for 42.5 % of the variance. In males, high PC1 scores were positively correlated with skull length and width, as well as cheek teeth and palate width, and distance between eye orbit and 1st molar and between the auditory bullae and the 2nd molar.

Table 2.13. Partial correlation between skull measures for male wolves from southwestern Manitoba and each of the rotated factors.

Measure	**Factor (principal component)**		
	1	2	3
1.length	0.804	0.495	0.047
2. zyg width	0.805	0.229	0.322
3. P1 - M2	0.246	0.785	-0.010
4. cheek	0.788	0.451	-0.184
5. p width	0.784	0.068	0.161
6. fr shield	0.639	0.029	0.615
7. M1-orbit	0.872	0.316	0.134
8. jugal	0.733	0.239	0.367
9. carnas.	0.284	0.874	-0.154
10. M2	-0.084	0.705	0.615
11. a-a	0.544	0.241	0.433
12. b-b	0.907	-0.028	0.011
13.g-g	0.139	-0.211	0.921
Eigenvalue after rotation	5.522	2.644	2.136
Percent of variance	42.5	20.3	16.4

Rotation Method: Varimax with Kaiser Normalization, rotation converged in 10 iterations.

The second principal factor (PC2) appeared primarily correlated with dentition. High PC2 values were positively correlated with upper carnassial teeth (P^4) length, second molar (M^2) width, and distances between the first premolar and the second molar (P^1 – M^2). The third principal factor (PC3) appeared associated with skull shape, and correlated strongly with variation in width of postorbital constriction (g – g; generally described as minimum braincase width behind the eye sockets). However, there was no clear relationship between braincase width and PC3 values, and skull shape appeared similar between latitude zones. Skull size (factor 1) appeared similar between males from different areas (Figure 2.14). Most Duck Mountain males had lower values for dentition (upper carnassial teeth (P^4) length, second molar (M^2) width) than Riding Mountain and Porcupine Mountain males, but 95% confidence intervals overlapped (Figure 2.15).

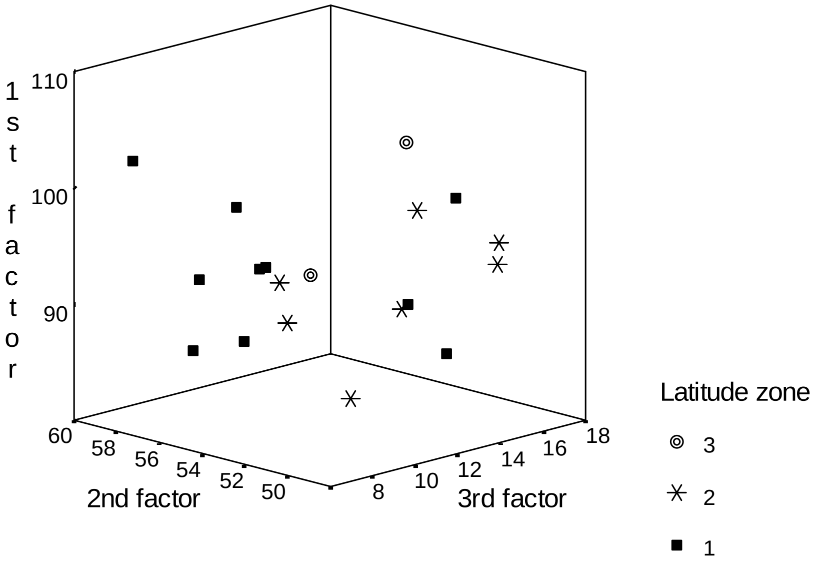

Figure 2.14. Male wolf scores for factors 1, 2, and 3 by latitude zone. Factor 1 generally correlated with skull size, 2 with dentition (P^4 and M^2 size), and 3 with skull shape. Zone 1 is Riding Mountain, zone 2 Duck Mountain, and zone 3 Porcupine Mountain.

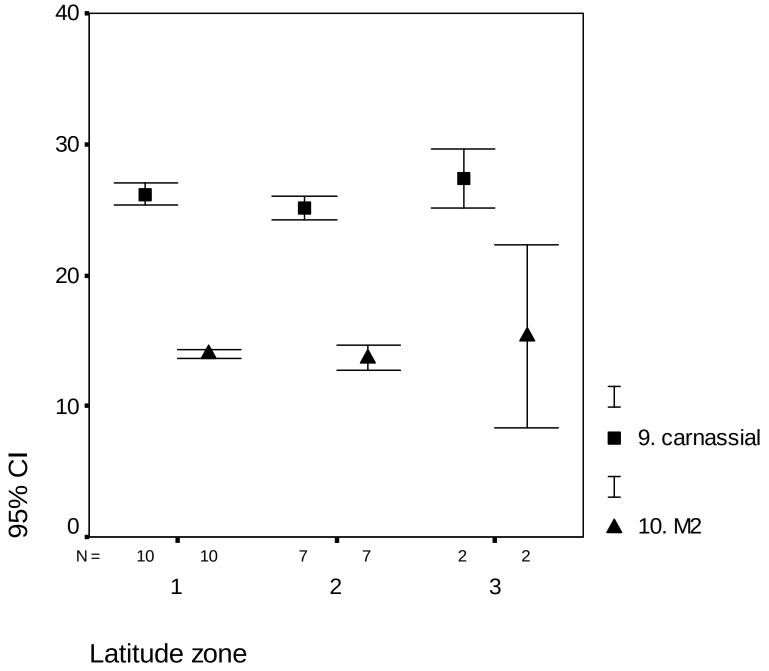

Figure 2.15. Mean value and 95% confidence interval for size of carnassial (P^4) and second molar (M^2) in male wolves. Zone 1 is Riding Mountain, zone 2 Duck Mountain, and zone 3 Porcupine Mountain.

Female wolves

Results for female wolves suggested that as with males, the first factor (PC1) was associated with skull size, and accounted for 45.8 % of the variance (Table 2.14). In females, high PC1 scores were positively associated with skull length and width, and distance between eye orbit and 1st molar and between auditory bullae and 2nd molar. Female PC1 scores were also positively associated with cheek width (distance between P^4), frontal shield width, and jugal width. In addition, I found a positive correlation with with $P^1 – M^2$ distance, which correlated highly with PC2 for males. As with males, the second principal factor (PC2) appeared primarily correlated with dentition, and high PC2 values were associated with P^4 length and M^2 width. In females, PC2 was also correlated with palatal width (distance between P^1).

Table 2.14. Partial correlation between skull measures for female wolves from southwestern Manitoba and each of the rotated factors.

Measure	**Factor (principal component)**		
	1	2	3
1.length	0.781	0.365	0.370
2. zyg width	0.881	0.129	0.372
3. P1 - M2	0.849	0.299	0.267
4. cheek	0.860	0.179	-0.257
5. p width	0.502	0.719	0.022
6. fr shield	0.821	-0.126	-0.072
7. M1-orbit	0.823	0.063	0.464
8. jugal	0.814	0.032	0.281
9. carnas.	0.225	0.890	0.013
10. M2	-0.276	0.767	0.316
11. a-a	0.063	0.098	0.890
12. b-b	0.739	0.372	0.330
13.g-g	0.405	0.147	0.598
Eigenvalue after rotation	5.953	2.359	2.070
Percent of variance	45.8	18.1	15.9

Rotation Method: Varimax with Kaiser Normalization, rotation converged in 10 iterations.

As in males, factor PC3 appeared associated with skull shape, but in females it seemed primarily correlated with braincase width (a-a) rather than postorbital constriction. Only four samples were available from Riding Mountain and these generally had smaller values for skull size than Duck Mountain females (Figure 2.16). Whereas Duck Mountain males generally had lower dentition values (PC2) than Riding Mountains males, values were opposite for females. Most Duck Mountain females had higher values for dentition (upper carnassial teeth (P^4) length, second molar (M^2) width) than Riding Mountain females, but 95% confidence intervals overlapped (Figure 2.17). Duck Mountain females generally showed higher values for PC3 and the one Porcupine Mountain skull also scored high on this value.

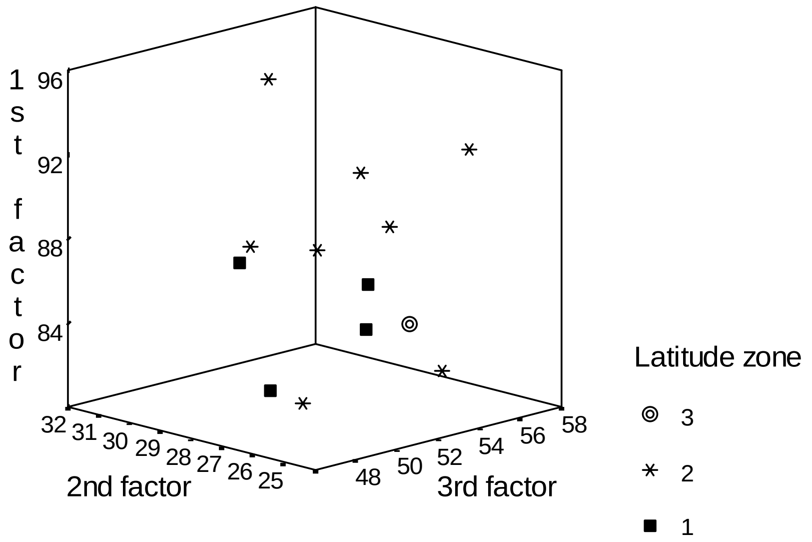

Figure 2.16. Female wolf scores for factors 1, 2, and 3 by latitude zone. Factor 1 generally correlated with skull size, 2 with dentition (P^4 and M^2 size), 3 with skull shape. Zone 1 is Riding Mountain, zone 2 Duck Mountain, and zone 3 Porcupine Mountain.

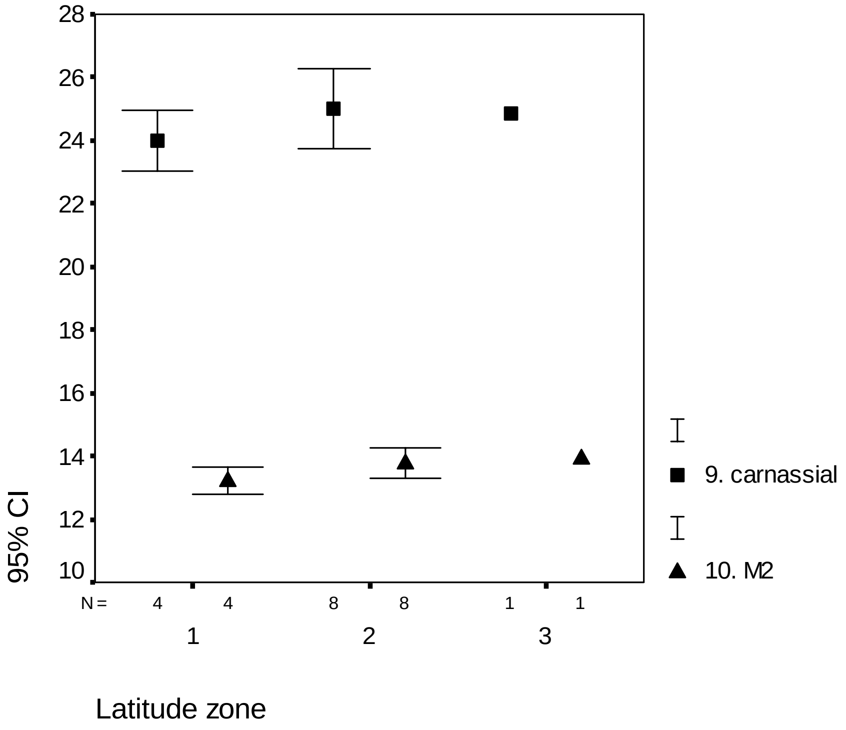

Figure 2.17. Mean value and 95% confidence interval for carnassial tooth (P^4) and second molar (M^2) in female wolves. Zone 1 is Riding Mountain, zone 2 Duck Mountain, and zone 3 Porcupine Mountain.

Discussion

Nuclear DNA extraction and individual identification

Most markers amplified successfully, and although errors were calculated from a small subset of re-tests, I consider errors to be random throughout. As expected, error rates and allelic diversity were generally positively correlated. When identifying matching samples from faecal material, I balanced the risk of counting an individual multiple times with that of combining several related (or wrongly genotyped) animals as one. The two known duplicate samples provided a limited test, but suggest the criteria of >75% allele sharing was sufficient. The identification of 221 individuals seems plausible considering the area and timespan over which samples were collected. Identification of forty-four percent as males is also a credible value. Although my sampling success varied throughout the study area, I believe my sample of individual wolves is representative for the years 2003 – 2005.

Gene flow and population genetic structure inferred from nuclear DNA

Overall microsatellite diversity

Levels of observed heterozygosity were lower than expected for all loci except one. Although comparisons may be difficult between studies using different markers (Ellegren et al. 1996), heterozygosity values appear similar to other North American wolf populations and allelic diversity seems relatively high[3] (Forbes and Boyd 1997, Thiessen 2007, Urton 2004, Carmichael 2006). The positive F_{IS} values, and Hardy

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[3] Excluding the Kettle Hill sample, which is likely composed of one or two packs.

Weinberg and linkage disequilibrium at many loci, are likely due to grouping of units that do not constitute a panmictic population (Frankham et al. 2002). Similar situations are reported from other wolf studies, and may be due to null alleles, inbreeding, Wahlund effect (additional undetected structure), non-random sampling, and presence of closely related individuals (Roy et al. 1994, Pilot et al. 2006, Thiessen 2007). I cannot rule out any of these factors but believe sample size is sufficient and representative for the study area. Only two markers are reported to be on the same chromosome (FH2017 and FH2088 on CFA15), so physical linkage is unlikely to have contributed significantly to the observed linkage disequilibrium values.

Population subdivision

Results are consistent with subtle genetic structure within the study area, and support division into at least three clusters. Moreover, genetic structure corresponds with fragmentation, as observed clusters are separated by a landscape matrix dominated by intensive agricultural development and a dense road network. As noted, violation of test assumptions may have caused “ghost” populations with no individuals assigned. Moreover, some individuals may have originated from areas not sampled for this study, such as the relatively undeveloped regions of central Manitoba. The three-dimensional FCA results indicated that some individuals have quite divergent genotypes and, thus, suggest presence of immigrants from outside the study area. Few samples were available from the areas surrounding RMNP and the Duck Mountains, and it is also possible that additional undetected structure exists within southwestern Manitoba.

The population divergence between the RMNP and Duck Mountain clusters (F_{ST} = 0.074, N_m = 3.13) is within the range (F_{ST} = 0.05 – 0.15) generally considered as moderate population divergence (Balloux and Lugon-Moulin 2002). A wolf study in the Canadian Rocky Mountains, using the same suite of markers with only a few exceptions, found smaller divergence values between populations separated by larger geographic distances (Thiessen 2007). Whereas biological interpretation of F_{ST} values is difficult (Balloux and Lugon-Moulin 2002), the RMNP – Duck Mountain divergence appears relatively high compared with the distances and geography separating the populations examined by Thiessen (2007).

The high number of loci pairs in linkage disequilibrium, positive F_{IS} values and heterozygosity deficit within clusters could again be due to the factors mentioned above. Similar findings within population clusters are reported from other wolf studies (Lucchini et al. 2002, Pilot et al. 2006, Thiessen 2007). A Wahlund effect would not contradict the observed clusters, but simply suggest presence of additional structure (Pilot et al. 2006). Testing within genetic clusters reduced the number of loci with possible null alleles and, with the exception of PEZ06, loci also differed between clusters. The presence of related individuals and null alleles also seems unlikely significantly to influence GENELAND results (Coulon et al. 2006).

Wolf numbers in RMNP may have declined to around 30 during the mid 1990s (RMNP unpub. data) which could have caused genetic drift. Recent landscape change and human-caused mortality could have further isolated the population (Fritts and Carbyn 1995, Parks Canada 2002). A founder effect in RMNP following a local extirpation (Carbyn 1980) or bottleneck could also result in significant structure in highly variable loci (Hedrick 1999), particularly if little gene flow occurred afterward. Bottlenecks are expected to create a signature of higher than expected heterozygosity while heterozygosity values adjust to reflect loss in allelic diversity (Luikart and Cornuet

1998). However, more than a decade (and 4 - 5 wolf generations) has passed since the putative bottleneck and effects may no longer be detectable. Furthermore, distinguishing relative contributions of bottlenecks and inbreeding toward loss of heterozygosity can be difficult (Eppley et al. 2007), and genetic drift could operate both in space and time for an isolated population and potentially create a Wahlund effect in both dimensions (Flagstad et al. 2003).

Spatial autocorrelation across loci suggests kinship is initially positively associated with distance, which can be expected in populations composed of territorial family groups. Aspi et al. (2006) found kinship positively correlated with distances up to 163 km, followed by significant isolation by distance on a limited spatial scale in a continuous Finish wolf population. A negative relationship between kinship and geographical distance occurred below 60 km in my study area. Sample sizes are relatively small and unevenly distributed with relatively large gaps, which make interpretation difficult. However, the overall results do not support significant spatial autocorrelation within the study area.

All individuals identified by nuclear alleles as putative immigrants to RMNP were found near the Park edge, and might not yet have established territories or been able to join a pack. We have field data from one identified immigrant, a male in good body condition found dead on the southern Park boundary. Necropsy revealed that he was killed by other wolves (T. Bollinger pers. comm.). Successful dispersal does not guarantee reproduction (Greenwood 1980, Riley et al. 2006), even where individuals succeed in joining new packs (Grewal et al. 2004). Consequently, territoriality and a social structure with low effective population size could present additional obstacles to gene flow, as relatively few individuals will be permitted to reproduce. This is a long-term concern for isolated protected areas where relatively few dispersers successfully negotiate the surrounding matrix. Moreover, kin-clustering favouring nepotistic interactions and subsequent local recruitment in birds (Watson et al. 1994, Temple et al. 2006) and mammals (Lambin and Yoccoz 1998) could influence dispersal in fragmented landscapes. I explore this further in Chapter 3. My overall findings support the matrix landscape acting as a filter to gene flow in the study area. Long-term monitoring is needed to establish whether differentiation between genetic clusters separated by the matrix landscape may be increasing.

MtDNA haplotype frequencies and sequence divergence

Most wolf haplotypes grouped with other North American and European gray wolves; of these, only haplotype 6 appeared markedly different from those identified in other studies. Haplotypes 6 and 8 were found in two wolves from a pack killed between RMNP and the Duck Mountains due to suspected livestock predation. This pack appears to have resided between the two areas and relatively close to the RMNP boundary. With the exception of haplotype 5, all RMNP samples support grouping the Park's wolf population with gray wolves and the high bootstrap value suggests the division between gray wolf and all other canid haplotypes is distinct. Uneven sample sizes for the various regions make it difficult to compare variation in haplotypes (see Pilot et al. 2006). However, RMNP and Prince Albert National Park are represented with 20 and 24 samples, respectively, and suggest more variation might be present in RMNP.

Several explanations are plausible for the individual with a RMNP wolf microsatellite signature and a haplotype (5) close to coyotes, eastern and red wolves.

The faecal sample was collected on a hiking trail in an area used by wolves, coyotes and dogs. However, a dog or coyote probably would have been identified as an immigrant to RMNP according to microsatellite alleles (Thiessen 2007). An offspring from a coyote female – wolf male breeding, the most likely route of introgression (Roy et al. 1994), could have crossed back into the wolf population. Though apparently rare, paternal mtDNA has also been reported in vertebrates (Avise 1991, Bromham et al. 2003). Moreover, paternally derived mtDNA has been found in cross-species hybrids, raising questions on whether development processes within the egg that normally destroy sperm mitochondria might possibly break down in hybrids (Bromham et al. 2003). Haplotype 5 was found in a faecal sample and it is therefore not possible to gather further information about the animal. Although inconclusive, haplotype 5 may represent the first known eastern wolf haplotype found within RMNP. Nonetheless, if eastern wolves do occur within the Park, they seem to be rare.

The finding of haplotype C3 in the Duck Mountains, as well as the closely related 12, supports Wilson et al. (2000) in extending eastern type wolves through southern Manitoba. The positions of C3 and 12 between haplotypes considered derived from gray wolves and those of red wolves and coyotes also seem consistent with their suggested origin for eastern wolves. A proposed taxonomy is that the eastern wolf and the red wolf *C. rufus* be considered one species, *C. lycaon*, which evolved on the North American continent independently of the gray wolf and shared an evolutionary lineage with the coyote *C. latrans* until 150 000 – 300 000 years ago (Wilson et al. 2000). The suggested distribution of eastern wolves currently includes Minnesota, Manitoba and northwestern Ontario (Wilson et al. 2000, Grewal 2001, Leonard and Wayne 2007). Information from intermediate haplotypes such as C3 and 12 may be combined with further understanding of wolf movement, ecology, and behaviour to clarify the coexistence in time and space of apparently different types of wolves. I recommend additional research on whether a relationship exists between degree of human-caused landscape fragmentation and the occurrence of different wolf types, and to what extent these may differ in their interaction with coyotes.

Gene flow and population genetic structure inferred from mtDNA

My findings are consistent with those of Wilson et al. (2000) and Grewal (2001) in suggesting that gene flow has occurred between northcentral Ontario and southern Manitoba. Furthermore, an eastern-derived haplotype (C3) occurs in Manitoba (Wilson et al. 2000, this study) and Abitibi-Temiscamingue in western Québec (Grewal et al. 2004), while a gray haplotype (C22) is found throughout Manitoba (this study), in the Northwest Territories and in northern Ontario and Québec (Wilson et al. 2000, Grewal et al. 2004). This suggests a wide genetic cline (Mayr 1970) extending from southwestern Manitoba to western Québec. This zone might be larger and could include more of the Great Lakes region (Grewal 2001, Leonard and Wayne 2007). My results, however, do not support the presence of eastern haplotypes in Saskatchewan. Despite sharing haplotype C23, the overall differences between RMNP and Duck Mountain haplotypes appear consistent with the finding of nuclear genetic structure between the two areas.

The major influences on wolf movement and distribution are thought to be glaciations and human-caused extirpation (Mayr 1970, Nowak 1983, Hewitt 2000), and the largest genetic distances found within species tend to be between regions believed to have acted as glacial refugia (Nichols 2001). If an eastern wolf evolved in North America (Wilson et al. 2000), it could be extending its range westward whilst the gray wolf, believed to be an immigrant from Eurasia (Kurtén and Anderson 1980), expanded eastward. Hence, an increasing zone of overlap might be a consequence of natural dispersal and mixture following retreat of the most recent ice sheets (Nowak 1983, Hewitt 1988, Kyle et al. 2006). Hybrid zones can be several hundred kilometers wide depending on dispersal range and time since contact (Hewitt 1988). The overlap between gray and eastern wolf haplotypes (Wilson et al. 2000, Grewal 2001, 2004, this study) seems consistent with wolf dispersal ability and could help explain the many revisions of wolf subspecies boundaries in the region (Brewster and Fritts 1995, Nowak 1995). Accordingly, the canids found between Manitoba and Québec might represent an evolutionary work-in-progress.

Wolf skull morphology

The results from the available skull samples do not support obvious divergence in skull shape or size between RMNP and the Duck Mountains, or wolf – coyote hybridization. Samples of Duck Mountain males had a slightly narrower nose (palatal and cheek teeth width) and narrower second upper molars than for RMNP. However, the difference is small and the opposite relationship was seen in females. Nowak (1995) found male Algonquin *lycaon* to have narrower palates than male wolves grouped with Western Ontario *lycaon* and *hudsonicus* (including northern Manitoba) but Algonquin wolves also had wider upper second molars. Previous comparison of Manitoba (Jolicoeur 1959) and RMNP (Skeel and Carbyn 1977) material with other regions of North America do not suggest distinct separation of groups. Skeel and Carbyn (1977) noted geographical trends suggesting incomplete panmixus, and found that skull size accounted for most of the variation between groups with boreal-subalpine individuals being the largest. They propose that differences between groups could be related to environmental variables such as prey composition and major vegetation and topographical features. Jolicoeur (1959) found a southwest to northeast cline where wolf skulls become shorter but broader.

It is uncertain how long eastern-type wolves have occurred in the Duck Mountains. Furthermore, it is not clear whether wolves carrying eastern-type mtDNA consistently have smaller skull features than wolves carrying gray-type mtDNA. Wolves in Mauricie National Park in Québec are considered eastern-type wolves (Villemure 2003). He reports that these wolves prey primarily on moose and that average weight of three collared males was 44.5 (+ 4.5) kg, whereas average weight of four females was 28.2 (+ 1.6) kg. In comparison, average weight of six collared RMNP males was 38.6 (+ 3.3) kg, and average weight of nine females was 35.5 (+ 3.6) kg. Preliminary data suggest that inbreeding may lead to reduced size in wolves (Fredrickson and Hedrick 2002). Further isolation of RMNP and subsequent inbreeding could therefore possibly result in smaller wolves. If eastern wolves and coyotes are more liable to interbreed due to similar size (Kolenosky and Stanfield 1975, Wilson et al. 2000), smaller RMNP wolves might be more likely to hybridize with other types of canids in and around the Park. We need a better understanding of coyote – wolf hybridization and on the extent of interbreeding between eastern and gray type wolves (Wilson et al. 2000, Grewal 2001, Wayne and Vilà 2003). Considering the size and diet of eastern wolves reported from Québec (Villemure 2003), similar canids might occur at the (reported) western edge of the *C. lycaon* range. Further research is needed where the three canid types overlap to examine whether human-caused fragmentation might be reducing niche diversity and divergent selection, hence promoting hybridization.

Overall assessment and the role of human-caused landscape fragmentation

Microsatellite genetic structure is evident between RMNP and the Duck Mountains and consistent with human-caused landscape fragmentation. Considering the history of landscape change in the RMNP region, my results suggest that a reduction in gene flow sufficient to generate distinct genetic clusters can occur quickly. Microsatellite allele frequencies might differ between eastern and gray type wolves, although Grewal (2001) found no correlation between mtDNA haplotypes and nuclear allele frequencies in Ontario. If divergent *C. lupus – C. lycaon* allele frequencies contribute to structure in my study area, the ultimate question still remains as to why eastern wolves are not (effectively) dispersing into RMNP.

Dispersal can be countered by divergent selection pressures between areas of close proximity, resulting in low effective gene flow (Frankham et al. 2002). Moose, a boreal forest species poorly adapted to and rarely reported from the matrix (Brook 2007), show heterozygote deficiency at neutral loci in RMNP (Wilson et al. 2003). However, wolf range (Kurtén and Anderson 1980), mobility (Mech and Boitani 2003), and diet breadth (Haight et al. 1998, Weaver et al. 1996) suggest genetic differentiation from behavioural ecology and selection alone to be unlikely within my study area. The observed haplotype differences between my study area and Prince Albert National Park, both located near the border of the Prairie and Boreal Plains ecozones (Environment Canada 1993), could be due to several reasons. Small and unrepresentative sample sizes (Cronin 1993), low gene flow following large-scale human extermination (Leonard et al. 2005), subsequent landscape fragmentation, and the possibility that eastern wolves are moving west but have yet to reach the Prince Albert region, might all be contributing factors. Nevertheless, wolf dispersal in biophysically similar landscapes (reviewed above) suggests that eastern-type DNA (mitochondrial and nuclear) should have reached RMNP soon after arrival in the Duck Mountains. I thus consider the mtDNA haplotype distribution an additional indication of cryptic genetic population structure due to human-induced landscape fragmentation. Dispersal from a patch is also expected to be influenced by local population dynamics (Kramer-Schadt et al. 2004). A combination of disease mortality within RMNP (Carbyn 1982a) and human-caused mortality in territories spanning the Park border (Carbyn 1980) could, to some extent, balance recruitment in RMNP wolves. In addition, yearly harvest removes an unknown number of Duck Mountain wolves and together with disease might balance recruitment in this area. Aside from any filters the landscape might impose, low dispersal and gene flow could, at least in part, be due to low recruitment and thus limited resource competition (Lidicker 1975). I explore this further in Chapter 4.

Overall, other processes known to cause genetic structure (mutation and selection) are not believed to have contributed significantly to the observed population differentiation in my study organism at this spatiotemporal scale. Ecological or behavioural factors, (including prey distribution and natal habitat-biased dispersal) or localized selection influenced by diseases such as bovine tuberculosis (Bovine TB, *Mycobacterium bovis*), might now influence gene flow. However, such potential influences are unlikely to have become established without prior fragmentation. Hence, human-caused fragmentation of a physically simple landscape can cause cryptic genetic population structure in vagile organisms on a fine spatiotemporal scale. Predicted effects of human landscape modifications (e.g. residential and agricultural development) on fine spatiotemporal scales can be quantified and incorporated into both theory and conservation practice. This has important implications for conservation, as detection of cryptic genetic structure can function as an early indicator in the long-term management of vagile and low-density species.

Human-caused fragmentation and isolation in the RMNP region may have significant implications for long-term conservation of the predator-prey system (Carbyn 1980). This includes both maintenance of genetic variation and an ecologically effective predator on large ungulates such as elk and moose. The morphological results do not suggest wolf – coyote hybridization. Why hybridization has not been documented to date in southwestern Manitoba is uncertain, and could be influenced by genetic factors as well as ecological niche (Templeton 1989, Crandall et al. 2000). Nevertheless, findings from in other regions (Grewal 2001, Leonard and Wayne 2007) suggest that hybridization and evolutionary change in regional canids is a future concern.

The Great Plains is now an intensely human-managed landscape (Guertin et al. 1997). Adaptive evolution can be critical for long-term population survival (Lande and Shannon 1996), and raises questions as to whether adaptation to altered (possibly degraded) habitats and disturbance should always be viewed as detrimental (Ashley et al. 2003, Kyle et al. 2006). The relationship between wolf size and prey size may be complex (Nowak 1983). However, the significance of maintaining viable wolf populations and predator – prey relationships includes the role wolves could potentially play in reducing ungulate populations and spread of diseases such as bovine tuberculosis (*Mycobacterium bovis*) between ungulates and livestock (Stronen et al. 2007, Appendix H). I discuss these potential implications in Chapter 5. More research is needed on whether local adaptive differences, potentially related to factors such as predator – prey relationships and disease resistance, could influence genetic and ecological exchangeability in the absence of physical barriers to dispersal.

