HYBRIDIZATION DYNAMICS BETWEEN WOLVES AND COYOTES IN CENTRAL ONTARIO

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ABSTRACT

HYBRIDIZATION DYNAMICS BETWEEN WOLVES AND COYOTES IN CENTRAL ONTARIO

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Eastern wolves (*Canis lycaon*) have hybridized extensively with coyotes (*C. latrans*) and gray wolves (*C. lupus*) and are listed as a 'species of special concern' in Canada. Previous studies have not linked genetic analysis with field data to investigate the mechanisms underlying *Canis* hybridization. Accordingly, I studied genetics, morphology, mortality, and behavior of wolves, coyotes, and hybrids in and adjacent to Algonquin Provincial Park (APP), Ontario. I documented 3 genetically distinct *Canis* types within the APP region that also differed morphologically, corresponding to putative gray wolves, eastern wolves, and coyotes. I also documented a substantial number of hybrids (36%) that exhibited intermediate morphology relative to parental types. I found that individuals with greater wolf ancestry occupied areas of higher moose density and fewer roads. Next, I studied intrinsic and extrinsic factors influencing survival and cause-specific mortality of canids in the hybrid zone. I found that survival was poor and harvest mortality was high for eastern wolves in areas adjacent to APP compared with other sympatric *Canis* types outside of APP and eastern wolves within APP. Contrary to previous studies of wolves and coyotes elsewhere, I hypothesized that all *Canis* types exhibit a high degree of spatial segregation in the Ontario hybrid zone. My hypothesis was supported as home range overlap and shared space use between neighboring *Canis* packs of all ancestry classes were low. Territoriality among *Canis* may increase the likelihood of eastern wolves joining coyote and hybrid packs and exacerbate hybridization. Canids outside APP modified their use of roads between night and day strongly at high road densities (selecting roads more at night), whereas they responded weakly at lower road densities (generally no selection). Individuals that survived exhibited a highly significant relationship between the difference in their night and day selection of roads and availability of roads, whereas those that died showed a weaker, non-significant response. My results suggest that canids in the unprotected landscape outside APP must balance trade-offs between exploiting benefits associated with secondary roads while mitigating risk of human-caused mortality. Overall, my results suggest that the distinct eastern wolf population of APP is unlikely to expand numerically and/or geographically under current environmental conditions and management regulations. If expansion of the APP eastern wolf population (numerically and in terms of its geographic distribution) is a conservation priority for Canada and Ontario, additional harvest protection in areas outside of APP may be required. If additional harvest protection is enacted, a detailed study within the new areas of protection would be important to document specific effects on eastern wolf population growth.

Key Words: *Canis*, coyotes, eastern wolves, hybridization, resource selection, survival, territoriality

ACKNOWLEDGMENTS

Although I led this research, I did not perform all tasks described herein. My use of the first person singular throughout is to reflect that I led the research, conducted all analyses and wrote this dissertation as a single-author document. However, I want to stress the collaborative nature of this research and explicitly acknowledge tasks that were done by others or with assistance from others. B. Patterson, T. Wheeldon, and P. Mahoney are co-authors on journal papers published or submitted from this work. B. Patterson led all research and data collection in the WMU47 and Kawartha Highlands study units. Tyler Wheeldon performed the bulk of the DNA laboratory work for this research. K. Beauclerc, E. Kerr, C. Kyle, and L. Rutledge also contributed to the DNA labwork. P. Mahoney was a valuable confidant and authored several important statistical codes that facilitated my analyses. K. Mills initiated data collection in WMU49 and captured the first 14 study animals in that study unit along with K. Downing. Helicopter captures were performed each winter during 2008-2010 by Heli Horizons, Pathfinder Helicopters, and Big Horn Helicopters. I led and was personally involved in all fieldwork and data collection in APP and WMU49 from 2008-2011. I conducted all analyses described herein. I thank B. Patterson who has served as my advisor throughout this work and has been a partner in all research efforts. I thank C. Wilson and J. Schaefer for their support as committee members and very helpful and positive discussions during committee meetings and elsewhere. I thank P. McLoughlin for very helpful comments on my original proposal. I thank all field staff. I thank G. Crawshaw, our collaborator at the Toronto Zoo for all the help supplying me with both medical equipment and veterinarians. I thank all volunteer vets that helped, especially M. Kummrow, C.

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GENERAL INTRODUCTION

Hybridization is the interbreeding of individuals from genetically distinct populations or species (Rhymer & Simberloff 1996; Allendorf *et al.* 2001). Introgression is the transfer of genetic material from one population into another via hybridization (Allendorf *et al.* 2001; Burke & Arnold 2001). Hybridization and introgression are natural processes that have played important roles in the evolution of plants and animals (Grant & Grant 1992; Dowling & Demarais 1993; Dowling & Secor 1997). However, hybridization can also be facilitated by human actions when formerly allopatric species or populations are brought into sympatry, either directly or by anthropogenic changes to the landscape that allow range expansion by one species (Rhymer & Simberloff 1997; Wolf *et al.* 2001). Hybridization can have serious conservation implications if one of the parental types is rare or endangered because such populations may decline or become extinct due to hybridization (Rhymer & Simberloff 1997; Wolf *et al.* 2001; Allendorf *et al.* 2001). If few reproductive barriers exist between the hybridizing populations, the rarer type may disappear as they mate with the more common type and hybrids. This process can be exacerbated if hybrid offspring are more fit than individuals of the rarer parental type because hybrids will become more numerous and may displace the rarer genotype (Rhymer & Simberloff 1996; Wolf *et al.* 2001). Recently it has been recognized that hybridization has the potential to lead to the rapid extinction of parental species or populations in only a few generations (Huxel 1999; Wolf *et al.* 2001). The importance and nature of the evolutionary and practical implications of hybridization are strongly influenced by the relative abundance and spatial distribution of hybrid and parental types (i.e. structure of hybrid zone), the relative fitness of hybrids compared with parental types, the presence and nature of reproductive barriers between species, and environmental conditions and resources that favor parental or hybrid genotypes.

Hybrid zones are areas where individuals from genetically distinct populations interbreed and produce offspring (Barton & Hewitt 1985). Studies of hybrid zones can provide significant contributions to the understanding of evolutionary processes, including factors that influence gene flow and reproductive isolation between species (Scribner 1993; Borges 2005), and have the potential to improve management of threatened or endangered species (Allendorf *et al.* 2001). Characterizing the structure of hybrid zones and identifying the processes that maintain them have been primary goals in studies of hybridization; however, developing a general theory of hybrid zone structure and maintenance has been elusive because each hybrid zone is a unique manifestation of the dynamics between the hybridizing populations and interactions with environment (Rand & Harrison 1989; Delport 2004; Vines *et al.* 2003). Several theoretical models have been developed which allow for the classification of hybrid zones by the selective forces that influence their maintenance and structure (Barton & Hewitt 1985; Turelli & Orr 1995; Barton 2001). A fundamental consideration when characterizing a hybrid zone is to identify whether it was shaped primarily by exogenous or endogenous selection (Burke & Arnold 2001). Exogenous selection refers to variation in fitness in relation to environmental factors (e.g., habitat types) that results in the maintenance of particular genotypes under certain environmental conditions. Endogenous selection means that fitness varies due to internal processes and often refers to genetic incompatibilities between interbreeding species that can result in selection against hybrids.

Exogenous selection in relation to environmental variation can result in hybrid zones structured in several ways depending on the spatial configuration of the conditions influencing the fitness of different genotypes. One possible outcome is bounded hybrid superiority, which can result in hybrid zones structured around ecotones if hybrids are favored in transitional habitats between habitat or landscape types where each parental type is favored (Moore 1977; Moore & Price 1993; Good *et al.* 2000). Another possibility is that the hybrid zone will be structured as a mosaic if conditions that favor parental and hybrid genotypes exist as a patchwork across the landscape, such that selection favors different genotypes in a more heterogeneous fashion (Rand & Harrison 1989; Costedoat *et al.* 2005). Many studies have found hybrid zones structured along environmental gradients or in relation to other patterns of environmental heterogeneity, but fewer have been able to demonstrate the environmentally-mediated mechanisms that influence relative demographic performance of hybrid and parental types (e.g., Good *et al.* 2000).

Alternatively, if the hybrid zone is shaped primarily by endogenous selection, it can be classified as a tension zone where the structure is determined by a balance between selection against hybrids and the homogenizing influence of dispersal by individuals of each parental type (Barton & Hewitt 1985). A key distinction between endogenous and exogenous models is that, with the tension zone model, the reduction in fitness of hybrids is intrinsic, for example through disruption of co-adapted gene complexes, such that selection is believed to be independent of environmental conditions

(Barton & Hewitt 1985). Examples of endogenous selection are well documented (Barton & Hewitt 1985) but it may be less prevalent than previously suggested (Arnold & Hodges 1995; Rieseberg 199; Fritsche & Kaltz 2000). Clearly, to understand the mechanisms underlying the structure and maintenance of a hybrid zone, it is important to determine the distribution and relative fitness of parental and hybrid genotypes and whether these vary in relation to environmental conditions, such as habitat features and other resources. Additionally, documenting social behavior and resource selection of individuals within the hybrid zone will lead to a greater understanding of dynamics between the interbreeding populations and provide insight into the mechanisms that structure the hybrid zone (Barton & Hewitt 1985).

The hybrid zone of wolves (eastern wolves, *Canis lycaon* and gray wolves, *C. lupus*) and coyotes (*C. latrans*) in Ontario, Canada, is conducive to investigating fundamental principles hybridization dynamics of vertebrates, and results from such studies will benefit wolf conservation efforts. Eastern wolves hybridize with both coyotes and gray wolves in Ontario (e.g., Kyle *et al.* 2006; Rutledge *et al.* 2010*a*; von Holdt *et al.* 2011), but the mechanisms underlying this hybridization are poorly understood. An understanding of the intrinsic and extrinsic factors that influence genotype-specific fitness of wolves and coyotes in the *Canis* hybrid zone, and that facilitate or inhibit hybridization, would allow for improved management of the genetically distinct eastern wolf population in and around Algonquin Park (APP; Rutledge *et al.* 2010*a*). Previously it was suggested that eastern wolves in Algonquin represented the southern portion of a larger metapopulation and that, in addition to Ontario, eastern wolves were distributed widely across portions of Quebec, Manitoba, and Minnesota (Wilson *et al.* 2000; Grewal *et al.* 2004; Wilson *et al.* 2009). However, recent research across much of the potential range of eastern wolves has failed to identify significant numbers of highly assigned eastern wolves outside of central Ontario (Wheeldon 2009; Rutledge *et al.* 2010*a*; Wheeldon *et al.* 2010; Rutledge *et al.* 2012). Recent analysis indicated that wolves within APP were mostly (78%) eastern wolves whereas canids in northeastern and southeastern Ontario were mainly admixed gray wolves and eastern coyotes, respectively (Rutledge *et al.* 2010*a*). These finding suggest that environmental conditions within the protected area of APP may favor eastern wolves and perhaps allow them to resist hybridization more effectively than in areas outside of the park. Understanding the influence of large protected areas on hybridization dynamics, and the ability of rare hybridizing species to persist in reserves when reproductive barriers are minimal or absent outside of the protected area, is critical to understanding the Ontario wolf-coyote hybrid zone. Unfortunately the influence of protected areas on hybridization dynamics has received little or no explicit research attention. Therefore, studying wolves and coyotes concurrently within and adjacent to the APP boundaries would be effective to address the role of protected areas in influencing hybridization between species. Specifically, determining the distribution of wolves, hybrids, and coyotes within and adjacent to APP, comparing demographic performance of individuals across genotype and landscape conditions, and investigating resource selection patterns and other behaviors that may influence fitness and hybridization, are necessary to gain a better understanding of the dynamics and conservation implications of this hybrid zone.

Eastern wolves are listed as a species of 'Special Concern' in Canada and Ontario and their conservation status is currently (2013) being reviewed by the Committee on the Status Endangered Wildlife in Canada (COSEWIC). The restricted distribution and potential threat of hybridization to the long-term persistence of eastern wolves in Ontario are principle concerns being considered with this review. Although debate remains regarding the evolutionary history and taxonomic classification of eastern wolves (e.g., von Holdt *et al.* 2011), there is little doubt that eastern wolves in APP represent a distinct wolf population with an extremely restricted distribution (Fain *et al.* 2010; Rutledge *et al.* 2010*a*; Mech 2011; Rutledge *et al.* 2012). I combined field study, genetic analysis, and experimental manipulation to provide a comprehensive assessment of wolf-coyote hybridization dynamics in and adjacent to APP. Specifically, I identified the spatial genetic structure of wolves, coyotes, and hybrids and associations between *Canis* genetic ancestry and landscape features (Chapter 2), compared intrinsic and extrinsic influences on genotype-specific survival of wolves, coyotes, and hybrids (Chapter 3), investigated spatial organization and territoriality among wolf, coyote and hybrid packs (Chapter 4), and modeled genotype-specific resource selection patterns influencing fitness of wolves and coyotes (Chapter 5). My results have important implications for eastern wolf conservation and contribute significantly to the general understanding of mechanisms and consequences underlying hybridization in wildlife populations.

STUDY AREA

I studied wolves and coyotes in central Ontario from October 2004- May 2011 in 4 study units in and around APP: 1) western APP and surrounding harvest ban area (APP, 2006-2011; 7780 km^2), 2) Wildlife Management Unit 49 (WMU49, 2006-2011; 2720 km^2), 3)

Kawartha Highlands (KH, 2009-2010, 1810 km^2), and 4) Wildlife Management Unit 47 (WMU47, 2004-2007; 1800 km^2; Figure 1.1). In Algonquin Park, and the surrounding harvest ban area (park + ban area = 15,623 km^2), wolf and coyote harvest was illegal (Figure 1.1). In the 3 study units adjacent to APP, wolf and coyote harvest by trapping and hunting was allowed, on a seasonal or year-round basis, except in several smaller areas within KH (Figure 1.1). However, all study animals I monitored outside of APP, including those using the smaller protected areas, were at risk of harvest as their movements and home ranges extended into unprotected areas. Details regarding vegetative cover types and habitat conditions in and adjacent to APP are available in Maxie *et al.* (2010).

CANIS TYPES AND TERMINOLOGY

Given the taxonomic uncertainty surrounding some *Canis* species and populations, it is important (but challenging) to use clear and consistent terminology when discussing wolves and coyotes in eastern North America (Cronin & Mech 2009). Hereafter, I refer to Algonquin-type eastern wolves (Rutledge *et al.* 2010*a*) as eastern wolves. Admixed gray wolves in the western Great Lakes Region and Ontario have experienced contemporary and/or historical hybridization (Koblmuller *et al.* 2009; Fain *et al.* 2010; Wheeldon *et al.* 2010; von Holdt *et al.* 2011) but I refer to them as gray wolves for simplicity. Eastern coyotes (hereafter coyotes) in the APP region cluster with southeastern Ontario coyotes in population genetic analyses (J. Benson & B. Patterson, unpublished data), and have a history of hybridization with eastern wolves (Rutledge *et al.* 2010*a*; Way *et al.* 2010). Thus, although I refer to animals in the study area as eastern wolves, gray wolves, and coyotes for simplicity, I do not suggest that the animals I

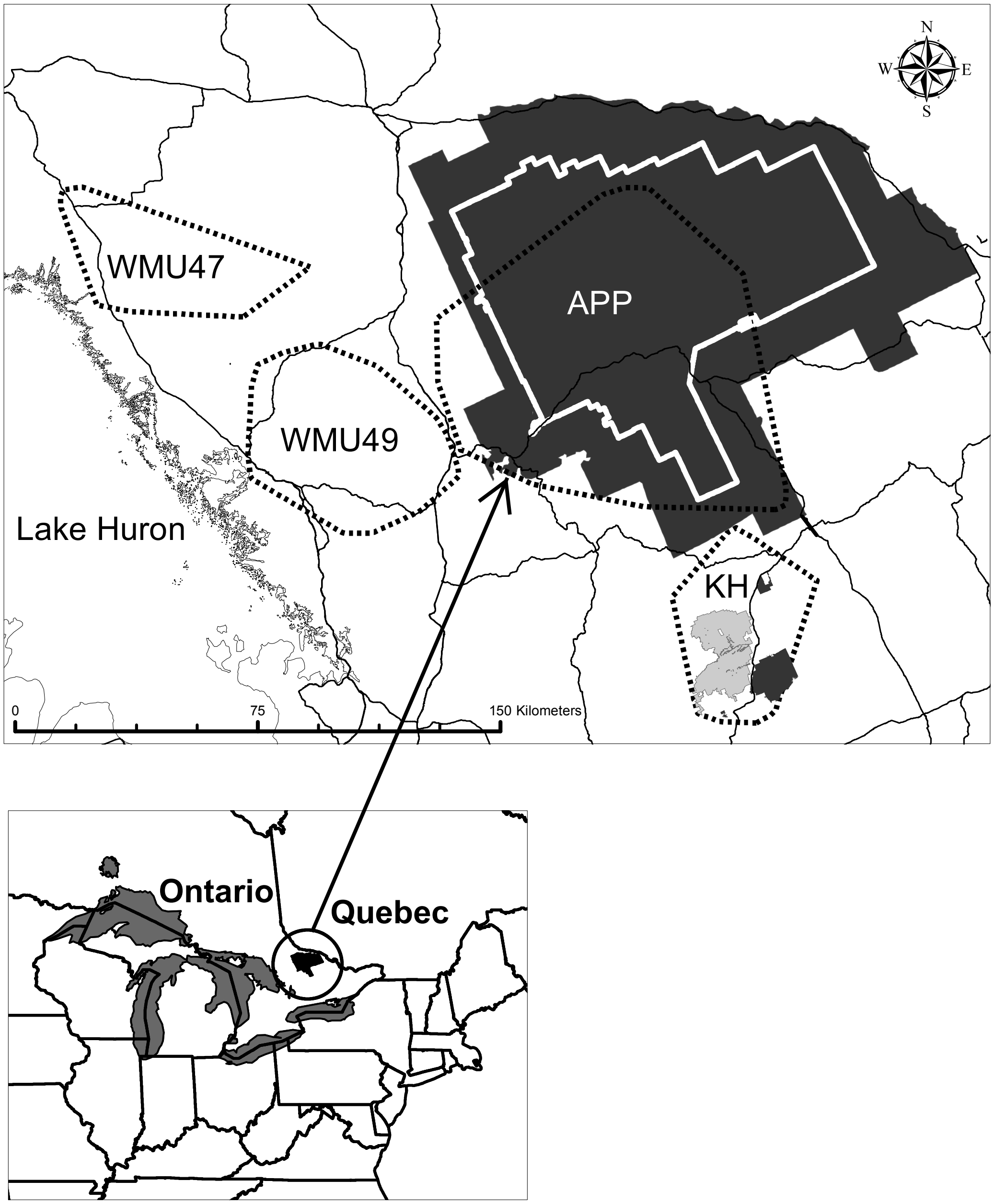

Figure 1.1 The 4 study units: Algonquin Provincial Park (APP), Wildlife Management Unit 47 (WMU47), WMU49, Kawartha Highlands (KH) in central Ontario denoted by minimum convex polygons (dashed outlines) created using telemetry data from study animals. Dark gray shading represents areas where wolves and coyotes were protected from harvest, whereas light gray shading indicates trapping (but no hunting) was allowed. White polygon shows the APP boundary and black lines represent major roads.

studied are “pure” representations of the ancestral genomes of these taxa, nor do my questions and inferences require acceptance of a specific evolutionary model. Rather, I acknowledge varying and uncertain levels of recent and historical gene flow between *Canis* populations in Ontario, and seek to provide insight into whether canids presently inhabiting the APP region are genetically and morphologically distinct, spatially structured, and associated with specific environmental conditions. Consistent with the recommendation of Cronin & Mech (2009), I argue that maintaining fit wolf populations is an important management goal and that more research should be directed towards understanding their ecological and demographic status.