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Chapter 3

Relatedness, parasite burden, and spatial overlap of wolves in the Riding Mountain National Park region, Manitoba

Abstract

Philopatry preserves locally-adapted gene complexes. In territorial organisms, philopatry and increased tolerance toward relatives could promote kin clusters, which could further encourage philopatry in patches within a landscape fragmented by humans. However, inbreeding can reduce disease resistance. I tested for correlations between parasite burden and 1) individual parental relatedness and 2) homozygosity in Riding Mountain National Park (RMNP) region wolves in Manitoba, Canada. I tested for correlations between spatial overlap and allele sharing between radio-collared wolves from RMNP packs. Eight of 18 blood samples indicated exposure to Canine Distempervirus and one wolf died from distemper. All 18 showed exposure to Canine Parvovirus. Mean internal relatedness for 221 wolves was 0.394 (SD ± 0.166, range 0.069 – 1). I found the gastrointestinal parasites *Alaria* sp., *Cryptosporidium* sp., *Giardia* sp., *Sarcocystis* sp., Taeniid, *Toxascaris* sp., and *Trichuris* sp. Neither individual parental relatedness, single-locus nor overall homozygosity were significantly correlated with parasite burden. Sample size was sufficient to detect a strong effect size (r = 0.5 with 0.75 power). However, power was low (0.10 – 0.30) and the effect is inconclusive. Allele sharing was not significantly correlated with overall spatial overlap. However, the relationship was negative in individuals tracked > 1 year and animals exhibiting exploratory behaviour showed a negative trend. Successful RMNP wolf dispersal to the Duck Mountains or other areas supporting wolf populations has not

been documented, and the inbreeding coefficient ($F_{IS} = 0.085$) suggests high kinship. Spatial overlap with genetically dissimilar conspecifics within the local patch might help balance dispersal risk and inbreeding avoidance.

Introduction

Philopatry preserves locally-adapted genes and gene complexes (Shields 1982) and delaying or abandoning dispersal is a central component in the altruistic and elaborate interactions characterizing many social species (Waser et al. 2001, Ross 2001). Nonetheless, factors promoting inbreeding avoidance seem central in the evolutionary forces acting on dispersal (Greenwood 1980, Clutton-Brock 1989, Keane 1990, Dieckman et al. 1999). Moreover, inbreeding can affect survival, reproduction and disease resistance enough to reduce viability in small and isolated populations (Keller and Waller 2002). Hence, there appears to be a balance between inbreeding and outbreeding (Shields 1982, Templeton 1986, Marshall and Spalton 2000).

Low dispersal rate or short dispersal distance may be due to several factors, including philopatry (Shields 1982) and unwillingness to cross inhospitable terrain (Wilcox 1980). Natal habitat-biased dispersal (preference for dispersal into familiar habitat) can also act as a natural selection mechanism for avoiding risk (Davis and Stamps 2004, Sacks et al. 2004). Furthermore, inbreeding may be unavoidable in many small and isolated populations (Keller and Waller 2002, Duarte et al. 2003) where even random mating can constitute inbreeding due to high levels of relatedness (Keller and Waller 2002). Importantly, natural selection may have removed alleles of large deleterious effect (such as hereditary blindness) from long-term small populations but retained alleles of small effect (Hedrick 2001, Munguia-Vega et al. 2007).

Increasing metapopulation configuration due to human-caused landscape fragmentation has evolutionary implications for many continuously distributed species (Dieckmann et al. 1999, Wiens 2001, Hanski 2001, Waser et al. 2001). Here, selection may promote long-distance dispersal (Wiens 2001) or adaptation to local conditions (Ashley et al. 2003). Effects of inbreeding in small populations of normally outbred organisms could therefore differ from that of long-term small populations with a history of inbreeding (Hedrick and Kalinowski 2000, Munguia-Vega et al. 2007, Reed et al. 2007a) and require further investigation.

Disease is of increasing concern for many isolated wilderness reserves (Aguirre et al. 1995) and now receives significant attention as a factor affecting wildlife conservation (Daszak et al. 2000). Inbreeding increases homozygosity (Frankham et al. 2002), which has been linked to reduced pathogen resistance (Coltman et al. 1999, Acedevo-Whitehouse et al. 2005, 2006). However, whereas individuals with high heterozygosity may better be able to resist infection and mount immune responses once infected (Acevedo-Whitehouse et al. 2005) other studies found no association between heterozygosity and fitness components (Whitlock 1993, Duarte et al. 2003).

Although parasite egg counts from faeces confirm parasite presence and permits a measure of parasite burden and fecundity (Coltman et al. 1999), egg shedding is variable and constitutes a relative measure. It is therefore difficult to document an individual's exact parasite load from non-invasive sampling of wild animals. Furthermore, eggs are counted in a small subsample from each faecal sample, and even if these accurately reflect level of infection, some parasites may go undetected. The statistical power to detect effects of inbreeding depression often requires large sample sizes (Slate and Pemberton 2002, Keller and Waller 2002, Acevedo-Whitehouse 2005).

Nonetheless, more research is required on the importance of genetic variation in small and isolated populations (Coltman et al. 1999, Reed et al. 2007a). If pathogens have more serious impacts in populations with low genetic variation (May 1988, Hess 1996, Reed et al. 2007b), further study is needed on normally outbred organisms in increasingly insular patches. Many organisms of conservation concern are territorial and wide ranging with small effective populations size (N_e) (Frankham et al. 2002), where only effects of high magnitude (large effect size) easily can be detected with statistical significance. However, biological and statistical significance often differ (Cherry 1998). Trends could therefore provide important information on the biological significance of reduced genetic variation, particularly if, from a conservation perspective, the primary concern is the failure to detect an effect (Type II error).

In territorial species, the risks associated with dispersal (Van Vuren 1998) could be augmented by the possibility of conflict with adjacent territory holders. The degree of relatedness between neighbours can influence their tolerance to each other, so that competition for space is reduced between kin, allowing increased density and survival (Watson et al. 1994, Lambin and Yoccoz 1998, Temple et al. 2006). In particular, territory holders exhibit higher tolerance for trespassing by familiar or related juveniles undertaking exploratory forays (Lambin and Yoccoz 1998). Juveniles may therefore spend more time exploring the surrounding territories occupied by close relatives.

Interactions where localized dispersal increases the number and size of kin clusters, further favouring local recruitment, could therefore influence long-term persistence of isolated populations (Temple et al. 2006). This scenario raises questions on how changes in the scale of environmental heterogeneity could influence the relative costs and benefits of philopatry (Shields 1982). I define a patch as a fragment of wilderness (or semi-wilderness) isolated by other such areas by a matrix (the areas surrounding reserves that are altered to various extents by human use). I hypothesize that within-patch dispersal (dispersal limited to the confines of the natal patch), and subsequent high relatedness within this area, has fitness-related costs and benefits. Expected costs are elevated parasite burdens in individuals with higher parental relatedness and levels of homozygosity. Expected benefits are increasing range overlap (and thus areas available for movement and feeding) for territorial organisms surrounded by related neighbours.

I make the following predictions:

A) *In normally outbred organisms with high dispersal capability, individual parental relatedness values are positively correlated with disease and parasite burdens.*

B) *In territorial organisms, degree of range overlap between neighbours is correlated with degree of relatedness.*

I test whether individuals with higher parental relatedness are disproportionally affected by parasitism. Subsequently, I test whether the spatial home range overlap is larger for individuals that share a higher number of alleles. I test the predictions in the Riding Mountain National Park (RMNP) region in southwestern Manitoba, Canada. Considerable human-caused landscape fragmentation has occurred in this region over the past 50 years (Walker 2001), and removal of forest cover to the Park edge (McNamee 1993) has resulted in a wilderness “island” within an agricultural region (Carbyn 1980, Noss 1995). I examine wide-ranging territorial animals, gray wolves *(Canis lupus*[4]*)*, for which the region constitutes a historically well-connected landscape

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[4] Mitochondrial haplotypes closely related to the proposed eastern wolf, *Canis lycaon*, (Wilson et al. 2000) also occur in part of the study area.

with high likelihood of gene flow (Kurtén and Anderson 1980, Haight et al. 1998, Vilà et al. 1999, Walker 2001). Genetic differentiation without human-caused landscape fragmentation, therefore, seems unlikely within this region (Chapter 2). Philopatry may offer benefits to wolves (Shields 1983); however, recent findings suggest behavioural adaptations limiting inbreeding (vonHoldt et al. 2007).

According to kin selection, non-breeding helpers benefit from assisting in the survival of siblings with whom they share on average 50% of their genes (Ridley 1993). Although strife may still occur, the budding of a new pack on the edge of an established range could benefit the original territory holders by replacing unrelated neighbours with known relatives and former pack mates (Meier et al. 1995). Wolves can show high tolerance to non-pack members when adjacent packs are highly related (Mech and Boitani 2003). Human-caused landscape change and mortality outside RMNP could also affect natural selection and result in adaptive strategies divergent from other wolf populations (Carbyn 1980).

Disease is now considered a central concern for carnivore conservation (Funk et al. 2000), including RMNP wolves (Carbyn 1982). More than 10 viral, bacterial and mycotic diseases are reported for wolves as well as over 70 species of helminth and ectoparasites (Brand et al. 1995). Significant infectious diseases include Canine Distempervirus (CDV), Canine Parvovirus (CPV) (Williams 2001, Barker and Parrish 2001), rabies (Johnson 1995), and infectious canine hepatitis (Kreeger 2003). Three major groups of helminth parasites infect wolves: trematodes (flukes), cestodes (tapeworms) and nematodes (roundworms) (Kreeger 2003). The most harmful ectoparasite on wolves appears to be the mite *Sarcoptes scabiei*, which causes sarcoptic mange (hereafter referred to as mange, Brand et al. 1995, Kreeger 2003). Disease, operating under various levels of food abundance and social stress, could play an important role in regulating the RMNP population (Carbyn 1982).

I correlate internal relatedness (degree of relatedness between an individual's two parents) with parasite burden, to examine whether genetic structure and inbreeding (Chapter 2) could present a cost in the form of elevated parasite load. Secondly, I compare parasite load for individuals homozygous and heterozygous at 13 nuclear loci. Finally, I correlate relatedness (measured as allele sharing) between wolves from different packs with degree of spatial overlap within RMNP, to test if philopatry could confer benefits through elevated tolerance by neighbouring territory holders.

Methods

Study Area

The study area for the genetic and disease component is described in detail in Chapters 1 and 2 and includes RMNP as well as Duck Mountain Provincial Park and Forest (hereafter the Duck Mountains) north of RMNP. For the study of spatial overlap, I examined radio-collared neighbouring wolf packs within RMNP.

Genetic analyses

I have data for 14 microsatellite nuclear markers from the study area, including 13 autosomal (non-sex chromosome) and one Y- chromosome loci. The DNA sampling, collection, extraction and amplification are described in Chapter 2. I collected samples (faecal, hair and tissue) during 2003 – 2005, and I also included some faecal and tissue samples from the period 1990 – 2003. Based on all samples, I identified altogether 221 wolves from the study area. To avoid male – female bias in relatedness calculations, I used only autosomal markers (as females would not have Y- chromosome alleles). I calculated internal relatedness for individuals and relatedness between individuals (kin structure). Parasites were counted in each faecal sample to quantify individual infection (Coltman et al. 1999) by noting the type(s) and number of eggs and oocysts (protozoa spore phase).

Radio collaring and tracking

Altogether, 19 wolves were captured by netgun from helicopter and 18 were radio-collared during 2003 – 2005. Capture and collaring protocols were prepared and approved by Parks Canada (RMNP unpub. reports), which contracted the work to a professional capture crew and veterinarians. Attempts were made to capture and collar at least two of the younger wolves in each pack; the individuals most likely to disperse (Mech and Boitani 2003). The aim was to collar an equal number of males and females, but focusing on young wolves meant that sex was difficult to determine for each animal until captured. Efforts were concentrated in the west end of RMNP where there are no highways bisecting the park (possibly affecting movement) and almost every pack territory borders the Park boundary (thus dispersers could leave RMNP without passing through any neighbouring territory). Dense forest in RMNP also makes it challenging to locate wolf packs. The gentler terrain and predominantly deciduous forest on the west side improved chances of locating and capturing wolves during wintertime compared with the coniferous forest and steeper terrain on the east side of the Park.

Wolves were weighed, sex was determined and they were fitted with standard VHF radio collars programmed to change frequency after eight hours of inactivity. Fur colour, health condition and approximate age (from tooth wear) were noted, and blood and hair samples were collected for disease and genetic analyses. One wolf died during capture. We did a fixed-wing tracking flight one or two days after each capture, and all 18 collared wolves were alive and with their packs. We tracked wolves every week of the year, weather permitting, using a Cessna 172 aircraft with antennas mounted on the wing struts and standard telemetry procedures (White and Garrott 1990). We obtained GPS positions using a handheld Global Positioning System (GPS) receiver, and noted habitat occupied by the animal (forest type, wetland etc.) and observation time.

Whenever we observed wolves, we noted behaviour, group size (including the number of pups), and other information such as kills and presence of other species nearby. Based on comparison of aerial and ground locations during retrieval of radio collars, location error was generally within 300 meters. However, average RMNP wolf territories size is 260 (SD ± 116) km^2 (Carbyn 1980) and I therefore consider this an acceptable error size for a study of spatial overlap. Wind conditions did not permit low level flying and better location accuracy on many occasions. Wolves rarely seemed bothered by aircraft, but additional time in overflight to improve location accuracy (when wolves were not visible) would have increased disturbance to wolves and other animals.

I was not able to obtain night time locations and this could bias the size of wolf home ranges, particularly if wolves may be more likely to leave the Park during the night and therefore move over a wider area than that suggested by daytime location data. However, I do not expect this possible bias to vary between different wolves. When wolves went missing, we searched widely in and around the Park, and repeated our searches for all missing animals at approximately monthly intervals. We investigated collars on mortality frequency as soon as possible from the ground by visiting the location and collecting the collar. We also collected the dead wolf if mortality was the reason for the stationary collar.

Disease and parasite load

Disease Analysis

All disease and parasite analyses were done in collaboration with the Canadian Cooperative Wildlife Health Centre (CCWHC) at the University of Saskatchewan. For faecal samples, parasite egg/oocyst counts were done to determine parasite type and degree of infection. For tissue samples (some collected during this study and others accessed from earlier research), I noted the presence or absence of mange where noted on the data tag accompanying the sample. Serology analyses on blood samples from radio collared wolves provided levels of Canine Parvovirus and Canine Distempervirus antibodies. Faecal samples were also analysed by virology to determine whether wolves were shedding the CPV virus. This would indicate current infections in the study area, as serology only indicates previous virus exposure by means of antibody levels.

Parasitology

Frozen faecal samples were transported to the CCWHC and placed at - 80˚C for three days to kill any *Echinococcus* sp. eggs present in the scat, as this parasite is dangerous to humans. Samples were thawed for one hour prior to processing, which used a modified Wisconsin technique (Cox and Todd 1962, Sallows 2007, Salb et al. 2008). Four grams of material from each sample were weighed into a labeled cup, 40 ml of water was added, and the mixtures filtered through a single layer of 50 weight cheesecloth into a second labeled cup. A 10 % aliquot (4 ml of the mixture) was taken from the filtrate using a 5 ml syringe, and placed into a test tube. Eight 8 ml of water was added to the test tube, which was centrifuged at 1500 rpm for 10 minutes. After pouring off the supernatant, the sediment was resuspended in Sheather's flotation solution, mixed, and the tube filled until there was a slightly convex meniscus in order to hold a slide cover slip in place. A cover slip was placed on top of the tube, and it was centrifuged again for 10 minutes at 1500 rpm. The cover slip was then placed on a labeled microscopic slide, and the types and numbers of parasites were counted. The parasites are reported at the genus level (except Taediid parasites reported at the family level).

For *Giardia* sp. and *Cryptosporidium* sp., we used immunoflorescent detection and the Cyst-a-Glo™ Comprehensive Kit. Samples were processed as outlined above. A thin layer was smeared on a slide, and Cyst-a-Glo™ antibody reagent added. The sample was incubated at room temperature for at least 40 minutes in a humid chamber, rinsed, and dried. Cover slips were placed on the slide, and we counted the number of *Giardia* sp. and *Cryptosporidium* sp. present. Prevalence was divided into four categories: 1 (1 – 50 oocysts present), 2 (51 – 250 oocysts), 3 (251 – 1000 oocysts), and 4 (> 1000 oocysts). For other parasites I report number of eggs/oocysts counted, with the exception of *Sarcocystis* sp. Because of the large numbers of this parasite in many samples, numbers of oocysts was at times noted as TNTC: Too Numerous to Count.

Statistical analyses

Sampling

The collection of faecal samples is outlined in Chapter 2. For genetic and disease data I tried to sample several wolves from as many packs as possible, considering that the RMNP population appears to consist of about 70 – 75 individuals in 10 – 12 packs (RMNP unpub. data). The Duck Mountains cover approximately the same area as RMNP and likely has a similar (but unknown) population. Identification of all individuals would have increased the chances of identifying as many alleles and immigrants as possible, although some likely would have been missed due to genotyping errors from non-invasive samples (Chapter 2). Also, some immigrants would probably carry alleles typical of residents. This is likely where differentiation between neighbouring populations is limited (Chapter 2). I focused on putative pack territories and known areas of wolf activity to sample enough individuals for an estimate of genetic diversity and the degree of gene flow. I then selected samples from all known or suspected territories for further analyses, based on apparent sample quality (fresh samples with a smooth surface). It was necessary to use samples of mediocre quality from some putative territories.

Power analyses

Large sample sizes may be needed to detect correlations between fitness and genome-wide or single-locus homozygosity (Coltman and Slate 2003). I would, for example, need a sample of 854 individuals to detect an effect size of 0.10 with 0.9 statistical power (Zar 1996, p. 380) which is not possible for my study area and organism. However, an effect size of $r = 0.5$ can be detected with 0.75 power by 31 individuals, suggesting I have sufficient sample sizes to detect strong effects and trends. I performed post-test power calculations for correlation coefficients using Zar (1996 p. 380).

Internal Relatedness

I calculated Internal Relatedness (IR, Amos et al. 2001) using IRmacroN3 (Amos 2005) as the degree of relatedness between an individual's two parents. For this measure, each genotype is weighted according to allele frequencies; thus, individuals homozygous for rare alleles receive higher weights (Amos et al. 2001). Using a formula adapted from Queller and Goodnight (1989), IR is calculated as $(2H - \sum f_i)/(2N - \sum f_i)$ where H is the number of homozygous loci, N is number of loci, f_i is frequency of the *i*th allele contained in the genotype (Amos et al. 2001).

Disease load

To determine the relationship between a measure of inbreeding and faecal parasite egg count (Coltman et al. 1999), I correlated IR for each individual with the number of parasite types found and with egg/oocyst counts for each parasite type. I used rank correlation for non-parametric data with Kendall's tau for large numbers of tied ranks (Zar 1996) in SPSS 9.0.0 (SPSS Inc. 1998). To test whether homozygosity at the different loci might be associated with infection of one or more parasites, I correlated presence of each parasite with heterozygosity status (0 = homozygous, 1 = heterozygous) at every locus. I also compared the number of parasite types for different internal relatedness values between RMNP and Duck Mountain individuals. To account for the chance of obtaining one or more significant results ($\alpha = 0.05$) during multiple tests I applied False Discovery Rate correction as outlined in Verhoeven et al. (2005).

Kin structure

Inbreeding is a relative measure that depends on the reference population (Keller and Waller 2002), and to measure relatedness between two individuals I calculated degree of allele sharing across the 13 autosomal nuclear markers. I do not have pedigree data, but for radio-collared individuals I know which pack they belonged to, although some wolves might have immigrated to the pack they associated with during the study (see Discussion). I do not have age data for individuals. Captured wolves were aged by physical examination (no immobilization was done and therefore no teeth were collected), and I could not obtain information on age from faecal or tissue material. Measuring allele sharing permits a comparison of relatedness between individuals without making assumptions about the nature of the relationships (such as whether two individuals are siblings or parent – offspring, both with expected relatedness of 0.5). All alleles did not amplify successfully in every individual. Allele sharing is therefore given as number of matching alleles divided by number of alleles amplified in both individuals, and given as a value between 0 and 1.

Wolf home ranges and degree of overlap

I used all wolf radio locations to calculate 100% Minimum Convex Polygon (MCP) home range for each collared wolf using ArcView 3.3. I did pair-wise comparisons of neighbouring wolves from within RMNP to determine spatial overlap. Home ranges vary in size, and the extent of overlap might cover 20% of the home range for one individual and 15% for the second. I therefore describe overlap using hectares. A MCP of 95% is often used in wolf home range analyses (see e.g. Messier 1985), but I used 100% MCP in order to consider all locations, including those I considered to be outside the usual home range and that likely constituted exploratory forays. Although wolves spend much time with their pack, individuals can undertake extensive exploratory movements on their own (Messier 1985). Importantly, wolves were at least half a year old when captured, and I can not exclude the possibility that they had immigrated to the territory where they were collared. For each of the two collared wolves in a pack, I considered the degree of overlap with neighbours to be independent of the other collared wolf and I calculated home range and overlap with neighbours for each wolf separately.

I correlated range overlap (in hectares) with degree of allele sharing (a value between 0 and 1) using rank correlation for nonparametric data and Kendall's tau for data with a high number of tied ranks in SPSS 9.0.0. The time spans that wolves carried collars varied due to timing of capture, death of wolves and lost collars. Some individuals carried collars simultaneously for several years, whereas the overlap in time for others was only a few months. I, therefore, did four separate analyses and started by comparing all pairs of neighbouring wolves within RMNP. I considered an individual's overlap with each of the other collared wolves, except for the member of the same pack ($n = 54$ pairwise comparisons). I then compared spatial overlap for wolves that had carried collars simultaneously for > 1 year ($n = 31$) and < 1 year ($n = 23$). Finally, I did pairwise comparisons ($n = 10$) of the wolves I considered to have undertaken exploratory forays from their home ranges. Whereas some collared wolves may have been breeders with little or no reason to undertake exploratory forays or dispersal, the behaviour of these five individuals suggested they were searching for a mate and a vacant territory.

Results

Disease, parasite load and relationship with internal relatedness

Antibodies to Canine Parvovirus (CPV) were found in 18/18 collared wolves; 8/18 showed exposure to CDV. One wolf died from CDV (T. Bollinger, pers. comm.), and was found dead in the central part of RMNP in April 2005. Neither of the two collared wolves from this pack showed exposure to CDV when captured in February 2005. No sign of CPV virus were detected in scats. For the 200 faecal samples analysed for 2003 – 2004, I found these parasites: *Alaria* sp. (in 15.0% of faecal samples), *Cryptosporidium* sp. (0.5%), *Giardia* sp. (49.0%), *Sarcocystis* sp. (36.5%), Taeniid (25.0%), *Toxascaris* sp. (2.5%), and *Trichuris* sp. (1.0%). With the exception of *Trichuris* sp., I found the same parasites in the 107 faecal samples analysed for 2004 – 2005: *Alaria* sp. (29.9%), *Cryptosporidium* sp. (3.7%), *Giardia* sp. (25.2%), *Sarcocystis* sp. (35.5%), Taeniid (29.9%), *Toxascaris* sp. (2.8%). Parasite counts were done for all these 307 faecal samples, but it was not possible to obtain genotype data for all. The subsequent analyses are therefore based on the faecal samples where I have both egg/oocyst counts and allele information from 9 or more markers. As noted above, the markers that amplified varied for each individual. The 307 faecal samples likely include more than one sample from some individuals. Where genotyping showed this to be the case, I selected the parasite count from the faecal sample with best quality (highest number of markers amplified). Where several samples attributed to one individual had amplified an equal number of alleles, I randomly selected one sample to represent the individual's parasite count. Mean IR for the 221 individuals identified (Chapter 2) was 0.394 (SD ± 0.166, range 0.069 – 1). The number of parasite types per individual were not significantly different between RMNP and the Duck Mountains (correlation coefficient 0.01, p = 0.43, and Figure 3.1). Spatial distributions of each parasite for each sampling year are in Appendix G.

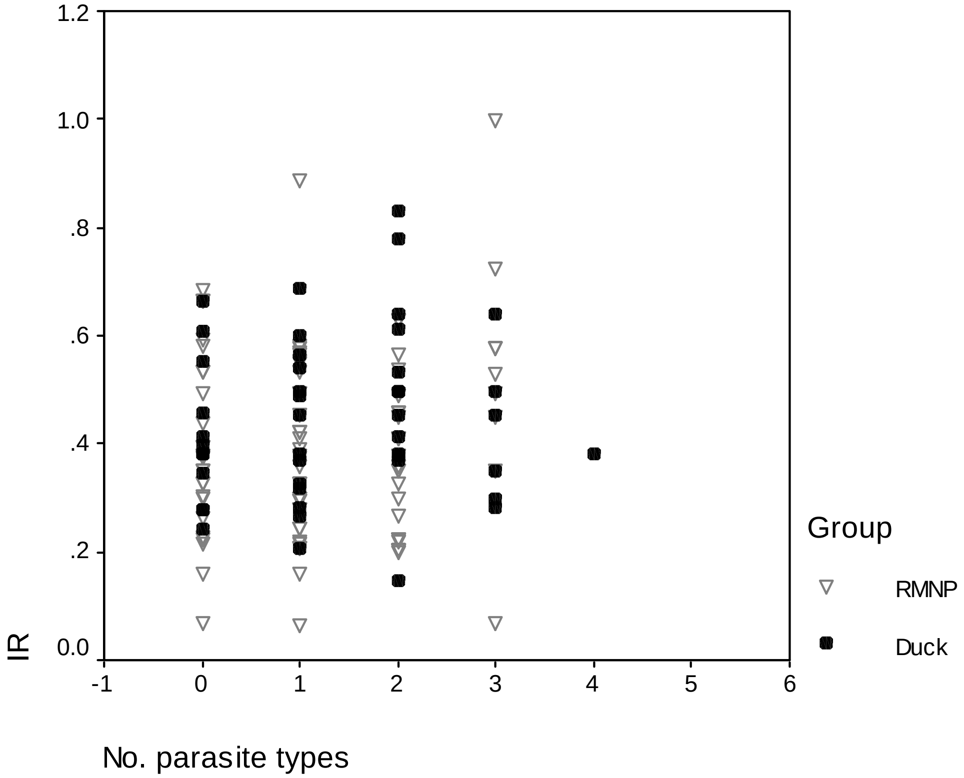

Figure 3.1. Number of parasite taxa (to genus or family level of classification) and internal relatedness (IR) for wolves in Riding Mountain National Park and the Duck Mountains, Manitoba, Canada.

The most common intestinal parasites are Taeniid, *Alaria* sp., *Sarcocystis* sp. and *Giardia* sp., and I used these to examine correlation between IR and parasite load.

Sarcocystis sp. oocysts were detected in 60 wolves of which 28 were TNTC, and *Giardia* sp. oocysts were detected in 50 wolves. The parasite burdens for individuals with *Giardia* sp. were distributed as: Category 1 (n = 23 wolves), 2 (n = 14), 3 (n = 8) and 4 (n = 3). Mean number of Taeniid eggs was 1143.98 (SD ± 4380.15, range 1 – 26 000, N = 44) and mean number of *Alaria* sp. eggs was 31.97 (SD ± 54.02, range 1 – 216, N = 34). Of 60 hair and tissue samples, mange was detected in 23 wolves. I indentified several genetic clusters of wolves (Chapter 2), but I here compare data from the two clusters that made up 190 of the 221 samples, RMNP and the Duck Mountains. Internal relatedness was similar for RMNP (Mean 0.401 ± 0.178, n = 130) and the Duck Mountains (0.422 ± 0.149, n = 60, Figure 3.2). One faecal sample from RMNP was homozygous for all alleles and consequently had IR = 1.

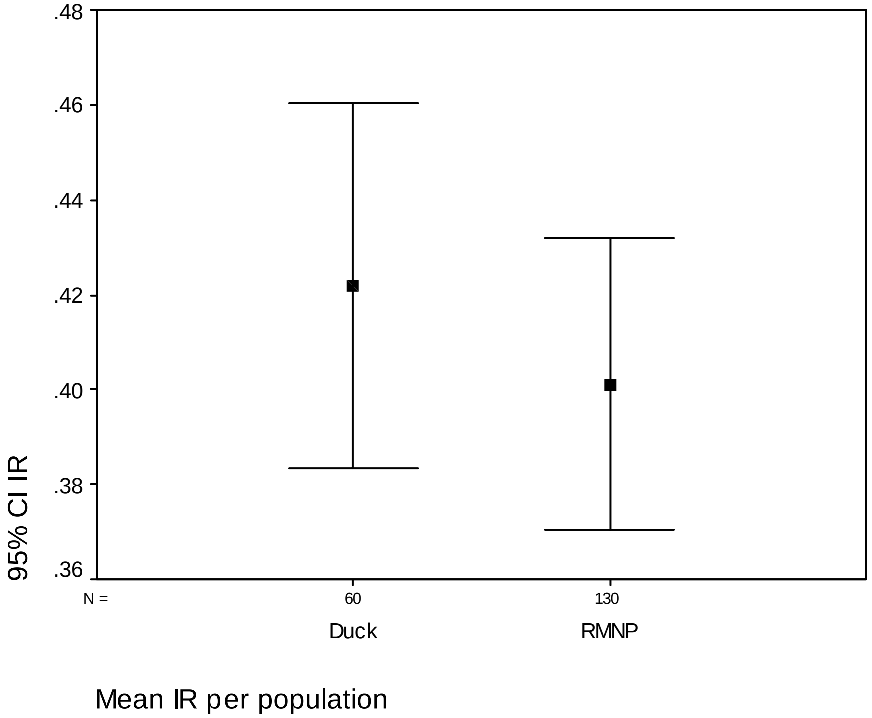

Figure 3.2. Mean Internal Relatedness (IR) with 95 % confidence intervals (CI) for the Duck Mountains and Riding Mountain National Park in Manitoba, Canada.

Internal relatedness at the 13 markers did not correlate significantly with number of parasites (Table 3.1). Mange, Taeniid, *Alaria* sp., *Sarcocystis* sp. or *Giardia* sp. were not significantly correlated with IR (Table 3.1, Figures 3.3 – 3.7).

Table 3.1. Correlation between IR and parasite presence for wolves in southwestern Manitoba, Canada, using Kendall's tau rank correlation for nonparametric data with a large number of tied ranks.

Factor 1	Factor 2	N	Correlation coefficient	Power	p-value
IR	Total # parasites*	221	-0.040	0.129	0.211
IR	mange	58	-0.148	0.295	0.087
IR	# taeniid eggs	44	0.076	0.123	0.233
IR	# alaria eggs	34	-0.119	0.164	0.167
IR	# sarcocyst eggs	60	0.052	0.106	0.293
IR	# giardia oocysts	50	-0.169	0.319	0.062

*Number of different parasite taxa (to family for Taeniids, otherwise to genus) found per individual.

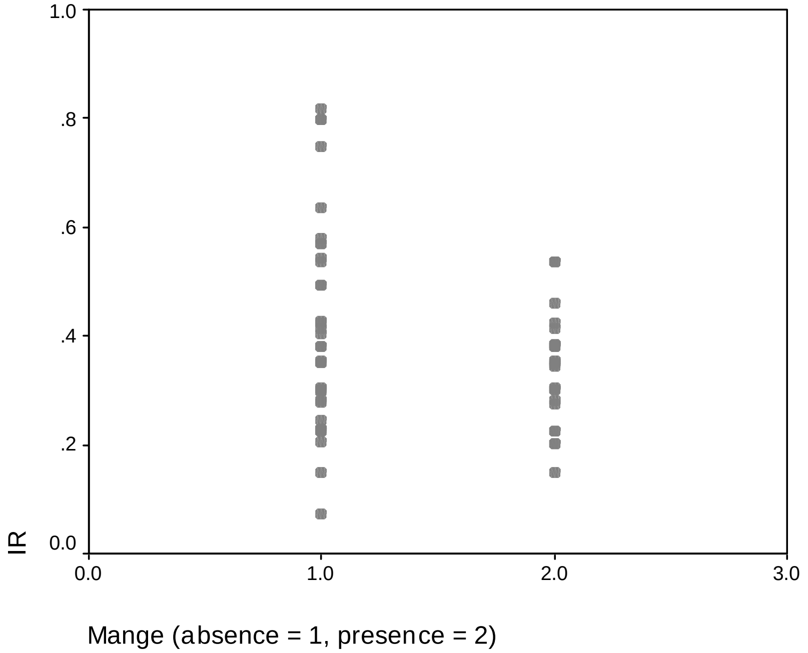

Figure 3.3. Internal relatedness (IR) and mange occurrence in southwestern Manitoba, Canada.

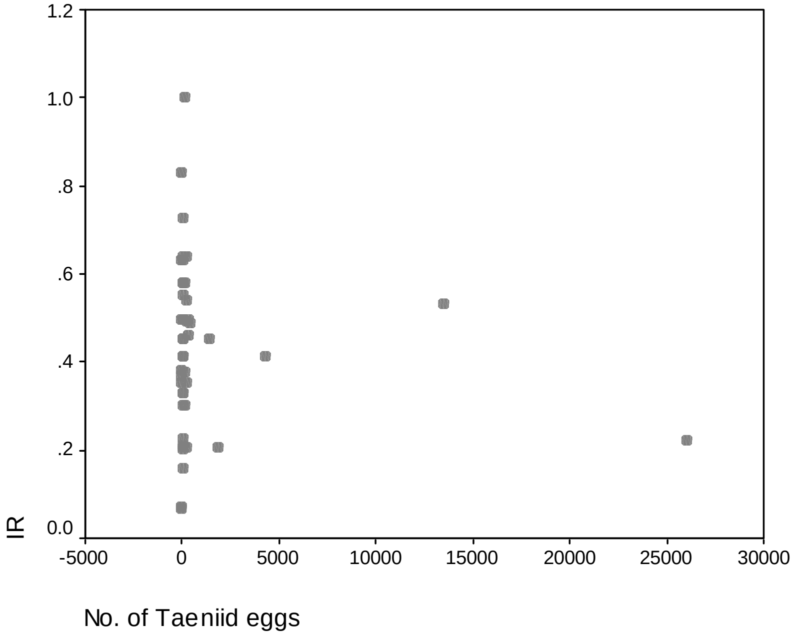

Figure 3.4. Internal relatedness (IR) and Taeniid egg count in in southwestern Manitoba, Canada.

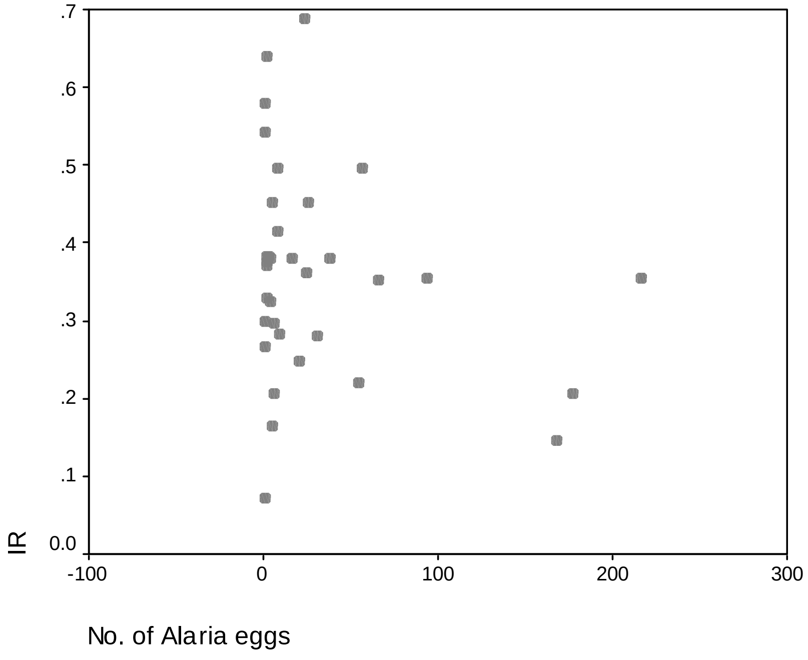

Figure 3.5. Internal relatedness (IR) and *Alaria* sp. egg count in in southwestern Manitoba, Canada.

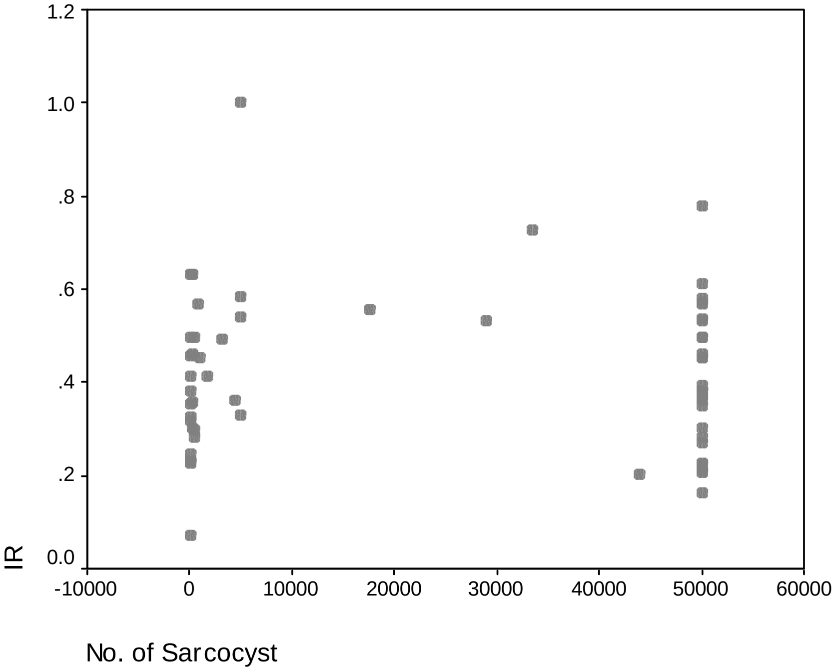

Figure 3.6. Internal relatedness (IR) and *Sarcocystis* sp. oocyst count in southwestern Manitoba, Canada. TNTC category (see Methods) is coded as 50 000 to allow display.

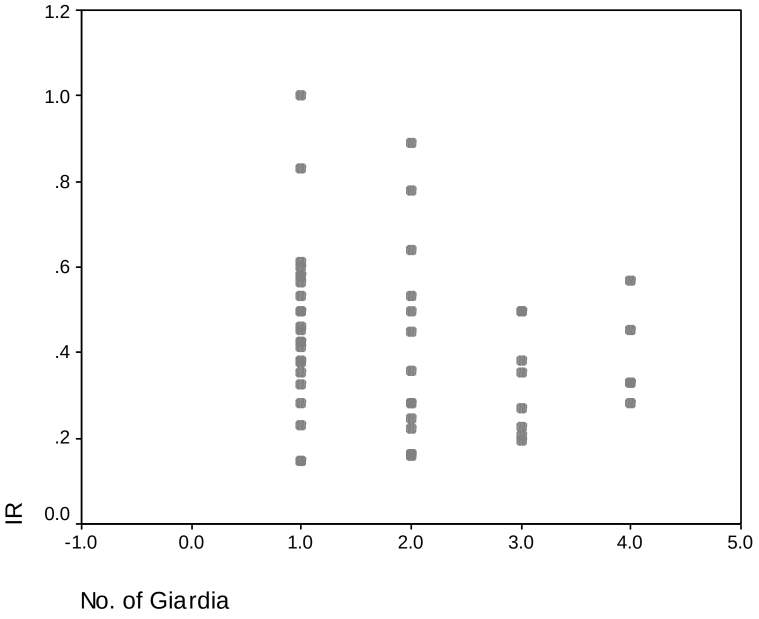

Figure 3.7. Internal relatedness (IR) and *Giardia* sp. oocyst count in in southwestern Manitoba, Canada. The categories 1 – 4 are described in Methods.

In contrast to overall IR values, single locus effects were significant for 12 loci – disease associations (Table 3.2), distributed among all diseases. Two loci (FH2017, PEZ19) remained significant after False Discovery Rate correction for multiple tests (Verhoeven et al. 2005), both for *Giardia* sp. Both tests had high post-test power: 0.94 for locus FH2017 and 0.99 for locus PEZ19.

Table 3.2. Correlation coefficient r (with significance values) between homozygosity at each locus and parasite presence using Kendall's tau for wolves in southwestern Manitoba, Canada. Significant values in bold; values with a star remained significant after FDR correction for multiple tests (Verhoeven et al. 2005).

Locus	Mange corr. coeff. (p-value)	Taeniid corr. coeff. (p-value)	Alaria corr. coeff. (p-value)	Sarcocyst corr. coeff. (p-value)	Giardia corr. coeff. (p-value)
FH2054	-0.139 (0.146)	0.020 (0.404)	0.022 (0.392)	**-0.142 (0.039)**	-0.044 (0.293)
FH2001	0.174 (0.096)	0.121 (0.064)	0.093 (0.120)	-0.024 (0.380)	**-0.146 (0.032)**
FH2096	0.006 (0.481)	-0.047 (0.282)	-0.124 (0.064)	-0.071 (0.192)	0.007 (0.463)
FH2010	0.072 (0.298)	-0.092 (0.125)	0.009 (0.457)	0.078 (0.165)	-0.042 (0.300)
FH2017	0.221 (0.056)	-0.028 (0.372)	-0.022 (0.401)	-0.130 (0.065)	**0.269 (0.001) ***
PEZ08	0.221 (0.071)	-0.064 (0.314)	**0.276 (0.019)**	-0.087 (0.255)	0.186 (0.080)
FH2088	-0.178 (0.093)	0.080 (0.157)	**-0.201 (0.006)**	0.041 (0.303)	-0.035 (0.332)
FH2422	**0.354 (0.004)**	**-0.150 (0.031)**	**0.204 (0.006)**	0.085 (0.146)	-0.014 (0.429)
FH3313	0.050 (0.359)	-0.029 (0.364)	**0.161 (0.026)**	-0.052 (0.265)	0.051 (0.269)
PEZ06	0.078 (0.283)	-0.035 (0.329)	-0.010 (0.449)	**-0.143 (0.036)**	-0.047 (0.279)
PEZ19	0.040 (0.383)	-0.032 (0.351)	**0.223 (0.003)**	-0.080 (0.168)	**-0.331 (0.000) ***
PEZ15	0.077 (0.284)	-0.078 (0.165)	0.117 (0.072)	0.059 (0.231)	0.024 (0.382)
FH3725	-0.135 (0.158)	-0.105 (0.096)	-0.001 (0.495)	0.036 (0.327)	0.070 (0.191)

Most individuals (110 of 137) were homozygous for locus FH2017 (Figure 3.8) and there were altogether four alleles at this locus. Fewer individuals were homozygous (59 of 146) for locus PEZ19 (Figure 3.9) and there were altogether 10 alleles at this locus. *Giardia* sp. infection was not associated with increased homozygosity at either locus. The majority of individuals surveyed for locus FH2017 did not show *Giardia* sp. infection (91 of 137 showed no infection), and the results were similar for locus PEZ19 (97 of 146 showed no infection).

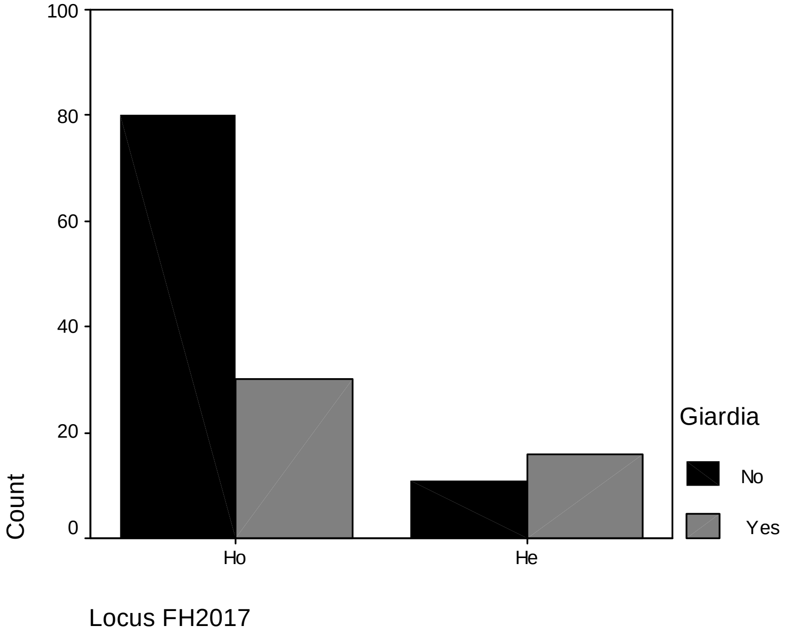

Figure 3.8. The number of wolves in southwestern Manitoba, Canada, with and without *Giardia* sp. infection versus homozygosity (Ho) and heterozygosity (He) for locus FH2017.

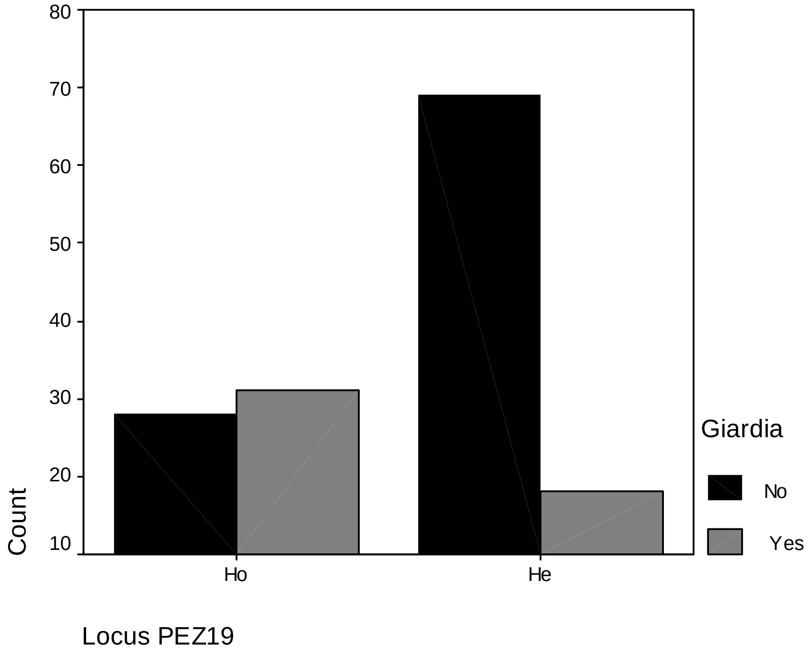

Figure 3.9. The number of wolves in southwestern Manitoba, Canada, with and without *Giardia* sp. infection versus homozygosity (Ho) and heterozygosity (He) for locus PEZ19.

Home range overlap and allele sharing

Average overlap (mean ± SD) in time and space for all wolves in western RMNP is 12 ± 7 months and 10 885 ± 14 488 ha, with 36 ± 15% of alleles in common (Table 3.3). The relationship between spatial overlap and allele sharing was not significant overall. When data were divided into wolves that had been followed simultaneously more than one year and less than one year, there was a significant correlation for the first group. The relationship was negative, indicating that individuals with greater spatial overlap shared fewer alleles (Figure 3.10). Spatial overlap was not significantly correlated with allele sharing for individuals considered to have undertaken exploratory forays, but this relationship was also negative.

Table 3.3. Correlation between allele sharing and spatial overlap for radio collared wolves from neighbouring packs in Riding Mountain National Park, Manitoba, Canada. Significant values in bold.

Pairwise comparison*	Average overlap (month)	Range	Average overlap (ha)	Range	Average allele sharing	Range	N**	Corr. coeff.	power	p-value
1	12	0-28	10 885	0-51 543	0.36	0.00-0.65	54	-0.016	0.063	0.436
2	17	11-28	16 794	0-51 543	0.41	0.13-0.60	31	**-0.253**	**0.390**	**0.029**
3	6	0-9	2921	0-22 198	0.29	0.00-0.65	23	0.000	N/A	0.500
4	17	11-28	30 717	0-51 543	0.38	0.13-0.55	10	-0.225	0.152	0.185

*Two wolves are radio collared per pack for six packs. Allele sharing and spatial overlap are compared pairwise between all wolves except for those from the same pack. Comparisons are done for four categories: 1) all wolves, 2) wolves that carried collar simultaneously for at least one year, 3) wolves that carried collars simultaneously for less than one year, 4) wolves that showed exploratory behaviour.

** Number of pairwise comparisons.

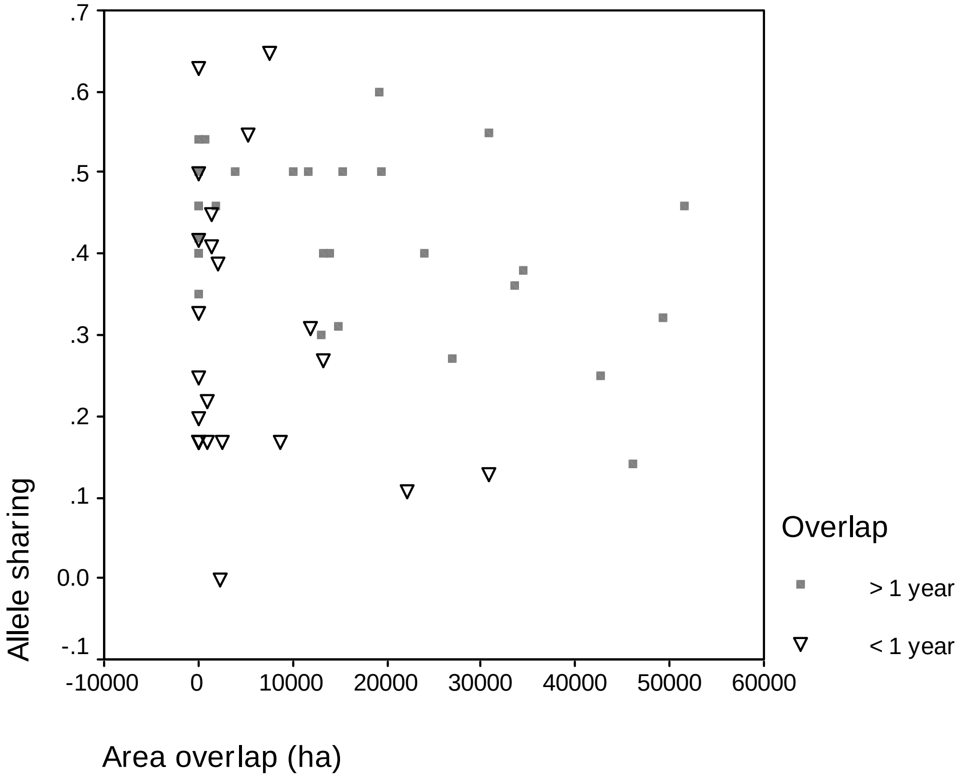

Figure 3.10. Allele sharing and spatial overlap between neighbouring wolves in Riding Mountain National Park, Manitoba, Canada, 2003 - 2005. Pairs of individuals marked with grey squares were tracked simultaneously for > 1 year (n = 31 pairwise comparisons), pairs with black triangles < 1 year (n = 23 pairwise comparisons).

Discussion

Disease, parasite burden and relationship with internal relatedness

Disease in the RMNP region

Serological results indicate only exposure to virus and not infection, but suggest CDV and CPV may be affecting wolves in the RMNP ecosystem. For two packs, one captured wolf showed CDV exposure whereas the other wolf did not. Wolves are social animals, but it is possible that only some pack members had been exposed. In both packs, the wolf that did not show exposure was believed to be the younger of the two; hence, the older wolf could have been exposed prior to the birth of the younger individual. It is also possible that all four were exposed, and that virus titers were subsequently reduced for some individuals over time. It is difficult to assess whether exposure to CPV is more prevalent than CDV, especially as CDV has caused four known mortalities in RMNP wolves (Carbyn 1982, this study).

Neither CPV-related wolf mortality nor faecal shedding of CPV has been documented in the region (Sallows 2007). However, local veterinarians have recorded several cases of CPV and CDV in dogs over recent years. Both may cause high pup moralities (Mech et al. 1986, Mech and Goyal 1995, Johnson et al. 1994), and unvaccinated dogs entering protected areas represent a continuing threat to populations of wolves, coyotes and foxes (Aguirre et al. 1995). Coyote and wolf ranges overlap throughout RMNP (Paquet 1992) and almost all wolf home ranges include the Park boundary. Infections in local dogs and coyotes are therefore a concern for RMNP wolves. Mange represents a similar concern, and park staff and trappers have reported this parasite from both RMNP and the Duck Mountains in recent years. Although the degree of mange infection varies between animals, a warden had to euthanize a RMNP wolf with severe mange in January 2006 (T. Hoggins, pers. comm.). High disease mortality in pups may reduce recruitment and, subsequently, resource competition and dispersal (Lidicker 1975, Waser 1985) from RMNP. I explore this further in Chapter 4.

Parasite burden and internal relatedness

Internal relatedness does not appear to be correlated with parasite burden in RMNP region wolves. My results suggest that a genome-wide relationship between heterozygosity and infection did not occur, or possibly was too weak to be detected with my available sample size, number of markers, and statistical power. Selection is expected to act against inbred individuals where parasites confer a significant fitness cost (Coltman et al. 1999). A more direct measure of fitness would be the number of offspring produced per animal. These data are difficult to collect for wolves as dens are often hard to locate in the forested environment and pups do not emerge for several weeks, at which point some pups might already have died. Furthermore, animals with heavy parasite loads might be in poorer physical condition (e.g. lower body weight) than animals with few or no parasites, but nonetheless have the same number of offspring.

Giardia sp. did not appear more common in homozygous individuals. Most individuals were not infected, and the significant results are likely due to chance effects and deviations from Hardy-Weinberg equilibrium with uneven distribution between homozygous and heterozygous individuals for loci FH2017 and PEZ19. Wolves may be able to live with *Giardia* sp. without adverse effects, but this and other parasites might predispose animals to CPV (Kreeger 2003).

Although IR for wolves ranged widely, the results indicate high overall relatedness (Chapter 2). Though I have only surveyed 13 microsatellite markers, mean IR value theoretically suggests high levels of inbreeding. This seems consistent with the findings of genetic population structure and relatively high inbreeding coefficient (F_{IS}) values (Chapter 2). It is plausible that typing more loci from this relatively limited number of individuals would increase resolution and change estimates of relatedness.

However, other studies have typically genotyped the same number or fewer loci (Marshall and Spalton 2000, Van Horn et al. 2004, Acevedo-Whitehouse et al. 2006, Temple et al. 2006).

Both number of loci and their mutation rates may affect the ability to detect heterozygosity – fitness associations (Slate and Pemberton 2002), and the degree of linkage disequilibrium may be important (Balloux et al. 2004). Analyses of Hardy-Weinberg and linkage equilibrium (Chapter 2) showed that overall, 13 of 14 loci had significantly higher homozygosity than expected, and 26 of 78 loci pairs showed linkage disequilibrium. Within RMNP, 22 of 78 pairs showed linkage disequilibrium and 6 loci (including FH2017) showed significantly less heterozygosity than expected. For the Duck Mountains, three loci (including FH2017 and PEZ19) showed significantly less heterozygosity than expected, and 10 of 78 loci pairs were in linkage disequilibrium. The high levels of homozygosity could have biased the analyses and may have increased the chance of finding homozygosity – parasite correlations. The linkage disequilibrium between these assumed neutral loci should not have any influence, but linkages between neutral loci and any loci involved in disease resistance could affect the results (Acevedo-Whitehouse et al. 2005).

Samples with no or very few parasite eggs/oocysts showed a wide range of IR values, and relatively few samples had large parasite burdens. This may have influenced the results. The many initial significant single-locus effects before FDR correction were likely chance effects, particularly as correlations varied between positive and negative for the same parasite at various loci. Other long-lived vertebrates show negative relationships between IR and reproductive success (Amos et al. 2001) and IR and disease resistance (Balloux et al. 2004). The relationship between heterozygosity measures and inbreeding is not clear, particularly the relative contributions of genome-wide heterozygosity versus single-locus effects (Balloux et al. 2004). Both general and single-locus effects appear to interact with bovine tuberculosis infection (Acevedo-Whitehouse et al. 2005), a disease diagnosed in RMNP wolves (Carbyn 1982).

Home range overlap and allele sharing

Though 100% MCP home ranges did not correlate significantly with overall allele sharing, the degree of allele sharing is relatively high at the 13 loci surveyed. The ability to detect significant differences in kin versus non-kin interactions may require higher spatial variation in relatedness between neighbouring territory holders (Watson et al. 1994, Van Horn et al. 2004). For example, red grouse (*Lagopus lagopus scoticus*), territorial males had fewer aggressive boundary disputes with close-kin neighbours than with less closely related neighbours (Watson et al. 1994). Territorial white-breasted thrasher (*Ramphocinclus brahyurus*) males may also benefit from tolerance and assistance offered by related neighbours (Temple et al. 2006).

The wolves that I consider to have undertaken exploratory forays from their home (likely natal) range, as suggested by field data, showed a negative trend between allele sharing and spatial overlap. Though behavioural mechanisms appear to limit inbreeding (Smith et al. 1997, vonHoldt et al. 2007), no successful dispersal of RMNP wolves to neighbouring areas has been documented to date and the inbreeding coefficient (F_{IS} = 0.0842) also suggests high kinship within the Park (Chapter 2). If dispersal from the local patch is dangerous (Carbyn 1980, Fritts and Carbyn 1995, Gros et al. 2006), and philopatric individuals benefit from kin-tolerance (Watson et al. 1994, Temple et al. 2006), this could affect dispersal costs and benefits for individuals in isolated patches. The risk of human-caused mortality as soon as individuals cross the RMNP boundary (Fritts and Carbyn 1995) might also result in locally adaptive strategies (Carbyn 1980). Although the wolf hunting season has been closed since 2001 in the areas surrounding RMNP (D. Chranowski pers. comm.), negative attitudes to wolves are widespread (Stronen et al. 2007, Appendix H) and shooting wolves on sight remains a common practice (Brook 2007). Individual wolves might choose to seek mates as genetically different as possible within their local patch rather than enter the surrounding landscape matrix.

Boundary strife in red grouse tended to rise following immigration (Watson et al. 1994). Some field observations from RNMP also suggest that territoriality and kin clusters could present additional obstacles to immigration and gene flow into an isolated patch. The Baldy wolf pack overlapped extensively with the adjacent Ranch Creek wolf pack, and allele sharing ranged from 0.27 – 0.60 (calculated from two wolves in each pack). All four wolves were assigned RMNP origin (Chapter 2). A male wolf was killed by other wolves (T. Bollinger, pers. comm.) on a section of the RMNP boundary used by the Baldy pack. Allele sharing between this male and Baldy wolves was 0.18, and he was assigned immigrant status (Chapter 2). The location strongly suggests that the Baldy pack killed the immigrant wolf, which may be consistent with the findings by Watson et al. (1994).

I also observed a male wolf from the Wilson River pack together with three members of the adjacent Ranch Creek pack. Unfortunately, I then lost contact with the Wilson River wolf. He shared none of eight alleles with the other collared Wilson River wolf, but allele sharing was 0.11 – 0.31 with the two collared Ranch Creek wolves. Because wolves were at least half a year old when radio-collared, I cannot exclude the possibility that some may have dispersed and joined new packs prior to capture. However, two wolves may also be born into the same pack and show low degree of relatedness if they have none or only one parent in common (Meier et al. 1995). The Wilson River male might have been tolerated by both groups as he travelled with the Wilson River pack for several months, and then spent time in the Ranch Creek home range in the weeks before I lost his signal.