GENERAL METHODS

Collection and Analysis of Field Data

Wolves, coyotes, and hybrids were captured using padded foothold traps, modified neck snares, and with net-guns fired from helicopters. I immobilized animals captured in traps and snares, whereas animals captured with net-guns were restrained manually without immobilizing agents. All capture and handling of animals was done in accordance with, and was approved by, Trent University (protocol no. 08039) and Ontario Ministry of Natural Resources (permit nos. 04-75 through 11-75) Animal Care Committees. I deployed mortality-sensitive Global Positioning System (GPS; Lotek Wireless, Newmarket, Ontario, Canada) or Very High Frequency (VHF; Lotek Wireless; Telonics Inc., Mesa, Arizona, USA; SirTrack, Havelock North, New Zealand) radio-collars on captured animals to monitor movements and survival. I programmed GPS collars to collect ~4000 fixes annually and to remain on the animals for approximately 1 year. I generally monitored collared animals 1-3 times/week from a fixed wing aircraft to track survival, space use, and pack associations. I estimated annual 95% fixed kernel home ranges (Börger *et al.* 2006) using the plug-in estimator to determine bandwidth (Sheather & Jones 1991) for all focal packs using GPS telemetry data. Each annual home range was estimated using data from 2-12 consecutive months. My fix schedules were variable within and across some months for some collars (range: 1 location/15 minutes to 1 location/6 hours) so I rarified data from collars with variable fix schedules such that the data used to estimate each home range were collected at regular intervals for each animal. I generally identified resident animals with home ranges estimated with GPS telemetry data and verified pack associations using aerial telemetry and visual sightings.

Environmental Variables

I estimated mean moose density across my study area, and within home ranges of wolves and coyotes, using aerial survey data collected by the Ontario Ministry of Natural Resources (OMNR) during 2003-2010. The data were collected by helicopter transects during January-March following a standardized protocol with the goal of counting every moose in 25 km^2 plots by scanning visually and investigating all fresh tracks. Plots were selected for survey during a given year using a stratified random design. In cases where individual plots were sampled in >1 year, I used the data from the survey that was closest to 2009 as this was the midpoint of the most intensive wolf-coyote telemetry study and I excluded overlapping data from other years.

After combining data from different years and areas, I performed a kriging analysis using the Geostatistical Analyst Wizard in ArcView 10 to estimate moose density. Kriging is an interpolation method which uses a set of linear regressions to predict values at locations without data based on data associated with known locations and the degree of spatial dependence between data points (Fortin & Dale 2005). I used ordinary kriging, and a stable semiovariogram with a prediction output, and set the lag size to 10,000 m, the number of lags to 12, and the maximum and minimum number of neighbors in each of 4 sectors of a moving window to 3 and 2, respectively. I used cross validation to assess the reliability of my kriging model, using the guidelines that: 1) prediction error should be unbiased if mean standardized prediction error is close to 0, and 2) variability in prediction was assessed correctly if the root-mean-square standardized error is close to 1 (Houlding 2000). Cross validation indicated that prediction error was unbiased as my standardized mean error was <0.001 (should be close to 0) and variability in prediction was assessed correctly as my standardized root-mean-square was 0.999 (should be close to 1). Thus, I considered my estimated moose density layer to be reliable for my analyses.

I intersected wolf and coyote home ranges with the moose density raster map produced from the kriging analysis using Geospatial Modeling Environment 3.1 to extract mean moose density for each home range. The KH study unit was located along the southern periphery of moose distribution in Ontario and, thus, moose surveys were not conducted south of KH. Portions of home ranges of 4 study animals in KH extended beyond the moose density layer and, thus, I restricted my estimation of moose density to the portions (mean = 83%, SE = 7%, n = 4) of these home ranges that overlapped with the layer. One additional home range did not overlap the moose density layer; however, much of this home range was actually surveyed for moose. The kriging analysis works on point data such that the density layer was truncated at the centroid of the southern-most moose sampling plots, which excluded this home range. In reality, the moose survey plots extended below this centroid and covered 71% of the home range in question, so I used the average number of moose seen within the home range to estimate moose density for this home range.

I estimated road densities (km/km^2) for each wolf and coyote range by developing 3 separate roads layers for primary, secondary and tertiary roads. I developed the primary and secondary roads layers by modifying the 2010 Ontario Roads Network layer (ORN; OMNR, Land Information Ontario, unpublished data) and supplementing this with park-specific roads layers for APP (OMNR, APP, unpublished data). Primary roads were paved roads with relatively high traffic volume classified as freeways, expressways or highways in the ORN. Secondary roads were generally paved and were classified as arterial, local/street, or collector roads in the ORN, except for a few major gravel logging roads in APP that received relatively high traffic volume and allowed for speeds of >50 km/ hour. I developed the tertiary roads layer with a trails layer developed by OMNR and supplemented this with a park-specific trails layer for APP. Tertiary roads were unpaved roads and trails that received light traffic, mostly from recreational vehicles and hikers. I intersected the resulting primary, secondary, and tertiary roads layers with wolf and coyote home ranges in ArcGIS 10 to calculate road densities for each pack.

CHAPTER 2. SPATIAL GENETIC AND MORPHOLOGIC STRUCTURE OF WOLVES AND COYOTES IN RELATION TO ENVIRONMENTAL HETEROGENEITY IN A *CANIS* HYBRID ZONE

Authors: John Benson, Brent Patterson, and Tyler Wheeldon

ABSTRACT

Eastern wolves have hybridized extensively with coyotes and gray wolves and are listed as a ‘species of special concern’ in Canada. However, a distinct population of eastern wolves has been identified in Algonquin Provincial Park (APP) in Ontario. Previous studies of the diverse *Canis* hybrid zone adjacent to APP have not linked genetic analysis with field data to investigate genotype-specific morphology or determine how resident animals of different ancestry are distributed across the landscape in relation to heterogeneous environmental conditions. Accordingly, I studied resident wolves and coyotes in and adjacent to APP to identify distinct *Canis* types, clarify the extent of the APP eastern wolf population beyond the park boundaries, and investigate fine-scale spatial genetic structure and landscape-genotype associations in the hybrid zone. I documented 3 genetically distinct *Canis* types within the APP region that also differed morphologically, corresponding to putative gray wolves, eastern wolves, and coyotes. I also documented a substantial number of hybrid individuals (36%) that were admixed between 2 or 3 of the *Canis* types. Breeding eastern wolves were less common outside of APP, but occurred in some unprotected areas where they were sympatric with a diverse combination of coyotes, gray wolves and hybrids. I found significant spatial genetic structure and identified a steep cline extending west from APP where the dominant genotype shifted abruptly from eastern wolves to coyotes and hybrids. The genotypic pattern to the south and northwest was a more complex mosaic of alternating genotypes.

I modeled genetic ancestry in response to prey availability and human disturbance and found that individuals with greater wolf ancestry occupied areas of higher moose density and fewer roads. My results clarify the structure of the *Canis* hybrid zone adjacent to APP and provide unique insight into environmental conditions influencing hybridization dynamics between wolves and coyotes.

INTRODUCTION

Identifying the spatial distribution of genotypes and phenotypes across hybrid zones has long been a goal of evolutionary ecologists seeking to infer the processes generating and maintaining hybrid zones (Mayr 1963; Endler 1977; Barton & Hewitt 1985). Hybrid zones may be spatially structured as clines, where genotypes and phenotypes transition along a gradient from one parental type to the other (Barton & Hewitt 1985; Rand & Harrison 1989). Alternatively, hybrid zones may be mosaic in structure, where a patchwork of alternating genotypes and phenotypes are distributed across the landscape, usually in relation to environmental heterogeneity (Rand & Harrison 1989; Britch *et al.* 2001). Most studies of hybrid zones have sought to provide theoretical insight into evolutionary processes such as speciation (Mayr 1963; Endler 1977; Barton & Hewitt 1985). However, increasing recognition of the practical implications of hybridization, as both a deleterious (e.g., reduction of rare species, Rhymer & Simberloff 1996; Allendorf *et al.* 2001) and creative (e.g., rapid adaptation to new environments, Seehausen 2004; Mallet 2005) evolutionary force means that understanding the structure of hybrid zones can also be an important conservation objective. Specifically, understanding spatial variation of rare genotypes and identifying environmental conditions underlying these patterns are important goals for developing sound management strategies for hybridizing species.

The colonization of northeastern North America by coyotes (*Canis latrans*) during the 20th century led to widespread hybridization between coyotes and eastern wolves (*C. lycaon;* Wilson *et al.* 2000; Kyle *et al.* 2006). This colonization was facilitated by human actions as forest-clearing and direct persecution reduced and eliminated wolves (*Canis spp*.) from much of the United States and southern Canada (Fritts *et al.* 2003), and may have also reduced reproductive barriers between wolves and coyotes (Kolenosky & Standfield 1975; Kyle *et al.* 2006). Eastern wolves also appear to have hybridized extensively with gray wolves (*C. lupus*) in the western Great Lakes Region and central Ontario (Fain *et al.* 2010; Wheeldon *et al.* 2010). Although considerable evidence suggests eastern wolves are a distinct species (e.g., Wilson *et al.* 2000; Kyle *et al.* 2006; Fain *et al.* 2010; Mech 2011) this designation remains controversial and an alternative viewpoint suggests intermediate sized wolves in eastern North America are the product of hybridization between gray wolves and coyotes (e.g., von Holdt *et al.* 2011). Eastern wolves are currently considered a subspecies of the gray wolf (*C. l. lycaon*) and are listed as a ‘species of special concern’ federally in Canada (COSEWIC 2001) and in the province of Ontario (COSSARO 2004). Regardless of uncertainty regarding their evolutionary history and distribution, eastern wolves are protected under Federal and Provincial Species at Risk Acts and a genetically distinct population of eastern wolves has been identified in Algonquin Provincial Park (APP) in Ontario (Rutledge *et al.* 2010).

A hybrid swarm has apparently replaced eastern wolves from many areas across Ontario such that few, if any, non-admixed individuals remain (Wilson *et al.* 2009; Rutledge *et al.* 2010*a*). However, most breeding wolves in APP are “Algonquin-type” eastern wolves and genetically distinct from both eastern coyotes in southeastern Ontario and admixed gray wolves (*C. lupus x lycaon*) in northeastern Ontario (Rutledge *et al.* 2010*a*). Although the APP population has been studied extensively within the park boundaries and compared with other populations across Ontario and beyond (Grewal *et al.* 2004; Rutledge *et al.* 2010*a*), the full extent of the Algonquin-type eastern wolf population remains unknown as the spatial genetic and morphologic structure of the hybrid zones in many areas immediately adjacent to the park have not been well studied. The conservation status of eastern wolves in Canada is being reviewed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) during 2012-2013; therefore, it is important identify the spatial distribution of *Canis* genotypes in areas adjacent to APP.

Across the broad *Canis* hybrid zone in northeastern North America, many studies have analyzed molecular data and made inferences regarding the genetic ancestry of individuals in wolf and coyote populations (e.g., Wilson *et al.* 2000; Grewal *et al.* 2004; Koblmuller *et al.* 2009; Fain *et al.* 2010; Rutledge *et al.* 2010; Wheeldon *et al.* 2010; von Holdt *et al.* 2011). Previous researchers have speculated that observed variation in genetic structure of *Canis* populations could be related to interactions between canid body size, prey availability, and genotype-specific responses to human disturbance (e.g., Wilson *et al.* 2000; Kyle *et al.* 2006; Koblmuller *et al.* 2009; Rutledge *et al.* 2010*a*; von Holdt *et al.* 2011). However, no previous studies have extended their molecular results by showing that the observed genetic distinctions manifested in morphological or ecological differences across hybridizing *Canis* types. Rutledge *et al.* (2010*a*) presented weights of animals from 3 regions of Ontario (including APP) inhabited by wolves and/or coyotes, but no genotype-specific analysis of morphology was conducted. Sears *et al.* (2003) conducted a detailed comparison of morphological characteristics of putative wolves, coyotes, and hybrids in areas within and adjacent to APP; however, they did not provide genetic profiles of these animals. Sears *et al.* (2003) also compared *Canis* diet from scat analysis and landscape attributes across study sites, but did not connect these data to individual animals or genetic ancestry. Thus, studies explicitly linking genetic inferences with morphological and ecological characteristics of individual animals are clearly needed to begin to elucidate the biological significance of wolf-coyote hybridization.

Accordingly, I studied genetics, morphology, and landscape associations of resident wolves and coyotes in and adjacent to APP with 3 main objectives and several associated questions and hypotheses. My first objective was to characterize the genetic structure of *Canis* populations in the hybrid zone in and adjacent to APP to 1) identify distinct *Canis* genetic types, 2) determine the extent of admixture between distinct types, and 3) investigate fine-scale spatial genetic structure. Specifically, I addressed the question of whether the hybrid zone adjacent to APP is structured as a cline or a mosaic, and whether the pattern varies across the region. Second, I hypothesized that variable environmental conditions related to prey availability, habitat fragmentation, and human disturbance would explain much of the variation in the distribution of wolf and coyote genotypes in and adjacent to APP. I predicted that wolves would be associated with areas of higher densities of large ungulates, whereas coyotes would be associated with areas of greater human disturbance. Third, I compared morphology of wolves, coyotes, and admixed individuals to determine if distinct genetic types also differed phenotypically with two hypotheses. First, I hypothesized that body size increases along a gradient from coyotes to eastern wolves to gray wolves, with distinct types exhibiting genotype-specific morphology. Second, I hypothesized that admixed individuals exhibit morphology intermediate to parental types. My results provide unique insight into the influence of a large protected area and variable environmental conditions in adjacent areas, on the structure of a hybrid zone between 3 putative *Canis* species.

METHODS

Sample Collection and Field Methods

I obtained DNA samples from 342 wolves and coyotes mostly from live capture (*n* = 272) using padded foothold traps, helicopter net-gunning, modified neck snares, and capture by hand (pups ≤ 6 weeks only). I weighed and measured captured animals, recording body mass (kg) and body length (cm; tip of nose to base of tail). I captured animals in APP during 2006-2011, WMU49 during 2006-2011, KH during 2009-2010, and WMU47 during 2004-2005. Blood was taken from the cephalic vein and deposited on FTA cards (GE Healthcare UK Ltd, Buckinghamshire, UK) which were stored at room temperature until processing. I collected non-invasive hair and scat (swabbed for DNA in the field, Rutledge *et al.* 2009) samples from kill, den, and rest sites of focal packs, and opportunistically while conducting field activities. I also collected tissue samples from road-killed animals. After processing, I compared successful non-invasive samples with those from captured animals to identify matching genotypes and family relationships. I identified 70 unique genotypes from non-invasive sampling beyond those that matched previously genotyped animals.

I had sufficient telemetry data to estimate home ranges for 79% (95 of 121) of the individuals included in the analysis. In the absence of GPS data, I determined residency status by tracking radio-collared animals (*n* = 20), by capturing pups or yearlings and subsequently obtaining non-invasive samples from parents (identified with pedigree analysis, see below) close (<1.2 km) to the capture sites (*n* = 4), or by capturing pups in obvious rendezvous sites (centers of activity for resident packs during pup rearing season, Argue *et al.* 2008; *n* = 2). During spring 2008-2011, I visited natal dens of focal packs within APP and WMU49 and captured neo-natal pups (3.5-6 weeks old) in or around these dens to collect DNA samples and implant them with VHF radio-transmitters to track survival and movements. I used similar methods to previous den work in APP (Mills *et al.* 2008). I used DNA from these pups, and pups captured later in the year in all study units, to identify or verify breeding animals in focal packs by determining parentage and other family relationships with a pedigree analysis (see below). Additionally, I assessed whether females had bred previous to capture by examining their nipples, which are enlarged and blackened in breeding animals (Mech *et al.* 1993).

Microsatellite Genotyping

12 autosomal microsatellite loci were amplified for each sample (Ostrander *et al.* 1993, 1995; cxx225, cxx2, cxx123, cxx377, cxx250, cxx204, cxx172, cxx109, cxx253, cxx442, cxx410, cxx147) as in Wheeldon *et al.* (2010). Genotyping was performed on an ABI3730 (ABI, Applied Biosystems) and alleles were scored in Genemarker v1.7 (Softgenetics LLC). Non-invasive (i.e. low template) samples were quantified based on

nuclear DNA (at locus cxx204) to ensure that ≥ 250-500 pg/ul of DNA was available before proceeding with microsatellite profiling. For non-invasive hair and scat samples, if extraction yielded <250 pg/ul microsatellite amplification was not attempted. PCR product was re-submitted at lower dilution and/or samples with low amplifying alleles were re-amplified to confirm homozygous genotypes or if there was any ambiguity in scoring alleles.

Mitochondrial DNA sequencing and Y-chromosome microsatellite genotyping

For all samples, published primers (Pilgrim *et al.* 1998; Wilson *et al.* 2000) were used to amplify a 343-347-bp fragment of the mitochondrial DNA (mtDNA) control region as in Wheeldon *et al.* (2010*a*). Sequencing was performed on an ABI3730 (Applied Biosystems) and sequences were edited and aligned in MEGA 5 (Tamura *et al.* 2007). Sequences were edited to 223-228 base pairs in length and haplotypes were assigned corresponding to previously described sequences (Wilson *et al.* 2000). For male samples, 4 Y-chromosome microsatellite loci (Sundqvist *et al.* 2001: MS34A, MS34B, MS41A, MS41B) were amplified as in Wheeldon *et al.* (2010). I used mtDNA and Y-chromosome haplotypes to assist in my pedigree analysis (as explained below). Haplotypes for individuals in the main analysis are presented in Appendix E & F.

Sample Information

I included microsatellite profiles from 121 and 146 individuals from the 4 study units in analyses assessing genetic structure for: 1) residents only, and 2) residents and transients, respectively. I also included samples from 40 northeastern Ontario (NEON) gray wolves as an outgroup (*n* = 40, Rutledge *et al.* 2010*a*), because I suspected admixed gray wolves existed in my dataset. Thus, for my main analysis, I included samples from 161 total individuals from APP (*n* = 40), WMU49 (*n* = 42), KH (*n* = 21), WMU47 (*n* = 18), and NEON (*n* = 40). Sample types included blood (*n* = 144), scat (*n* = 5), hair (*n* = 4), and tissue (*n* = 1). I also reconstructed genotypes from 7 breeding individuals for inclusion in my analyses based on genotypes of 1 known breeder (identified with pedigree analysis, see below) and ≥4 known offspring from a single litter. I reconstructed these genotypes using the alleles of the known breeder and those of the pups following principles of Mendelian inheritance implemented in the program Gerud 2.0 (Jones 2005). I limited my main analysis to resident pack animals (*n* ≥ 57 packs) because I was interested in assessing genetic structure of the resident, breeding units of wolves and coyotes across the study area. Based on radio-telemetry data, I excluded non-resident animals (*n* = 25) that were solitary (not with a pack) and did not exhibit home range behavior. I monitored reproductive status of all radio-collared females in my study each spring using telemetry to identify natal den sites, but did not document reproduction by non-residents. Thus, my findings were similar to other studies which have indicated that breeding by non-resident wolves is extremely rare (Mech & Boitani 2003).

I excluded all direct offspring of breeding animals from my analyses (*n* = 144), unless I did not have samples for both breeding animals in a given pack (see below). Most (78%) of these offspring were captured as neonatal (3-6.5 weeks old) pups in natal dens as part of a companion study of pup survival. When both parents were included in the analysis, I also excluded offspring identified by my parentage analysis from samples obtained by other captures (i.e., not at den sites, 19%) or by non-invasive sampling (3%). I excluded these offspring to avoid unstable and potentially spurious results from population genetic analyses, which can arise because relatedness among family members may be difficult to distinguish from population structure (Camus-Kulandaivelu *et al.* 2007). Given that I sampled offspring unequally from my focal packs (range 0-15 pups per pack), including all offspring would have biased my analyses by over-representing the genotypes of packs sampled more intensively. In many cases, I had both breeding animals genotyped from focal packs (*n* = 60 breeders from 30 packs) and both were included in my analyses. When I had 0 or 1 breeders genotyped, and reconstruction of the other breeder was not possible, I included 2 offspring (*n* = 5) or a breeder and an offspring (*n* = 3), respectively, to represent the parental genotypes. In other cases (*n* = 13) I included a single adult from a pack. I also included any resident individuals (*n* = 2) that were unrelated (not direct offspring or siblings) to the breeding animals in focal packs. The only instances in which I included direct offspring of breeding pairs in my analyses were cases where these offspring joined or formed other packs later in the study, or became breeding animals themselves within their natal packs. Finally, I did not include non-invasive samples (*n* = 55) if I was unable to link them to focal packs via pedigree analysis and/or field data. This was because I had no way of knowing whether these animals were residents or non-residents, and could have simply been dispersing through the area. The exclusions noted above decreased overall sample size, but strengthened inferences by ensuring that results were unbiased and directly relevant to the resident, breeding *Canis* population in the study area.

Pedigree Analysis

To determine parent-offspring relationships, I conducted parentage assignments using a hierarchical, multi-analysis approach. First, if I found mismatches between mtDNA and Y-chromosome haplotypes, I ruled out a parent-offspring relationship. Second, (if haplotypes matched), I used exclusion to identify plausible parent-offspring relationships (Jones *et al.* 2010). Third, I used a categorical allocation approach implemented in the program Cervus 3.0 (Kalinowski *et al.* 2007) to assign parents with 80% or 95% confidence. Cervus uses robust likelihood methods and allows for genotyping errors that could exclude real parents if exclusion alone were used. Fourth, I used the maximum likelihood approach of ML-RELATE (Kalinowski *et al.* 2006) to test specific hypotheses about parent-offspring or sibling relationships if previous results were ambiguous. I assigned parents using the following protocol: 1) if >2 microsatellite allele mismatches were detected using exclusion, that individual was excluded as being the potential parent, 2) if ≤2 mismatches were detected and Cervus assigned parentage with 95% confidence, I considered this to be the parent, 3) if Cervus assigned parentage with 80% confidence, I tested this parent-offspring relationship specifically with ML-RELATE to confirm or refute this assignment at $\alpha = 0.05$. Many mother-offspring relationships were clear from capturing live pups in dens of presumed breeding females; however, I verified all such relationships with the above methodology. I also inferred breeding status when there was only a single adult animal of a given sex in a pack and from examining nipples of females for evidence of prior breeding. In cases where direct offspring were excluded from my analyses because both parents or a full-sibling were genotyped but individual assignments were needed for subsequent analyses, I assumed a 50%-50% contribution from the parents, and/or that their genotype was identical to that of full siblings.

Genetic Structure Analyses

I obtained autosomal microsatellite genotypes based on 10 (n = 1), 11 (n = 2), or 12 (n = 158) loci for the main analysis. I analyzed autosomal genotype data in several ways to assess population structure and investigate sources of genetic variation in wolves and coyotes across the study area. First, I used a Bayesian approach, implemented in the program Structure (v.2.3.3, Pritchard *et al.* 2000) to identify genetic clusters and to estimate genetic origin of individuals using microsatellite allele frequencies. The Structure analysis allows for estimation of admixture proportion (Q) which is an estimate of the proportion of an individual's genome derived from a given genetic population (Falush *et al.* 2003). I ran the admixture model of Structure, assuming correlated allele frequencies and inferring the parameter alpha, for $K = 1$ to $K = 7$ with five repetitions of 10^6 iterations following a burn-in period of 250, 000 iterations for each K. I calculated the posterior probability (Ln P[D]) for each K by averaging Ln P[D] across the five runs. I evaluated relative support for each value of K based on the mean Ln P[D] (Pritchard *et al.* 2000) and ΔK (Evanno *et al.* 2005), and I also considered the biological significance of each potential number of clusters. I conducted a second Structure analysis in which I included non-resident animals ($n = 25$) and replaced 1 pup with their mother or father for packs ($n = 4$) where I only had one breeder sampled and had included 1 or 2 pups to represent genotypes of the breeding animals. I conducted this second analysis to obtain individual assignments for all breeding and non-resident animals not included in the main analysis for use in subsequent analyses.

Next, I conducted a centered Principal Components Analysis with the autosomal microsatellite allele data using the 'adegenet' package v. 1.3-1 (Jombart 2008) in R v. 2.13.1 (R Development Core Team, 2011) to corroborate inferences from the Structure analysis by arranging individuals in the study area along axes of variation based on their microsatellite allele genotypes (Patterson *et al.* 2006). After running the PCA, I calculated the percentage of the total variance explained by each component and calculated 95% confidence ellipses for groups of individuals, organized by study unit of residency, to assess the genotypic composition of each study unit. PCA is also an effective dimension reducing method to prepare microsatellite data for an alternative clustering procedure, *K*-means, which partitions genetic variation into between-group and within-group components and attempts to minimize the latter in order to find cohesive clusters (Lee *et al.* 2009; Jombart *et al.* 2010). *K*-means, when used with Bayesian Information Criteria (BIC) to determine the best supported model, has been shown to perform similarly or better than Structure (with LnP[D] and ΔK) in terms of determining the number of clusters in genetic data (Liu & Zhao 2006; Lee *et al.* 2009; Jombart *et al.* 2010).

Individual Assignments

I repeated the Structure procedure at the highest, strongly supported value of K for 10 repetitions and averaged Q-values across the 10 runs for use with individual assignments. I classified individuals of $q \geq 0.8$ as belonging to a specific cluster and individuals with all $q < 0.8$ as being admixed, consistent with previous *Canis* research (Verardi *et al.* 2006; Rutledge *et al.* 2010; Wheeldon *et al.* 2010). Although analysis of microsatellite data with program Structure has been recommended for individual assignment and detecting hybrids (e.g., Manel *et al.* 2005; Vähä & Primmer 2006), I verified the assignments using PCA. I employed this additional step due to the inherent difficulty of detecting backcrossed hybrids between closely related species with a recent history of admixture (Randi 2008) and because PCA does not require genetic assumptions that, if violated, can compromise accuracy of individual assignment in Structure (Paschou *et al.*

2007). Furthermore, I recognize the arbitrary nature of *q*-value thresholds for determining hybrid status (Vähä & Primmer 2006), making it important to verify assignments with additional analysis subsequent to the application of the threshold criteria. Thus, after placing individuals into genotype classes, I used PCA to calculate 95% confidence ellipses with individuals grouped by their assigned cluster, to evaluate whether the original assignments agreed with the clustering of individuals along axes of variation in the PCA. If the PCA indicated that an individual was within, intersecting, or beyond the 95% confidence ellipse of another group (highly assigned or admixed groups), I assumed that individual belonged in that cluster. Additionally, I used PCA to clarify ancestry of 4 individuals whose *Q* scores suggested possible admixture between > 2 clusters. My approach follows Cegelski *et al.* (2003) and Bohling & Waits (2011) by using multiple analytical approaches to improve confidence in individual genetic assignments, which is particularly important for studies with management implications.

Spatial Genetic Structure

I conducted a spatial principal components analysis (sPCA) to investigate spatial genetic patterns among wolves and coyotes in and adjacent to APP and to identify areas in this landscape where eastern wolves persist. sPCA utilizes Moran's I, an index of spatial autocorrelation, to compare allele frequencies observed in individuals at given spatial locations with those of individuals at neighboring sites (Jombart *et al.* 2008). Jombart *et al.* (2008) developed 2 multivariate tests for use with sPCA to detect global (e.g., clines and patches) and local structure. Significant global structure is identified when individuals that are geographically close are also similar genetically (positive spatial autocorrelation), whereas significant local structure is identified when individuals that are spatially close are dissimilar genetically (negative spatial autocorrelation). For spatial locations, I used the centroid of the home range for all animals in packs with sufficient GPS telemetry data (*n* = 95). For other animals, I used the mean center of all telemetry locations (*n* = 15), capture location (*n* = 6), sample location (for non-invasive samples, *n* = 4), or den site location (*n* = 1). To facilitate this analysis, I developed a Gabriel's graph (Legendre & Legendre 1998) as a connection network to model the spatial relationships between individuals. In contrast to the previous analyses (Structure, PCA, *K*-means) I did not include the NEON out-group because I were interested in investigating spatial genetic relationships only within the study area.

I used generalized additive mixed models (GAMMs; Lin & Zhang 1999; Wood 2006) implemented with the packages 'gamm4' v. 0.1-6, 'mgcv' v. 1.7-6, and 'lme4' in R to further investigate spatial genetic structure surrounding APP. A GAMM is simply a generalized linear mixed model (GLMM) in which part of the linear predictor is specified in terms of smooth (non-linear) functions of covariates (Lin & Zhang 1999). No adjustment is required to GLMM methods (beyond the inclusion of the smooth term[s]) to fit a GAMM (Wood 2006). GAMMs are extensions of generalized additive models (GAMs; Hastie & Tibshirani 1986) in which ≥1 random effect is included in addition to fixed effects (Wood 2006). GAMs and GAMMs are appropriate for analyzing spatial genetic patterns because they are flexible, semi- or non-parametric regression models that can be used to model complex, non-linear relationships between response and predictor variables (Snäll *et al.* 2004). Predictor variables are specified in terms of smooth functions, in this case these were thin-plate regression splines, for which the exact parametric form is unknown (Wood 2006). The smoothing functions (splines) fit curves to non-linear trends between the response and predictor variables; however, if the relationship with a given predictor variable is better modeled as linear (i.e. estimated df = 1) the term can be included as a parametric fixed effect (Wood 2006). I used GAMMs (rather than GAMs) to allow for the inclusion of pack as a random effect, to account for the social structure of wolves and coyotes in the models. Mixed models are increasingly being used to analyze ecological data that is hierarchical in nature, such as when individuals are sampled from groups, to avoid violating the assumption of independence among samples required for regression (Bolker *et al.* 2009). Previous studies of wolf ecology have also adopted mixed-modeling regression approaches by specifying pack as a random effect (e.g., Hebblewhite *et al.* 2008).

The GAMMs differed from the sPCA in that I used % eastern wolf ancestry of resident animals as the response variable to explicitly investigate the spatial distribution of these highly assigned and admixed wolves in and around APP. Specifically, I investigated whether the hybrid zone extending out from APP into adjacent areas: 1) showed a cline, a mosaic pattern, or elements of both, and 2) whether the pattern was similar in shape and steepness to the west, south, and northwest of APP. I predicted that if the hybrid zone adjacent to APP was clinal, the relationship between space and wolf ancestry would be approximately linear and a simple distance variable would explain most of the variation. However, if the pattern was a mosaic, I predicted that the relationship would be modeled better by a spatial variable that allowed for more complex, discontinuous patterns between space and genotype.

I transformed the proportional response variable using the logit transformation (ln $y[1-y]$) to map admixture proportions monotonically to the whole real line ($-\infty, \infty$) and to meet assumptions of regression modeling (Warton & Hui 2011). I conducted these spatial analyses with 2 model sets. First, I used distance from the center of APP (hereafter distance) to the centroid of each animal's home range as the continuous independent variable, entered into the model as a smooth (non-parametric) predictor of % eastern wolf ancestry. Next, I substituted the distance variable for a smooth interaction term between easting and northing metric spatial coordinates (hereafter space) to assess whether this variable improved model fit and identified more complex spatial-genotype patterns. Spatial coordinates can be included in regression models as independent variables to detect (and account for) spatial autocorrelation in the response variable (Beale *et al.* 2010). Thus, I used the spatial covariate to model spatial genetic structure of eastern wolf ancestry. I also included a random effect of pack in all models to account for the fact that I sampled (1-4) individuals from different packs across the study area. All GAMMs (and underlying GLMMs) were estimated using restricted maximum likelihood (REML) methods which produce less biased estimates of variance components for random effects in mixed models than traditional maximum likelihoods (Wood 2006; Bolker *et al.* 2009). I conducted an overall analysis (all study units) to compare models with the distance and space variables, and then conducted analyses with data from APP and each of the adjacent study units separately to model the genotypic patterns extending from APP into each adjacent area with distance and space, and to consider differences in these patterns. All distance and space models contained only a single predictor term (distance or space) and I assessed fit between pairs of models with the space or distance variables using Akaike's Information Criteria corrected for small samples (AIC_c) and the difference between AIC_c values (ΔAIC_c, Burnham & Anderson 2002). Models with

$\Delta AIC_c < 2$ are generally considered to be plausible competing models (Burnham & Anderson 2002). I determined the amount of variation explained by each model using adjusted R^2 values. I included all data from my main population genetics analyses, except that I substituted single breeding males or females for sibling pairs ($n = 4$) and removed single pups from packs that also contained a parent ($n = 3$) or a sibling ($n = 1$). This was done to avoid including any closely related animals from the same pack in the analyses to further ensure independence between samples.

Landscape Analysis

Next, I extended the GAMMs to test hypotheses regarding the influence of prey availability (moose [*Alces alces*] and deer [*Odocoileus virginianus*]) and fragmentation/human disturbance (road densities) on the distribution of genotypes in the APP area to investigate the environmental conditions underlying spatial genetic structure. I estimated mean moose density across the study area, and within home ranges of wolves and coyotes, using aerial survey data collected by the Ontario Ministry of Natural Resources (OMNR; see estimation details in Supporting Methods). I used a Geographic Information System (GIS) layer of deer wintering areas, compiled and digitized by OMNR, and intersected these with wolf and coyote home ranges to calculate the proportion of the home range comprising deer wintering habitat as an index of winter deer availability. I estimated road densities (km/km^2) for each wolf and coyote range by developing separate layers for primary, secondary and tertiary roads. Primary roads were paved roads with relatively high traffic volume classified as freeways, expressways or highways. Secondary roads were mostly paved and were classified as arterial, local/street, or collector roads, except for a few major gravel logging roads in APP that received relatively high traffic volume and allowed speeds of > 50 km/hr. Tertiary roads were unpaved roads and trails that received light traffic, mostly from recreational vehicles and hikers.

Harvest was illegal within APP and the surrounding buffer area, and I found no evidence of illegal harvest within the APP study unit despite monitoring survival and cause-specific mortality of >100 radio-instrumented canids in APP between 2006-2011 (J. Benson & B. Patterson, unpublished data). Given that tertiary roads were smaller, unpaved roads, I assumed their effect on wolves and coyotes would be mostly by providing access to hunters and trappers into otherwise remote areas outside of APP. Therefore, I included an interaction term between tertiary roads and harvest protection status to test the hypothesis that access to harvest (via tertiary roads) would influence wolf-coyote occurrence and ancestry differently in areas with (APP) and without harvest protection (other study units). I included tertiary road density as a non-parametric (smooth) variable and modeled the interaction with a categorical, parametric term for study unit (bivariate term, with study units outside of APP pooled) using the ‘by’ command in the ‘mgcv’ package in R. I also included study unit as a parametric main effect in all models retaining the interaction term to account for the fact that smooth terms are subject to a centering constraint, which was not required in this case due to the interaction with a factor variable (Wood 2006). I included pack as a random effect in all landscape models to account for the fact that I sampled 1-4 individuals from different packs.

I used % eastern coyote ancestry (logit transformed) of adult, resident animals as the response variable (inverse was % wolf), under the assumption that eastern and gray wolves would be more similar in their environmental associations than gray wolves and coyotes. This allowed me to primarily compare landscape associations of eastern wolves and coyotes (from which most ancestry of individuals in my sample was derived), without excluding data from individuals with gray wolf ancestry (which represented a smaller proportion of *Canis* ancestry). I conducted 2 landscape analyses in a hierarchical manner because I was interested in modeling landscape-genotype relationships: 1) across the entire study area including APP, 2) across all areas outside APP. APP is inhabited primarily by eastern wolves (Rutledge *et al.* 2010) and has higher moose densities, fewer deer wintering areas, and lower primary and secondary road densities than surrounding areas (McLoughlin *et al.* 2011). Thus, the analysis restricted to study units adjacent to APP focused on areas characterized by a greater diversity of *Canis* genotypes and more heterogeneous landscape conditions, such that the results would not be influenced by the more homogenous, protected wolf population and landscape of APP.

In addition to the prey availability and road density variables, I included the spatial covariate (interaction term between easting and northing spatial coordinates, described above) to account for spatial autocorrelation inherent in spatial datasets (Beale *et al.* 2010). I started with the full model (all variables included) and decided which variables to drop following methods of Wood & Augustin (2002) and Parra *et al.* (2011), modified slightly as I used AIC_c scores rather than Generalized Cross Validation scores. First, I sequentially considered variables as candidates to be dropped based on estimated degrees of freedom (edf) near the lower limit of 1. Second, I assessed whether zero was included in the confidence interval across the entire range of the predictor variable. Third, I re-ran the model without the variable being considered to determine if a lower

AIC_c score was achieved (indicating improved model fit). If all 3 criteria were met, I dropped the variable and considered additional variables that were candidates for removal. The landscape analyses were restricted to adult (≥ 2 years old) animals (n = 85) in packs (n = 47) for which I had sufficient GPS telemetry data to reliably estimate home ranges. I had sufficient telemetry data to estimate home ranges, and associated landscape variables, for only 2 packs in WMU47; thus, the landscape analyses modeled ancestry-landscape relationships primarily in APP, WMU49, and KH.