Overall assessment

I did not find a significant correlation between increased parental relatedness and parasite burden. Other studies have shown similar results (Whitlock 1993, Duarte et al. 2003). Nevertheless, two factors are important to consider. Non-invasive methods can show high variability in parasite egg counts (Coltman et al. 1999). Counts of gastrointestinal parasites from dead specimens would improve accuracy but killing animals would be incompatible with conservation goals. Moreover, I examined common gastrointestinal parasites. Whereas wolves appear to tolerate a wide range of parasites, other pathogens such as viral diseases can have more severe effects (see review in Kreeger 2003). I recommend further research on heterozygosity-fitness correlations in wild species by examination of 1) a larger number of markers, 2) more severe pathogens (than parasites) such as CDV and CPV, and 3) a direct fitness measure such as number of offspring per individual.

The impacts of reduced genetic variation on disease susceptibility and resistance may be difficult to quantify until the impacts become irreversible, and loss of heterozygosity at loci involved in disease resistance such as the major histocompatibility complex (MHC) is a particular inbreeding concern (Funk et al. 2006). Low rate of effective dispersal between RMNP and surrounding areas (Chapter 2) could increase local extinction risk (May 1988) in the future due to cumulative impacts of small-population processes (Hedrick 2001, Frankham 2005) and disease exposure from the surrounding matrix (Aguirre et al. 1995).

I did not find a significant overall correlation between parental relatedness and spatial overlap as predicted. Individuals might be related to most of their neighbours and show low differentiation in tolerance. Alternatively, low wolf density and high food availability (Carbyn 1980, RMNP unpub. data) might permit spatial overlap irrespective of kinship status. Improved resolution of movements using GPS collars could improve understanding of dispersal costs and benefits in isolated patches surrounded by human-dominated landscapes. I recommend future meta-analyses using multiple isolated patches. Individual animals function as habitat patches and dispersal agents for pathogens (Dobson and Grenfell 1995) and dispersal response to fragmentation might therefore have disease implications on a range of scales (Bolker et al. 1995). Future research could help clarify whether individuals affected with diseases not immediately causing mortality, such as mange, might be more or less likely to disperse.

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Chapter 4

Landscape resistance to dispersal: The Great Plains agricultural matrix

Abstract

Human-caused fragmentation can affect dispersal and gene flow in highly mobile organisms across physically-simple landscapes. Spatially-explicit population models (SEPM) permit investigation of factors believed to influence dispersal, including stochastic events such as disease epidemics. Major human-caused fragmentation and road development has occurred in the region surrounding Riding Mountain National Park (RMNP) in southwestern Manitoba, Canada. Wolves (*Canis lupus*) in RMNP now exhibit genetic differentiation from the nearest neighbouring population about 30 km away. I collected data on spatial and demographic variables believed to affect wolf survival and movement, and simulated dispersal in HexSim to investigate potential long-term effect of these factors on wolf emigration and disperser success. I predicted that road, disease, and negative attitude-associated mortality (25%, 50% and 75%) would reduce dispersal, and have cumulative effects. I simulated disease impact through mortality in wolf pups to explore possible consequences of reduced emigration. I examined mortality from roads and negative human attitudes as landscape resistance to dispersal. Mortality associated with negative human attitudes and disease mortality did not seem to affect dispersal, but increasing levels of simulated road mortality was associated with reduced dispersal distance. Scenarions with all three variables showed dispersal distances similar to that of the road mortality level included. Seven of fifteen scenarios included dispersal > 30 km. Although simulated individuals travelled

extensively, lack of straight-line movements limited net position change. This seems consistent with genetic and radio-tracking results. Pup disease mortality may have primarily compensatory effects, and thus limited impact on emigration. Even roads that do not constitute barriers (no fencing, low volume) could act as movement filters and reduce dispersal and gene flow over time. Mortality from negative human attitudes in the immediate surroundings of farm homesteads may have little effect on dispersal. Importantly, however, I could not examine whether negative experience with humans might influence movement decisions near roads and farms, potentially restricting movement in the matrix landscape despite high dispersal capability.

Introduction

Dispersal mediates individual response to altered circumstances (South et al. 2002) and is a key behavioural process with consequences at the population level (Levin 1992, Van Vuren 1998, Ferreras et al. 2004). Study of individual-level responses to human-caused landscape fragmentation can therefore help predict ecosystem change at higher scales (Levin 1992, Van Vuren 1998). Areas surrounding reserves and altered to various extents by human use, commonly referred to as the landscape matrix, play a critical role in connectivity (Franklin 1993). The matrix receives less attention than reserves and corridors and we need better understanding of its role in ecosystem conservation (Lord and Norton 1990, Franklin 1993). Particularly, we need information on how steep ecological gradients could influence movement between patch and matrix (Lord and Norton 1990, Franklin 1993).

Human-caused fragmentation occurs at multiple scales (Lord and Norton 1990, Lindenmayer and Fischer 2006) and involves co-varying factors (Levin 1992, Ewers and

Didiham 2007). The landscape matrix plays at least three important roles in biodiversity conservation, by i) providing habitat at smaller spatial scales (the small forest patches surronding farms might e.g. provide habitat for several birds and small mammals); ii) in many cases increasing reserve effectiveness (where semi-natural areas around reserves adds to effective reserve size), and iii) controlling landscape connectivity, including dispersal between reserves (Franklin 1993). The costs and benefits of dispersal decisions therefore need further study at a scale that incorporates reserves as well as the surrounding landscape (South et al. 2002). If resource competition is paramount in dispersal then individuals should move to the nearest vacant home range (Waser 1985). However, sex-biased dispersal in many organisms supports inbreeding avoidance and predicts that dispersers may bypass suitable vacancies near their natal home range (Koenig et al. 1996).

Four components of human-caused landscape fragmentation believed to be important are fragment size, isolation, edge effects and vulnerability to extrinsic disturbances (Lord and Norton 1990). Fragmentation is relative to the organism in question, and how species perceive the matrix will depend on mobility and habitat specialization (Wilcox 1980, Wiens 2001, Lindenmayer and Fischer 2006). Generalists (flexible in usable structures and diet) are generally less affected by fine scales of landscape fragmentation than specialists (narrow range of usable structures and diet) operating at the same spatial resolution (Lord and Norton 1990).

If dispersal from the local patch involves high risk (Fritts and Carbyn 1995, Van Vuren 1998, Gros et al. 2006) and philopatric individuals benefit from kin-tolerance (Watson et al. 1994, Temple et al. 2006), the costs and benefits of emigration from small and isolated populations could be affected over time. Moreover, juveniles may undertake low-risk exploratory forays inside territories of related neighbours (Lambin and Yoccoz 1998) and could benefit from biding for local vacancies in high-quality territories surrounded by tolerant relatives (Temple et al. 2006).

Fragmentation is rarely open to experimental manipulation and data on dispersal mortality and distance may involve high degree of error (Kareiva et al. 1996). Some information may be nonspatial (Kareiva et al. 1996) and parameters such as dispersal rate from a patch will, at least in part, depend on its local population dynamics (Kramer-Schadt et al. 2004). Modelling is a helpful tool in exploring consequences of factors that can not be easily separated experimentally (Levin 1992) and allows inclusion of periodic events such as disease epidemics (Haydon et al. 2002).

Most temperate landscapes are subject to human alterations (Franklin 1993), and the Great Plains is now an intensely human-managed landscape (Guertin et al. 1997). Here, the attitudes and actions of humans occupying the matrix may contribute to source-sink dynamics that can not be understood from habitat variables alone (Brook 2007). Conservation management aimed at making the overall landscape less hostile for movement is a connectivity approach which is different from that of corridors (Franklin 1993, Mladenoff et al. 1995). Spatially-explicit population models (SEPMs) permit assessments at the scale of regional landscape modifications and are therefore useful for investigating how such changes may affect dispersal behaviour (South et al. 2002).

Metapopulations resulting from recent landscape modification may be far from equilibrium (Kareiva et al. 1996, Hanski 1997). Whereas molecular studies reveal genetic consequences of successful dispersal, they seldom explain the factors influencing dispersal success (Van Vuren 1998). Wolves *(Canis lupus)* show high behavioural plasticity in food acquisition (Weaver et al. 1996) and are considered primarily limited by food availability (Haight et al. 1998). Wolves are capable of dispersing several hundred kilometres (Fritts 1983, Gese and Mech 1991, Wydeven et al. 1995). However, the large area requirements for viable wolf populations may conflict with land uses such as livestock production (Carroll et al. 2006), and wolves in some reserves rely on immigration from surrounding unprotected regions (Callaghan 2002, Villemure 2003). Human tolerance in the broader landscape may therefore be the most important factor influencing the long-term viability of wolves (Fritts and Carbyn 1995, Boyd and Pletscher 1999).

In southwestern Manitoba, Canada, wolves in Riding Mountain National Park (RMNP) show genetic differentiation from the nearest neighbouring population about 30 km away (Chapter 2). A combination of disease and human-caused fragmentation and mortality could threaten long-term survival of the Park wolf population (Carbyn 1982, Fritts and Carbyn 1995, Parks Canada 2002). High disease mortality within RMNP and human-caused mortality outside the Park could balance local recruitment and result in low dispersal from RMNP.

I investigate three factors believed to affect emigration rate and landscape resistance between patches in the matrix. I assess impacts on dispersal from simulated mortality due to 1) roads, 2) disease and 3) negative human attitudes. I predict that these factors will reduce dispersal, and also show cumulative effects. Roads, negative attitudes and disease are likely to reduce both the tendency to disperse and the success of animals that attempt to do so. For this analysis, disease reduces tendency to disperse because pup mortality leaves more resources for the remaining pack members. However, the model does not consider genetic factors that might predispose animals to leave in order to avoid inbreeding (Greenwood 1980, vonHoldt et al. 2007). Roads and human attitudes likely also influence tendency to disperse as most pack territories include the Park boundary. However, for the purpose of this analysis roads and human attitudes only affect dispersal success and not tendency to disperse.

Methods

Study Area

Reserve size and survival ability in the landscape matrix determines species persistence in many parks (Wilcove and May 1986, Newmark 1995). Because of an emphasis on balancing biodiversity protection and human use, national parks and adjoining biosphere reserves (UNESCO 2007) are valuable settings in which to examine cross-boundary conflicts between conservation and local development (Schonewald-Cox et al. 1992). The region surrounding RMNP has experienced major human-caused landscape fragmentation (Walker 2001). Agricultural development has removed forest cover to the Park edge and the RMNP boundary is now visible from satellite imagery (McNamee 1993). Several mammalian species have been extirpated from the area and RMNP is considered a wilderness “island” within an agricultural region (Carbyn 1980, Noss 1995).

The region is located at the transition of the Prairie and Boreal Plain ecozones (Environment Canada 1993), and includes Duck Mountain Provincial Park (1424 km^2) and Forest (3760 km^2, hereafter jointly referred to as the Duck Mountains) and Riding Mountain Biosphere Reserve (15 000 km^2). The biosphere reserve encompasses the core 2974 km^2 protected area of RMNP and 15 surrounding rural municipalities. Land clearance was greatest north and east of RMNP, particularly in the area that previously connected RMNP and the Duck Mountains (Walker 2001). An almost continuous forest corridor existed between RMNP and the Duck Mountains until the 1950s but only 14% remained by 1991, with intense development of farmland in the center effectively severing RMNP from other forested areas (Walker 2001). Agriculture is the dominant land use and occupies approximately 58% of the area (35% cropland and 23% rangeland); managed crown land (including parks) makes up 16% (Parks Canada 2004). Wolves have been present in southwestern Manitoba at least 5000 years (Goulet 2000). High dispersal capability and annual productivity suggest resilience to modest levels of human disturbance if refugia are distributed across the landscape within distances of approximately < 196 km (see review in Weaver et al. 1996). Hence, for wolves, the region constitutes a historically well-connected landscape with high likelihood of gene flow.

Model Variables

I collected data on spatial and demographic variables believed to affect wolf dispersal (Table 4.1), and created an initial map of the study area (Figure 4.1) using ArcView 3.3 (ESRI Inc.). Data were transferred to a raster format in ArcGIS 9.2 (ESRI Inc.) using a pixel size of 100 m. A raster calculator was then used to compute overlay of variable values. Every pixel is thus classified either as patch (suitable habitat), matrix, water, road or negative attitude (Table 4.1). This layer was converted to a bitmap using ArcView 3.3 for import to the HexSim program (previously called PATCH, Schumaker 1998) version 1.0.1.5.

Table 4.1. Variables for predicting wolf dispersal in the Riding Mountain National Park region, Manitoba, Canada.

Variable	**Description**	**States (Values)**	**Source**
Occupancy	Known species landscape occupancy	Habitat (breeding habitat); Matrix (move through, not suitable for breeding)	RMNP, Manitoba Conservation, this study
Water	Major lakes	Water No water	RMNP
Landscape fragmentation	Provincial highway network with 300m buffer	Main road No road	Manitoba Department of Transportation
Attitude	Farmer attitude expressed upon seeing species on private land, with 300m buffer	Negative attitude No attitude	R. Brook survey data (see Appendix H, Figure 1.)
Disease	Pup survival within wolf pack territories	No disease, max survival = 0.46 Disease, max survival = 0.20	Max survival from Carroll et al. 2006. Disease simulated stochastically, based on observed pup numbers (RMNP unpub. data)

Terms such as landscape and matrix may be interpreted in several ways (South et al. 2002). I here refer to *landscape* as the study area (Figure 4.1), *habitat* as areas supporting breeding, and *matrix* as the surrounding areas not supporting breeding (Wiens 1997). A *patch* is an area of habitat capable of supporting at least one breeding pair (here a wolf pack) separated by matrix from other such areas (South et al. 2002).

Occupancy

I classified national park (RMNP) and provincial park and forest lands (the Duck Mountains) as occupied habitat. These two areas represent the patches in my study area (Figure 4.1) and have been continuously occupied by wolves for many decades (RMNP and Manitoba Conservation, unpubl. data). They are protected against most forms of development and thus considered relatively stable. Moreover, I collected DNA samples within the two patches and estimated movement between them based on genotype frequencies (Chapter 2). Hence, I can compare HexSim simulation results with the number of migrants per generation (N_m) and putative dispersers identified from DNA analyses (Chapter 2). Territory persistence in the regional agricultural matrix seems strongly dependent on local human attitudes to wolves, and shooting wolves on sight remains a common practice around RMNP (Brook 2007). Almost the entire matrix is covered by a 1 mile x 1 mile grid road system, which exposes animals to vehicle collision risk and negative attitudes. I therefore classified agricultural land as unsuitable for occupancy (Carroll 2006), and simulated individuals in HexSim can travel throughout the matrix but not establish territories. Wolves at times attempt to establish territories in the matrix, but such packs (or pairs) are vulnerable to human mortality and seem to have low persistence (RMNP unpubl. data). I therefore considered it reasonable to classify agricultural lands as unsuitable for long-term occupancy.

Human-caused landscape fragmentation

The influence of roads on movement of wide-ranging animals such as wolves is complex, and depends on factors such as traffic mortality, wolf harvest management, ease of travel, and human use and attitudes (Mladenoff et al. 1995, Haight et al. 1998, Fuller et al. 2003, Whittington et al. 2005). The area surrounding RMMP now has a road density of 0.7 km of road per km^2 (Parks Canada 2004). In 2001, an estimated 10 690 km occurred within the biosphere reserve (Canadian Parks and Wilderness Society 2004). This is a 2% increase from 1948, and most of these roads have therefore been in place for over 50 years (Canadian Parks and Wilderness Society 2004). At least one access point to RMNP exists per mile of Park boundary (Parks Canada 2004). Roads reach the edge of the Park at all these loations, but with a few exceptions the roads do not extend into RMNP. There are approximately 80 km of paved roads within RMNP, most of which are Provincial Highway 10 that bisects the Park from north to south and is a main transportation route between the city of Brandon and the town of Dauphin (Parks Canada 2004). For this analysis I created a 300 meter buffer around all provincial roads, being the approximate range of a hunting rifle.

Water

Within the two patches, wolves show extensive use of certain water bodies during winter and I did not exclude these from occupied habitat. However, I considered all major lakes in the matrix as unavailable. I consider individuals not to be able to move across the large lake system connected to Lake Winnipegosis in the north-western corner of the study area (the top right corner of Figure 4.1; lakes are not shown to avoid covering data on roads and negative human attitudes). Inability to disperse across large lakes during winter provides a conservative estimate of movement, but considering the popularity of these large lakes for snowmobiling and ice-fishing and the vulnerability of wolves, I consider this classification to be realistic for the study area.

Disease

Canine distempervirus (CDV) and canine parvovirus (CPV) could negatively affect wolves in the RMNP ecosystem, and four mortalities in RMNP have been attributed to CDV (Carbyn 1982, Chapter 3). CPV and CDV may cause high pup mortalities (Mech et al. 1986, Mech and Goyal 1995, Johnson et al. 1994). Local veterinarians have recorded several cases of CPV and CDV in dogs over recent years, and unvaccinated dogs entering parks represent a continuing threat to wild canids (Aguirre et al. 1995). Although little is known about pup mortality rates, simulations suggest that 40% pup mortality reduced immigration rate between patches (Haight et al. 1998). Park staff and trappers have reported wolves with sarcoptic mange (caused by the mite *Sarcoptes scabiei*) in RMNP and the Duck Mountains in recent years. Mange infection occurs in pups (G.Goulet, pers. comm.), and a warden had to euthanize a young RMNP wolf with severe mange in January 2006 (T. Hoggins, pers. comm.).

Whereas RMNP wolves carried antibodies to CPV and CDV (Chapter 3), little is known about pup mortality due to disease in the study area. Average numbers of RMNP pups per pack are considered six or less (Carbyn 1980). However, RMNP packs are at times seen with only 1 – 2 pups (RMNP unpub. data). I have therefore used a standard value of 5 - 6 pups per pack, and then introduced reductions in survival to 1 – 2 pups per packs in order to simulate mortality due to CDV, CPV and mange. Food shortage and birth defects (congenital or hereditary) can cause similar mortality. Nonetheless, this simulation represents a means to explore, over time, the possible impacts of disease in a population not believed to be limited by food (Carbyn 1980).

Human attitudes

Dispersal can be dangerous (Van Vuren 1998), and local attitudes to wolves in the RMNP region are often negative (Brook 2007, Stronen et al. 2007, Appendix H). Furthermore, human-caused wolf mortality is high (Carbyn 1980, Fritts and Carbyn 1995, RMNP unpub. data). I included farm locations where residents had reported negative attitudes to seeing wolves on their land (n = 244) during a survey on attitudes to wolves and other wildlife (Brook 2007, Stronen et al. 2007, Appendix H). Negative

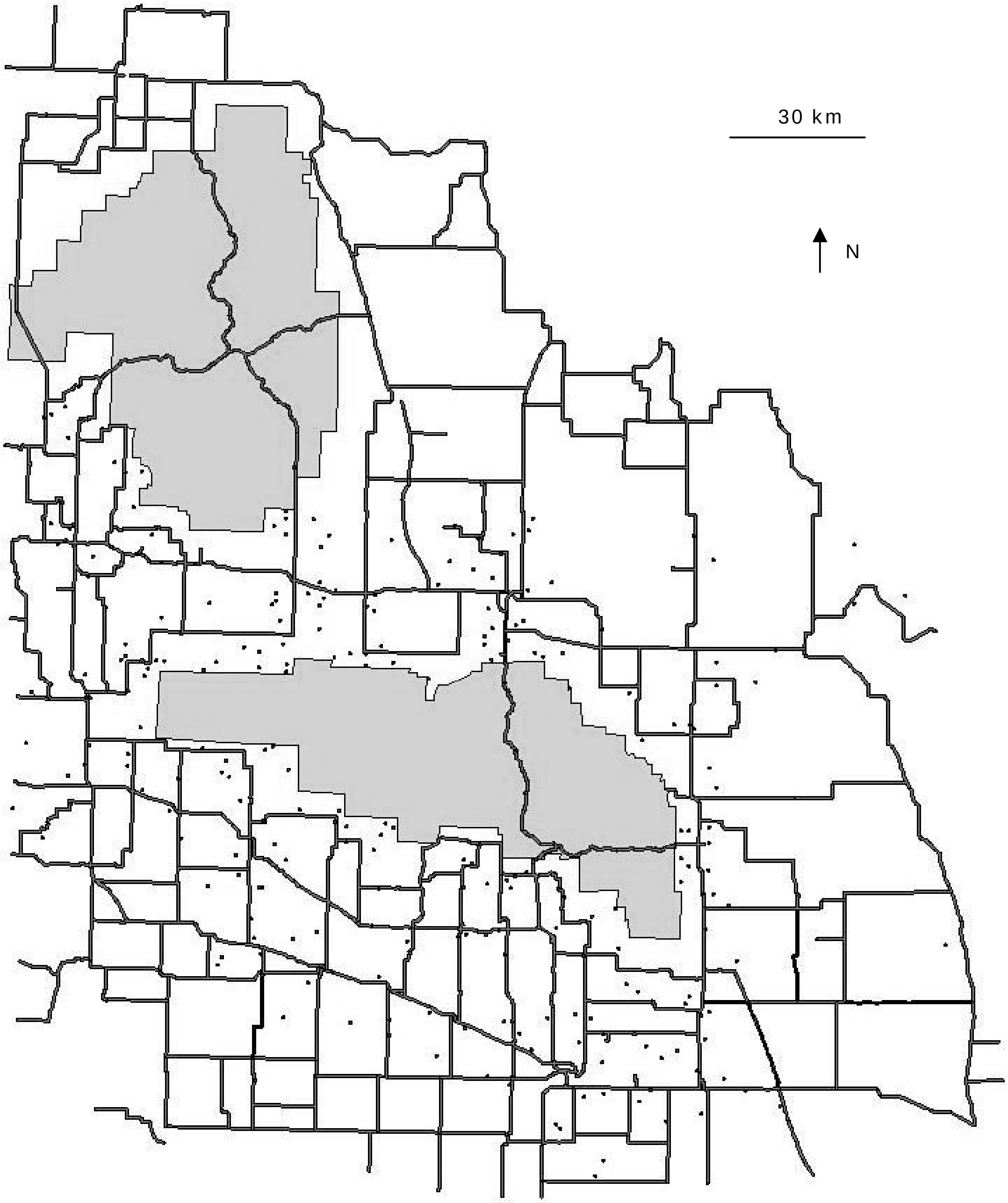

Figure 4.1. Patches (grey colour, occupied) and matrix (white colour, not occupied) in the Riding Mountain National Park region, Manitoba, Canada. Dark grey lines represent provincial highways surrounded by 300 meter buffers. Dark grey points show farms surrounded by 300 meter buffers where residents reported negative attitudes toward wolves.

attitudes do not necessarily translate into action. However, since 2001, killing of wolves in the hunting zones surrounding RMNP is illegal except in the defence of property (D. Chranowski, pers. comm). It is thus unlikely that residents would report their farm location in the survey as well as any actions potentially considered illegal. I created 300 meter buffers around UTM coordinates for farms where survey respondents reported a negative attitude, and consider this to represent the approximate range of a rifle shot from a farm yard.

HexSim simulations

I used HexSim version 1.0.1.5 to test predictions on how simulated disease mortality, occurring at different intervals, influences emigration from a patch. I also tested predictions on how mortality associated with landscape features (roads and negative human attitudes) affects dispersal success for individuals attempting to negotiate the human-dominated agricultural matrix. Finally, I combined these factors to test predictions on their cumulative impacts on dispersal.

I defined dispersal as a movement from one territory (area defended by an individual or family group) where the organism was born to another territory where it will establish and might breed (South et al. 2002). Territories are occupied by family groups (wolf packs), and individual movements can take place within and between habitat patches. HexSim is a spatially-explicit population model (SEPM) suitable for modelling effective dispersal in territorial animals, where breeding requires possession of a territory (Schumaker 1998). The classical metapopulation model requires repeated extinction and colonization of patches, and this is possible but not required in HexSim (Schumaker 1998).

The model permits examination of population viability within a landscape by combining spatial landscape data on habitat patches with information on the organism's response to various habitat types (Carroll 2003). Survival and reproductive rates are obtained from field data and published studies, and GIS habitat data are assigned weights based on survival and fecundity levels expected for each habitat class (Carroll et al. 2006). The model links survival and fecundity of territory holders [here wolf packs] to GIS data by intersecting the habitat data with hexagonal cells (Schumaker 1998). The width of one hexagon is 569 meters, and the distance between RMNP and the Duck Mountains is about 30 km or approximately 52 hexagons if travelling in a straight line. Population estimates are assumed to be in winter when pack size is at its minimum value for the year (Carroll 2003), and the breeding pulse in HexSim occurs prior to the dispersal of juveniles (Schumaker 1998). I focused on movement between the two identified patches (Wiegand et al. 2004) as long-term RMNP questions (Carbyn 1980) seem to center on why, given the abundant food, there are not a) more wolves, and b) more sign of dispersal between RMNP and nearby wilderness areas.

Spatial autocorrelation in the HexSim model determines the linearity of an individual's search path during dispersal, and 100% autocorrelation would cause movement in one direction only. However, no autocorrelation would result in random movement and exploration of sites already visited (N. Schumaker pers.comm.), which is likely to be biologically unrealistic. I therefore used a 50% spatial autocorrelation value to model the possibility of an animal continuing in the same direction.

I used a mean dispersal of 99 (± 116) km, based on average dispersal distances found from wolves in biophysically similar landscapes (Fritts and Mech 1981, Fuller 1989, Mech 1987, Gese and Mech 1991, Wydeven et al. 1995). Based on the HexSim simulations, I report 1) mean dispersal length (with standard deviation), and 2) 'mean explored area', which is defined as the exploration a disperser undertakes once it stops dispersing and sets out to establish a territory. I then report 3) 'mean position change' from start point to end location, which is often shorter than mean dispersal length if animals have spent time exploring in different directions and not moved in a straight line. Finally, I report 4) 'mean number of floaters' which represents lone wolves that do not have a territory and therefore can not reproduce (Fuller et al. 2003).

I set maximum wolf territory size (range in HexSim) as 457 km^2 (Carbyn 1980). He found RMNP mean pack size to be 8.3 (SD ± 3.8) wolves, and as HexSim is a females-only model I set a maximum number of five females per territory. Wolf population data are not available from the Duck Mountains, but based on RMNP population surveys (RMNP, unpub. data) and the similar size of the two patches, I set a total population of 70 females at the start of each simulation. I used a population with four stage classes and survival and fecundity values as outlined in Carroll et al. (2006). Most wild wolves are unlikely to live longer than 4 – 5 years (Fuller et al. 2003), and those that do probably show survival and reproduction values corresponding to the fourth stage class. Survival is 0.46 for the first year, 0.86 for the second year, and subsequently 0.96. Fecundity is zero for the first two years. Third year fecundity is 2.29 and subsequent values are 3.21.

I altered simulated pup survival rate to explore effects of disease mortality on dispersal. I simulated disease by approximately every 10th, 5th, and 3rd year allowing survival of only 1 – 2 pups per pack. Mamimum survival is thus reduced to approximately one of five pups (0.2). The model will apply this value throughout all packs on chosen years. Although mortality is likely to vary between packs within years, these simulations permit assessment of disease events that could include cumulative effects from CPV, CDV, and mange. For the purpose of the model, and because food is not considered limiting (Carbyn 1980), disease mortality in pups was considered compensatory.

I treated human attitudes and provincial highways as potential barriers to movement within the matrix. Upon encountering a barrier, the individual will either cross the barrier (transmission in HexSim), turn back (deflection from the barrier) or die (mortality). Transmission, deflection and mortality probabilities always sum to one. I was primarily interested in exploring the effects of mortality, and therefore set barrier probabilites as: Transmission = Deflection = 0.5* (1 – Mortality). I explored mortality values of 0.25, 0.50, and 0.75 [25%, 50% and 75% chance of mortality upon encountering road and negative attitude barriers]. I examined various barriers (Table 4.2) and one scenario without barriers or disease mortality. I also combined barriers and examined scenarios with disease as well as barrier mortality.

Table 4.2. Dispersal variable codes for wolves in the Riding Mountain National Park Region, Manitoba, Canada.

Variable	Description
None	No variables
A1	Attitude mortality = 0.25
A2	Attitude mortality = 0.50
A3	Attitude mortality = 0.75
R1	Road mortality = 0.25
R2	Road mortality = 0.50
R3	Road mortality = 0.75
D10	Disease mortality every 10 years (pup survival reduced from 0.46 to 0.20)
D5	Disease mortality every 5 years (pup survival reduced from 0.46 to 0.20)
D3	Disease mortality every 3 years (pup survival reduced from 0.46 to 0.20)

Local hunting, trapping, land clearing and poisoning appear to have caused a local extirpation of the RMNP wolf population around 1900 (Carbyn 1980). However, reports from Park wardens and residents confirmed that wolves had returned by the 1930s, possibly via immigration from the Duck Mountains (Fritts and Carbyn 1995). The RMNP population appears to have been reduced to around 30 animals during the 1990s (RMNP unpub. data), but has numbered approximately 70 - 75 individuals in late winter over the past 5 years (RMNP unpub. data). HexSim simulations require some time to stabilize (N. Schumaker pers. comm.). I used 3 replicates of 40 years each, and modelled periods of altogether 120 years to compare simulations to the approximate time since (assumed) extirpation of the previous RMNP population. I then examined the last 10 years of each replicate to determine variation within the period of a decade. I used SPSS 9.0 (SPSS Inc.) to examine the mean and variation in dispersal parameters for the various scenarios.

Results

Dispersal during scenarios using 25%, 50% and 75% chance of negative attitude mortality, and disease mortality (pup survival simulated as 0.2) every 10th, 5th or 3rd year were similar to scenarios based on dispersal with no barriers or disease (Table 4.3). Dispersal during scenarios with increasing road mortality (25%, 50% and 75%) was associated with decreasing mean dispersal distance and to some extent with less overall position change. Mean dispersal distance and area explored during scenarios that included all three variables (simulated disease, road and negative human attitude mortality) were similar to results from scenarios with the corresponding road mortality value (Figure 4.2).