Morphological analysis

I applied a correction factor to standardize body mass data because study animals often gained substantial weight during winter in my study area, which has been noted previously for wolves and coyotes (Poulle *et al.* 1995). The mean increase in weight from non-winter (April-November) to winter (December-March) captures for individuals captured during both periods was 3.9 kg (SE = 0.86, n = 11). Thus, I subtracted and added 2 kg to winter and non-winter weights, respectively, to standardize weights across seasons for the analyses. I included weights only from adults (≥2 years old) in my analyses. For body length, I also included data from yearlings because skeletal growth ceases between 12-14 months for wolves (Kreeger 2003). I conducted 2 separate Analysis of Variances (ANOVA) to compare mass and length between genotype classes. Genotype classes in the analysis included eastern wolves, coyotes, and eastern wolf × coyote hybrids. The response variables were mass or length and I tested for effects of sex, genotype class, and sex * genotype class interactions. If interactions between sex and genotype class were not significant, I conducted post-hoc testing between different genotype classes with Tukey's HSD. I considered all tests to be significant if $P < 0.05$

and marginally significant if 0.10 > *P* > 0.05. All statistical tests for morphological analyses were done using R. I excluded data from highly assigned and admixed gray wolves from ANOVA tests because of small and unbalanced sample sizes. Although remaining data were also unbalanced between some levels, the 'TukeyHSD' command in R incorporates an adjustment for mildly unbalanced data. The potential consequence of severely unbalanced data with ANOVA is a lack of power (Farraway 2002), which may have made my tests conservative in some cases.

RESULTS

Number of Genetic populations: Structure and PCA

The Bayesian analysis in Structure provided support for 3 genetic clusters in the APP region (Figure 2.1) and indicated admixture between all 3 (Figure 2.2). I interpreted the 3 clusters as distinguishing between gray wolves, eastern wolves, and coyotes. There was also strong support for 2 genetic clusters, which I interpreted as the distinction between gray wolves and eastern wolves/coyotes (Figures 2.1, 2.3). The *K*-means procedure, following PCA, also showed strong support for $K = 3$ (Figure 2.1, Appendix A). The PCA results showed 2 main axes of variation, which explained 6.7% and 4.4% of the variation respectively (Figure 2.3a). I interpreted these 2 axes to represent variation between gray wolves and eastern wolves/coyotes (PC1) and eastern wolves and coyotes (PC2; Figure 2.3). Remaining axes each explained ≤3.6% of remaining variation and were not easily interpreted biologically.

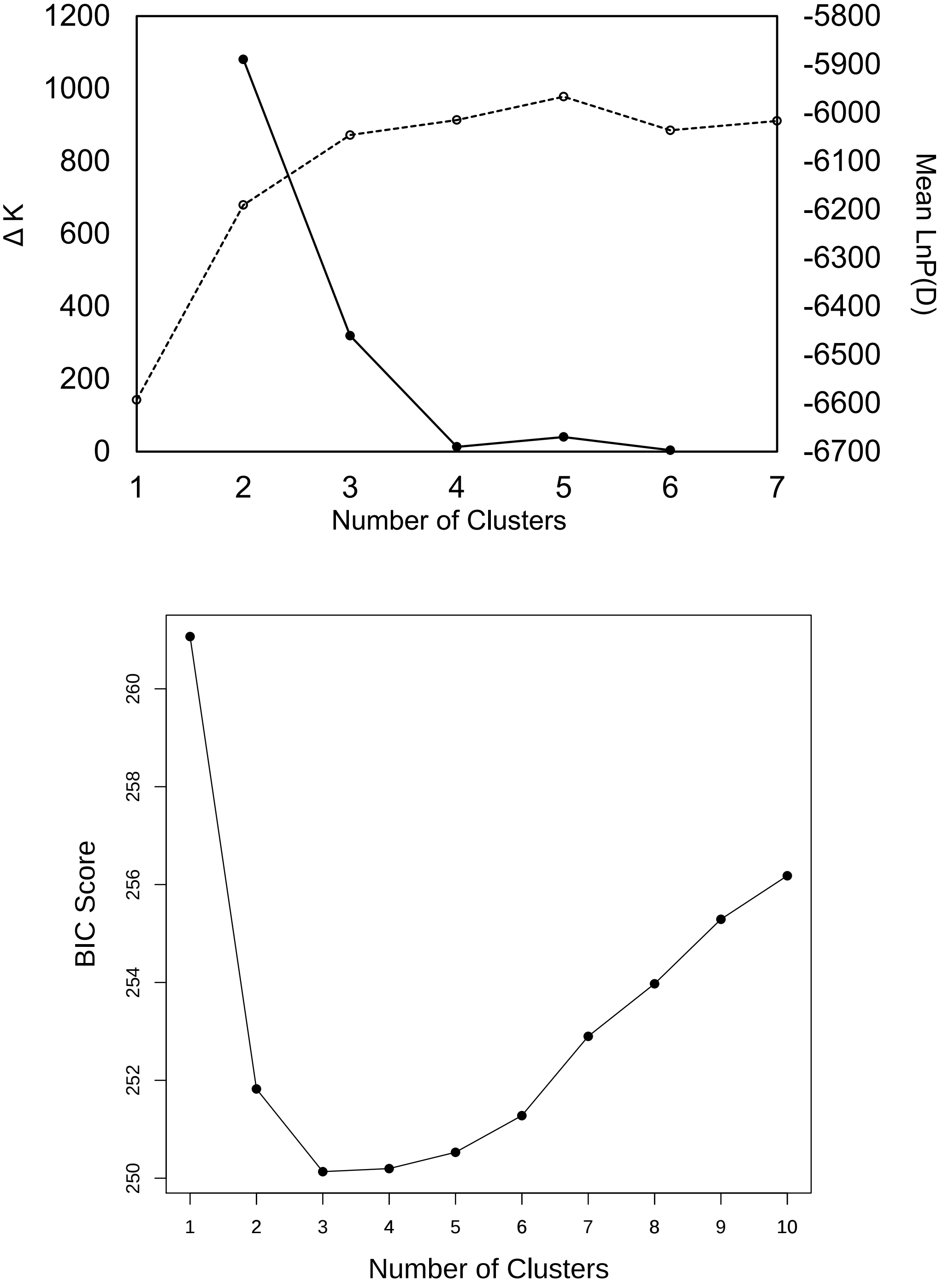

Figure 2.1a-b. Results of 2 genetic structure analyses to evaluate support for the number of genetic populations (*K*) in the data. a) Program Structure analysis showing mean Ln P[D] (dotted line) and Δ*K* (solid line) for *K* = 1-7, b) *K*-means procedure showing Bayesian Information Criteria (BIC) for *K* = 1-10. The strongest supported number of clusters should be the *K* with the minimum number of clusters after which the BIC value increases, or decreases by a negligible amount (Jombart *et al.* 2010).

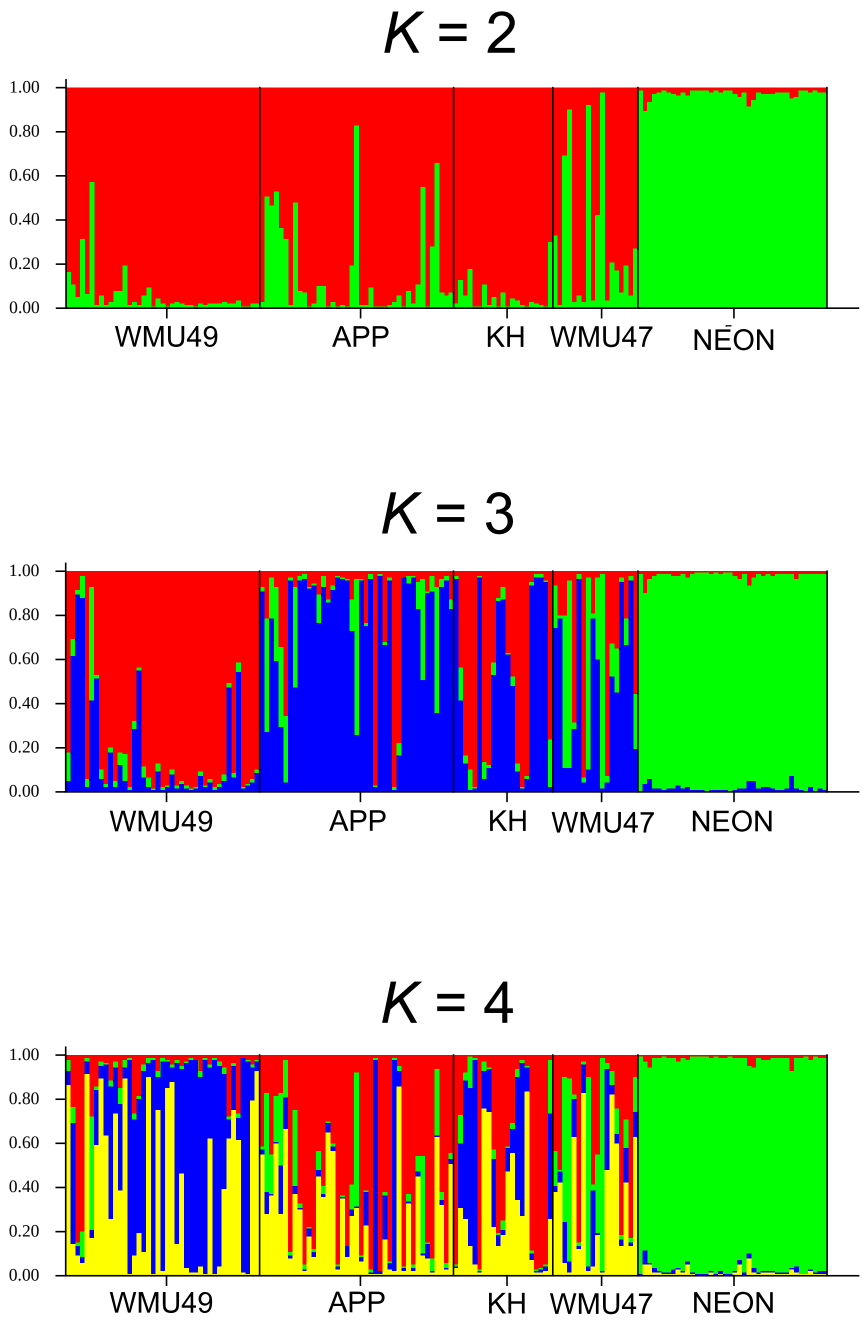

Figure 2.2a-c. Bar plots from Structure individual assignments at a range of potential number of genetics clusters (*K* = 2-4). Each bar represents an individual and individuals are grouped by study units (WMU49, APP, KH, WMU47) and the outgroup (NEON). a) At *K* = 2, I interpret red and green portions of bars as identifying eastern wolf/coyote and gray wolf ancestry respectively. b) At *K* = 3, I interpret blue, red, and green portions of bars as identifying eastern wolf, coyote, and gray wolf ancestry, respectively. c) My analyses did not strongly support *K* = 4 and I do not speculate on individual assignments with this number of clusters.

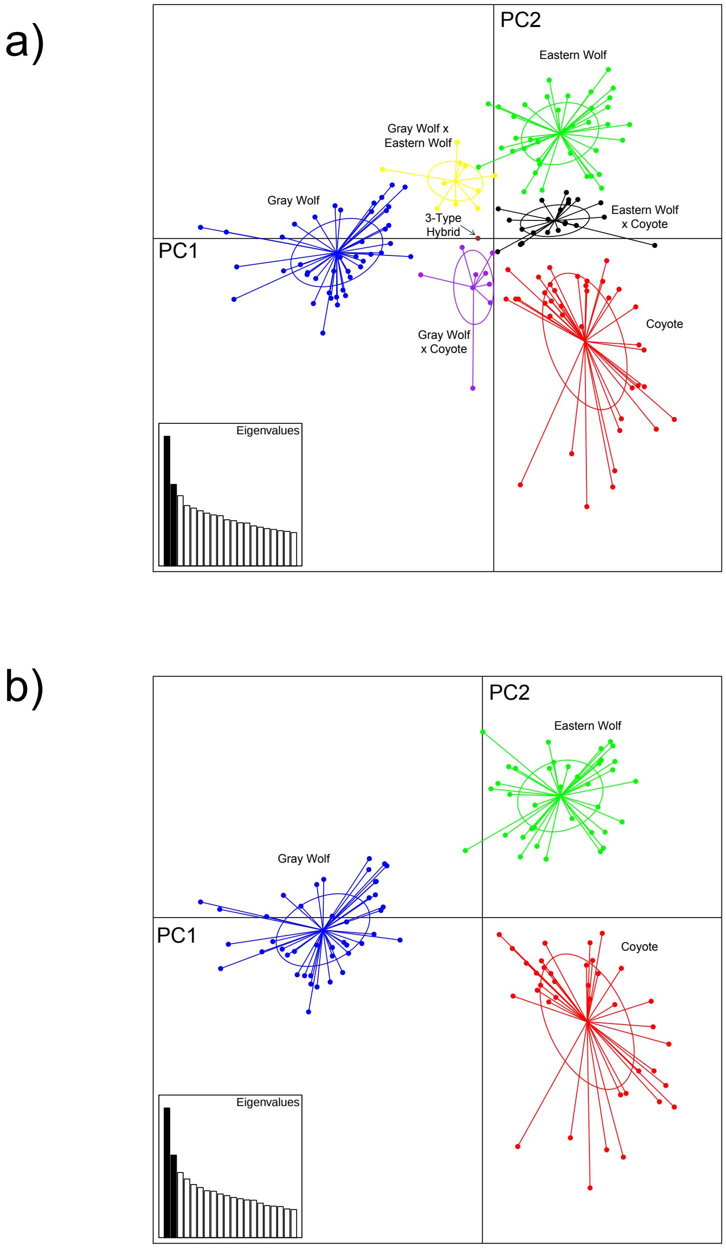

Figure 2.3a-b. Individual wolves, coyotes, and hybrids in and adjacent to APP (*n* = 121) and the NEON outgroup (*n* = 40) arranged along axes 1 and 2 of Principal Components Analysis (PCA), which explained 6.7% and 4.6% of the total variation, respectively. PCA was used to corroborate individual assignments to genetic clusters and admixed categories made at *K* =3 with results from program Structure. Different genotype classes are represented with different colors and 95% confidence ellipses are shown for each class. The 2 plots show: a) all individuals in main analysis; b) all individuals highly assigned to distinct clusters (*n* = 119) to provide graphical representation of the distinct gray wolf, eastern wolf, coyote clusters at *K* = 3.

Individual Admixture

Individual admixture proportions of resident individuals in the APP area (and the NEON outgroup) at $K = 2$, $K = 3$, and $K = 4$ are shown in Figure 2.2. Given the support for both 2 and 3 genetic clusters in the data, I made individual assignments at $K = 3$. These assignments allowed me to address subsequent hypotheses regarding morphology, spatial genetic structure, and landscape associations of gray wolves, eastern wolves, and coyotes. PCA corroborated 90% of the original assignments made based on Structure Q-values (at $K = 3$) and the 80% threshold criteria. I also used the PCA results to reclassify 12 animals from their original assignment (Figures 2.3a). Using this procedure, 8 animals were moved from highly assigned to admixed classes, 3 animals were moved from admixed to highly assigned classes, and 1 animal was moved from an admixed class between 2 clusters to the admixture class between 3 clusters (Figure 2.3a). PCA indicated that no animals should be moved from 1 highly assigned class to another. Additionally, 4 animals had Q-scores of < 0.8 for all groups and either > 0.2 for all groups ($n = 2$) or < 0.2 for 2 groups ($n = 2$), which could have suggested admixture between 3 groups. PCA indicated these were admixed between 2 groups ($n = 3$) or were highly assigned to a single group ($n = 1$; Figure 2.3). I also re-ran the PCA after excluding all individuals that were classified as hybrids to provide graphical representation of genetic variation contained in PC1 and PC2 for only the 3 distinct *Canis* types: gray wolves, eastern wolves, and coyotes (Figure 2.3b).

Animals in APP were predominantly eastern wolves with smaller numbers of coyotes and hybrid animals, mostly with gray wolf admixture (Table 2.1, Figure 2.4).

Table 2.1. Proportions and numbers of resident and breeding animals of each genotype in Algonquin Provincial Park (APP), Wildlife Management Unit 49 (WMU49), Kawartha Highlands (KH), and WMU47 in Ontario, 2004-2010.

	APP				WMU49				KH				WMU47			
	Residents		Breeders†		Residents		Breeders†		Residents		Breeders†		Residents		Breeders†	
	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
Eastern Wolf	62.5	25	62.5	20	2.4	1	3.3	1	38.1	8	44.4	4	16.7	3	50.0	3
Coyote	5.0	2	6.3	2	64.3	27	60.0	18	33.3	7	22.2	2	11.1	2	0	0
Gray Wolf	0	0	0	0	0	0	0	0	0	0	0	0	16.7	3	16.7	1
Coyote x Eastern Wolf	7.5	3	3.1	1	23.8	10	26.7	8	19.0	4	22.2	2	27.8	5	33.3	2
Gray x Eastern Wolf	17.5	7	18.8	6	4.8	2	6.7	2	0	0	0	0	11.1	2	0	0
Gray wolf x Coyote	7.5	3	9.4	3	4.8	2	3.3	1	9.5	2	11.1	1	11.1	2	0	0
3-Way Hybrid	0	0	0	0	0	0	0	0	0	0	0	0	5.6	1	0	0

†Not all breeding animals were identified; % is proportion of all known breeders

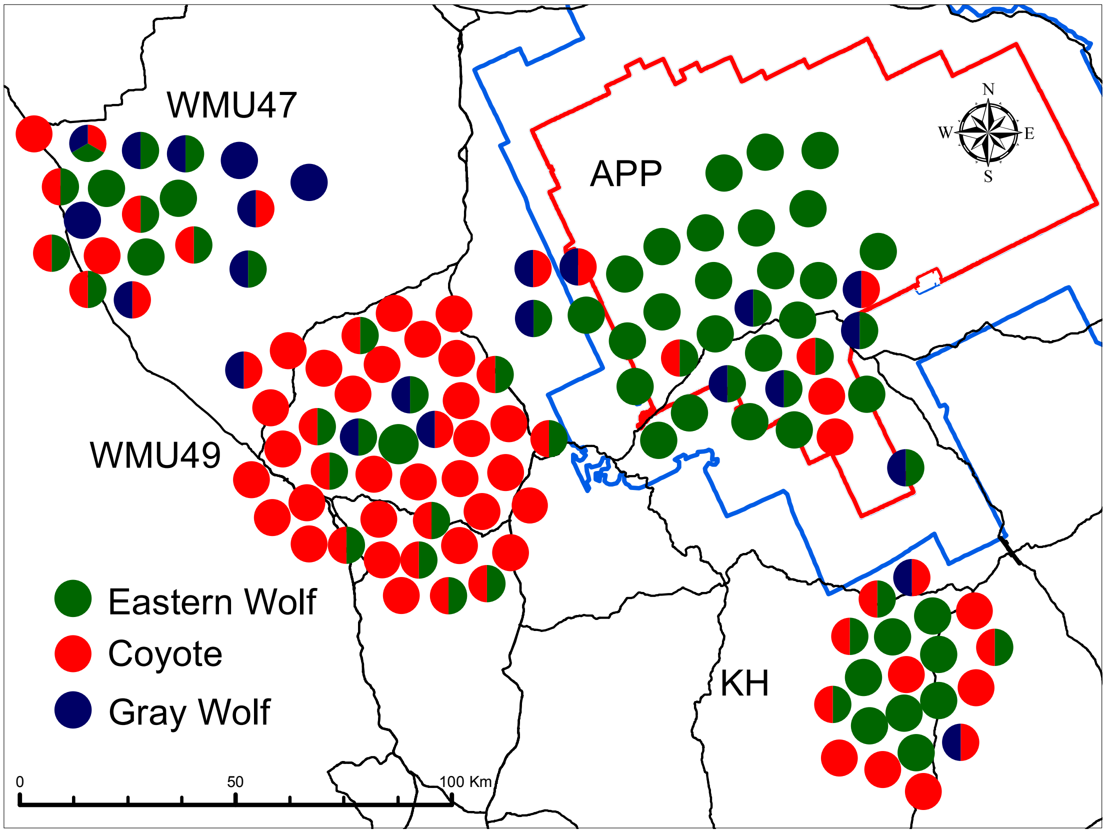

Figure 2.4. Study area with resident individuals plotted (approximately) at home range centroids with pie charts showing genotypes based on individual assignment to genetic clusters using Structure and PCA. >1 color in pie charts indicates admixture. Pie charts are simplified to show 100% ancestry for highly assigned individuals, and 50%-50% or 33%-33%-33% for individuals admixed between 2 or 3 parental clusters, respectively. Also shown are major roads (black lines), APP boundary (red line), and harvest ban buffer area boundary (blue line).

Conversely, animals in WMU49, approximately 25 km to the west, were predominantly coyotes and eastern wolf × coyote hybrids, with fewer gray wolf × eastern wolf hybrids ($n = 2$), gray wolf × coyote hybrids ($n = 2$), or eastern wolves ($n = 1$, Table 2.1, Figure 2.4). Animals in KH were a relatively balanced mix of eastern wolves, coyotes, and admixed individuals between these 2 groups (Table 2.1, Figure 2.4). Finally, WMU47 had a mixture of highly assigned and admixed individuals from all 3 genetic clusters (Table 2.1, Figure 2.4). Genotype frequencies differed across the 4 study units ($P < 0.001$, Fisher's exact test; highly assigned and admixed gray wolves pooled due to small samples).

The 90% credible regions calculated in Structure were wide for some admixed individuals, even ranging from 0 to 1 in some cases (Appendix B), a phenomenon noted by previous studies of wolves and coyotes (Wheeldon *et al.* 2010; Bohling & Waits 2011). However, highly assigned animals generally had much narrower credible regions and many ranged from > 0.8-1.0 for their group of assignment (Appendix B). Previous testing of Bayesian credible regions from Structure with individuals of known ancestry suggested they may be overly conservative in terms of overstating uncertainty of q-value estimates (Bohling & Waits 2011); nevertheless, the wide credible regions from Structure for some individuals highlighted the importance of corroborating individual assignments with additional analysis. Q-scores (mean and standard deviations from 10 runs at $K = 3$), 90% credible regions, and original and final assignments for all individuals in the main analysis are provided in Appendix B. I also provide assignments for all individuals at $K = 2$ based on the run with the highest Ln(P)D and lowest variance (Appendix C).

Spatial genetic structure: sPCA and spatial modeling

Using sPCA, I found significant global (P = 0.002) but not local (P = 0.780) structure across the study area. The global structure revealed the spatial genetic patterns extending from APP to the west, south, and northwest (Figure 2.5). Genetic differentiation to the west between APP and WMU49 was great and the cline was very steep, however the differentiation was much more gradual to the south (into KH) and northwest (into WMU47; Figure 2.5).

Eastern wolf ancestry was not well modeled by distance from the center of APP (distance) across the study area (R^2 = 0.12, AIC_c = 516.9, n = 113; Figure 2.6b). The interactive term with easting and northing spatial coordinates (space), improved model fit (R^2 = 0.32, AIC_c = 507.4, n = 113) and indicated there was significant spatial genetic structure in eastern wolf ancestry throughout the study area (Figure 2.6a). When considering study units separately, the model with data from WMU49 and APP suggested a steep cline of decreasing eastern wolf ancestry extending west from APP with distance as a predictor of ancestry (R^2 = 0.34, AIC_c = 328.6, n = 75). However, spatial structure from APP to WMU49 was better modeled with space (R^2 = 0.48, AIC_c = 321.7, n = 75) than distance. Model fit with distance was poor for KH (R^2 = 0.05, AIC_c = 277.5, n = 59) and improved with space (R^2 = 0.29, AIC_c = 273.6, n = 59), suggesting that the spatial structure was not well modeled as a cline into KH. WMU47 was not well modeled with distance (R^2 = 0.09, AIC_c = 249.9, n = 57) or space (R^2 = 0.19, AIC_c = 251.6, n = 57). Although the model with space explained more variation in eastern wolf ancestry in WMU47 than the model with distance, $\Delta\mathrm{AIC}_c$ was < 2 suggesting that neither model was substantially better.

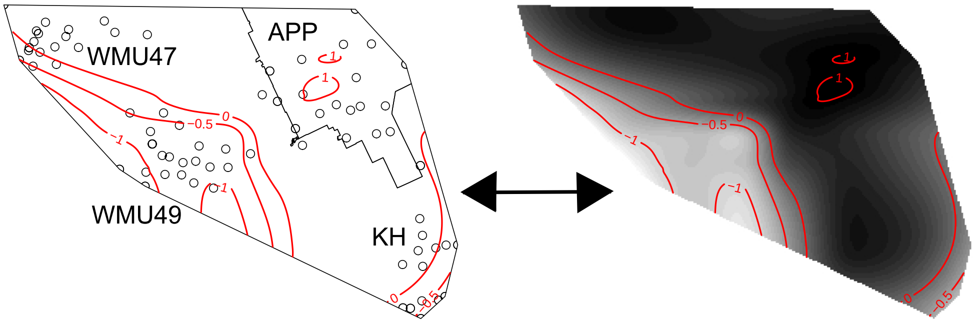

Figure 2.5. Maps of spatial genetic structure from sPCA analysis for 121 resident wolves and coyotes in APP region. Both maps are of same area and represent results of same analysis, shown at different scales of genetic differentiation. Map on left shows sample locations (circles), APP boundary, and red contour lines of major genetic differences. Map on right shows contours of finer genetic differences in dark to light shading.

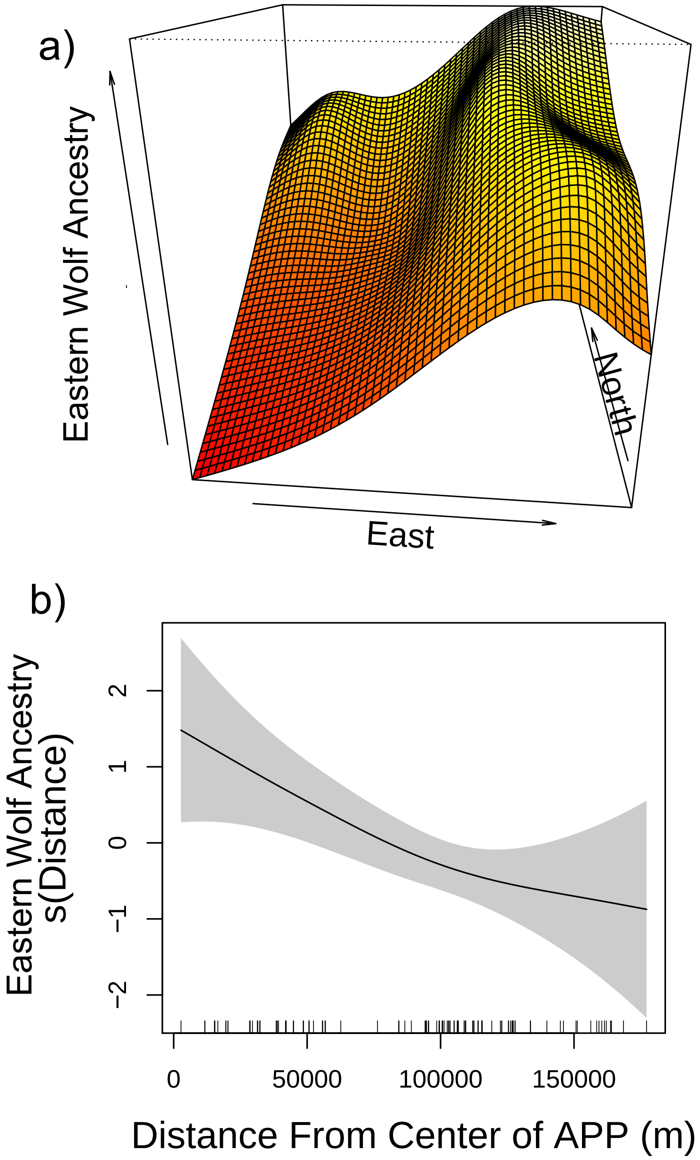

Figure 2.6a-b. Results of 2 competing spatial genetic models of eastern wolf ancestry in the APP region based on generalized additive mixed models (GAMMs). a) 3-D perspective plot of spatial GAMM (R^2 = 0.32, edf = 7.9, P = 0.001, n =113) showing % eastern wolf ancestry (z-axis) as function of a smooth interactive term with easting (east; x –axis) and northing (north; y-axis) spatial coordinates. Yellow color and higher peaks represent greater levels of eastern wolf ancestry, orange is intermediate, and red is lower levels of eastern wolf ancestry. WMU47, APP, KH, WMU49 study units are in NW, NE, SE, SW portions of the plot, respectively. b) Distance GAMM (R^2 = 0.12, edf = 1.5, P = 0.015, n = 113), showing % eastern wolf ancestry as a smooth function (s) of distance from APP center (in meters, x-axis) with data from all study units. On y- axis, eastern wolf ancestry is centered on 0, with positive values indicate increasing eastern wolf ancestry, negative values indicate decreasing eastern wolf ancestry. Shaded area is 95% confidence interval around predicted trend in ancestry and vertical bars on x-axis indicate sample locations.

Landscape Analyses

The best landscape model explaining variation in coyote ancestry in resident, adult animals across the entire study area (including APP, R^2= 0.57, n = 85) retained additive, linear effects of moose density (F = 6.9, edf = 1, P = 0.010, Figure 2.7) and the spatial covariate (F= 1.5, edf =2, P = 0.216), as well as the positive, non-linear effect of secondary road density (F = 5.1, edf = 1.7, P = 0.013; Figure 2.8). There was also a significant interaction between tertiary road density and harvest protection, as there was a positive, linear effect of tertiary road density outside of APP (F = 5.0, edf = 1, P = 0.029), but inside of APP there was not a significant relationship between tertiary road density and coyote ancestry (F = 1.2, edf = 2.4, P = 0.312; Figure 2.9). When data from APP were excluded, the best model explaining variation in the degree of coyote ancestry in resident, adult animals (R^2 = 0.40, n = 51) retained the positive, non-linear effect of secondary road density (F = 4.2, edf = 1.9, P = 0.024, Figure 2.10) on coyote ancestry. The negative linear effect of moose density (F = 4.4, edf = 1, P = 0.041), the positive linear effect tertiary road density (F = 6.2, edf = 1, P = 0.016, Figure 2.11), and the spatial covariate (F = 1.9, edf = 2, P = 0.168) were also retained.

Morphological Analyses

Body mass and length were different between males and females (Weight: $F_{65,1}$ = 25.8, P < 0.001; Length: $F_{92,1}$ = 7.8, P = 0.007) and between genotype classes (Weight: $F_{65,2}$ = 32.3, P < 0.001; Length: $F_{92,2}$ = 26.2, P < 0.001), but there was not a significant interaction between sex and genotype class for mass ($F_{63,2}$ = 0.3, P = 0.713) or length ($F_{90,2}$ = 0.1, P = 0.873). Eastern wolves were heavier than both coyotes (P < 0.001) and coyote × eastern wolf hybrids (P < 0.001, Table 2.2). However, there were not

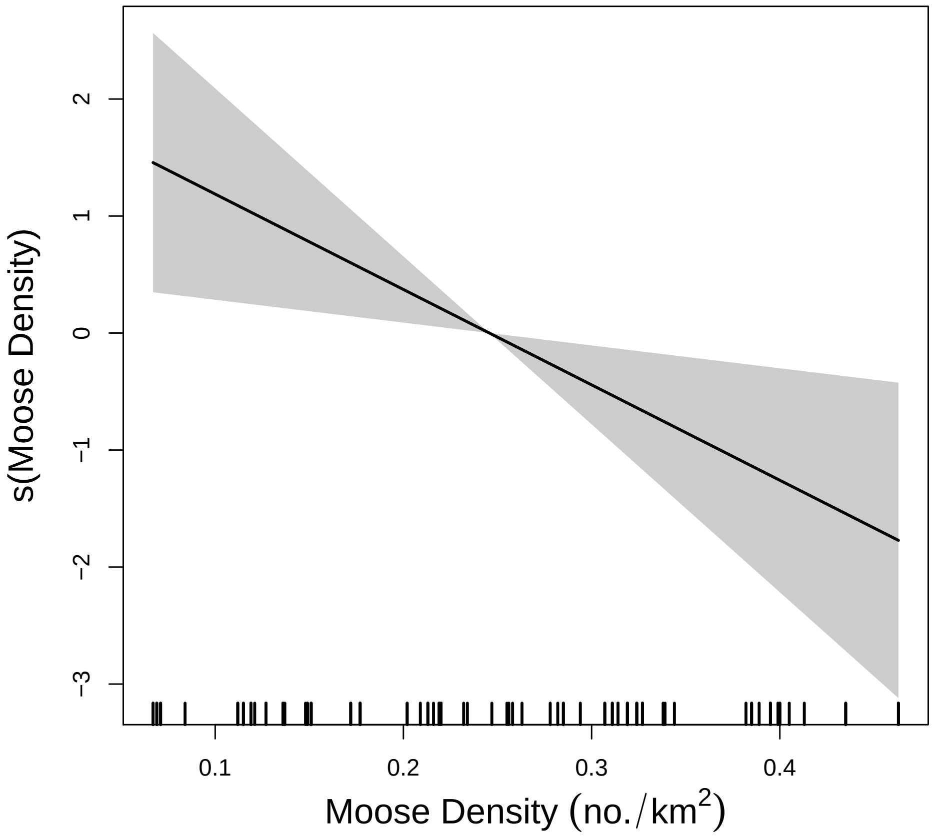

Figure 2.7. Relationships between % coyote ancestry and mean moose density (*P* = 0.01, *n* = 84) throughout the home ranges of resident adult wolves and coyotes across the study area as predicted by generalized additive mixed models. Y-axis shows % coyote ancestry as a smooth function (s) of the independent (environmental) variables centered on 0 where positive values indicate increasing coyote ancestry, negative values indicate decreasing coyote ancestry. Shaded area is 95% confidence interval around predicted trend and vertical bars on x-axis indicate sample locations.

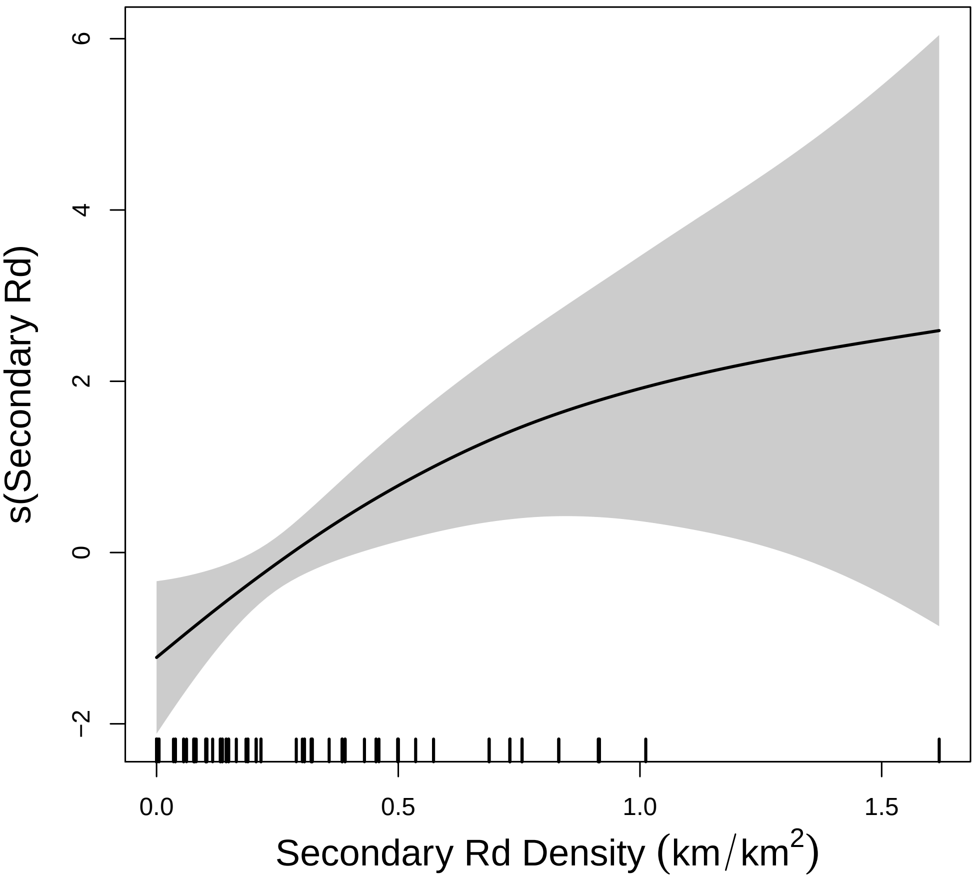

Figure 2.8. Coyote ancestry as a smooth function of secondary road density (*P* = 0.013, *n* = 85) across the study area (including APP) as predicted by generalized additive mixed models. Y-axis shows % coyote ancestry centered on 0 where positive values indicate increasing coyote ancestry. Shaded area is 95% confidence interval around predicted trend and vertical bars on x- axis indicate sample locations.