Simulations with high road mortality (75%) showed the most impact on mean explored area. All road mortality simulations showed a marked reduction in the mean number of floaters (individuals without territories) and in most cases reduced the number of floaters to zero. Simulations with negative human attitudes also showed a reduction in the number of floaters, whereas simulations with disease mortality did not appear to have an effect on this parameter. Because these are exploratory analyses I performed no significance tests.

Table 4.3. Dispersal simulation results for spatial and demographic variables in the Riding Mountain National Park region, Manitoba, Canada. For variable codes see Table 4.2.

Variables	Mean dispersal length (km) ± std.*	Mean explored area (km^2) ± std. **	Mean position change (km) ± std.***	Mean number of floaters ± std.****
None	103 (± 3.3)	475 (± 2.2)	23 (± 1.5)	20 (± 3.6)
A1	101 (± 4.5)	436 (± 14.0)	23 (± 1.9)	10 (± 3.2)
A2	101 (± 4.7)	416 (± 19.0)	22 (± 1.5)	8 (± 2.0)
A3	101 (± 3.7)	410 (± 18.2)	22 (± 1.3)	8 (± 2.1)
R1	90 (± 4.1)	276 (± 32.0)	21 (± 1.5)	0 (± 0.5)
R2	88 (± 4.0)	256 (± 28.9)	21 (± 1.5)	0 (± 0.3)
R3	84 (± 5.6)	240 (± 41.3)	20 (± 2.0)	0 (± 0.4)
D10	104 (± 3.6)	475 (± 4.7)	22 (± 1.7)	22 (± 6.9)
D5	103 (± 3.9)	473 (± 5.8)	22 (± 1.4)	18 (± 6.2)
D3	103 (± 3.6)	470 (± 9.7)	22 (± 1.7)	18 (± 4.7)
D10A1R1	90 (± 4.9)	272 (± 26.5)	21 (± 1.1)	0 (± 0.5)
D10A3R3	81 (± 6.5)	231 (± 35.0)	20 (± 2.0)	0 (± 0.3)
D5A2R2	85 (± 4.6)	246 (± 27.1)	20 (± 1.4)	0 (± 0.4)
D3A1R1	91 (± 5.7)	264 (± 47.5)	21 (± 1.9)	0 (± 0.3)
D3A3R3	84 (± 5.7)	233 (± 37.4)	21 (± 1.5)	0 (± 0.2)

*Mean dispersal distance

**Exploration undertaken once a wolf stops dispersing and starts to establish a territory

***Mean change in distance from start to end (often shorter than actual dispersal distance)

****Wolves that do not possess a territory and therefore can not reproduce.

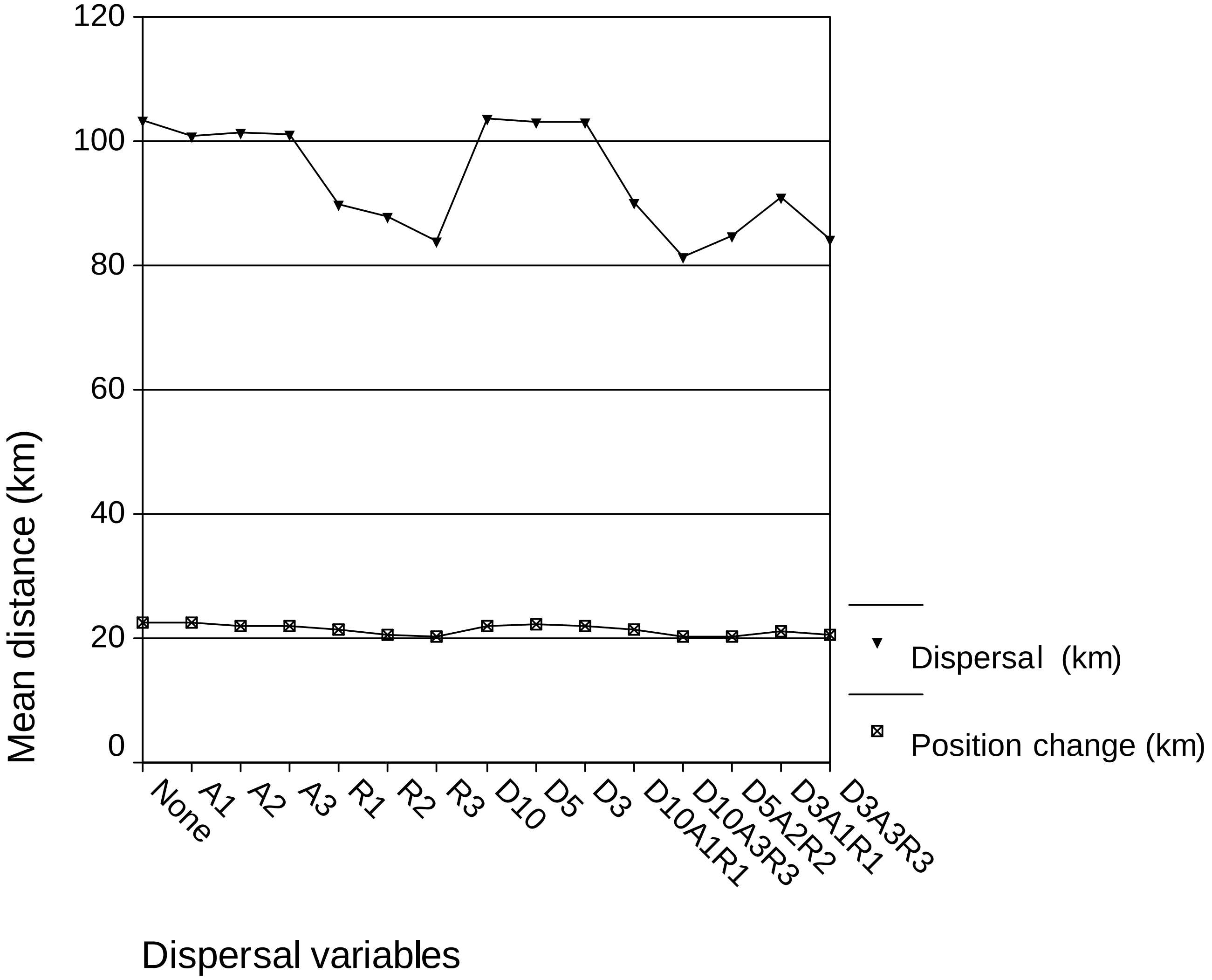

Figure 4.2. Mean dispersal length (top) and position change (bottom) in kilometers for different variable combinations simulated for the Riding Mountain National Park region, Manitoba, Canada. For variable codes see Table 4.2.

An individual can move around in the matrix for some time (several time steps in HexSim) without having travelled far from its natal patch (Figure 4.3 and 4.4). Simulated individuals had no prior knowledge of road and human attitude barriers until they encountered the features, which resulted in transmission, deflection or mortality. The road network limited the possibilities of moving in a straight line without crossing roads, and Figure 4.3 illustrates the difference between explored area and actual position change. All scenarios resulted in dispersal > 30 km, the distance between RMNP and the Duck Mountains. Mean position change did not exceed 30 km in any scenario, although dispersal events during some years did exceed this distance.

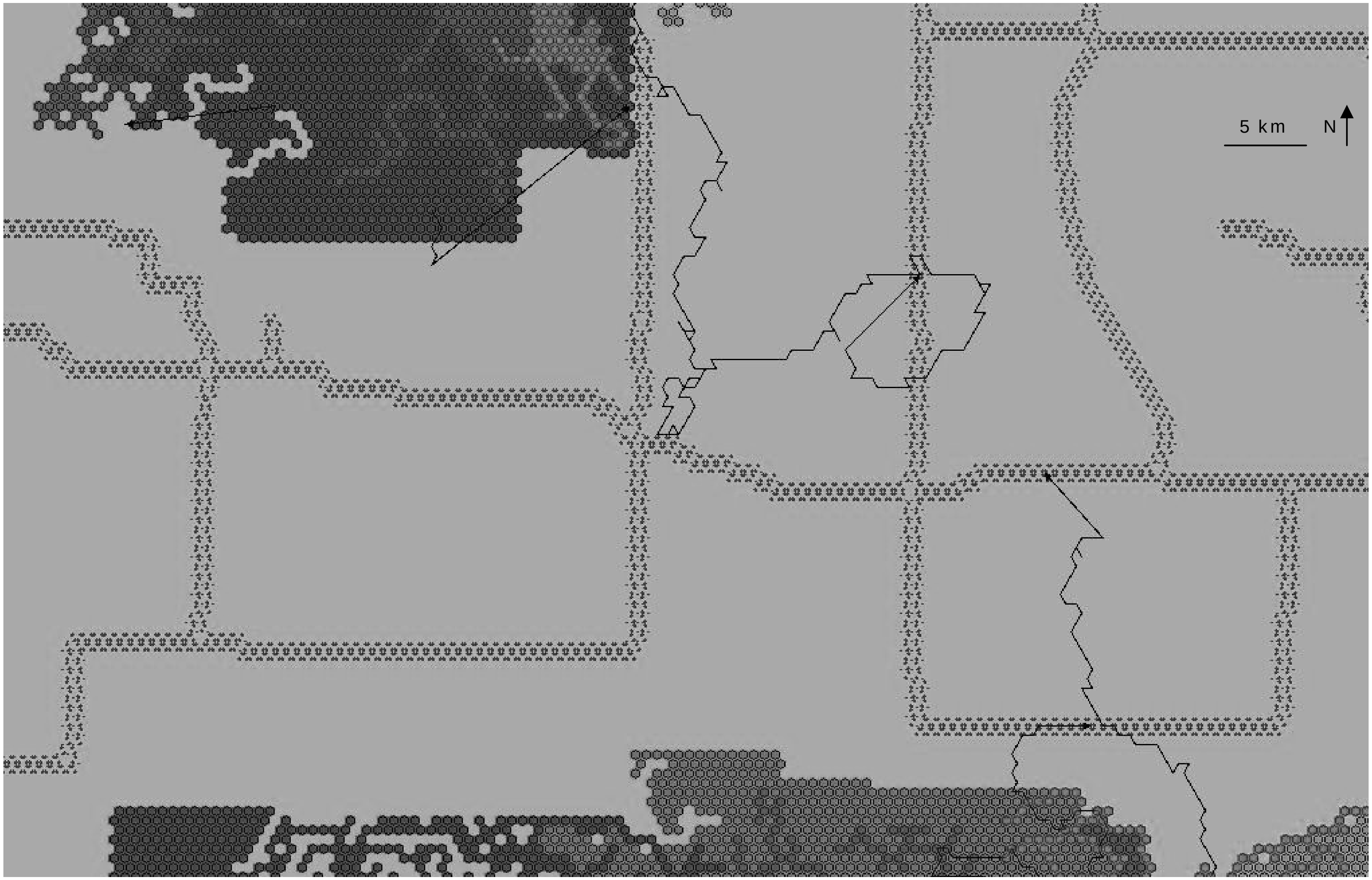

Figure 4.3. Individual exploration movement (black line) in the landscape matrix between Riding Mountain National Park (bottom dark gray shaded area) and the Duck Mountains (top gray shaded area) in southwestern Manitoba, Canada, using the HexSim simulation model. Dotted light grey lines show provincial roads surrounded by 300 m buffers.

Figure 4.4. Individual exploration movement (black line) in the landscape matrix between Riding Mountain National Park (bottom dark gray shaded area) and the Duck Mountains (top gray shaded area) in southwestern Manitoba, Canada, using the HexSim simulation model. Black dots show farms (surrounded by 300 m buffers) where residents identified a negative attitude to seeing wolves on their land.

Discussion

Predicted dispersal in the RMNP region

Although mean position change did not exceed 30 km in any scenario and thus the distance between RMNP and the nearest neighbouring wolf population in the Duck Mountains, seven of fifteen scenarios included dispersal events > 30 km. Lack of straight-line movements seems to limit net dispersal distances and the difference between wolf travel distance and net dispersal seems consistent with radio tracking results from RMNP. At least five radio-collared wolves undertook what appeared to be extraterritorial forays in RMNP during 2003 – 2005 (Chapter 3). Their net travel distances were longer than the distance between RMNP and the Duck Mountains, but although they travelled extensively within their assumed natal patch, none were found (at the time of last contact) to have left the Park.

Population differentiation estimated from genetic data (F_{ST}) was 0.074 and the number of migrants per generation (N_m) between RMNP and the Duck Mountains was 3.13 (Chapter 2). These calculations are based on equilibrium population assumptions and reflect historical processes; therefore, they may not accurately represent current gene flow (Frankham et al. 2002). The HexSim simulations may be too conservative in the estimates of net position change. Nonetheless, spatial genetic structure is evident between the two patches (Chapter 2) and more than 10 immigrants per generation might be needed to prevent genetic differentiation (Vucetich and Waite 2000). Furthermore, successful immigration does not guarantee subsequent survival and reproduction (Grewal et al. 2004, Chapter 2). Within-patch dispersal promoting local kin clusters in territorial animals could also reduce the integration of immigrant genes (Chapter 3). I identified seven immigrant individuals to RMNP (Chapter 2). Their contribution to the RMNP gene pool is inconclusive, but caution should be used when translating movement into gene flow. Importantly, new genetic techniques can detect the immediate consequences of removing as well as adding barriers to dispersal (Waser and Strobeck 1998). However, even if these seven individuals represent recent gene flow, landscape fragmentation has been able to cause genetic differentiation between RMNP and the Duck Mountains over the past decades (Chapter 2). Whereas a portion of the Park edge appears to delineate the boundary for most RMNP wolf pack territories, the seven individuals were all found close to the boundary. This area has long been considered relatively marginal habitat (Carbyn 1980), and wolves near the boundary have been killed by shooting, (coyote) trapping and poisoning during the course of this study (RMNP, unpub. data).

The value of these simulations is more in the relative comparison of scenarios than in actual parameter values predicted such as mean dispersal distance. Because no RMNP wolf dispersal has been documented (Chapter 1), I did not have actual dispersal data with which to test the simulation results. Nevertheless, I consider my results useful for evaluating the relative importance of potential mortality factors in the local landscape. My simulations suggest that human features such as roads can reduce dispersal distances across physically simple landscapes such as the Great Plains, which have few obvious barriers to the movement of large mammals. Below I discuss the results from each simulated mortality factor.

Disease mortality

The results suggest that simulated mortality from disease alone did not have obvious effects on dispersal. Mech and Goyal (1995) found an inverse relationship between CPV titers in a Minnesota population and pup recruitment the following year. They suggest that CPV mortality could be compensatory, and that more pups likely die from starvation in years with low or no disease mortality. Disease mortality alone might therefore have limited influence on dispersal. Haight et al. (1998) found that two immigrants per year were sufficient to maintain high site occupancy in a spatially non-explicit simulation model with 40% CDV pup mortality. Nonetheless, they concluded that in addition to prey availability, long-term trends in factors such as human-caused mortality and immigration are important for the survival of disjunct populations. Compensatory disease mortality could result in less overall emigration from patches during some years, as there would be less competition for resources within packs (Waser 1985). Hence, additional food would likely be available to low ranking subadult wolves in packs with few pups. Both philopatric behaviour (Chapter 3) and disease mortality in pups could lead subadult wolves to remain longer in their natal packs.

Roads as measure of human-caused landscape fragmentation

Simulated road mortality was associated with reduced dispersal distances, as well as area explored after individuals stopped dispersing. The low quality of matrix habitat means that individuals exploring this portion of the study area will tend to explore widely, and hence frequently encounter roads. Roads will therefore necessarily interrupt exploration of this low-quality habitat, although this may not have much impact on the overall population (N. Schumaker, pers.comm). Although wolf mortality due to motor vehicle-collisions has not been frequently reported in the RMNP region, this is a major mortality source in other areas (Callaghan 2002, Whittington et al. 2005). I believe that road mortality in the matrix surrounding RMNP region is more likely to be associated with animals near roads being shot from vehicles than being hit by vehicles. Moreover, such mortality is likely to remain unreported. Although this mortality would be caused by negative human attitudes, it differs from mortality around farm yards in that the latter more easily can be argued to represent defence of property, which is permitted (Stronen et al. 2007, Appendix H).

The actual permeability related to features such as negative human attitudes, roads and agricultural landscapes are uncertain, as are behavioural considerations such as the possibility of long-lived organisms learning to avoid human features (Whittington et al. 2005). Findings from similar landscapes suggest that more positive human attitudes allow wolves to live with higher road density than first thought (Fuller et al. 2003). The high road density combined with lack of tree cover in the matrix surrounding RMNP likely reduces wolf movement, particularly for individuals that might have learned to associate roads, humans and vehicles with danger (Whittington et al. 2005).

A reserve system positioned within the dispersal capabilities of a species will still fail if the majority die during the attempt to move between reserves (Van Vuren 1998). In national parks and surrounding areas in the Canadian Rocky Mountains, human-caused death constituted 75% of total mortalities and 67% of these were outside protected areas (Callaghan 2002). Moreover, wolves in territories entirely within reserves survived longer than wolves in territories spanning reserve boundaries (Callaghan 2002). Rivard et al. (2000) concluded that Canadian national parks do not yet seem to have suffered evident species loss from habitat loss or fragmentation, but that this could change in the future due to lagged response or further landscape change.

Negative human attitudes

The zone in which simulated negative human attitudes influence wolf mortality occupies a small portion of the matrix, and my results do not suggest that negative human attitudes reduced dispersal success. Nonetheless, negative attitudes to wolves are likely underreported, as the survey only involved active farm operations and not other residents, and many farmers did not return the survey (R. Brook, pers. comm.). Whereas animals probably have a higher risk of being shot when approaching farms, negative attitudes are not limited to a 300 m buffer around farmyards. Hence, negative human attitudes are likely to affect wolves across more of the matrix area than what I have simulated in these analyses. Human attitudes could therefore be an important factor for animal movement around RMNP over the long term (Brook 2007). Wolf survival in rural agricultural areas is disproportionately influenced by the actions of people who depend on the productivity of the landscape for their livelihood (Musiani et al., 2004). Conservation plans for the agricultural matrix must consider local human attitudes to wildlife, and negative attitudes to some species could reduce the value of otherwise suitable habitat (Brook 2007).

Survival of carnivores is often relatively low, and most mortality is caused by humans through hunting, trapping and vehicle mortality (Van Vuren 1998). Although 51% of farmers in the RMNP region felt they had never experienced serious damage from wolves, 44% of all farmers surveyed did not enjoy seeing wolves on their land (Stronen et al. 2007, Appendix H). They had noticeably different attitudes toward other wild species: despite similar reports of no serious damage by elk (55%), deer (50%) or moose (60%), only 15%, 8% and 10% of farmers, respectively, disliked seeing these three species on their land (Brook 2007). A review of 38 North American and European quantitative studies during 1972 – 2000 on attitudes toward wolves across social groups also shows that rural residents and farmers and ranchers had the lowest percent positive attitudes to wolves (Williams et al. 2002). Wolf observations reported by residents in the RMNP matrix suggest that forest cover was a better predictor of wolf occurrence than proximity to protected areas (Brook 2007). Forest cover has been considerably reduced in the RMNP region during the past 50 years (Walker 2001). This increases the vulnerability of wolves to negative attitudes where such attitudes might exist, as lack of tree cover augments the likelihood that wolves are seen and shot at by humans. Future land clearing for agricultural, residential or other purposes may therefore increase risk of human-caused mortality of wolves and other wildlife species in the RMNP region.

Overall assessment of HexSim simulations for the RMNP region

Uncertainties in parameters such as dispersal distances can significantly influence model predictions (Kareiva et al. 1996), and accurate estimates of dispersal distances are difficult to achieve even for common species (Van Vuren 1998). Inappropriate representations of dispersal can include disregarding organism 1) knowledge of local surroundings, 2) dispersal decision making, and 3) response to the landscape (South et al. 2002). I explicitly chose a model organism where distance between patches would not be a limiting factor, as wolves can move over 70 km in a day (Mech and Boitani 2003). Whereas models present simplifications of reality, there are advantages in carefully investigating the behaviour of a relatively simple model (South et al. 2002). I examined a physically-simple landscape without obvious dispersal barriers for large mammals, and wolves in the RMNP region do not appear limited by food (Carbyn 1980, RMNP unpub. data).

Spatially-explicit population model (SEPM) connectivity depends on the quality of source habitat and on landscape permeability (Carroll et al. 2006). For the RMNP region, there are uncertainties in both factors. At least three radio-collared wolves from this study died from human causes (shooting, coyote trapping) in the matrix. For nine wolves, the radio signal was lost or the collar malfunctioned. I do not know if these wolves survived and whether any may have dispersed, but extensive aerial searches were unsuccessful. All simulation scenarios resulted in mean dispersal distances of > 30 km, but lack of straight-line movements seems to limit net position change, which appears consistent with radio-tracking results for at least five RMNP wolves (RMNP unpub. data) and the lack of documented dispersal between RMNP and the Duck Mountains (Chapter 1). The model results appear to suggest less movement between patches than the genetic estimates (Chapter 2) and may therefore be too conservative. Nonetheless, not all immigrants survive and establish or join packs in their new location, and the effective number of migrants will therefore be smaller than the observed number of animals assigned immigrant status (Chapter 2).

Overall, the simulations suggest that mortality due to human features such as roads is capable of reducing dispersal between patches within physically-simple landscapes, even where patches are separated by relatively short distances (Weaver et al. 1996). Even roads that do not constitute barriers (no fencing, low volume) could thus act as filters to gene flow over time. Mortality from negative human attitudes in the immediate surroundings of farm homesteads may have little effect on dispersal.

Importantly, however, I could not examine whether negative experience with humans might influence movement decisions near roads and farms, potentially restricting movement in the matrix landscape despite high dispersal capability. Pup disease mortality may have primarily compensatory effects, and thus limited impact on emigration and gene flow. However, adult wolves can also die from Canine Distemper (Chapter 3). The potential effects of isolation, small population size and rapid evolution of new strains in Canine Parvovirus since its recognition in 1978 (Barker and Parrish 2001) also suggests that an interaction between disease and fragmentation could affect gene flow over the long term.

I here investigated factors believed to affect both emigration and landscape resistance between patches in the agricultural matrix, even if there are insufficient data to test the model at this time. Although the study is exploratory, my results indicate that persistence of small populations is uncertain in patches surrounded by a potentially high-risk matrix. The results might inform future data collection and testing of alternative models (Stephens et al. 2005), and in Chapter 5 I further explore possible evolutionary responses to the landscape matrix. Although large scale studies are needed to gain better understanding of the effects on real-world populations, fragmentation is rarely open to experimental manipulation (Kareiva et al. 1996). Future HexSim model development will permit further study of the sensitivity of barrier mortality. Increased resolution of life history events including reproduction, survival, and aging will also allow further simulations of how disease might influence emigration from a patch and dispersal behaviour.

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Chapter 5

Synthesis and Conclusions

The influence of movement barriers on dispersal and gene flow is relatively well studied. However, the effects of human-caused fragmentation in physically-simple landscapes: areas where movement barriers and natural ecological discontinuities (e.g. climate, mountain-lowland transitions, prey distribution) can, as far as possible, be eliminated as causes for population genetic structure, is less understood. I tested the prediction that human-induced fragmentation of a physically-simple landscape can reduce dispersal and gene flow, resulting in genetic population structure. I then assessed possible evolutionary consequences of human-induced landscape change and potential consequences of within-patch dispersal and high relatedness in normally outbred and vagile organisms (wolves) within patches embedded in the matrix. Finally, I explored predictions regarding factors believed to affect movement in the landscape matrix and, therefore, effective dispersal between patches, using a spatially-explicit simulation model. My findings showed that:

- Microsatellite genetic structure is evident ($F_{ST} = 0.074$) and consistent with fragmentation. Previous information on gene flow in the study area is not available, but landscape data and the ecology of the study organism suggest that gene flow was high prior to the 1950s. Neither mutation nor natural selection is expected to have contributed significantly to structure at this spatiotemporal scale (30 km, 60 years). Although ecological or behavioural factors (including prey distribution and natal habitat-biased dispersal) or localized selection influenced by diseases such as Bovine TB might now influence gene flow, such potential influences are unlikely to have become established without prior fragmentation. Hence, human-caused fragmentation of a physically-simple landscape can reduce gene flow and cause cryptic genetic population structure in vagile organisms on fine spatiotemporal scales. Previous studies have shown moderate short-distance genetic structure in mobile species such as wolverines, lynx and coyotes in areas without obvious barriers (Chapter 1). However, natural and/or long-standing ecological discontinuities, potentially combined with the shy nature of some species and subsequent low tolerance of humans, played a major role or could not be excluded. This study contributes new knowledge on the spatiotemporal effects of human-caused fragmentation, by showing moderate genetic structure in a vagile species that is 1) not influenced by barriers or natural/historical ecological discontinuities in the study area, and that is, if tolerated by humans, 2) able to live relatively close to human settlements. These findings are significant, by showing that human-caused fragmentation can have more profound consequences for gene flow that previously thought. Importantly, this influence can act rapidly. Conservation planning for wide-ranging and low-density species in physically-simple landscapes modified by human development should thus include more conservative predictions of gene flow to isolated sites.

- There is to date no evidence of interbreeding between the three canids in the study area (gray wolves *C. lupus*, coyotes *C. latrans* and the proposed eastern wolf *C. lycaon*). However, I found one RMNP faecal sample with an mtDNA haplotype intermediate between canid types.

- Individual parental relatedness and homozygosity was not significantly correlated with parasite burden. Allele sharing was not significantly correlated with overall spatial overlap, but the relationship was negative in individuals tracked > 1 year. Spatial overlap with genetically dissimilar individuals in patches surrounded by a landscape fragmented by humans might help balance dispersal risk and inbreeding avoidance.
- Spatially-explicit population modelling suggest that even roads that do not constitute barriers (no fencing, low volume) could act as movement filters and reduce dispersal and gene flow over time in physically-simple landscapes such as the Great Plains.

The primary contribution of my research is to show that human-caused fragmentation of physically-simple landscapes without dispersal barriers can limit gene flow in vagile organisms on fine spatiotemporal scales. Loss of genetic variation through drift therefore presents a higher risk to the maintenance of long-term genetic integrity and evolutionary potential in small and isolated populations than first thought. Predicted effects of human landscape modifications (e.g. residential and agricultural development) on fine spatiotemporal scales can be quantified and incorporated into both theory and conservation practice. Detection of cryptic genetic structure can therefore function as an early indicator in the long-term management of vagile and low-density species. Human tolerance of wolves is often limited, and many local residents may feel that there are ‘too many wolves’ (Stronen et al. 2007, Appendix H) while effective dispersal in the landscape matrix remains low (Chapter 2). This study demonstrates that these two situations can occur at the same time, which has important implications for long-term conservation of carnivores. Low-density and vagile species should therefore be conserved and managed with increased attention toward the need for gene flow to supply isolated sites within the landscape matrix. As an example, I discuss this further in Appendix J, which outlines my conservation recommendations submitted to Parks Canada, provincial conservation managers, local trapper and hunter associations and other residents. In this chapter, I summarise my findings and discuss how the agricultural matrix might influence dispersal and ecological niche over time.

Effects of human-induced landscape change

Human-induced landscape change is now pervasive (Ashley et al. 2003, Despommier et al. 2007). This can influence individual dispersal decisions and therefore, over time, genetic population structure (Van Vuren 1998). Humans alter landscapes by rescaling patterns in space and time (Urban et al. 1987), and the results raise fundamental questions on the human role in selection (Ashley et al. 2003). Recent examples are harvest effects on size of sheep (Coltman et al. 2003), bill modification in birds after food source extinction (Smith et al. 1995), and plant adaptations to pollution (Bone and Farres 2001). I examined the Riding Mountain National Park (RMNP) and Duck Mountain Provincial Park and Forest (hereafter the Duck Mountains) region in southwestern Manitoba, Canada. The Great Plains is now highly modified by humans (Guertin et al. 1997), and only forest fragments remain in the RMNP region (McNamee 1993, Walker 2001) at the intersection of the Great Plains and the boreal forest (Environment Canada 1993).

I used gray wolves *(Canis lupus)* as a test organism due to their wide range (Kurtén and Anderson 1980), high dispersal ability (Fritts 1983, Wydeven et al. 1995), behavioural plasticity in food acquisition (Weaver et al. 1996, Haight et al. 1998) and sensitivity to human tolerance in human-dominated landscapes (Fritts and Carbyn 1995, Boyd and Pletscher 1999, Carroll et al. 2006). Mammalian carnivores within the *Canidae* family are also valuable for examining human-induced change, as recent research has raised questions concerning the history, future, and role of central and eastern North American canids (Lehman et al. 1991, Roy et al. 1994, Wilson et al. 2000, Kyle et al. 2006, Leonard and Wayne 2007). These studies suggest that hybridization has become common after coyote immigration. Although the canids believed to be involved vary regionally, they include gray wolf, coyote *(C. latrans)*, red wolf *(C. rufus)*, and eastern wolf *(C. lycaon)*[5]. This has created a conservation challenge (Fascione et al. 2001, Allendorf et al. 2001, Grewal 2001, Murray and Waits 2007).

Canid niche and the emerging concerns over infectious disease in wild ungulates (Tessaro et al. 1990, Williams and Miller 2002, Lees 2004) raise further questions regarding long-term effects of human-modified selection within the matrix. Isolated patches at the edge of a species' distribution range can preserve important predator – prey relationships (Carbyn 1980). Dispersal and selection at range margins take on special importance (Bridle and Vines 2006), and I summarize my findings in view of the current wolf ecological niche as a pursuing predator of large ungulates. The discussion springs from two questions I have asked during the years of fieldwork in the RMNP region:

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[5] I refer to *C. rufus* in the eastern US (Phillips et al. 2003), *C. lycaon* in Canada from Quebec to Manitoba (Wilson et al. 2000) and *latrans – lycaon* hybrids (Tweed wolves, eastern coyotes) for southern Ontario (Kolenosky and Stanfield 1975, Grewal 2001, Grewal et al. 2004, Sears et al. 2003).