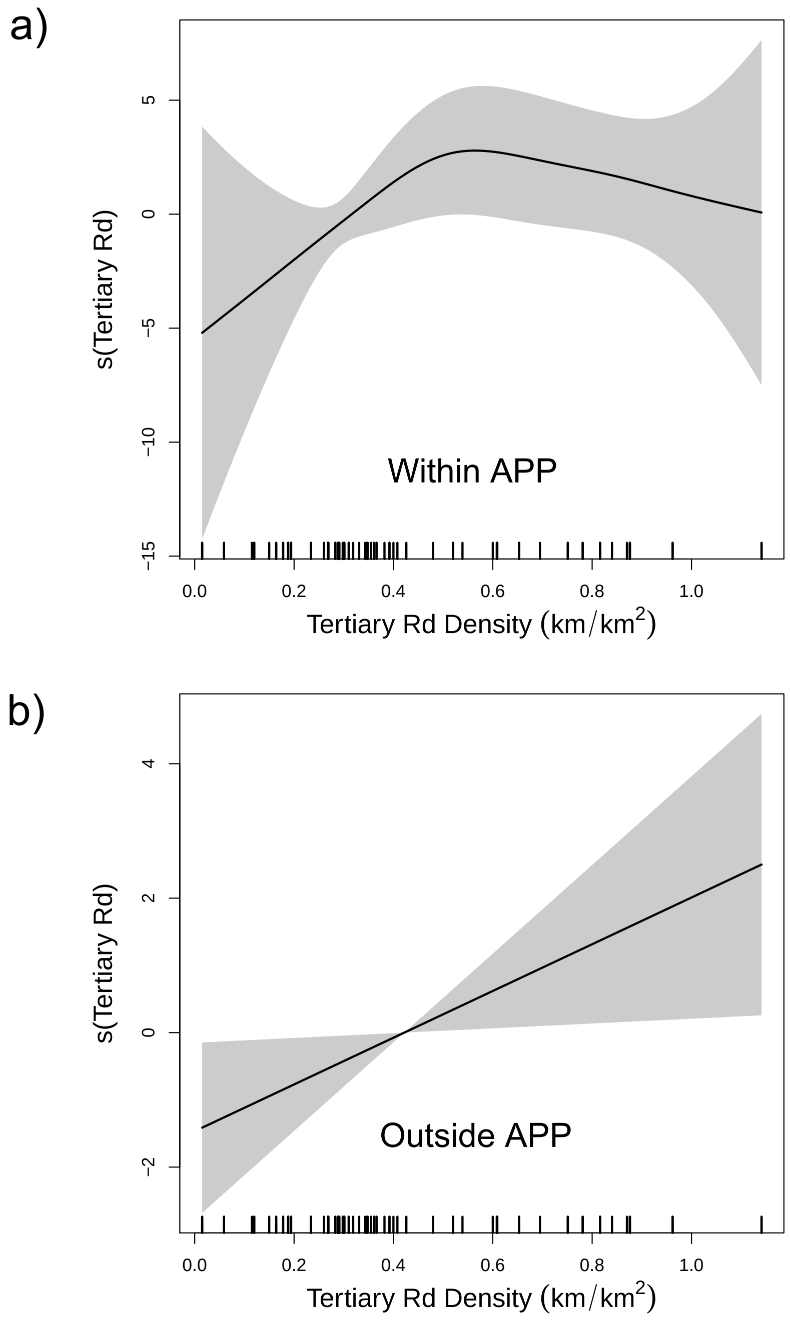

Figure 2.9. Coyote ancestry as a smooth function (s) of tertiary road density showing the significant interaction between tertiary road density and harvest protection as predicted bygeneralized additive mixed model. Shown are the relationships between coyote ancestry and tertiary road density, a) in APP (*P* = 0.312, *n* = 34) and b) outside of APP (*P* = 0.029, *n* = 51). Y- axis shows % coyote ancestry centered on 0 where positive values indicate increasing coyote ancestry. Shaded area is 95% confidence interval around predicted trend and vertical bars on x axis indicate sample locations.

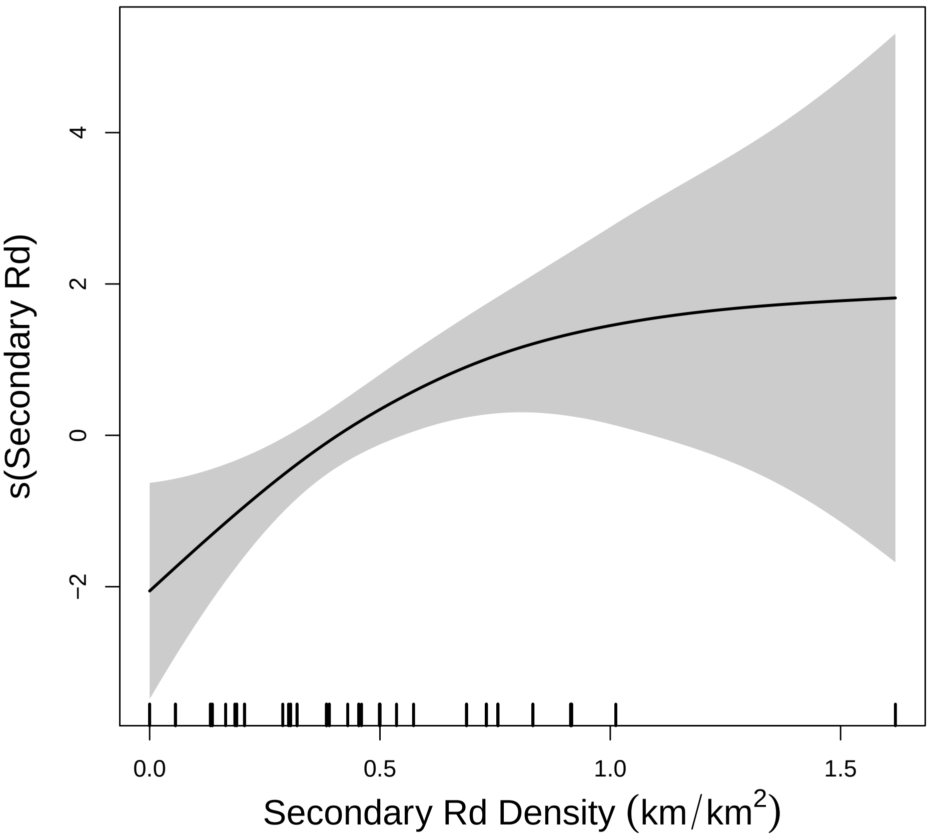

Figure 2.10. Coyote ancestry as a smooth function of secondary road density (*P* = 0.02, *n* = 51) throughout the home ranges of resident wolves and coyotes in study units adjacent to APP as predicted by generalized additive mixed models. Y-axis shows % coyote ancestry as a smooth function (s) of the independent (environmental) variables centered on 0 where positive values indicate increasing coyote ancestry, negative values indicate decreasing coyote ancestry. Shaded area is 95% confidence interval around predicted trend and vertical bars on x-axis indicate sample locations.

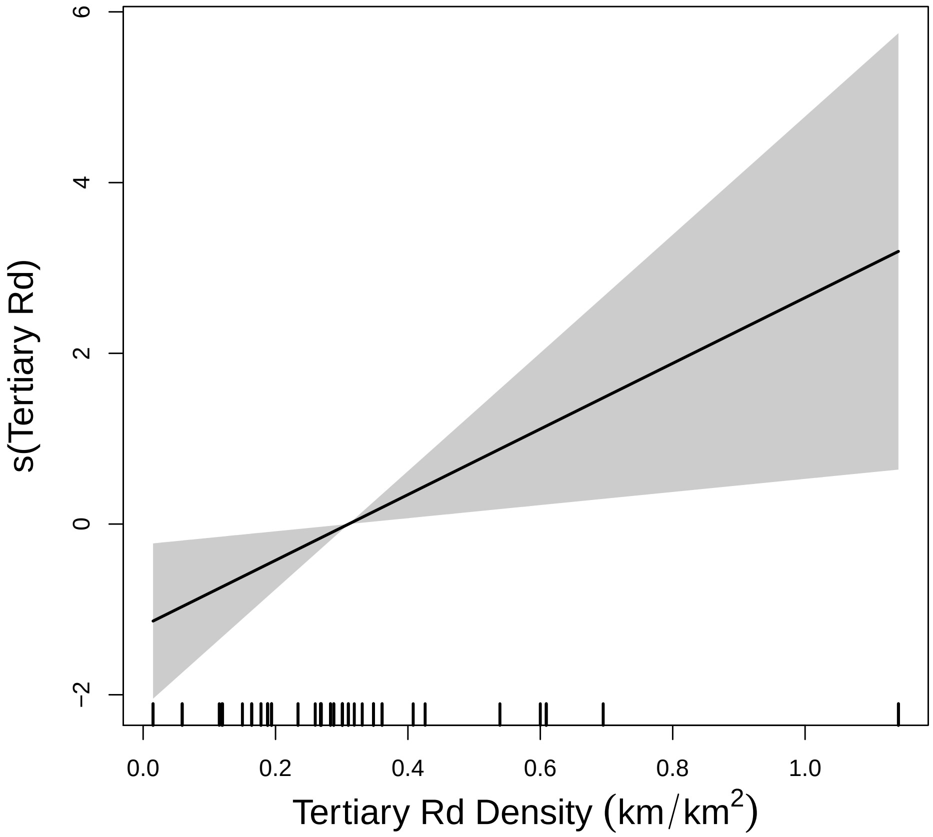

Figure 2.11. Coyote ancestry as a smooth function of tertiary road density (*P* = 0.02, *n* = 51) throughout the home ranges of resident wolves and coyotes in study units adjacent to APP as predicted by generalized additive mixed models. Y-axis shows % coyote ancestry as a smooth function (s) of the independent (environmental) variables centered on 0 where positive values indicate increasing coyote ancestry, negative values indicate decreasing coyote ancestry. Shaded area is 95% confidence interval around predicted trend and vertical bars on x-axis indicate sample locations.

Table 2.2 Mean body mass and length of wolves and coyotes from in and adjacent to Algonquin Provincial Park in Ontario, Canada, 2004-2010. Also shown are standard errors (SE) and sample size (*n*). ND = No Data.

	Mass (kg)†						Length (cm)‡					
	Females			Males			Females			Males		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Coyote	17.8	0.6	11	22.0	1.1	10	97.3	1.6	17	102.2	1.9	14
Eastern Wolf × Coyote	19.6	0.8	7	24.3	1.0	7	103.0	2.4	10	105.8	1.6	11
Eastern Wolf	25.0	0.8	19	28.2	1.0	15	109.3	1.3	24	113.0	1.8	21
Gray × Eastern Wolf	24.7	2.0	3	34.4	1.0	5	111.0	2.2	6	120.7	1.7	5
Gray Wolf	ND	--	--	36.8	3.2	3	ND	--	--	119.0	0	1
Gray Wolf × Coyote	ND	--	--	28.2	2.7	5	98.0	0	1	113.3	3.8	5

†I subtracted and added 2 kg to winter and non-winter weights, respectively, to account for increases in weight during winter (mean increase = 3.9 kg)

‡Length from tip of nose to base of tail

significant differences in mass between coyotes and coyote × eastern wolf hybrids (P = 0.121). Eastern wolves were significantly longer than both coyotes ($P < 0.001$) and coyote × eastern wolf hybrids (P = 0.001), and coyote × eastern wolf hybrids were significantly longer than coyotes (P = 0.032, Table 2.2). When considering all genotype classes (including gray wolves and admixed gray wolves), mean body mass and length for each genetic cluster followed a decreasing gradient from gray wolf to eastern wolf to coyote, with associated hybrids generally exhibiting intermediate mean morphological characters, providing additional (non-statistical) support for my hypothesis (Table 2.2).

DISCUSSION

I have demonstrated that 3 *Canis* types inhabit the region in and adjacent to APP: Algonquin-type eastern wolves, eastern coyotes, and admixed gray wolves. My genetic analyses showed support for 2 and 3 genetic clusters in the sample of resident individual wolves and coyotes, which were distributed in home ranges spanning a mostly contiguous area across western APP and adjacent areas to the west, northwest, and south. I interpret support for 2 genetic clusters in the Structure analysis as identifying broad structure between gray wolves and eastern wolves/coyotes. However, the analyses also clearly supported an additional level of population structure at $K = 3$, which I interpret as identifying the additional distinction between eastern wolves and coyotes. The clusters of the 3 highly assigned *Canis* types are easily visualized by examining axes of genetic variation identified with PCA (Figure 2.3). The PCA also identified a relatively high proportion (36%) of admixed individuals that were arranged along the axes at intermediate positions between the distinct clusters (Figure 2.3a). The presence of multiple, valid layers of genetic structure in a given dataset is not unusual, and indeed, many previous studies of *Canis* genetic structure have found support for multiple *K*-values in single datasets (e.g., Koblmuller *et al.* 2009; Fain *et al.* 2010; Rutledge *et al.* 2010; von Holdt *et al.* 2011). I based subsequent analyses on assignments made at $K = 3$ because, after showing support for this number of clusters in the molecular data, this level of genetic structure allowed me to test morphological and ecological hypotheses regarding eastern wolves, coyotes, and gray wolves in the APP hybrid zone. At $K = 2$ all highly assigned eastern wolves and coyotes were assigned to a single class (Appendix C). Thus, the morphological and ecological differences I documented between eastern wolves and coyotes clearly support the contention that an additional, biologically meaningful level of *Canis* population structure exists in the APP region.

Elucidating genetic structure in hybrid zones between closely related species and populations is a difficult and uncertain endeavor. My use of 12 microsatellite loci provided lower resolution than would have been possible with larger numbers of loci, or perhaps different markers, in terms of correctly identifying the ancestry of individuals with genotypes resulting from complex hybridization patterns. Greater numbers of microsatellite loci (≥48) are sometimes necessary to distinguish between parental types and advanced backcrossed individuals in hybrid zones (Vähä & Primmer 2006). Additionally, use of Single Nucleotide Polymorphisms (SNPs) is increasing and may provide researchers with much greater resolution for untangling complex population structure, as analysis of thousands of loci, or even whole-genome analyses are becoming possible (Morin *et al.* 2004; Helyar *et al.* 2011; von Holdt *et al.* 2011). Although using additional loci or markers may have allowed me to more accurately identify genetic ancestry of individuals, my study also employed many powerful field techniques (e.g., GPS telemetry, aerial tracking) that are rarely used in combination with detailed molecular analysis. I limited genetic inferences to resident, breeding packs and showed that genetic distinctions, observed and corroborated with multiple analyses, manifested in both differences in morphology and associations with different landscape attributes. Thus, although I used a relatively modest number of microsatellite loci, my overall approach, using genetic, morphologic, demographic, and behavioral data, represented a more balanced and powerful approach to molecular ecology than studies employing large numbers of loci, but with no means of assessing whether the inferences were biologically meaningful. For studies seeking to provide practical information for the conservation and management of wild populations, verifying that genetic inferences are biologically significant is an important, if often overlooked, consideration.

Despite extensive admixture, highly assigned individuals of the 3 *Canis* types exhibited genetic and morphologic differences within the relatively limited geographic area in and adjacent to APP. The portion of the hybrid zone within my study area contained mostly eastern wolves, coyotes, and admixed individuals with varying levels of eastern wolf, coyote, and gray wolf ancestry. However, there were also resident, breeding gray wolves in the northernmost study unit (WMU47), suggesting that gray wolves likely disperse into the APP region via northeastern Ontario. Resident, admixed gray wolves were present in the other 3 study units, but highly assigned gray wolves were not found. The southernmost study unit (KH) was inhabited primarily by eastern wolves, coyotes, and coyote × eastern wolf hybrids, suggesting that south of APP the hybrid zone mostly comprises animals whose ancestry is derived from only 2 genetic clusters.

My analyses provide novel information regarding the spatial structure of areas adjacent to APP and have important implications for conservation of eastern wolves in unprotected landscapes. The steep cline I identified from APP into WMU49, over which the dominant genotype changed abruptly from eastern wolf to coyote and hybrid, shows the dramatic influence this large protected area exerts on fine-scale *Canis* genetic structure. The genotypic patterns to the south and northwest appear clinal in the transition zone between APP and the surrounding matrix, a perception that was likely accentuated by considering hybrid zone structure along an abrupt change in environmental conditions (Bridle *et al.* 2002; Ross & Harrison 2002). However, there were patches in KH and WMU47 that were more similar genetically to APP than to WMU49 indicating that the hybrid zone may be better characterized as a mosaic in these areas. Thus, my results show the importance of APP as the population core for eastern wolves, but also indicate that eastern wolves can inhabit unprotected landscapes where suitable environmental conditions exist.

The 3 genetically distinct *Canis* types I identified also differed in morphology, as I found a decreasing gradient in body mass and length from gray wolves to eastern wolves to coyotes. I also documented intermediate morphology for hybrids, which is common in animal hybrid systems (e.g., Grant & Grant 1994; Mavarez *et al.* 2006; but see Ackermann *et al.* 2006), although differences in mean body mass were not statistically significant between coyotes and eastern wolf × coyote hybrids. Regardless, my results indicate that highly assigned eastern wolves, gray wolves, and coyotes retain morphological differences despite extensive hybridization in central Ontario. Additionally, the morphological results are important because they correspond closely with the individual genetic assignments and were consistent with assumptions regarding ancestry of the distinct *Canis* types.

Previous studies have provided detailed analysis of the protected and relatively homogenous eastern wolf population within the boundaries of APP, and also compared it with populations across Ontario and Quebec (Grewal *et al.* 2004; Rutledge *et al.* 2010*a*). Grewal *et al.* (2004) compared APP with a study unit to the west referred to as the Magnetawan region, which appeared to overlap with areas in WMU49 and WMU47 (based on their Figure 2), although little specific information is provided regarding the sample locations. Regardless, Grewal *et al.* (2004) concluded that genetic differentiation between canids in APP and Magnetawan was lower than between APP and the Frontenac Axis (FRAX) southeast of APP. In contrast, I found a steep cline between APP and WMU49 to the west, which was characterized mostly by coyotes and hybrids. Rutledge *et al.* (2010*a*) analyzed a subset of the same FRAX samples from Grewal *et al.* (2004), identified them as primarily eastern coyotes, and confirmed the earlier results with respect to genetic differentiation between APP and FRAX. I note that the westernmost sample collected in FRAX by previous studies was collected approximately 25 km from the home range centroid of the easternmost individual in the sample from KH, and yet my findings were quite different. I found KH to be inhabited by a relatively balanced combination of eastern wolves, coyotes, and hybrids, whereas the earlier studies found canids in FRAX to be genetically distinct from APP and to be mostly eastern coyotes and hybrids (Grewal *et al.* 2004; Rutledge *et al.* 2010*a*). Differences in my results may be explained simply by the different sample locations, as despite the proximity of KH and FRAX, there was no overlap between these study units. These different results may also provide additional evidence of the mosaic distribution of the *Canis* hybrid zone in relation to environmental heterogeneity in areas adjacent to APP. However, the samples analyzed by these earlier studies were collected during 1995-1998 (see details in Sears *et al.* 2003) which was ≥10 years before I sampled DNA from individuals in KH in 2009-2010. This leaves open the possibility that eastern wolf presence has increased in adjacent areas south of APP since the collection of the samples analyzed by Grewal *et al.* (2004) and Rutledge *et al.* (2010*a*), perhaps as a result of increased harvest protection in the buffer area around APP since December 2001. Collection and analysis of contemporary samples from the FRAX region could evaluate this speculation.