1) Why have we no evidence of wolf – coyote hybridization, if wolf-coyote hybrids are successful in the agricultural matrix in other regions?

2) If human-induced changes modify current wolf niche, which ecological consequences might this have for predator – prey relationships and infectious disease transmission?

Human-caused landscape fragmentation can increase selection for long-distance dispersal (Wiens 2001), but also facilitate selection for adaptation to local conditions (Ashley et al. 2003). Nuclear microsatellite alleles can identify fine-scale population structure (Sunnucks 2000), which is now evident in the RMNP region (Chapter 2). Moreover, divergence between RMNP and Duck Mountain wolves appears high considering the distance of less than 30 km ($F_{ST} = 0.074$, $N_m = 3.13$) and seems consistent with the landscape matrix acting as a filter to dispersal.

Local adaptation of dispersal strategies seems possible in patches above a certain size (Gros et al. 2006) and could affect RMNP (Carbyn 1980). Philopatry can eliminate dispersal risks (Bekoff et al. 1984) and, for RMNP wolves, particularly the threat of being killed by humans in the agricultural matrix (Fritts and Carbyn 1995, Brook 2007). I tested whether increased relatedness might be correlated with high parasite burden (Acevedo-Whitehouse et al. 2003) and high tolerance to relatives in territorial organisms (Lambin and Yoccoz 1998). I found examples of heavy parasite burdens and high relatedness, but no significant correlations. However, I could not assess potentially more severe diseases such as Canine Distempervirus in the study area. Nuclear allele sharing was not significantly correlated with home range overlap, and for some wolves I found a trend toward a negative relationship. Though speculative, individuals in patches isolated by a high-risk matrix (Fritts and Carbyn 1995) might seek to minimize inbreeding (Smith et al. 1997, vonHoldt et al. 2007) by exploring home ranges within their natal patch where occupants are maximally genetically differentiated from themselves.

Maternally inherited mtDNA markers are well suited for taxonomy (Sunnucks 2000) and I examined mtDNA haplotypes from Manitoba and Prince Albert National Park, Saskatchewan, and compared these with canid haplotypes from other regions of North America and Europe. Most Manitoba and all Prince Albert wolf haplotypes grouped with *C. lupus*. *C. lupus* and *C. lycaon* co-occur in the Duck Mountains, but *C. lycaon* seemed absent or rare in RMNP. However, one RMNP haplotype was close to *C. lycaon, C. latrans* and *C. rufus*. No *C. lycaon* haplotypes were found in Prince Albert. Possibly, *C. lycaon* type genes might be moving westward but have yet to reach this region.

I conducted no experiments and my results provide correlative evidence only. Moreover, differences in neutral molecular markers do not necessarily reflect adaptive differences with implications for conservation management (Hedrick 1999, Crandall et al. 2000). Divergent adaptive selection in the study area, perhaps related to disease resistance, might limit effective dispersal. However, I found genetic population clusters across a simple landscape and at a distance my test organism can travel in one day (Mech and Boitani 2003). Wolf ecology suggests that genetic differences between animals in neighbouring patches at this spatial scale are unlikely to have become established without prior fragmentation (Chapter 2).

Dispersal modeling did not suggest that simulated mortality from roads, negative human attitudes and disease had additive effects (Chapter 4). Most scenarios showed dispersal events longer than the 30 km separating RMNP and the Duck Mountains, but net dispersal distance (net position change between natal territory and end point in the simulated movement) rarely exceeded this distance. Although the current rate of gene flow is not known, the model estimates for between-patch dispersal appeared to be too conservative (Chapter 2 and 4). Nonetheless, exploratory behaviour with frequent changes of direction and limited net movement (Chapter 4) seem consistent with RMNP radio-tracking results (Chapter 3).

If the Park was still well connected to neighbouring wilderness areas I would not expect genetic population structure at this spatial scale. My main findings are not that RMNP wolves are genetically different from wolves 30 km farther north in the Duck Mountains, but, more importantly, that human modification and fragmentation of a physically simple landscape seems capable of influencing dispersal and genetic structure even in highly mobile animals.

Intensely human-managed landscapes such as the Great Plains (Guertin et al. 1997) raise key questions about adaptation to human-dominated environments (Allendorf et al. 2001, Reznick and Ghalambor 2001, Ashley et al. 2003, Despommier et al. 2007) as rapid evolution toward coexistence with humans may at times be the only option left (Ashley et al. 2003). Dispersal-related traits may evolve slowly (Bone and Farres 2001), although further understanding is needed on selection for reduced dispersal rate and distance (Cody and Overton 1996). Such possible adaptations to landscapes fragmented by humans require further long-term research. The mtDNA haplotype distribution also indicates that interactions between genotype, ecology and niche merits further study. An important question for long-term conservation of canids and other taxa is not only *if* hybridization occurs, but *why* this is taking place and how current processes might influence local ecosystems. Environmental conditions facilitating canid hybridization are poorly understood (Wayne and Vilà 2003) and could influence ecological relationships (Fascione et al. 2001, Kyle et al. 2006). Adaptation to human-modified landscapes may therefore provide additional habitat, but also initiate niche modifications with long-term implications for local ecosystems.

Canid taxonomic relationships on the Canadian prairies

Dispersers to RMNP will likely arrive from the immediate surroundings, although long-distance dispersers have been recorded in the region (Fritts 1983) and could reach RMNP. The Duck Mountain *C. lycaon* haplotypes found to date (Wilson et al. 2000, Chapter 2) are positioned between *C. lupus* and those identified as *C. rufus* and *C. latrans*. It is nonetheless unclear whether *C. lycaon* and *C. lupus* haplotypes imply biological differences in Duck Mountain wolves. Smaller wolves have been reported by some residents, but I found no obvious differences in skull features between the two areas. However, canid hybridization is a future concern for RMNP (Carbyn 1980). Why hybridization has not been documented to date is uncertain, and could be influenced by genetic factors as well as ecological niche (Templeton 1989, Crandall et al. 2000).

There is inherent difficulty in delineating species when they must, at the same time, be treated as evolutionary entities that are constantly adapting to their environment (Hey et al. 2003, Isaac et al. 2004). When assigning conservation status for geographically separate but genetically similar units, it is important to assess possible local adaptations as well as genetic uniqueness caused by drift (Murray and Waits 2007, Kyle et al. 2007). RMNP and Mexican wolves *(C. l. baileyi)* have shown specific mtDNA haplotypes (Wayne et al. 1992). Nonetheless, the Mexican wolves' presently isolated status and genetic differentiation from other gray wolves reflects human-induced changes that could be mitigated by again allowing intergradation with northern populations (Leonard et al. 2005). Yellowstone wolves have dispersed into Colorado (Smith and Ferguson 2005, p.184), and the Mexican wolf and northern gray wolf population ranges might naturally re-connect in the future. Genetic differences due to drift would then likely diminish, whereas differences due to local adaptation (such as resistance to local parasites) have a higher likelihood of persistence, particularly during modest levels of gene flow (Hedrick 2001).

If eastern wolves now occur around the Great Lakes and in western Manitoba (Wilson et al. 2000, Chapter 2) and have resided in the region > 100 years (Leonard and Wayne 2007), then the apparent rarity of these haplotypes in RMNP (Chapter 2) and Pukaskwa National Park (Grewal 2001) is unexpected. If *C. lycaon* arrived in Manitoba and the Great Lakes region via natural range expansion (Kyle et al. 2006) with ability to hybridize with both gray wolves and coyotes (Grewal 2001), its potential to bridge the ecological niche between sympatric *C. lupus* and *C. latrans* merits further research. The matrix landscape appears to favour *C. latrans* and *C. lycaon - latrans* hybrids (Gier 1975, Lehman et al. 1991, Sears et al. 2003, Kyle et al. 2006). The seven putative immigrants to RMNP that I identified were in forested areas adjacent to the southern Park boundary and may have been lone wolves (Carbyn 1980, Chapter 2), which could have several important consequences. First, dispersal to RMNP does not guarantee reproduction, and at least one immigrant was killed by conspecifics (Chapter 2). In organisms that defend territories, aggression toward trespassers can reduce effective dispersal between small populations in isolated patches (Chapter 2). With current inbreeding (RMNP $F_{IS} = 0.084$, Duck Mountain $F_{IS} = 0.074$) and a potentially inverse relationship between allele sharing and spatial overlap, wolves residing near the Park boundary might in the future choose to mate with any unrelated hybrids.

Random choice of dispersal direction can cause high losses for reserves located at the periphery of an organism's range (Van Vuren 1998). RMNP is at the edge of the Great Plains and dispersers heading south have limited chance of finding a mate. Several recent mortalities (RMNP unpub. data) suggest that the area south of RMNP is now a dispersal sink. Dispersers that can not find a mate of the same species might also choose to pair with a hybrid.

Canids in the landscape matrix: Implications for ecological niche?

Templeton's (1989) cohesion species concept emphasizes the distinction between genetic exchangeability (gene flow) and ecological exchangeability (shared ecological niche), and the role adaptation and natural selection play in the latter. Coyote range benefited greatly from human agricultural expansion (Gier 1975), and their niche overlaps with wolves in the RMNP area where both prey on medium-sized species such as beavers and invasive white-tailed deer (hereafter deer, Meleshko 1986, Paquet 1992). Coyote-like morphology and medium- to small-prey diet was correlated with fragmentation and road density in *C. lycaon – latrans* hybrids in southern Ontario (Sears et al. 2003), and similar landscape types occur around RMNP (Walker 2001).

Reproductive success linked to food choice in birds was found to be higher in individuals combining adaptive traits from parental bird species (Good et al. 2000), and a hybrid buffer zone could have played a major role in swamping the remnant *C. rufus* population in the southeastern US (Gier 1975). Hybrid canids preying primarily on deer and beaver (Sears et al. 2003) could thus become superior competitors in the RMNP region matrix due to higher reproductive success (Arnold and Hodges 1995, Good et al. 2000). This might extend into RMNP if hybrids have higher fitness than either parental species within the Park and swamp the local population (Arnold and Hodges 1995, Bridle and Vines 2006).

Moderate-sized wolves previously common on the prairies [*C. lupus nubilus*] preyed on the largest North American ungulate, bison *(Bison bison)*, which suggests the relationship between canid size and prey type may not be direct (Nowak 1983). Moreover, wolves in Mauricie National Park in Québec are considered eastern wolves, but can be relatively large and prey primarily on moose (Villemure 2003). The extent to which a hybrid canid might prey on elk is nevertheless uncertain. Forbes and Theberge (1996) suggest that smaller Algonquin wolves may be ineffective predators on moose and, hence, focus on deer and beaver. Deer appear much more common than elk and moose in the matrix surrounding RMNP (Brook 2007) and many farmers feel the beaver population in and around the Park is too high (Stronen et al. 2007, Appendix H). Hybrids focusing on medium- and small size prey could therefore potentially be highly successful in the matrix. Due (at least in part) to prey choice, hybrids would also be predicted to show coyote-like behaviour such as smaller group and territory sizes, which facilitate living near humans (Gier 1975).

Human-modified ecotones are increasingly associated with infectious disease (Daszak et al. 2000, Despommier et al. 2007). Infectious diseases in elk and deer have major human health and economic implications (Tessaro et al. 1990, Simonetti 1995), and include bovine tuberculosis *(Mycobacterium bovis*, hereafter bovine TB*)* in Canada (Nishi et al. 2006), and brucellosis *(Brucella abortus)* and Chronic Wasting Disease (CWD) in the US and Canada (Thorne and Herriges 1992, Williams and Miller 2002).

Elk, deer and cattle have been diagnosed with bovine TB in the RMNP region (Lees 2004, RMNP, unpub. data). Wolves are primary predators on elk in this area and conserving predation processes is vital for Park ecological integrity (Parks Canada 2002). Disease can predispose ungulates to wolf predation (Joly 2001, Williams and Miller 2002, Krumm et al. 2005), and the two could interact in affecting large ungulate abundance (Joly and Messier 2004).

Wolf predation could remove CWD-infected deer from a population more effectively than human harvest, as the latter is more likely to be random in respect to presence of disease (Krumm et al. 2005). Human health concerns may also erode hunting participation in disease-affected areas, with possible negative effects for free-ranging cervid populations (Williams and Miller 2002). Farmers around RMNP indicate that elk come out to the farmland to calve and seek protection from predation (Carbyn 1980, Stronen et al. 2007, Appendix H), which could increase disease transmission risk between wild ungulates and livestock. The agent causing bovine TB has also been associated with infections in humans (Despommier et al. 2007), and multi-drug resistant strains can pose serious threats to public health (Samper et al. 1997).

Nonetheless, negative attitudes toward wolves are common around RMNP (Brook 2007). Though 26% of farmers felt the regional wolf population was too high, 60% of these farmers were extremely concerned about bovine TB in wild elk, which suggests the potentially mitigating role of predators in limiting spread of disease may not be widely recognized (Stronen et al. 2007, Appendix H). Although co-evolution between ungulates and their pursuing predator may only date to the Pleistocene (Janis and Wilhelm 1993), predation has played a major role for modern ungulates (Dawkins and Krebs 1979).

Only two RMNP wolves have been diagnosed with bovine TB (Carbyn 1982), suggesting that adaptive disease resistance might influence genetic diversity (Altizer et al. 2003) and local predator – prey interactions. Whereas wide-ranging predators will need to negotiate the agricultural landscape to supply isolated reserves such as RMNP, their presence could also play a subtle role within the matrix. The scale of many predator – prey relationships do not easily allow experimental manipulation, and the role of a pursuing predator is difficult to elucidate in regions that have not experienced its extended absence.

Speciation processes are one of the least understood major features of evolution (Schluter 2001), and new species taxa can be considered hypotheses that might be supported with new data or require future revision (Hey et al. 2003). Templeton's (1989) cohesion species concept suggests that a group of organisms can share both genetic drift and adaptations through the processes of genetic exchange and ecological equivalence (Hey et al. 2003). Increasing homogenization of the matrix landscape likely relaxes divergent selection (Seehausen et al. 2008). Interbreeding canids in the RMNP region could therefore converge on a form intermediate in size between wolves and coyotes, which feeds on medium sized prey such as white-tailed deer and beavers abundant in agricultural landscapes. If this should be the case, human landscape change might create a feedback-loop between gene flow and shared ecological niche.

The viability of a functional large-predator niche within patches embedded in human-modified landscapes will require effective dispersal. Moreover, such a niche might entail a balance between continued exposure to matrix-associated diseases and selection preserving the ecology of a large ungulate predator. Disease resistance could play an important role in maintaining genetic diversity within small and potentially locally adapted populations. Although dispersers can act as disease vectors (Robertson et al. 2006), the ecological and evolutionary benefits of landscape connectivity will likely outweigh the risks posed by dispersal (Altizer et al. 2003). Further research should examine 1) whether there are adaptive genetic differences between wolves carrying *C. lycaon* and *C. lupus* genes; 2) whether *C. lycaon* type wolves readily hybridize with *C. latrans* across their range, and 3) how hybridization in canids and other organisms within human-modified landscapes affects ecological niche.

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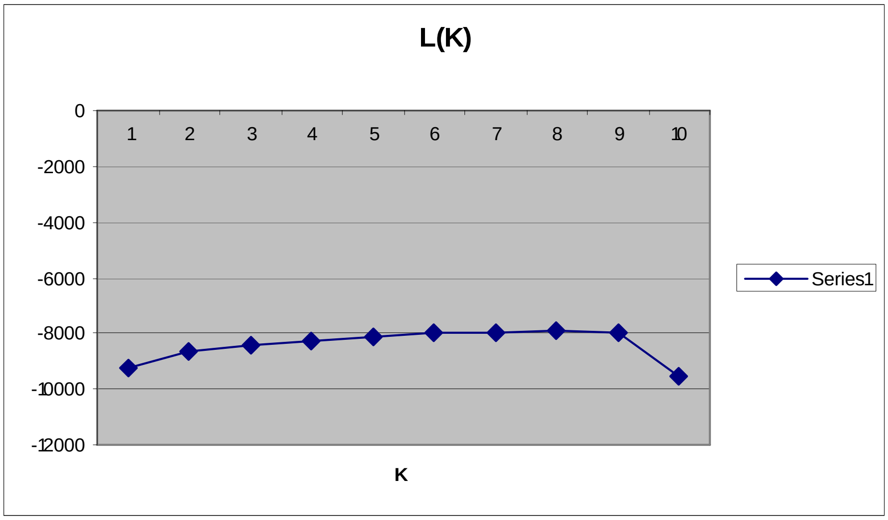
Appendix A. Canid microsatellite primer sequences forward (F) and reverse (R). For primer references see Methods in Chapter 2.

Marker	Chromosome	Forward primer	Reverse primer
FH2096	cfa11	CCGTCTAAGAGCCTCCCAG	GACAAGGTTTCCTGGTTCCA
FH2001	cfa23	TCCTCCTCTTCTTTCCATTGG	TGAACAGAGTTAAGGATAGACACG
FH2422	cfa10	TTGCCCGTCCTATACTCCTG	CCACATGATTTCACTTGTATATGG
FH3313	cfa19	TGCACACCCAAAAAGTAAGC	CAATCTGAAGCCAATCTCATC
FH2054	cfa12	GCCTTATTCATTGCAGTTAGGG	ATGCTGAGTTTTGAACTTTCCC
MS41b	Y	TCCTCTAATTTTCCCCTCTC	CTGCTCGACCCTCTTCTCTG
FH2017	cfa15	AGCCTCTATAATCACGTGAGCC	CCCAGTACCACCTTCAGGAA
FH2088	cfa15	CCCTCTGCCTACATCTCTGC	TAGGGCATGCATATAACCAGC
PEZ6	cfa27	ATGAGCACTGGGTGTTATAC	ACACAATTGCATTGTCAAAC
PEZ8	cfa17	TATCGACTTTATCACTGTGG	ATGGAGCCTCATGTCTCATC
FH3725	cfa14	GAAAGAACTCACTCAAAACTTCC	AAATGTTACTTCAGAAAAGCTGG
FH2010	cfa24	AAATGGAACAGTTGAGCATGC	CCCCTTACAGCTTCATTTTCC
PEZ19	cfa20	GACTCATGATGTTGTGTATC	TTTGCTCAGTGCTAAGTCTC
PEZ15	cfa05	CAGTACAGAGTCTGCTTATC	CTGGGGCTTAACTCCAAGTTC

Appendix B. Results from genetic structure analysis for wolves in southwestern Manitoba, Canada, using STRUCTURE (Pritchard et al. 2000).

I calculated results using K (number of genetic clusters) ranging from 1 – 10, as I considered this to include all plausible values for the study area. I used the options of population admixture and allowed allele frequencies to be correlated, which are considered the best approach where genetic structure is expected not to be strongly differentiated (Falush et al 2003). I did initial runs using
1 500 000 iterations and burnin of 10 000, and because values for the estimated logarithm of probability for the data [ln Pr(X|K)] did not differ markedly from results with 10 000 iterations and burnin of 10 000, I used the latter parameters for the calculations.

The figure shows the results for K = 1 – 10. Here, higher (less negative) values for K have more support (Pritchard and Wen 2004). The highest value was observed at K = 8. However, this resulted in several clusters within Riding Mountain National Park, and some of these corresponded with known family groups (wolf packs).



STRUCTURE (Pritchard et al. 2000) values of proposed number of genetic clusters L(K) in southwestern Manitoba, Canada, for K = 1 – 10.

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Appendix C. Microsatellite allele frequencies per cluster for Kettle Hills (n = 8), Central Manitoba (n = 14), Riding Mountain (n = 123) and Duck Mountain (n = 71).

Locus	**Allele**	**Kettle Hills**	**Central Manitoba**	**Riding Mountain**	**Duck Mountain**
FH2054	**142**	0.313	0.036	0.000	0.077
	146	0.000	0.321	0.345	0.141
	150	0.000	0.036	0.084	0.035
	154	0.000	0.000	0.155	0.232
	156	0.000	0.000	0.042	0.014
	158	0.000	0.107	0.000	0.007
	162	0.000	0.107	0.029	0.007
	166	0.375	0.071	0.210	0.155
	170	0.000	0.250	0.092	0.218
	174	0.313	0.071	0.042	0.113
FH2001	**127**	0.000	0.000	0.012	0.000
	128	0.375	0.036	0.246	0.092
	130	0.000	0.071	0.000	0.000
	134	0.000	0.036	0.111	0.106
	138	0.000	0.143	0.012	0.169
	142	0.313	0.679	0.443	0.444
	146	0.000	0.036	0.012	0.113
	150	0.000	0.000	0.061	0.035
	152	0.313	0.000	0.102	0.042
FH2096	**95**	0.571	0.143	0.336	0.214
	99	0.000	0.536	0.424	0.514
	103	0.429	0.321	0.240	0.271
FH2010	**220**	0.000	0.214	0.004	0.063
	224	0.500	0.321	0.339	0.704
	228	0.000	0.179	0.208	0.141
	232	0.500	0.214	0.449	0.085
	236	0.000	0.071	0.000	0.007
FH2017	**260**	0.000	0.000	0.130	0.038
	264	0.750	0.885	0.870	0.894
	268	0.250	0.077	0.000	0.061
	272	0.000	0.038	0.000	0.008
PEZ08	**213**	0.000	0.050	0.000	0.025
	217	0.000	0.100	0.000	0.000
	221	0.000	0.100	0.018	0.163
	225	0.500	0.550	0.544	0.225
	229	0.000	0.100	0.044	0.075
	231	0.000	0.050	0.000	0.000
	233	0.000	0.000	0.079	0.163
	237	0.000	0.000	0.009	0.025
	239	0.000	0.000	0.035	0.013
	241	0.000	0.000	0.211	0.200
	245	0.500	0.050	0.018	0.000
	247	0.000	0.000	0.035	0.050
	253	0.000	0.000	0.009	0.063
FH2088	**92**	0.000	0.000	0.000	0.028
	108	0.000	0.036	0.071	0.113
	112	0.000	0.000	0.092	0.000
	116	0.625	0.607	0.483	0.739
	120	0.000	0.214	0.038	0.035

	124	0.375	0.107	0.313	0.085
	132	0.000	0.036	0.004	0.000
FH2422	**174**	0.000	0.143	0.004	0.086
	176	0.000	0.000	0.008	0.000
	178	0.000	0.107	0.017	0.157
	182	0.000	0.000	0.021	0.064
	186	0.000	0.214	0.174	0.057
	190	0.563	0.214	0.587	0.479
	194	0.000	0.000	0.021	0.014
	198	0.000	0.179	0.099	0.036
	202	0.438	0.000	0.000	0.007
	225	0.000	0.000	0.000	0.007
	226	0.000	0.036	0.008	0.057
	230	0.000	0.000	0.062	0.014
	238	0.000	0.071	0.000	0.021
	242	0.000	0.036	0.000	0.000
FH3313	**337**	0.000	0.000	0.000	0.015
	349	0.000	0.143	0.082	0.022
	353	0.000	0.036	0.014	0.000
	361	0.000	0.036	0.000	0.000
	365	0.000	0.000	0.018	0.007
	369	0.167	0.107	0.282	0.321
	373	0.000	0.036	0.186	0.037
	377	0.000	0.000	0.132	0.097
	381	0.000	0.036	0.014	0.045
	385	0.333	0.071	0.164	0.007
	389	0.000	0.107	0.000	0.000
	393	0.000	0.036	0.000	0.015
	397	0.167	0.107	0.105	0.172
	399	0.000	0.000	0.000	0.015
	401	0.000	0.143	0.000	0.037
	405	0.333	0.143	0.000	0.194
	409	0.000	0.000	0.000	0.007
	413	0.000	0.000	0.000	0.007
	425	0.000	0.000	0.005	0.000
PEZ06	**164**	0.000	0.036	0.054	0.000
	168	0.188	0.179	0.038	0.176
	170	0.000	0.000	0.004	0.000
	172	0.000	0.071	0.000	0.000
	176	0.000	0.143	0.000	0.070
	180	0.313	0.000	0.113	0.070
	182	0.000	0.071	0.008	0.049
	184	0.188	0.214	0.196	0.493
	186	0.000	0.071	0.217	0.070
	188	0.000	0.000	0.208	0.000
	190	0.313	0.143	0.142	0.014
	192	0.000	0.000	0.000	0.028
	194	0.000	0.036	0.000	0.028
	198	0.000	0.036	0.021	0.000
PEZ19	**186**	0.000	0.036	0.005	0.022
	190	0.000	0.000	0.005	0.000
	194	1.000	0.464	0.432	0.471
	196	0.000	0.036	0.000	0.044
	198	0.000	0.071	0.266	0.250

	200	0.000	0.000	0.063	0.037
	202	0.000	0.214	0.221	0.154
	204	0.000	0.143	0.000	0.022
	214	0.000	0.036	0.009	0.000
PEZ15	**204**	0.375	0.000	0.085	0.035
	208	0.000	0.036	0.410	0.085
	212	0.000	0.000	0.077	0.035
	216	0.000	0.393	0.291	0.232
	220	0.188	0.000	0.004	0.028
	224	0.000	0.036	0.017	0.063
	228	0.000	0.000	0.004	0.000
	232	0.000	0.036	0.000	0.000
	234	0.000	0.000	0.009	0.007
	236	0.000	0.179	0.000	0.077
	238	0.000	0.071	0.004	0.007
	240	0.000	0.036	0.013	0.007
	244	0.000	0.036	0.030	0.000
	246	0.000	0.036	0.000	0.021
	248	0.000	0.107	0.000	0.000
	250	0.250	0.000	0.000	0.007
	276	0.000	0.000	0.004	0.042
	280	0.188	0.000	0.009	0.345
	282	0.000	0.036	0.000	0.000
	284	0.000	0.000	0.043	0.007
FH3725	**130**	0.000	0.000	0.000	0.029
	132	0.000	0.000	0.000	0.043
	134	0.438	0.308	0.370	0.164
	138	0.000	0.231	0.042	0.100
	142	0.375	0.000	0.172	0.014
	144	0.000	0.077	0.118	0.000
	154	0.000	0.000	0.004	0.000
	158	0.000	0.000	0.000	0.007
	160	0.000	0.000	0.063	0.007
	164	0.125	0.000	0.025	0.136
	166	0.000	0.115	0.000	0.007
	170	0.000	0.077	0.000	0.000
	174	0.063	0.000	0.092	0.386
	176	0.000	0.038	0.013	0.036
	180	0.000	0.000	0.025	0.000
	184	0.000	0.000	0.046	0.000
	188	0.000	0.000	0.000	0.007
	190	0.000	0.000	0.000	0.029
	194	0.000	0.154	0.029	0.036

Missing data per locus and per population:

FH2096: 12% in Kettle Hills.

FH2017: 25% in Kettle Hills, 7% in Central Manitoba, 15% in Riding Mountain, 7% in Duck Mountain.

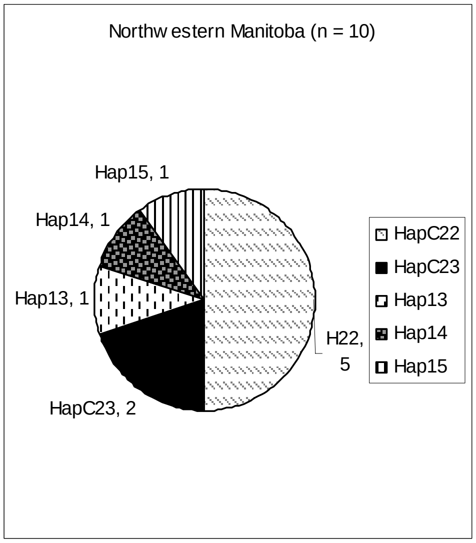
PEZ08: 87% in Kettle Hills, 28% in Central Manitoba, 53% in Riding Mountain, 43% in Duck Mountain.

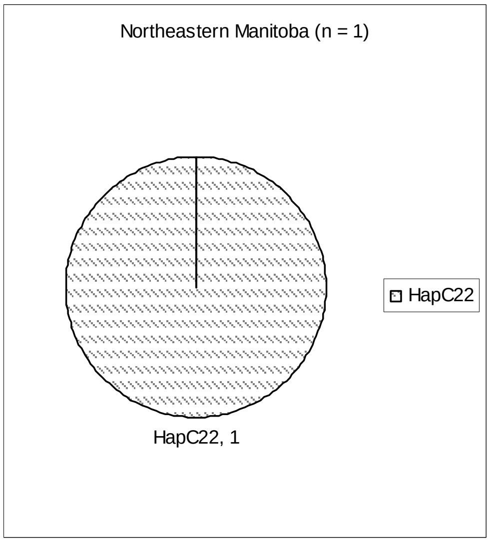
FH3313: 25% in Kettle Hills, 10% in Riding Mountain.

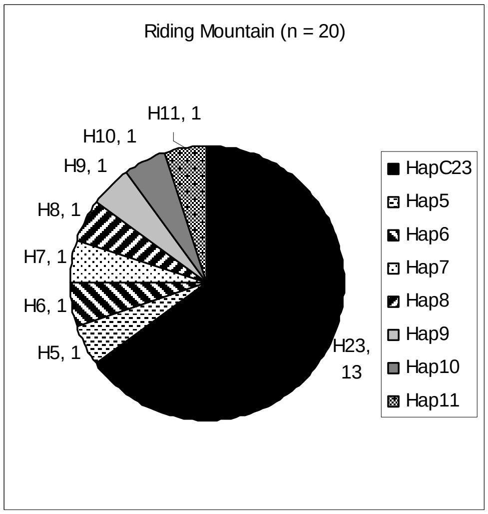
PEZ19: 9% in Riding Mountain.

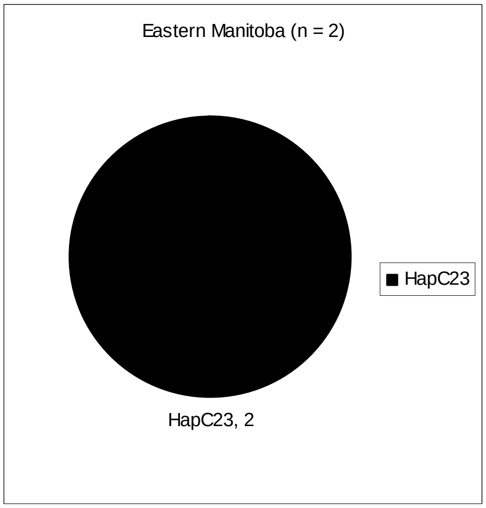
FH3725: 7% in Central Manitoba.

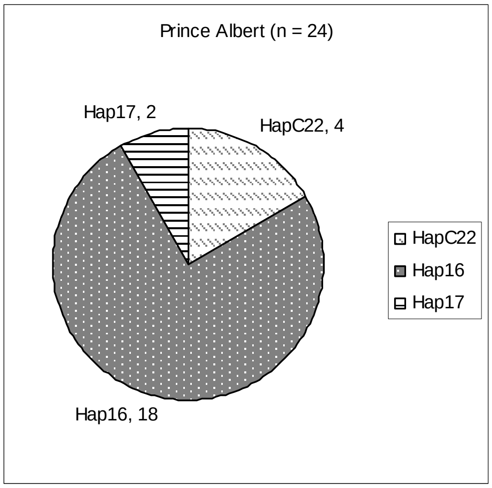
Appendix D. Haplotype frequencies within regions in Manitoba and Saskatchewan found in this study.

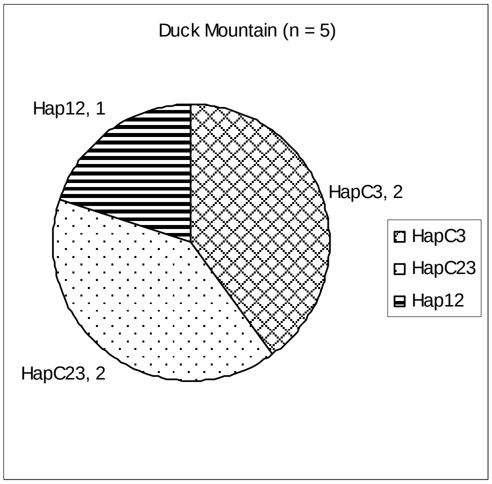




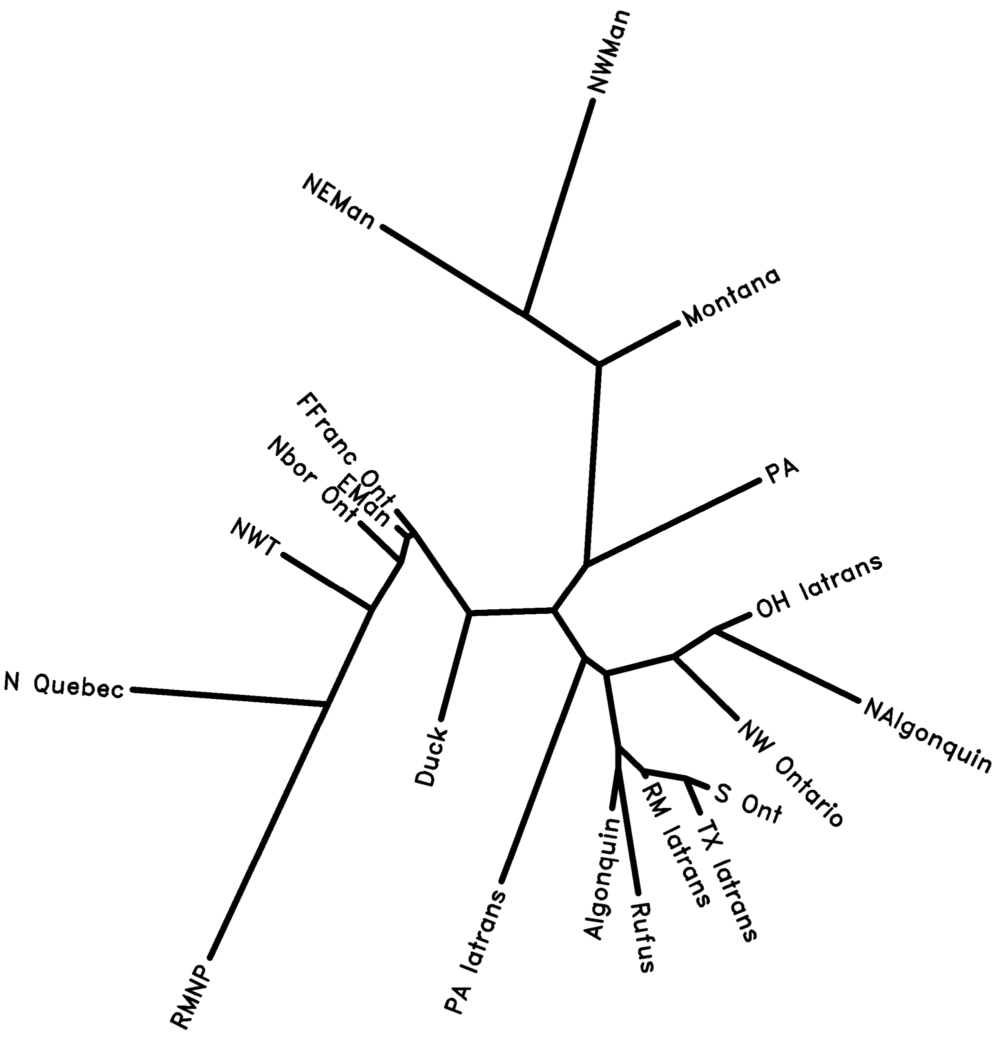






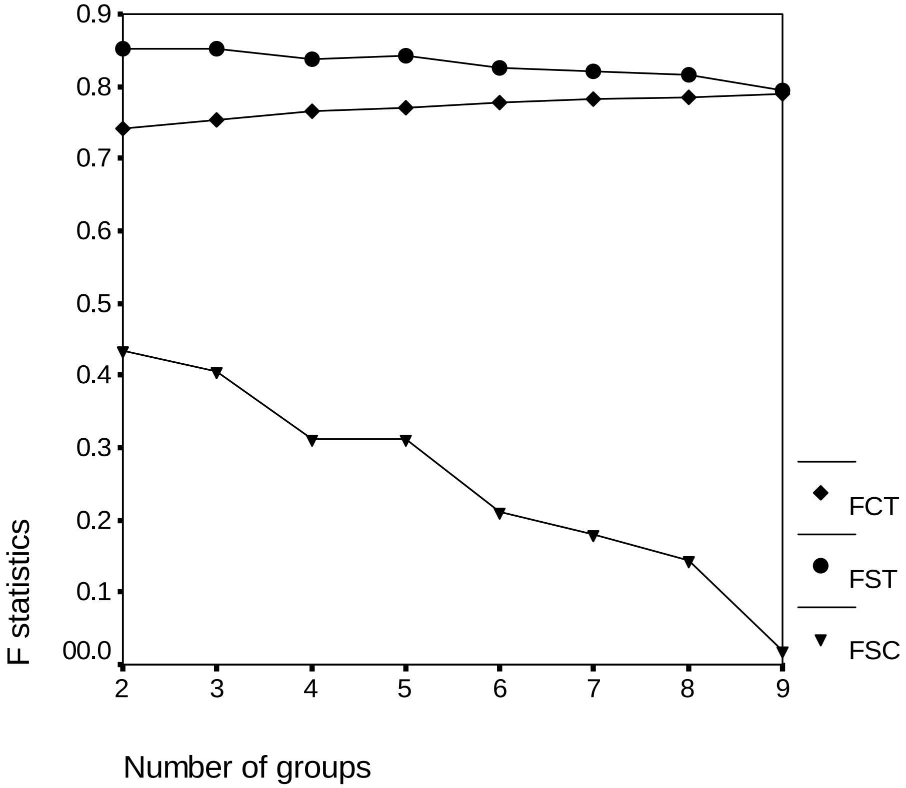


Appendix E. Gene flow and population genetic structure inferred from mtDNA.



Unrooted Neighbour-joining tree of relationships between haplotypes from 20 North American canid locations. For details on sample groups, see Table 2.12. Gene flow and population genetic structure inferred from mtDNA could be strongly affected by uneven distribution of haplotypes, but indicate that higher-order groupings may to some extent be based on geographical proximity.

Appendix F. Population clusters of haplotypes supported by SAMOVA.

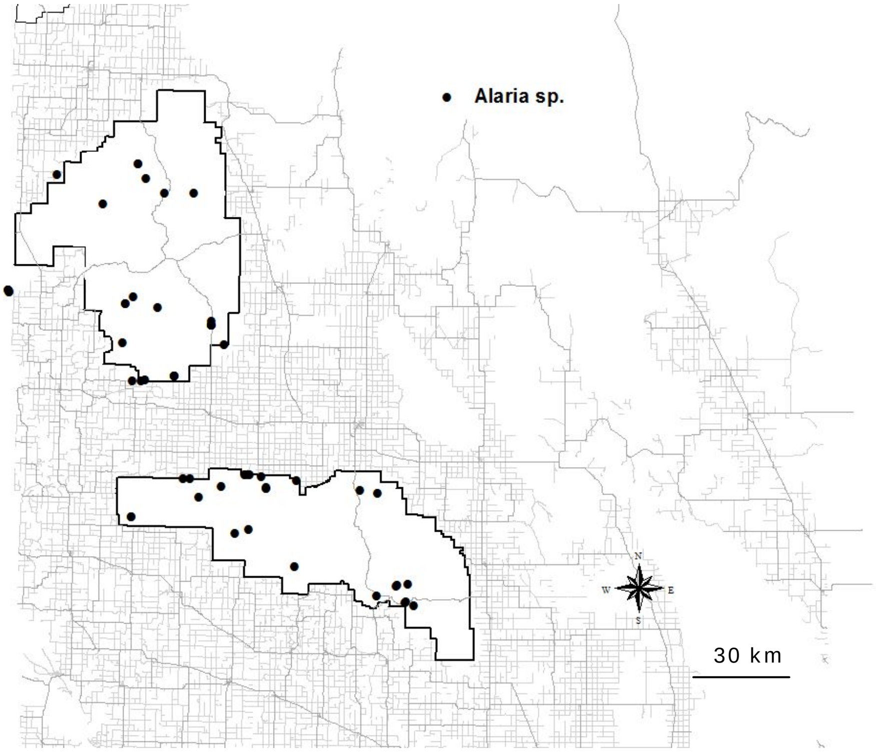


Changes in F-statistics for spatial analysis of molecular variance (SAMOVA) for North American canids with number of groups representing maximally differentiated populations. F_{CT} shows variation among groups, F_{ST} variation within populations, and F_{SC} variation within groups among populations. I have included both wolves and coyotes from the RMNP and Prince Albert regions, and I therefore did not organize organize populations into geographically proximate groups (Pilot et al. 2006).

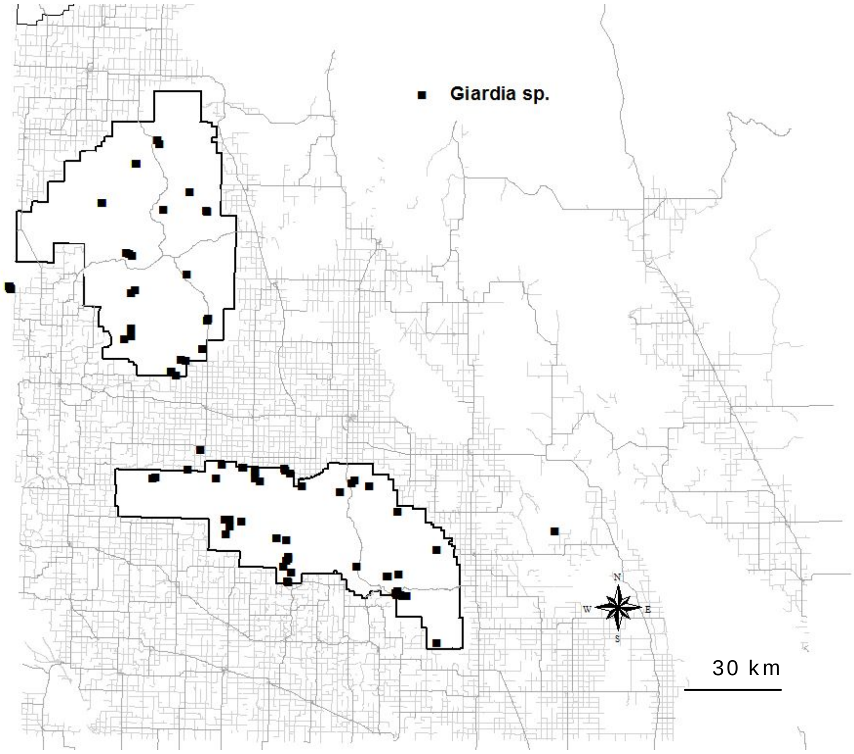
Best SAMOVA clusters for North American canids with k = 9 groups. Gene flow and population genetic structure inferred from mtDNA could be strongly affected by uneven distribution of haplotypes, but indicate that higher-order groupings may to some extent be based on geographical proximity.

SAMOVA cluster	**Populations in cluster**
1	RMNP *latrans*, Ohio *latrans*, Texas *latrans*, southern Ontario
2	PA *latrans*
3	*Rufus*
4	Algonquin, North of Algonquin
5	NW Ontario
6	NW Manitoba, N Québec, NE Manitoba
7	East Manitoba, RMNP, NWT, north boreal Ontario, Fort Francis Ontario
8	Duck Mountain
9	PA, Montana

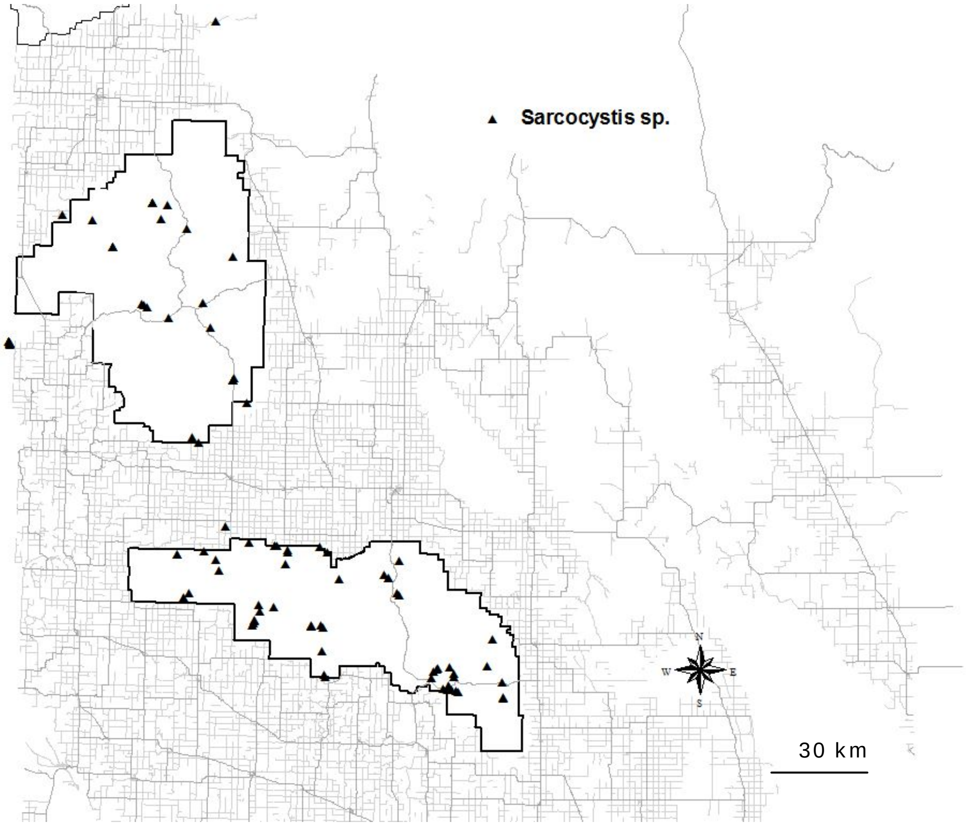
Appendix G. Gastrointestinal parasites found in wolves in the study area.



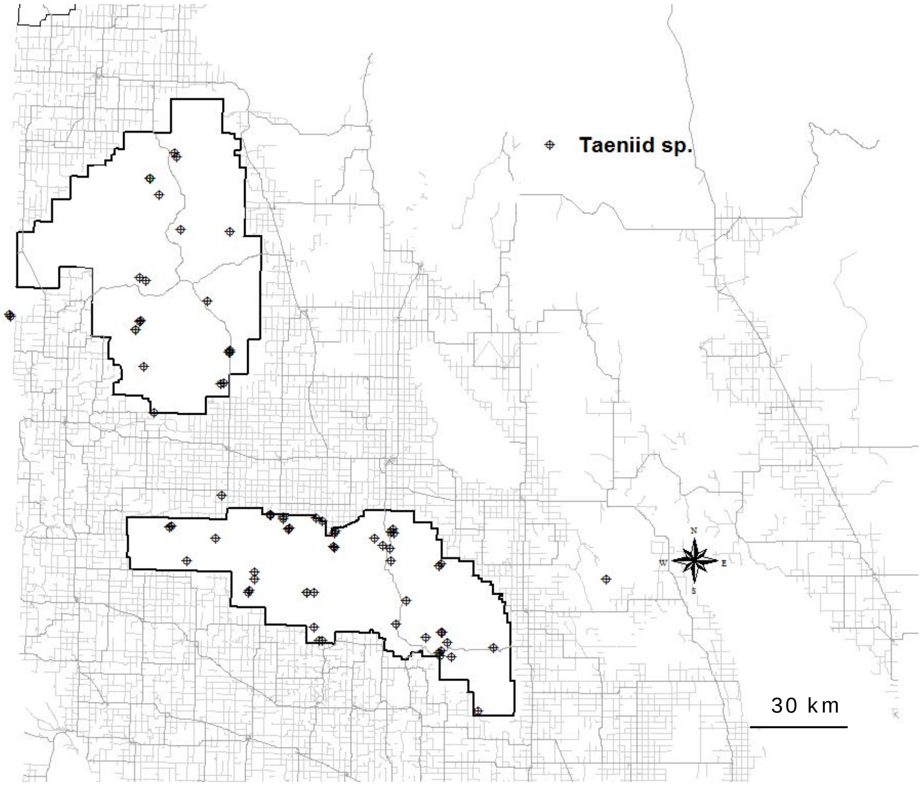
Spatial distribution of *Alaria* sp. occurrence in the study area.



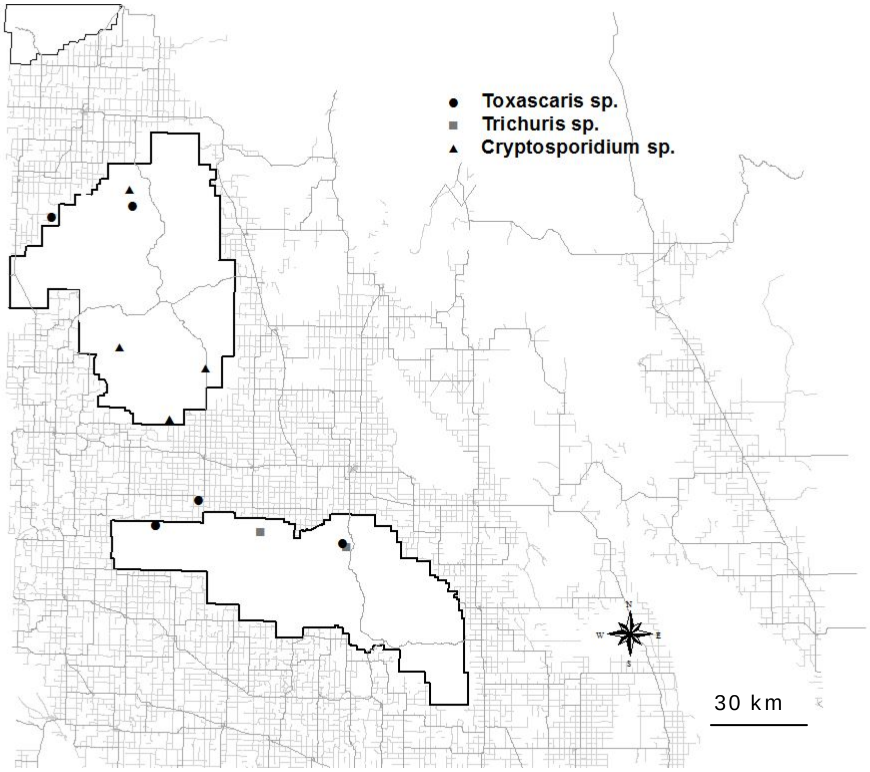
Spatial distribution of *Giardia* sp. occurrence in the study area.



Spatial distribution of *Sarcocystis* sp. found in the study area.



Spatial distribution of *Taeniid* sp. found in the study area.



Spatial distribution of *Toxascaris* sp., *Trichuris* sp., and *Cryptosporidium* sp. in the study area.

Appendix H. Farmer Attitudes toward Wolves: Implications for the Role of Predators in Managing Disease[6,7]

Abstract

The potential for disease transmission between wild and domestic animals may interfere with wildlife and habitat conservation on lands surrounding protected areas. Recently, possible transmission of bovine tuberculosis (*Mycobacterium bovis*) from wild ungulates to domestic livestock has affected the Riding Mountain National Park region in Manitoba, Canada. Wolf *(Canis lupus)* predation on ungulate populations may help lessen the risk of disease transmission to livestock. We conducted an exploratory analysis of causal factors associated with farmer attitudes toward observing wolves on their farms. A survey to 4 220 farms within 50 km of the Park resulted in an adjusted response rate of 25%. We constructed several logistic regression models with factors hypothesized to influence whether farmers agreed with the statement "I enjoy seeing wolves on my land," and three candidate models received reasonable support. Factors most affecting attitudes were, in order of importance, perceived wolf population size, frequency of seeing wolves, perceived seriousness of wolf damage, distance to Park boundary and number of beef cattle (*Bos taurus*) owned. The factors least influential on attitudes were education and age. Concern over bovine tuberculosis in wild elk also had minimal influence. Of respondents who perceived the wolf population as "too high",

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[6] Reprinted with permission from Biological Conservation, Vol. 135, Stronen, A., R.K. Brook, P.C. Paquet, S.M. McLachlan. Farmer attitudes toward wolves: Implications for the role of predators in managing disease. Pages 1 – 10, Copyright (2007), with permission (Appendix I) from Elsevier.

[7] Contributions to this multi-authored paper; primary contributor listed first: i) Research Design: Stronen, Brook, McLachlan ii) Data Collection: Brook, iii) Data Analysis: Stronen, Brook, iv) Manuscript Preparation: Stronen, Brook, Paquet, McLachlan.

60% were extremely concerned about bovine tuberculosis in wild elk. Although the role of wolf predation as a potential natural regulator of disease in wild ungulates might not be widely recognized in many areas, we believe this provides a unique opportunity to re-examine the significance of maintaining viable wolf populations.

Introduction

The role of wildlife disease in ecosystem conservation is gaining increasing recognition (May, 1988; Aguirre et al., 1995; Hess, 1996), as is the realization that large scale management of protected areas will require cooperation with local landowners (Schonewald – Cox, 1988; Irby et al., 1997; Naughton-Treves et al., 2003a; Maehr, 2004). Whereas many core wildlife areas are public lands, the lands surrounding and connecting these areas are often privately owned (Ruediger, 2004). The attitudes, concerns and values of landowners who manage this land are increasingly recognized as relevant, especially regarding transboundary issues related to wildlife (Laubhan and Gammonley, 2001; Beedell and Rehman, 1999; Mattson, 2004). Although use of private land by wild species can be beneficial to wildlife and landowners, it often results in overlapping ranges between wild and domestic animals, which can facilitate disease transmission among them (Foreyt and Jessup, 1982; Simonetti, 1995). North American examples of this situation are elk (*Cervus elaphus*) and bison (*Bison bison*) infected with brucellosis (*Brucella abortus*) using areas surrounding Grand Teton and Yellowstone National Parks in the U.S. (Thorne and Herriges, Jr., 1992), and bison infected with brucellosis and bovine tuberculosis (*Mycobacterium bovis*, bovine TB) in Wood Buffalo National Park in Canada (Tessaro et al., 1990; Joly and Messier, 2004). Bovine tuberculosis was also discovered in Michigan white-tailed deer (*Odocoileus virginianus*) in 1994 (Schmitt et al., 1997) and the disease has been found in 32 cattle (*Bos taurus*) herds in the state (VanderKlok, 2004).

Bovine tuberculosis presents major challenges for the protection of human and animal health, economic sustainability of agriculture, and the conservation of wildlife (Tessaro et al., 1990; Schmitt et al., 1997; Dorn and Mertig, 2005). Wildlife-agriculture interactions associated with bovine TB transmission have therefore become a contentious issue in the region surrounding Riding Mountain National Park (RMNP) in southwestern Manitoba, Canada (Lees, 2004). Over the last 15 years bovine TB has been found in 31 wild elk and seven white-tailed deer in and around RMNP, as well as in 12 cattle herds near the Park. The disease was also identified in two RMNP wolves (*Canis lupus*) in 1979 (Carbyn, 1982). Movement of infected wildlife from Parks to surrounding areas has intensified concerns that diseases such as bovine TB and brucellosis are spreading from wildlife to domestic cattle and has raised questions of whether Parks such as Yellowstone and RMNP are acting as reservoirs of disease (Simonetti, 1995; Lees, 2004).

Although lands surrounding Parks are vital for conservation of wide-ranging mammals, the risk of disease transmission and other costs such as crop damage may discourage landowners from permitting wildlife use of their lands (Simonetti, 1995). Farmer acceptance of wildlife use may vary depending on the species, and farmers may be willing to tolerate some wildlife impacts in exchange for the presence of ungulates such as elk and deer (Irby et al,. 1997). However, increasing public attention to disease in ungulate populations in and around Parks also pose important questions for the ecological role of predators such as wolves and the role of predation in disease management. Although several recent studies suggest wolves may have complex ecosystem effects (Dekker at al., 1996; Ripple and Larsen, 2000; Ripple et al., 2001), hostility toward all predators is strong in many rural areas and human-caused mortality rates of wolves leaving Parks to use surrounding areas are often high (Carbyn, 1980; Forbes and Theberge, 1996; Callaghan, 2002). A review of 38 North American and European quantitative studies on attitudes toward wolves across social groups from 1972 – 2000 shows that rural residents and farmers and ranchers had the lowest percent positive attitudes (Williams et al., 2002). Whereas 55% of respondents in a random sample of all residents had positive attitudes toward wolves, only 45 % of rural residents and 35 % of ranchers and farmers had positive attitudes.

Although the financial costs of living with wolves are relatively easy to calculate, there may also be benefits that are more difficult to express in monetary terms (Estes, 2004). Livestock losses to wolves and the risk of livestock losses are, at times, direct costs to farmers on lands surrounding Parks. However, the risk of disease transmission also directly affects some farmers near protected areas (Simonetti, 1995). These farmers may benefit from wolves as predators on wild ungulate populations. Higher density of a host species can sometimes lead to increased disease prevalence because of increased transmission rates (Scott, 1988), and wolves may therefore play a positive role in managing diseases such as bovine TB by decreasing the size of prey populations. In addition, wolves have been found to reduce average group sizes of social ungulates such as elk, which effectively reduces elk density (Creel and Winnie, Jr., 2005). Because farmer attitudes toward wildlife have important implications for persistence of many species (Irby et al., 1997), understanding how farmers perceive wildlife is important, especially in regions where protected areas are becoming increasingly isolated. Whereas public attitudes toward wolves may be overall positive, people living and interacting with wolves can have very different opinions (Ericsson and Heberlein, 2003), and wolf survival in rural agricultural areas is disproportionately dependent on the actions of people who depend on the productivity of the landscape for their livelihood (Musiani et al., 2004). Riding Mountain National Park is surrounded by agricultural lands and considered an isolated reserve (Noss, 1995; Parks Canada, 2002). The insular configuration of RMNP combined with recent concerns over bovine TB transmission between elk and cattle on private lands surrounding the Park provide a valuable opportunity to:

1) Assess farmer attitudes toward observing wolves on their land and the factors that influence these attitudes;
2) Determine whether farmers more concerned over disease in wild ungulates have more positive attitudes toward wolves;
3) Discuss whether the ecological role of wolves may benefit farmers in their efforts to minimize impacts from infections disease on livestock operations.

This analysis is part of a comprehensive study examining wildlife-agriculture interactions around Riding Mountain National Park (Brook and Mclachlan, in press).

Methods

Study Area

Our study area is located in southern Manitoba, Canada, and includes the agriculture-dominated area within 50 km of Riding Mountain National Park. It represents a broad transition zone between the prairies and the more northerly Boreal Plains. The Park comprises 2 974 km^2, extending 115 km from east to west and 60 km from north to south, and is dominated by the Manitoba Escarpment, which rises 475 m above the surrounding, largely flat, landscape. The Park represents a core area of relatively undisturbed wilderness surrounded by agriculture, which is dominated by canola (*Brassica napus)*, wheat (*Triticum* sp.), and hay production. Approximately 50 000 beef cattle are raised in the region (Statistics Canada, unpublished data). The Riding Mountain TB Eradication Area, which was established by the Canadian Food Inspection Agency around RMNP, requires increased bovine TB surveillance and a permit to move cattle out of the area. Wildlife is abundant in the study area, with a regional elk population of approximately 5 000 elk, 2 500 moose, and more than 5 000 deer (RMNP, 2005 unpublished data). Over the past 3 years, the RMNP wolf population has numbered approximately 70 - 75 individuals in late winter (RMNP, 2005 unpublished data). Other large predators include lynx (*Lynx canadensis*), black bears (*Ursus americanus*), and coyotes (*Canis latrans*). Climate is continental and typical of the Canadian prairies, with warm summers and cold winters. Mean temperatures for July and January are about 20 C and -19.5 C, respectively. Growing season is short (mean = 65 days, range 43-106) and snow cover persists for approximately five months (Keck, 1975). Approximately 546 mm of precipitation falls annually, 160 mm as snow.