The patchwork of eastern wolf genotypes and ancestry I documented outside of APP was influenced by heterogeneous environmental conditions, as animals with higher proportions of wolf ancestry were associated with lower levels of human disturbance (i.e., roads) and higher densities of large ungulate prey (i.e., moose). Across the study area, the environmental conditions and management regulations of APP appear to represent the most suitable current habitat conditions available for eastern wolves. APP was characterized by a lesser degree of anthropogenic habitat fragmentation and road densities than other study units, supported the highest moose densities across the study area (Appendix D), and prohibited harvest of wolves and coyotes in the park and surrounding buffer area. APP may be difficult for coyotes to colonize given that smaller prey, such as deer, are relatively scarce in summer and largely absent in winter in western APP (Cook *et al.* 1999). Also, alternative foods such as garbage or the remains of hunted animals are presumably rare relative to adjacent areas. In contrast to the findings of previous studies, my results suggest that gray wolf admixture may currently be more prevalent in APP than admixture with coyotes. This apparent discrepancy may be due to the fact that I mostly sampled western APP, whereas previous studies sampled extensively in eastern APP (Wilson *et al.* 2000; Grewal *et al.* 2004; Rutledge *et al.* 2010*a*). Gray wolf admixture could be more prevalent in western APP because of differences in prey base favoring gray wolves, as moose densities are higher in that portion of the park. Western APP is also in closer proximity to northeastern Ontario, a likely source of gray wolf immigration into the APP region. Alternatively, gray wolf admixture may have increased within APP since these earlier studies and continued genetic sampling of the population should investigate this possibility.

Moose density was likely a better predictor of *Canis* ancestry than deer availability because both wolves and eastern coyotes prey extensively on deer (e.g., Messier *et al.* 1986; Patterson & Messier 2003), whereas wolves are more effective predators of moose (Mech & Peterson 2003; Loveless 2010). Alternatively, as I relied on an index of deer availability, rather than directly estimating density of deer across the study area, greater uncertainty in this variable could have affected my ability to detect significant relationships. The negative relationship between coyote ancestry and moose density predicted by the models, although significant, was likely weakened by the fact that a few animals outside of APP with relatively high proportions of coyote ancestry occupied areas of high moose density. These areas may have contained alternate, smaller prey, but coyotes may also benefit in such areas by feeding on moose as carrion (Boisjolly *et al.* 2010) and perhaps also by occasional opportunistic predation. In unprotected landscapes, patches with suitable prey availability for wolves may be occupied by coyotes, at least temporarily, as human-caused mortality of wolves may create vacant areas that can be occupied by coyotes, particularly in areas like WMU49 where wolves are rare. In contrast to western wolf-coyote systems where spatial overlap of resident sympatric wolves and coyotes is high (Berger & Gese 2007), resident wolves, coyotes and hybrids in my study area exhibit a high degree of spatial segregation, likely due to more subtle differences in body size and resource use (Chapter 4, J. Benson & B. Patterson, unpublished data). Thus, areas of suitable wolf habitat occupied by coyotes may be difficult to colonize by dispersing wolves attempting to settle outside of APP.

Previous studies have found wolf densities to be negatively associated with road densities, particularly in areas where wolf harvest is allowed (Mech *et al.* 1988, Mladenoff *et al.* 1995), whereas coyotes are often abundant in areas with high road densities and associated human disturbance (Riley *et al.* 2003; Gehring & Swihart 2003). However, my findings are unique in indicating that hybridization dynamics between these species are influenced by the density of secondary and tertiary roads. The interaction between tertiary road density and harvest protection, in terms of its effect on *Canis* ancestry, can be understood by considering how secondary and tertiary roads affect wolves and coyotes. Secondary roads likely affect wolves and coyotes both by fragmenting the habitat, and also by providing access for hunters and trappers (Thiel 1985; Fuller *et al.* 2003). Tertiary roads probably affect wolves and coyotes primarily by providing access for harvest, as these unpaved roads and trails are likely not a significant source of fragmentation or mortality from vehicle collisions. Where wolves are protected, wolf presence may actually be positively associated with tertiary roads because wolves use linear features such as roads to facilitate rapid movement across rugged terrain (James & Stuart-Smith 2000; Whittington *et al.* 2005). Tertiary road density in my study area was significantly associated with greater coyote ancestry only in areas where harvest was legal, suggesting that eastern wolves may be more susceptible and/or less demographically resilient to trapping and shooting mortality than coyotes. This would be consistent with previously observed relationships regarding the sensitivity of wolves and resilience coyote populations to human persecution (e.g., Sterling *et al.* 1983; Fritts *et al.* 2003). Indeed, past introgression from coyotes into the APP wolf population has been linked to high harvest pressure during wolf culls in APP during the 1960's (Rutledge *et al.* 2011). My results suggest that lower levels of harvest, such as those occurring presently in areas adjacent to APP, also may influence hybridization dynamics between wolves and coyotes. Future studies should compare genotype-specific survival and cause-specific mortality of radio-collared wolves and coyotes to directly test the hypothesis that wolves are more susceptible to harvest than coyotes in unprotected areas adjacent to APP.

Conclusions

Although hybridization between eastern wolves, coyotes, and gray wolves has been extensive in the APP region, it is notable that many (64%) individuals were highly assigned to distinct *Canis* types. Thus, the hybrid zone is not truly bimodal (i.e., mostly genotypes resembling parental types) or unimodal (i.e., mostly hybrids), but is better described as an intermediate or "flat" hybrid zone with a more balanced mix of highly assigned and admixed individuals (Harrison & Bogdanowicz 1997; Jiggins & Mallet 2000). Bimodal hybrid zones suggest that the species involved possess well-developed, but incomplete, pre-reproductive isolation mechanisms, whereas unimodal hybrid zones indicate these mechanisms are weak and/or that selection against hybrids is absent

(Jiggins & Mallet 2000; Rubidge *et al.* 2004). It follows that an intermediate hybrid zone, such as the one I studied, would have characteristics of both. Indeed, pre-reproductive barriers likely explain the dominance of eastern wolf genotypes within APP, perhaps with assortative mating as the mechanism (Rutledge *et al.* 2010*a*). However, it is unknown whether eastern wolves in APP generally breed with eastern wolves due to an innate preference, or because the environmental conditions favorable to eastern wolves in APP result in a relatively homogenous population where mating opportunities with other genotypes are limited. Investigating the mating patterns of wolves and coyotes outside of APP would be informative, as eastern wolves would encounter fewer conspecifics and a more diverse range of prospective mates. Also, increased human-caused mortality of wolves and coyotes in harvested areas outside of APP may result in higher rates of mate turnover. Understanding whether the pre-reproductive mechanisms that have maintained the distinct population in APP are intrinsic or environmentally mediated, and whether they are also exhibited by the patchily distributed eastern wolves in unprotected landscapes, would provide insight into whether these wolves represent viable extensions of the APP population. If these mechanisms are absent at lower densities, the occurrence of highly assigned eastern wolves outside of APP may be ephemeral and largely maintained by regular dispersal from the park.

CHAPTER 3. GENOTYPE × ENVIRONMENT INTERACTIONS INFLUENCE CANIS MORTALITY RISK AND HYBRID ZONE DYNAMICS

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ABSTRACT

It is widely recognized that protected areas can strongly influence ecological systems, and that hybridization is an important and enigmatic conservation issue. However, previous studies have not explicitly considered the influence of protected areas on hybridization dynamics between species. Eastern wolves are a species of special concern and their distribution is largely restricted to a protected population in Algonquin Provincial Park (APP), Canada. I studied intrinsic and extrinsic factors influencing survival and cause-specific mortality of canids in the 3-species hybrid zone between eastern wolves, eastern coyotes, and gray wolves in and adjacent to APP. I found that mortality risk for eastern wolves (annual survival [$\hat{s}$] = 0.39) in areas adjacent to APP was significantly higher than 1) other sympatric *Canis* types outside of APP ($\hat{s}$ = 0.55-0.66), and 2) eastern wolves within APP ($\hat{s}$ = 0.85). Outside of APP, the annual mortality rate of all canids by harvest (24%) was higher than for other causes of death (4- 7%) and eastern wolves were significantly more likely to die from harvest relative to other *Canis* types. Survival was also more negatively influenced by increased road density for eastern wolves compared with other *Canis* types, further highlighting their sensitivity to human disturbance. Source-sink survival and hybridization make it unlikely that the genetically distinct APP eastern wolf population will expand significantly in the unprotected matrix adjacent to APP. I have identified an important demographic mechanism underlying the spatial genetic structure of this *Canis* hybrid zone and demonstrate that the large protected area of APP strongly influences hybridization

dynamics between wolves and coyotes. These results suggest that protected areas can allow rare hybridizing species to persist even when reproductive barriers to hybridization are largely absent elsewhere.

INTRODUCTION

Understanding demographic consequences of hybridization is a central goal for both evolutionary ecology and conservation biology as the theoretical and practical implications of interbreeding between species are strongly influenced by the relative fitness of parental and hybrid genotypes within a hybrid zone (Burke & Arnold 2001; Allendorf *et al.* 2001). An important consideration when studying hybrid zones is to determine whether demographic performance of individuals is driven primarily by exogenous or endogenous factors (Barton & Hewitt 1985; Ross & Harrison 2002). Fitness of admixed individuals relative to parental types may vary due to intrinsic qualities, as hybrids may exhibit increased fitness due to heterosis or decreased fitness due to genetic mismatches between parental types (Burke & Arnold 2001). Alternatively, fitness in hybrid zones is often influenced more strongly by environmental conditions that vary over time (Grant & Grant 1992) or space (Moore 1977). Identifying environmental conditions influencing genotype-specific survival and reproduction improves our mechanistic understanding of hybrid zone structure and can provide critical information for the conservation of rare hybridizing species.

Protected areas have become crucial to the persistence of species that are sensitive to environmental perturbation and human disturbance (Diamond 1975; Soulé & Simberloff 1986). In addition to their practical importance, studies of ecological systems in and adjacent to protected areas often provide effective frameworks within which to understand the effects of human disturbance on a wide range of ecological processes including population dynamics (e.g., Knight & Eberhardt 1985), animal behavior (e.g., Schtickzelle & Baguette 2003), and community structure (e.g., Shears & Babcock 2002). Hybridization has been identified as an important and enigmatic issue impacting conservation, with potentially positive and negative outcomes for the persistence of species (Allendorf *et al.* 2001; Seehausen 2004). Rates of hybridization are often increased in disturbed areas (Anderson 1948; Lamb & Avise 1986), and hybrids sometimes thrive in habitats that are marginal for parental species (Moore 1977), so it follows that hybridization should be more prevalent outside of protected areas. However, despite recognition of the important practical and theoretical implications of hybridization, and the potentially strong influence of protected areas on the structure and function of ecological systems, I am unaware of previous studies explicitly considering the role of protected areas in influencing hybridization and hybrid zone structure

The diverse hybrid zone between eastern wolves (*Canis lycaon*), eastern coyotes (*C. latrans*) and gray wolves (*C. lupus*) in and around Algonquin Provincial Park (APP), Ontario, Canada, is an excellent study system within which investigate the influence of a protected area on hybridization dynamics. Eastern wolves are a ‘species of special concern’ in Ontario and Canada and their current distribution appears to be largely restricted to a genetically distinct population within APP (Rutledge *et al.* 2010*a*; Chapter 2). Eastern wolves also inhabit some unprotected landscapes in the hybrid zone immediately adjacent to APP, although eastern wolf ancestry declines sharply in resident canids outside the protected area where the hybrid zone comprises a mosaic distribution of eastern wolves, coyotes, gray wolves and hybrids (Chapter 2). Wolf and coyote ancestry in resident animals was negatively and positively associated, respectively, with road densities outside of APP (Chapter 2). This suggests wolves are more sensitive to human disturbance than other canids in the APP region, consistent with the widespread elimination of wolves and increase in coyotes across North America in the 20th century that was concurrent with intense human persecution and habitat alteration (Fritts *et al.* 2003). However, demographic rates of eastern wolves, and other canids, have not been evaluated in unprotected landscapes adjacent to APP. Thus, it remains unclear whether the hybrid zone is structured by genotype-specific habitat preference or spatially varying fitness among *Canis* types.

Accordingly, I modeled and estimated survival and cause-specific mortality of radio-collared wolves, coyotes, and hybrids by combining telemetry, genetic, and environmental data from areas inside and adjacent to APP to 1) investigate the influence of multiple intrinsic and extrinsic factors on *Canis* mortality risk, 2) compare mortality risk of eastern wolves, coyotes, gray wolves, and hybrids in and out of the protected area, and 3) identify and evaluate the relative importance of the main causes of mortality in protected and unprotected areas. Based on the limited and patchy distribution of eastern wolves outside of APP, and their negative association with areas with greater access for trapping and hunting (Chapter 2), I made 3 hypotheses. First, eastern wolves outside of APP survive poorly compared with sympatric *Canis* types adjacent to APP and eastern wolves within APP. Second, eastern wolves survive poorly in areas of greater human presence (i.e. at higher road densities). Third, eastern wolves are more susceptible to harvest mortality than other *Canis* types outside of APP. My results will clarify whether patchily distributed eastern wolves in unprotected landscapes adjacent to APP can contribute positively to viability of this genetically distinct wolf population. More broadly, this study elucidates mechanisms by which a large protected area can influence hybridization dynamics between species.

METHODS

Field Methods

I captured 147 canids using padded foothold traps and professional capture crews (see Acknowledgments) caught canids with nets fired from helicopters. I deployed mortality sensitive Global Positioning System (GPS) or Very High Frequency (VHF) radio-collars on captured animals. I targeted locations within our study units for trapping to capture animals in areas not covered by our active telemetry collars. In the central Ontario hybrid zone, canids are territorial with each other regardless of genetic ancestry, such that all resident wolves, coyotes, and hybrids are spatially segregated (Chapter 4). Thus, when I successfully captured and collared resident animals in a given area (1-4 per pack), I relocated our trapping efforts to new areas. With this strategy I was successful at capturing individuals from a high proportion of the resident canid packs across the study units as evidenced by the relatively contiguous arrangement of territories that resulted from GPS telemetry data (Chapter 4). Additionally, I captured non-resident (dispersing or transient animals) opportunistically. I monitored survival and movements of radio-collared animals ≥ once per week by fixed-wing aircraft. I investigated mortalities and retrieved carcasses promptly (generally within 24 hours of detection). I assigned cause of death using field evidence and/or with necropsies by experienced veterinarians and pathologists (Canadian Cooperative Wildlife Heath Center, Guelph, Ontario). I estimated age classes of captured animals using tooth wear (Gipson *et al.* 2000) and staining to classify animals as pups (0-1), yearlings (1-2), or adults (>2). Pups were susceptible to different mortality risks than yearlings and adults and survival data from 17 radio-collared pups were excluded from our analyses (J. Benson & B. Patterson, unpublished data). However, all radio-collared pups that became yearlings during the study (*n* = 9) were entered into our models when they reached 1 year of age. Thus, data from 139 adult and yearling canids were used for the analyses. I created a dichotomous adult variable to test for differences in survival between adults (coded 1) and yearlings (coded 0).

Ancestry, Residency and Harvest Protection

All study animals were assigned to one of the following genetic ancestry classes 1) Algonquin-type eastern wolves (hereafter eastern wolves), 2) eastern coyotes (hereafter coyotes), 3) coyote × eastern wolf hybrids, or 4) admixed gray wolves based on genetic analysis of blood samples from captured animals described in detail in Chapter 2. The admixed gray wolf class included gray wolves, gray wolf × eastern wolf hybrids, gray wolf × coyote hybrids, and hybrids admixed between all 3 *Canis* types, which I combined into a single ancestry category due to relatively small sample sizes. I dummy-coded the ancestry variables by coding each animal with a 1 for their assigned genotype of eastern wolf, coyote, eastern wolf × coyote hybrid, or admixed gray wolf and 0 for all other genotypes. I included all 4 dummy coded ancestry variables in the mortality risk models and considered all models that retained 0-3 of these variables by allowing the reference category to change depending on the relative mortality risk of each group (Table 3.1). This strategy allowed me to explicitly test the hypotheses regarding eastern wolf survival in relation to harvest and human disturbance (see Introduction). Additionally, as the relative fitness of admixed and parental types in hybrid zones can be

Table 3.1. Discrete and continuous variables included in analyses with all data (Overall) and data from only residents (Residents) for models of mortality risk of radio-collared adult and yearling wolves, coyotes, and hybrids in and adjacent to Algonquin Provincial Park, 2004-2010. We considered models with all possible combinations of ≤4 variables for both model sets.

		Model Set		
		Overall	APP & Non-APP	Residents
Discrete Variables	Reference Group	Included?	Included?	Included?
Residency Status	Non-Residents	Yes	Yes	No
APP	Non-APP	Yes	No	Yes
Male	Female	Yes	Yes	Yes
Adult	Yearling	Yes	Yes	Yes
2010	2004-2009	Yes	Yes	No
Eastern Wolf	Varied†	Yes	Yes	No
Eastern Wolf × Coyote	Varied	Yes	Yes	Yes
Hybrid	Varied	Yes	Yes	Yes
Admixed Gray Wolf	Varied	Yes	Yes	Yes
Continuous Variables				
Moose Density	NA	No	No	Yes
Deer Availability	NA	No	No	Yes
2° Road Density‡	NA	No	No	Yes
Interactions				
2° Road Density × Eastern Wolf	Other genotypes	No	No	Yes

† Reference group changed depending on which genotype variables were retained in a given model, ‡ Secondary road densit

highly variable and difficult to predict (Burke and Arnold 2001), my strategy also allowed me to objectively identify alternate scenarios if they were better supported by the data.

I created a resident variable by classifying all animals as residents (1) or non-residents (0). Residents were associated with social groups (packs) and restricted movements to well-defined home ranges, whereas non-residents were solitary and exhibited transient or dispersing behavior. I created an APP variable by classifying all radio-collared animals that largely restricted movements to APP and the surrounding harvest ban area as APP (coded 1) and all radio-collared animals outside of APP as Non-APP (coded 0). APP animals were fully protected from harvest, whereas Non-APP animals were not. Small portions of annual home ranges (5% and 11%) of 5 animals from 2 resident packs in APP extended into unprotected areas but I classified them as protected given that they were mostly not at risk of legal harvest. One resident animal that was captured on the periphery of APP had a home range that was primarily (75%) outside of the protected area and I classified this animal as unprotected. Twelve animals dispersed in or out of APP during the study and I reclassified their APP and Non-APP variables accordingly.

Landscape Variables

Moose and deer are important prey for canids in and adjacent to APP (Forbes & Theberge 1996; J. Benson & B. Patterson, unpublished data). I estimated mean moose density (number/km^2) within home ranges of resident canids, using aerial survey data collected by the Ontario Ministry of Natural Resources (OMNR). I intersected a Geographic Information System (GIS) layer of deer wintering areas with canid home ranges to estimate the proportion of each home range comprising deer wintering habitat as an index of winter deer availability. These continuous variables of prey availability allowed us to test the hypotheses that moose density and/or deer availability influenced survival of resident canids. I estimated road densities (km/km^2) for each wolf and coyote range by developing a GIS layer for secondary roads to test the hypothesis that these roads increased mortality risk for canids. Secondary roads were mostly paved roads that were classified as arterial, collector or local roads. Secondary roads can influence wolf and coyote survival directly through collisions with vehicles or indirectly by allowing access for harvest and/or through effects of fragmentation (Thiel 1985; Fuller *et al.* 2003). I did not include primary or tertiary roads in my models to reduce the number of variables and prevent over-fitting models. Preliminary analyses (not shown) indicated secondary roads influenced mortality risk more than primary or tertiary roads. See additional details regarding prey and road variables in General Methods.