Wolves have been present in southwestern Manitoba for at least 5 000 years (Goulet, 2000) and occupied the Riding Mountain region until a combination of hunting, trapping, land clearing and poisoning likely caused a local extirpation around 1900 (Carbyn, 1980). By the 1930s, reports from Park wardens and residents confirmed that wolves had returned. After years of no provincial designation under the Manitoba Provincial Wildlife Act and predator designation under the Predator Control Act, wolves were classified as a big game species in a 1980 revision of the Wildlife Act (Stardom, 1983). Since 2001, the wolf-hunting season has been closed in areas that surround RMNP, although landowners within this area may shoot wolves in defense of property (D. Chranowski, personal communication). The area closest to RMNP supporting a wolf population is Duck Mountain Provincial Park and Forest (hereafter referred to as the Duck Mountains), approximately 35 km north of RMNP.

Data Collection

This study focused on rural residents living on farms within 50 km of RMNP. The Joint-Faculty Human Subject Research Ethics Board at the University of Manitoba approved our study design. Using Canada Post mailing lists we identified 4 220 rural households within our study area, and mailed all listed farm operations a questionnaire on 18 April 2002, and included a self-addressed, stamped envelope. On 18 May 2002 we sent a reminder. All surveys returned before 31 August 2002 were included in subsequent analyses. To test for response bias, we telephoned a sample of 65 survey recipients who did not respond to the survey. We then asked a subset of questions from the original questionnaire to compare responses of respondents and non-respondents.

We designed the mail-out questionnaire to determine farmer attitudes toward bovine TB in wildlife and livestock and identify the influence of socio-demographic variables on attitudes toward wildlife. While attending seven town hall meetings throughout the study area between January and April 2002, we documented comments from over 500 local agricultural producers, which we used to design the survey. We also gained insights from discussions with staff from federal and provincial agencies, as well as other special-interest groups. We pre-tested the questionnaire on 15 highly knowledgeable farmers, as well as researchers and government staff. The final version was nine pages, contained 257 data variables, and took about 30-40 minutes to complete. Respondents rated statements on a seven-point likert scale ranging from “strongly disagree” to “strongly agree”. Respondents were asked to report the location of their farms, provide written comments on all aspects of the survey, and list any other concerns that they had. Comments were recorded verbatim, and systematically assessed and identified with underlying themes. They were then incorporated with the quantitative results as complementary information. Overall mail survey results for the variables age, education, and farm size were compared with data from the 2001 Agriculture Census of Canada to assess the representative nature of the questionnaire data from this study (Statistics Canada 2002).

Data Analyses

We measured minimum distance of each farm to the RMNP boundary using Arcview GIS 3.2 (ESRI). We identified a set of 13 independent variables believed to influence whether farmers agreed with the statement “I enjoy seeing wolves on my land” based on literature review of attitudes to wolves and conversations with local residents (Table 1). Because only 9 % of respondents were female and because many surveys were likely filled out by both women and men, we chose not to include sex as a variable in the analyses. We used Spearman rank correlation to assess correlation among variables and identified any group of variables with r >0.7.

We designated the lowest and highest 33% of responses, thus the most positive and most negative choices on the likert scale, as a binary response variable in logistic regression to model the probability that farmers enjoy seeing wolves on their land. Because this analysis does not permit missing data for any variable, 191 responses were usable. We ran all possible combinations of logistic regression models with the 13 independent variables hypothesized to influence farmer attitudes toward wolves. Akaike's Information Criterion with small sample adjustment (AICc) and Akaike weights (*w*) were calculated to assess model fit (Chamberlin, 1965; Akaike, 1973; Burnham and Anderson, 2002). Cumulative AICc weights were calculated for each independent variable by summing the AICc model weights for all models containing that variable (Burnham and Anderson, 2002). Variables with the highest cumulative AICc weights have the greatest relative influence on farmer attitude toward seeing wolves on their land, allowing the variables to be ranked from most important to least important (Flanders-Wanner et al., 2004). Based on the cumulative AICc scores, we created 16 candidate models using combinations of the most important variables that we hypothesized to influence farmer attitude toward wolves.

Results

Socio-demographic Composition

Average respondent age was 52 years (range 18-85). This is consistent with the 2001 Agriculture Census of Canada for this region (Region 3, Division 15), which determined average age of operators to be 50 (Statistics Canada 2002). Most respondents (92%) had lived at the current location for five or more years and most (81%) were raised on a farm. The average farm size was 467 ha (range 16-5.666 ha), which compares favorably with the overall average farm size of 419 ha for this region

(Statistics Canada 2002). Seventy-one percent (71%) of respondents reported the location of their farm. Sixty-five percent (65%) of respondents owned livestock, and more than half (55%) of survey respondents had at least some cattle, with 45% owning more than 20 cattle. Education level varied; <1% had no formal education whereas 20% had completed grade school, 35% had high school education and 40% had college, university, or technical training. The 2001 agriculture census for all of Manitoba indicated 31.5% of male and 43% of female farm operators have college, university, or technical training and 53.1% of male and 49.8% of females had high school, which is generally consistent with our results.

We received 786 completed useable surveys by mail as well as 62 refusals and 584 surveys that indicated the recipient did not operate a farm. In addition, we received 94 telephone calls. Using the response data, telephone calls received, and telephone calls made to follow-up with non-responders, the overall adjusted response rate was 25%. Although this figure is low, it corresponds with recent findings of declining response rates in natural resource based-surveys (Connelly et al. 2003), which suggests that response rates of less than 30% are no longer uncommon. Because rural addresses are unavailable for purchase in Manitoba we used non-addressed mail, which may have given the survey low priority with some recipients. We did not identify any significant differences between respondents and non-respondents.

Attitudes toward wolves

Although 51% of farmers felt they had never experienced serious damage from wolves, 44% of all farmers did not enjoy seeing wolves on their land (Tables 2 and 3). More than half of livestock owners (52%) disagreed with the statement ‘I enjoy seeing wolves on my land', whereas only 26% agreed with the statement. For the 26 % of farmers that reported not owning any livestock, the results were opposite, with 26% disagreeing with the statement and 54% enjoying seeing wolves on their land. Twenty-six percent of respondents felt that the regional wolf population size was 'too high' (Table 3). Twenty-four percent felt the wolf population was 'about right', whereas 10% found the population 'too low'. Respondents expressed a diversity of views on management of wildlife populations in and around RMNP. Some respondents had positive attitudes to wolves, and indicated that they had a right to be present:

> *"Elk, deer, moose, wolves were here first and are part of this country and we should manage around them" [Respondent #455]*

Others expressed less positive attitudes to wolves:

> *"The wolves should be dealt with in the park. They are too many and are chasing the elk out of the park" [Respondent #457]*

Some also had less positive attitudes to other species of wildlife:

> *"We have too many deer, wolves, beavers in this area" [Respondent # 336]*

Although most respondents around RMNP did not feel they had experienced serious damage from wolves, livestock predation is a concern for many farmers. Some comments reflected the concern that farmers will not be granted compensation if there is no physical evidence of livestock predation, and that livestock losses may thus be underreported:

> *"Wolves and bears and coyotes cause very heavy damage to my cattle and I am unable to collect compensation due to lack of proof you can not find dead calves taken by bears" [Respondent #404]*

Of respondents that lived within 10 km of RMNP, 7% reported seeing wolves ‘regularly, most years’, or ‘regularly, all years’. For those that lived 11 – 20 km and 21 – 30 km away from the park border, the numbers were 4% and 3%. Altogether 49% of respondents described their concern over bovine TB in wild elk as ‘extremely high’. Twenty-six percent of respondents felt the regional wolf population was ‘too high’. Within this group, 60% were extremely concerned about bovine TB in wild elk, whereas 13% had low or moderate concern about the disease. Some responses from the survey also addressed the role of wolves in regulating the RMNP elk population:

> *”Talk to rural people about the Park. The only people that know the Park are the people who live near it. People in Ottawa [Canada’s capital] should not be listened to. If you kill the elk off the wolves will starve, etc. Let nature take its course in the park” [Respondent #484]*

Importantly, the potential role of wolves in disease management was also noted:

> *”Monitor the Park but don’t interfere. If you kill wolves the elk will increase and then disease strikes” [Respondent #484]*

The factors most important for farmer response to seeing wolves on their land were, in order of importance, perceived wolf population size, frequency of seeing wolves, perceived seriousness of wolf damage, distance to RMNP or the Duck Mountains, and number of beef cattle owned (Table 4). The least influential factors were education and age. Concern over bovine TB in wild elk, number of beef calves owned and number of hunter days also had minimal influence. Because all models within 2 units of the minimum delta AICc value should be considered when making inferences (Burnham and Anderson 2002), three candidate models received reasonable support (Table 5). No models based on any single factor were supported. Positive attitudes toward wolves were associated with less frequent sightings of wolves:

> *“We have heard of wolf sightings more this winter so they could become a problem” [Respondent # 308]*

as well as with less perceived damage from wolves and perceived lower wolf population size:

> *”I have no problem with wildlife provided their numbers are kept in low numbers” [Respondent #401]*

More positive attitudes were also associated with increasing distance from RMNP or the Duck Mountains, and with owning fewer beef cattle. Positive and negative attitudes were often found on neighboring farms and did not show any clear geographical pattern (Figure 1).

Discussion

Wolf predation and wildlife disease

Many respondents were extremely concerned over bovine TB in wild elk and, at the same time, felt the wolf population was too high. This suggests that the role of wolf predation as a potential natural regulator of elk in the RMNP ecosystem may not be widely recognized or valued by farmers in the area. Wolves likely affect ungulate population dynamics, and wolf predation appears to reduce interactions of density and environmental factors (such as disease and food competition) on population dynamics of species such as elk (Seip, 1995; Hebblewhite et al., 2002). However, long-term monitoring is needed to better understand the relative influence and interactions between various factors such as climate and predation (White and Garrott 2005). Elk is the most important prey species for wolves in RMNP (Carbyn, 1980; Meleshko, 1986; Paquet, 1989, 1992) and in both summer and winter wolves showed a preference for elk over other prey species (Carbyn, 1980; Meleshko, 1986; T. Sallows, unpublished data). Although the relationship between wolves and bovine TB in wild ungulates is not clear, pathogens such as bovine TB and brucellosis may increase wolf killing success to some degree through debilitation of prey (Joly, 2001). Lower wolf predation rates may thus increase elk density in RMNP and possibly prevalence of bovine TB, as the disease generally transmits from animal to animal by inhalation and ingestion (Clifton-Hadley et al., 2001). Disease and predation may also interact in affecting ungulate abundance (Joly and Messier, 2004), and recent research in Yellowstone also suggests that wolf presence and predation risk may affect ungulate distribution (Ripple and Beschta, 2004). Thus, the effects of wolves may extend beyond direct mortality of ungulates, and affect both crop damage and transmission of diseases such as bovine TB. As hunting is not permitted within the National Park, wolf predation, along with winter severity and hunting outside the Park, will likely continue to be important regulators of elk population size both inside RMNP and, indirectly, on the surrounding farmland.

With recent attention on potential disease transmission between wildlife and domestic animals, it is increasingly important to understand how farmers view movements of wild ungulates and their predators on private land surrounding protected areas. Indeed, the frequency of seeing elk on farms was the primary cause of concern regarding bovine TB (Brook and McLachlan, in press). Our results suggests that farmer attitudes to seeing wolves on their land around RMNP improve when they perceive wolf populations to be low, rarely see wolves, and when they feel wolves do little damage.

Farmers with few or no beef cattle were also more accepting of wolves on their land. While Ponech (1997) found that most respondents, including farmers, favored having wolves in RMNP, Daley et al. (2004) concluded that landowner attitude to wildlife is closely related to reliance on the land for direct economic income. Farmers, especially those that own livestock, may be more positive toward wolves occupying the Park, or wolves in general, than wolves observed on their own land. Should this be the case, this attitude may compromise the long-term viability of the RMNP wolf population. Although attitudes toward wolves may be generally positive as long as animals remain inside the Park boundary, wolves will need to disperse between RMNP and surrounding areas to maintain genetic variation in the Park population. Although dispersal carries the risk of individuals acting as disease vectors between populations (Robertson et al. 2006), increased isolation and inbreeding can also increase an animal's susceptibility to disease and parasites (Acevedo-Whitehouse et al., 2003), which could further reduce viability of the RMNP wolf population.

The relationship between wolf predation and wildlife disease could also be affected by other ecological links. Many farmers in the region feel that the beaver population in and around the Park is too high, and that beaver flooding have forced elk out onto agricultural lands (Schroeder, 1981; Menzies, 1998; Brook and Mclachlan, in press). Wolves are important predators on beavers in RMNP, and beaver remains were found in 33 % of summer wolf scat (Meleshko, 1986; T. Sallows, unpublished data). Although elk may leave the Park to find forage, farmland may also provide them with a refuge from predators such as wolves, which are generally discouraged or controlled in agricultural areas (Thompson and Henderson, 1998). In the Canadian Rocky Mountain National Parks, zones with high human activity often exclude or limit wolf presence

(Callaghan, 2002) and elk tend to congregate in higher density in these areas (White et al., 1998). Our conversations with local landowners indicate that elk are also coming out to the farmland surrounding RMNP to calve. If attitudes to wolves and other predators were more positive in the landscape surrounding RMNP, this could reduce the refuge status these lands may currently provide and possibly reduce the number of elk using the farmland around the Park.

Whereas human-wolf conflicts associated with livestock predation continue to pose significant problems for global conservation of wolves (Fritts et al., 2003; Woodroffe et al., 2005), perceived wolf damage alone did not provide a good predictor for attitudes to wolves in the RMNP region. The overall risk of livestock predation in the RMNP area is low, and Manitoba farmers have received compensation for livestock killed or injured by wolves and other carnivores since 1997 (Wilcox, 2004). Ponech (1997) also reported that most respondents in all groups disagreed with the statement "Wolves have a significant impact on the livestock industry around RMNP". Although the relationship between actual and perceived levels of damage may be unclear, perceptions are important for farmer attitudes to wildlife (Conover, 1998). We asked farmers to what degree they felt wolves had caused financial damage on their land, so answers likely reflect both confirmed and suspected losses. While financial damage alone was not a good predictor, the threat of predation also creates stress for livestock producers (Fritts et al., 2003), and there are hidden costs involved in livestock predation that are difficult to calculate, including loss of valuable breeding animals and the emotional costs of finding dead and wounded livestock (Wålberg 1987; Hafer and Hygnstrom, 1991). Increasing distance from RMNP or the Duck Mountains was also associated with a more positive attitude to wolves. Although farmers close to RMNP reported more wolf observations and damage, we received a number of observations > 30 km outside RMNP or the Duck Mountains. We believe that some observations may actually have been of coyotes and not wolves. However, it is important to realize that the essential factor is not whether the farmer actually saw and/or experienced financial damage from a wolf, but whether the person *believe* they did.

Social and cultural factors

Williams et al. (2002) report that, overall, those with higher education have more positive attitudes toward wolves while age is negatively correlated with attitudes. In contrast, we found education and age to have no influence on farmer attitudes toward wolves. Beliefs and perceptions are the factors primarily affecting tolerance of wolves (Boitani, 2003; Fritts et al., 2003), and occupation and social identity might be more powerful predictors of tolerance than personal experience (Kellert et al., 1996; Naughton-Treeves et al., 2003b; Chavez et al., 2005). Our respondents all operate farms and live in rural areas, and a high percentage of our respondents are multi-generational farmers. If perceptions of family and community are key factors shaping attitudes to wolves, age and education may be relatively unimportant variables in our survey.

Whereas Ponech (1997) reports that most respondents were not afraid to hike in RMNP knowing that wolves are in the Park, Tucker and Pletscher (1989) and Lohr et al. (1996) report that positive attitudes to wolves was associated with less fear for human safety. The following excerpt from a local newspaper (The Dauphin Herald, 8 October 2002), shows that the fear of wolves is still present in the RMNP region and that it is sometimes still perpetuated by the media:

> *"And now, something not quite so exciting - Timber Wolves. I would caution local nature lovers to be very careful on their long walks. There has been a pack of seven timber wolves spotted in the area and even a bear is suspected of killing a calf. Some domestic animals are missing - and presumed buffet for these beasts."*

This news item also links missing livestock to predation by wolves and bears, and it helps to reinforce many people's perceptions that humans cannot be safe in the presence of wolves, and that wolves and bears are frequent livestock predators in the RMNP region.

Recent research on innovative preventative techniques to minimize and in some cases eliminate livestock predation (Breck, 2004; Musiani et al., 2004; Treves et al., 2004) could reduce costs and limit controversy over predators such as wolves. The species should be conserved and managed as part of a working ecosystem (Mech and Boitani, 2003). However, debates that, on the surface, are about wolves often reflect conflicting human values (Nie, 2004), and urban residents may not sympathize with or understand challenges faced by farmers and other rural residents (Ericsson et al., 2003; Fritts et al., 2003; Mech and Boitani, 2003; Skogen, 2003). Norton (2000) concludes that "private land is important not only because of its indigenous biodiversity, but also because … it is here that most people encounter nature". The sense of personal attachment to the land has long been recognized as instrumental to the support for conservation (e.g. Leopold, 1949), and the importance of this connection is still emphasized (e.g. Van Tighem, 2000). Because wolves have high ability to disperse and exist in a variety of habitats, human tolerance of wolves in the broader landscape may be the most important factor in ensuring their long-term viability (Boyd and Pletscher, 1999; Fritts and Carbyn, 1995).

Recommendations

We believe the potential role of wolves in reducing ungulate populations, and thus possibly mitigate spread of bovine TB to livestock, provide a unique opportunity to re-examine the significance of maintaining viable wolf populations. While the relationship between wolves and ungulate disease is not clear, we recommend further research into the ecological role of wolves and other predators in managing infections disease in prey populations. Increasing exchange of information between rural residents, researchers and various levels of government will be important. The increasing number of forums involving special interest groups established to discuss wildlife and disease concern in the RMNP area is encouraging, as are periodic open-house sessions on this topic.

Many residents have expressed interest learning more about research in our study area, and several noted that results are not widely publish locally and may be difficult to obtain. Publication and dissemination of research is often focused exclusively on professional and scientific journals with the consequence that local residents may feel left out. We believe increased exchange with local residents about research objectives and findings would offer many benefits. While this does entail extra work for researchers, it could provide a non-confrontational means to address many of the common misconceptions about wolves, especially related to livestock predation and human safety. This approach also provides a means for residents to communicate with and therefore educate researchers about local knowledge and concerns. This is something we have benefited from and have been able to incorporate into our research on elk, wolves and human-wildlife relationships in the Riding Mountain region.

The view of wolves as an integral component of a working ecosystem, as opposed to a species to like or dislike, is important in improving tolerance of the species outside protected areas. Because perceived wolf damage alone was a poor predictor of attitudes in our study, we recommend further research on how farmers calculate potential costs and benefits of having wolves on their land, and on how values and perceptions about wolves interact with personal experience in determining attitudes. This could help identify farmer concerns and facilitate long-term management of ungulate populations and disease around protected areas. While livestock predation by wolves can have significant impact, it is important to consider the role of wolves and other predators in the evolution of ungulates, and thus the help wolves may provide in managing diseases such as bovine TB. Future research might help clarify the role of wolves, and could afford an opportunity to mitigate impacts of bovine TB and other infections diseases on livestock operations while simultaneously promoting conservation of a wide-ranging carnivore.

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Table 1. Variables used to analyze farmer attitudes to wolves.

Abbreviation	Variable description
population	perception of current wolf population (too low, about right, too high)
damage	seriousness wolf damage (1997-2001) (never, seldom, some years, all years)
wolfsee	wolf observations on farm (never, rarely, …. regularly on all years)
distance	minimum distance from farm to RMNP or Duck Mountain Provincial Forest (km)
beefcattle	size of cattle herd >1 year old (0, 1-20, 21-40, 41-60, 61-80……>160)
beefcalves	size of cattle herd < 1 year old (0, 1-20, 21-40, 41-60, 61-80……>160)
huntdays[a]	Total number of hunting days for elk and deer on farm
TBconcernelk	Level of concern regarding TB in elk (of no concern…neutral…of great concern)
horses	number of horses on farm (0, 1-20, 21-40, 41-60, 61-80……>160)
farmsize	size of farm (hectares)
income	% of total income derived from farming
education	education of respondent (grade school high school college/university)
age	age of respondent (years)

[a] Hunting for wolves around RMNP is illegal, but wolves can be shot in defense of property.

Table 2. Percentage distribution of responses regarding livestock ownership versus the statement "I enjoy seeing wolves on my land". N = 786.

"I enjoy seeing wolves"	**Own livestock**			
	No	Yes	No response	**Total**
Disagree	7	34	3	**44**
Neutral	4	10	2	**16**
Agree	14	17	3	**34**
I don't know	1	2	0	**3**
No response	0	2	1	**3**
Total	**26**	**65**	**9**	

Table 3. Percentage distribution of perceived financial damage by wolves versus perceived wolf population size. N = 786.

Perceived wolf population size	**Perceived financial damage by wolves**						
	Never serious	Seldom serious	Most years	All years	I don't know	No response	**Total**
Too high	8	3	2	1	1	5	**26**
About right	15	3	0	0	1	4	**24**
Too low	7	0	0	0	0	3	**10**
I don't know	17	1	0	0	3	6	**28**
No response	4	1	0	0	0	6	**11**
Total	**51**	**8**	**2**	**1**	**5**	**24**	

Table 4. Cumulative AIC_c[a] weight of variables.

Variable[b]	Cumulative AICc weight[c]
population	1.00
damage	0.98
wolfsee	0.97
distance	0.78
beefcattle	0.76
beefcalves	0.43
huntdays	0.41
TBconcernelk	0.31
horses	0.29
farmsize	0.28
income	0.27
education	0.26
age	0.25

[a] AICc = Akaikes's Information Criterion with small-sample bias adjustment (Burnham and Anderson 1998).

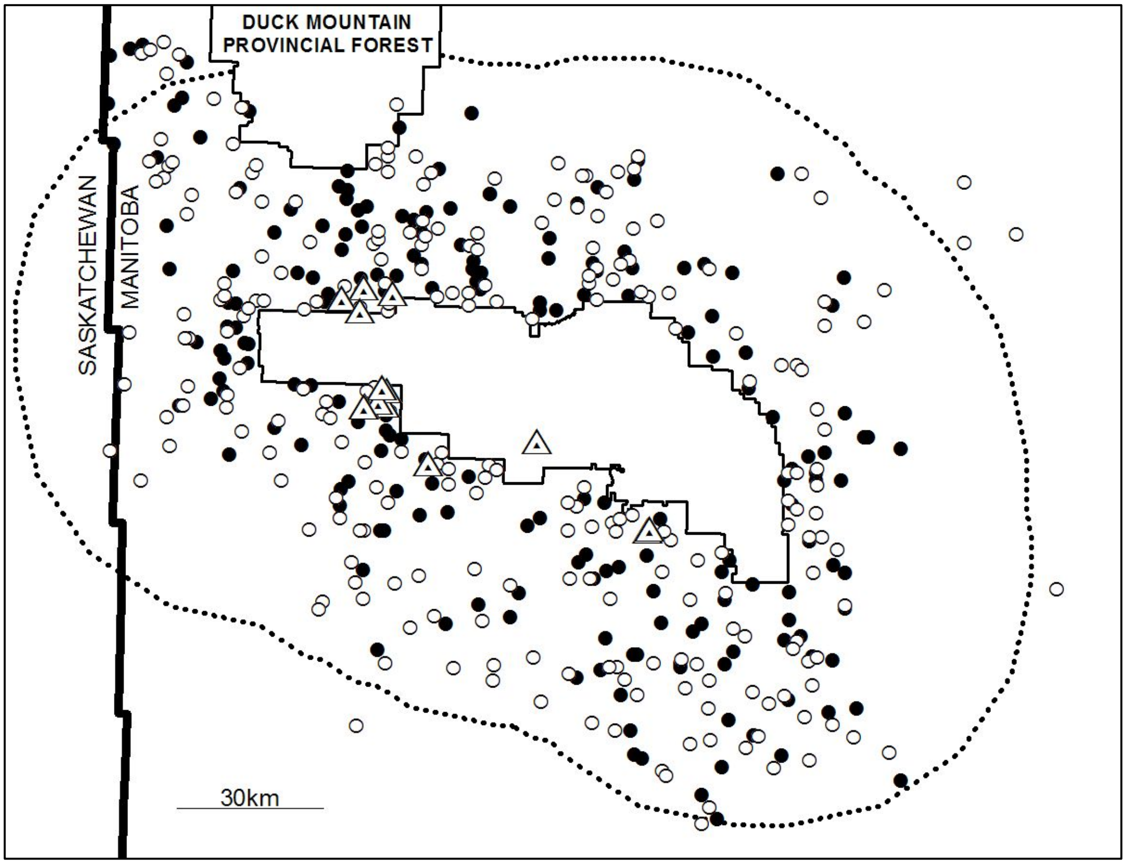
[b] Variables are described in table 1.

[c] Cumulative AICc weight of a variable = the percent of weight attributable to models containing the at particular variable and is calculated by summing the AICc model weights of every model containing that variable.

Table 5. Selected set of candidate models for farmer attitudes to wolves.

Model Structure	*-2Log(L)*	*k*	ΔAIC_c	AIC_c *w*
population + damage + wolfsee + beefcattle	161.95	5	0.0	0.648
population + damage + wolfsee + distance + beefcattle	161.95	6	2.0	0.238
population + damage + wolfsee + distance + beefcattle + damage * wolfsee + distance * wolfsee	159.48	8	3.5	0.111
population + damage + wolfsee + distance	173.61	5	11.7	0.002
population + damage + wolfsee	177.85	4	13.9	0.001
Population + damage + population*damage	181.86	4	17.9	<0.001
population * wolfsee + population * distance + wolfsee * distance + population * damage + population * beefcattle	185.84	6	25.9	<0.001
population	195.90	2	27.9	<0.001
wolfsee + damage + beefcattle + damage* beefcattle	207.59	5	45.6	<0.001
damage + wolfsee + beefcattle^2	209.80	4	45.8	<0.001
damage	224.22	2	56.3	<0.001
damage + wolfsee	222.38	3	56.4	<0.001
beefcattle	242.64	2	74.7	<0.001
distance	261.35	2	93.4	<0.001
wolfsee	261.46	2	93.5	<0.001
population * damage + wolfsee	259.81	4	95.9	<0.001

Figure 1. Farmer response to the statement “I enjoy seeing wolves on my land” in the Riding Mountain National Park region, Manitoba, Canada - 2004. Black circles indicate farmers who agreed with the statement, white circles farmers who disagreed. Triangles indicate documented cases of bovine tuberculosis in cattle or wild ungulates. A 50 km buffer around Riding Mountain National Park is represented by a dashed line.



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Appendix J. Management recommendations based on wolf research in southwestern Manitoba, Canada, 2003 - 2006:

- **Maintain regional genetic diversity and promote movement and gene flow between RMNP, the Duck Mountains and surrounding areas.** The gray – eastern wolf type mixture in the RMNP – Duck Mountain region may represent contemporary canid evolution.
- **Continue the designation of no general wolf hunting and trapping in the hunting zones around RMNP.** Genetic differentiation between RMNP and Duck Mountain wolves is not strong but nonetheless evident. This indicates that successful wolf dispersal and gene flow across the 30 km of mostly agricultural lands is infrequent. The few suggested RMNP immigrants I identified seem to spend time near the Park boundary. This makes them vulnerable to human-caused mortality even if they live on wild prey. Such individuals could contribute important genetic variation to the Park and should not be removed at random. In the event of livestock predation or other concerns regarding certain wolves or wolf packs, I recommended dealing with these case by case. If landowner/government staff conclude that non-lethal measures are insufficient and decide to remove one or more wolves, a site-specific approach would increase the likelihood of targeting only the individual(s) of concern. Avoid attracting wolves and remove dead livestock whenever possible to discourage habits of feeding on domestic animals.
- The southern Duck Mountains is likely the closest source of immigrants to RMNP. **Reduced trapping and hunting pressure in the southern Duck Mountains** could increase the number of dispersers and encourage movement across to RMNP.
- **Document all harvest where permitted, and collect local knowledge of wolf disease.** Wolves are difficult to observe and count, and with their pack structure and low density a combination of harvest and disease could potentially cause rapid population decline. Records from hunters and trappers of all animals taken, including discarded pelts, and any diseased animals seen or caught, would help keep track of local wolf numbers and warn of any decline. Recording all harvest also promotes consideration of wolves as an animal that should be conserved and valued as part of the local ecosystem.
- Differences in neutral genetic markers between populations do not automatically translate into biologically meaningful divergence. Further research could help clarify:
    1. **Whether there are biologically significant differences between eastern and gray type wolves**. More research on the eastern wolf haplotypes in the Duck Mountains could help clarify the coexistence in time and space of apparently different types of wolves. Also, whether eastern-type wolves appear to be a relatively new arrival in the Duck Mountains, and to what extent they seem to be expanding their range westward. Continue to monitor wolves in RMNP, and also survey the Duck Mountains to keep track of population change and the health and distribution of wolves. If possible, DNA analyses from the reported "brush wolves" intermediate between wolves and coyotes would be valuable to determine relationship with other wild canids.
    2. **Whether there are biologically significant differences between RMNP and Duck Mountain wolves,** such as locally adaptive traits related to predator – prey relationships and disease resistance. The genetic differentiation across relatively short distance could be due to landscape fragmentation and isolation, but it is also important to establish if RMNP and/or Duck Mountain wolves hold adaptive genetic variation that should be preserved to maintain long-term evolutionary potential in the prairie region.

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