Survival Models

I modeled survival and investigated factors influencing mortality risk using the Anderson-Gill (AG) extension to Cox proportional hazards regression modeling (Therneau & Grambsch 2000). I used a 365 day (recurrent) time scale to model the baseline hazard (Fieberg & DelGiudice 2009). I standardized the recurrent time scale to a biological year beginning on May 1 (approximate mean birthdate for canids in the study area) and ending on April 30 the following year. Newly captured animals were entered into the models the day following capture and, if still alive, were right-censored on April 30 and re-entered into the models on May 1 the following year. I right-censored animals whose radio-collars dropped off, failed or if I otherwise lost telemetry contact (due to dispersal outside of the study area) on the last day I recorded an active signal. I assumed that animals that I lost radio-contact with were not more or less likely to die than other animals. As an informal check on this assumption some of the animals I lost contact with for various reasons (*n* = 51; Table 3.2) were recaptured later in the study (*n* = 10). Additionally, 2 animals that I censored (1 dropped collar and 1 unknown) were detected via non-invasive DNA and identified as being parents of 3 and 2 litters, respectively, after I lost radio-contact with them through the genetic analyses conducted by Chapter 2. Thus, although I could not know the ultimate fates of all animals beyond their monitoring periods, I documented that 24% of these animals were still alive later in the study suggesting the assumption was valid. I only lost contact with 6 animals for unknown reasons: 4 of these likely dispersed out of the study area and for the remaining 2 it was unclear whether they dispersed or if their collars failed.

To accommodate state-changes for the resident and APP variables, I censored animals on the day prior to detecting the state change and re-entered them into the model with their new covariates on the day of detection. All other time-varying covariates (i.e., age-class, landscape variables associated with annual home ranges) varied on an annual basis. I did not have sufficient data for a detailed treatment of temporal trends in survival; however, I observed higher mortality for radio-collared animals in 2010 (in terms of raw number of deaths) compared to previous years. Thus, I included a dichotomous temporal variable (2010) that separated data from 2010 (coded 1) with data from earlier years of the study (coded 0) to test and account for the potentially lower survival in 2010.

Table 3.2. Fates of radio-collared wolves, coyotes, and hybrids in and adjacent to Algonquin Provincial Park, Ontario, 2004-2010

All Data	*n*	Mortality	Collar-Failure†	Planned Drop‡	Lost Contact§	Unplanned Drop
Eastern wolves	49	18	8	9	4	2
Coyotes	38	16	3	5	1	1
Eastern wolf × Coyote	27	15	4	4	1	0
Admixed Gray Wolves	25	9	7	2	0	0
Total	139	58	22	20	6	3

Resident	*n*	Mortality	Collar-Failure	Planned Drop	Lost Contact	Unplanned Drop
Eastern wolves	37	10	8	8	2	0
Coyotes	26	8	4	3	0	1
Eastern wolf × Coyote	14	5	3	3	0	0
Admixed Gray Wolves	11	2	4	2	0	0
Total	88	25	19	16	2	1

†Mostly GPS collars (expected battery life 1 year, *n* = 21), but I also assumed collar failures when I lost contact with VHF collars > 5 years after deployment on resident, adult animals (*n* = 2).

‡GPS collars were programmed to drop off ~1 year following deployment

§ Either dispersed out of study area or premature radio-failure

First, I conducted an overall analysis with data from all radio-collared animals (*n* = 139) to confirm that mortality risk was greater in areas adjacent to APP compared with the protected area. Next, I modeled APP and Non-APP separately with data from all radio-collared animals from each area included (residents and non-residents). I separated data from APP and Non-APP due to differences in genetic structure and environmental conditions between these areas that would have necessitated the inclusion of multiple interactions to test the hypotheses. The separate model sets reduced model complexity and provided results that were easier to interpret within the protected and harvested portions of the study area. Finally, I conducted an analysis restricted to resident animals in packs for which I had sufficient GPS telemetry data to estimate home ranges and associated environmental variables described above (*n* = 87). I could not include the environmental variables (roads and prey availability) in the overall analyses because these variables could not be estimated for non-residents as they did not restrict their movements to definable home ranges. In the global model set for residents, I included an interaction between eastern wolf and secondary road density to test the hypothesis that eastern wolf survival was more negatively influenced by human disturbance than other *Canis* types. For all model sets, I considered models with all possible combinations of the variables relevant to my hypotheses (Table 3.1). However, I did not consider individual models with >4 variables to avoid over-fitting models. I ranked models using Akaike's Information Criteria corrected for small samples (AIC_c; Burnham and Anderson 2002), with the number of mortalities as the sample size in the calculation of $AIC_{c.}$ Using number of mortalities as the sample size further emphasized parsimony in the model selection process. I considered models with $\Delta AIC_c < 2$ to have strong empirical support and calculated Akaike model weights following Burnham & Anderson (2002). I assessed significance of variables retained in supported models with robust *z*-tests, hazard ratios (hazard; exponentiated β coefficients), and 95% confidence intervals for hazard ratios (shown in brackets after each hazard ratio; Therneau & Grambsch 2000). For categorical variables, the hazard ratio provides an estimate of the ratio of the instantaneous risk of mortality relative to the reference group. For continuous variables, I report the hazard ratios corresponding to a one unit (0.1) change in the covariate. I selected increments of 0.1 to provide hazard ratios that were easily interpreted biologically as differences of this magnitude in the estimates of prey availability and road density were common among individuals in our dataset. I estimated robust (‘sandwich’) standard errors for parameter estimates based on data clustered by individual (for the overall analysis) or pack (for resident analysis; Therneau & Grambsch 2000). To assess the relative importance of individual variables based on the model section results, I summed Akaike model weights across all models retaining a given variable (Burnham & Anderson 2002). Finally, I also estimated annual survival rates for harvest protection (APP and Non-APP) and genetic ancestry categories using the Kaplan Meier product limit estimator, modified for staggered entry (Pollock *et al.* 1989). I provide these estimates as intuitive measures of annual survival, but restrict inferences to the results of the Cox AG models which are more powerful and appropriate for assessing the influence of multiple covariates on survival (Therneau & Grambsch 2000).

Cause-Specific Mortality

To model and estimate the relative importance of different mortality agents affecting wolves and coyotes, we estimated cause-specific mortality rates using the nonparametric cumulative incidence function estimator (CIF; Heisey & Patterson 2006). We attributed mortality of radio-collared adults and yearlings to 1 of 4 causes: 1) vehicular collisions, 2) harvest (trapping or shooting, 3) natural, or 4) unknown. Natural causes of death included death associated with mange, starvation, intraspecific aggression, prey defense (kicked by ungulate), and unknown natural causes (i.e., necropsy failed to determine cause of death, but harvest and hit by vehicle were ruled out). Next, I combined all non-harvest mortalities into a single cause to identify classes of animals outside of APP that were more or less likely to die of harvest using Cox-proportional hazards modeling (at $P < 0.05$) following methods described by Lunn & McNeil (1995) and Heisey & Patterson (2006). Specifically, I created multiple records for each individual (one set for each cause of death) with an associated stratum variable indicating the specific cause. Next, I fit models that included this stratum identifier in the model statement to allow fitting of separate hazard functions for harvest and non-harvest mortality. Finally, I included interactions between covariates of interest and the cause of death/stratum identifier to allow the effect of covariates to differ for harvest and non-harvest mortality. I conducted all survival and cause-specific mortality analyses using the ‘survival’, ‘MASS’, and ‘gtools’ packages in R version 2.15.1 (R Development Core Team 2011).

RESULTS

Overall Survival Analyses

Overall I documented 58 deaths of radio-collared canids across the 4 study units during 2004-2010. The top model predicting adult and yearling mortality risk with data from all radio-collared canids retained APP, resident, male and eastern wolf variables (Table 3.3).

Table 3.3. Candidate models of mortality risk of radio-collared adult and yearling wolves, coyotes, and hybrids in and adjacent to Algonquin Provincial Park (APP), 2004-2010, from model sets with all data (Overall), residents and non-residents outside APP (Outside APP), and residents in and out of APP (Residents). We show the number of variables retained (*K*), AIC for small samples ($\mathrm{AIC_c}$), and $\mathrm{AIC_c}$ differences ($\Delta\mathrm{AIC_c}$), for all models with strong empirical support ($\Delta\mathrm{AIC_c} < 2$) and also the null model.

Overall	*K*	AICc	ΔAICc
Resident + APP† + Eastern Wolf + Male	4	557.16	0
Resident + APP + Eastern Wolf	3	557.65	0.49
Resident + APP + Eastern Wolf + 2010‡	4	558.09	0.93
Null Model	0	579.70	21.54

Outside APP	*K*	AICc	ΔAICc
Resident + Eastern Wolf	2	428.74	0
Resident + Eastern Wolf + Male	3	429.59	0.84
Resident + Eastern Wolf + Adult	3	429.89	1.14
Resident	1	430.39	1.64
Resident + Eastern Wolf + Hybrid§	3	430.39	1.65
Resident + Eastern Wolf + 2010	3	430.45	1.71
Resident + Eastern Wolf + Coyote	3	430.67	1.93
Resident + Eastern Wolf + Male + Adult	4	430.71	1.96
Null Model	0	441.58	12.83

Residents	*K*	AICc	ΔAICc
Sec. Rd + Deer¶ + 2010 + Eastern Wolf × Secondary Rd	4	211.41	0
Sec. Rd + Deer + 2010	3	214.67	3.26
Sec. Rd + Deer + 2010 + Eastern Wolf	4	215.59	4.19
Null Model	0	227.45	16.05

†Coded 1 for animals in APP, 0 for animals outside
‡Coded 1 for data from 2010, 0 for data from 2004-2009
§Eastern wolf × coyote hybrid
¶ Index of deer availability within home ranges of resident canids

Based on the top model, animals in APP survived better than animals outside of APP (z = -4.4, *P* < 0.001, hazard = 0.18 [0.09, 0.39]. I tested subsequent hypotheses with the subset model sets (for APP and non-APP) which were simpler to interpret. The top model outside of APP (*n* = 49 deaths) retained the resident and eastern wolf variables (Table 3.3). Based on the top model, residents survived better than non-residents (z = -4.2, *P* < 0.001, hazard = 0.34 [0.21, 0.56]), whereas eastern wolves survived poorly relative to other *Canis* types (z = 3.1, *P* = 0.002, hazard = 2.12 [1.32, 3.38]). No other variables included in the overall analysis outside of APP significantly influenced survival of adult and yearling canids (Appendix G). Parameter estimates, confidence intervals and significance tests were very consistent for individual variables across supported models (Appendix G).

In APP, I failed to identify variables substantially influencing mortality risk of radio-collared canids as the null model was strongly supported ($\Delta AIC_c = 0.96$). Thus, there was little evidence that any of the variables considered influenced survival of adults and yearlings in APP. Annual survival rates for canids outside and inside of APP are provided in Table 3.4. Variable weights from model selection of overall analysis outside of APP and for residents are provided in Table 3.5

Survival of Residents

The top model for mortality risk of radio-collared adult and yearling residents (*n* = 25 deaths) retained the main effects of 2010, secondary roads, and deer availability, as well as the interaction between eastern wolf and secondary roads (Table 3.3). Residents survived poorly in 2010 compared with other years (z = 3.4, *P* < 0.001, hazard ratio = 4.9 [1.95, 12.55]). Secondary road density within home ranges negatively influenced

Table 3.4. Estimated annual Kaplan-Meier survival rates ($\hat{s}$), standard errors (SE) and number of animals tracked for different *Canis* genetic types in study units outside and inside of Algonquin Provincial Park, Ontario, 2004-2010. Ancestry did not influence survival in APP so all canids are pooled.

Outside APP	$\hat{s}$	SE	*n*
Eastern wolves	0.388	0.12	15
Coyotes	0.662	0.07	35
Eastern Wolf × Coyote	0.551	0.09	22
Admixed Gray Wolves	0.625	0.1	20
APP	$\hat{s}$	SE	*n*
All Canids	0.852	0.05	58

†Eastern wolves = 39, coyotes = 3, eastern wolf × coyote = 8, admixed gray wolves = 8.

Table 3.5 Variable weights for all predictor variables included in the overall analysis outside of APP and resident survival analyses calculated by summing the Akaike model weights across all models retaining a given variable (Burnham and Anderson 2002).

	Outside APP	Residents
Resident	0.97	NA
Eastern Wolf	0.56	0.10
Male	0.34	NA
Adult	0.29	NA
Coyote	0.28	0.08
2010	0.25	0.97
Admixed Gray Wolf	0.22	0.05
Eastern Wolf - Coyote Hybrid	0.22	0.05
Deer Availability	NA	0.96
All Secondary Road Density Variables†	NA	0.94
Secondary Road Density	NA	0.81
Eastern Wolf × Secondary Rd Density	NA	0.62
All Eastern Wolf Variables†	NA	0.70
APP	NA	0.10
Moose Density	NA	0.06

† Included as a main effect or interaction

survival (z = 5.0, *P* < 0.001, hazard ratio = 1.22[1.13, 1.32]), whereas deer availability within home ranges positively influenced survival of residents (z = -3.4, *P* < 0.001, hazard = 0.34[0.19, 0.63]). The significant interaction between eastern wolf ancestry and secondary roads (z = 4.8, *P* < 0.001) indicated that resident eastern wolves survived worse at increasing secondary road density than all other genotypes combined. To investigate the relationships between eastern wolf mortality risk at increased secondary road density and that of each of the other 3 ancestry groups individually, I reversed the reference group for this interaction (i.e. from all other genotypes to eastern wolves). Coyotes (z = -5.0, *P* < 0.001, hazard = 0.57 [0.46, 0.71], coyote × eastern wolf hybrids (z = -3.9, *P* < 0.001, hazard = 0.62 [0.48, 0.79], and admixed gray wolves (z = -2.4, *P* = 0.017, hazard = 0.57 [0.35, 0.90] each survived better than eastern wolves as road density increased (Figure 3.1). I confirmed this result by repeating the analysis while sequentially removing data from each resident eastern wolf that died during the study to ensure it was not unduly influenced by single mortality events (Appendix H).

Cause-Specific Mortality

Across the study area, the mortality rate due to harvest (CIF = 15.8%, *n* = 29, SE = 2.7, 95% CI [11.3, 20.2]) was greater than the rate due to natural deaths (CIF = 6.6%, *n* = 12, SE = 1.9, 95% CI [3.6, 9.7]), vehicular collisions (4.9%, *n* = 9, SE = 1.5, [2.0, 7.5]), or unknown causes (4.8%, *n* = 8, SE = 1.7, 95% CI[1.6, 6.7]). Outside of APP the mortality rate due to harvest (CIF = 24.0%, *n* = 29, SE = 3.9, 95% CI [17.6, 30.5]) was also greater than for all other causes (Table 3.6). No harvest mortality was documented in APP (Table 3.6). Cause-specific mortality rates are summarized for in and outside of APP in Table 3.6. Outside of APP, eastern wolves (z = 3.0, *P* = 0.003, hazard = 3.45[1.52, 7.84])

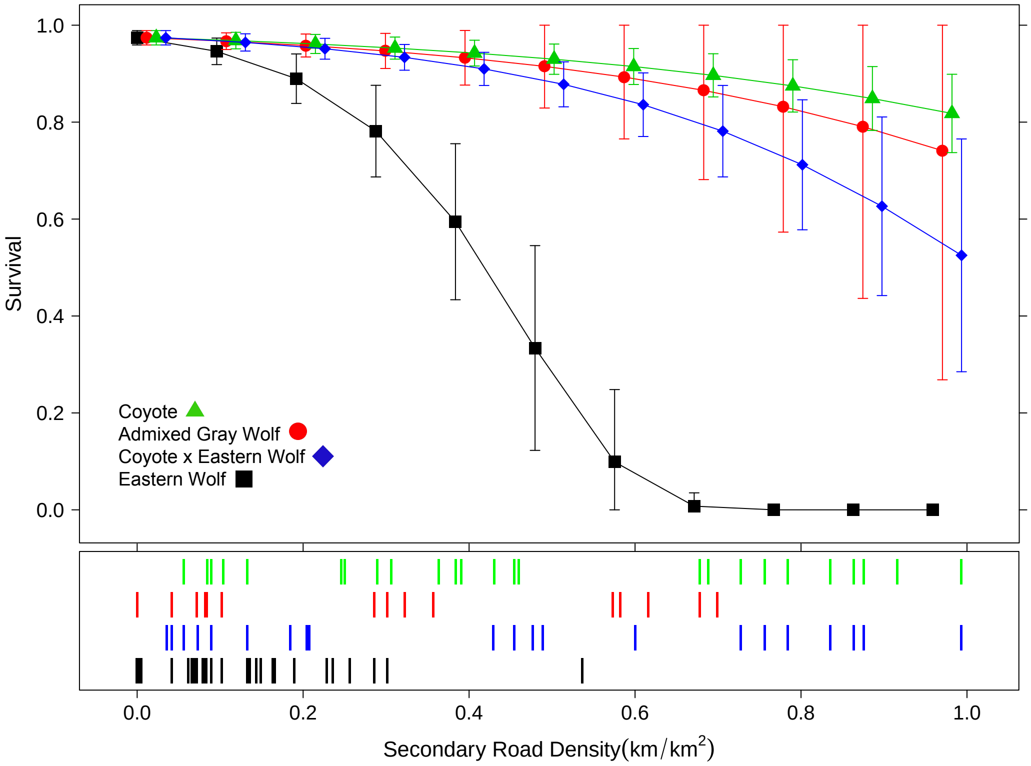

Figure 3.1. Genotype-specific survival rates with increasing secondary road density predicted by model of mortality risk for resident radio-collared *Canis* in and adjacent to Algonquin Provincial Park, 2004-10. Survival rates (± robust SE) predicted at a range of secondary road densities (km/km^2) between 0 and 1.0. Road densities within home ranges of each individual are indicated below the x-axis with colors corresponding to those used to show survival trends for each *Canis* type.

Table 3.6. Cause-specific mortality rates calculated with Cumulative Incidence Function (CIF) for radio-collared canids outside and inside of Algonquin Provincial Park, Canada, 2004-2010. Shown are CIF rates, number of mortalities (n), standard errors (SE), and 95% confidence intervals (CI) for each cause of death.

	Outside of APP					Inside of APP				
				95% CI					95% CI	
Cause	CIF (%)	*n*	SE	Lower	Upper	CIF (%)	*n*	SE	Lower	Upper
Harvest	24.0	29	3.9	17.6	30.5	---	0	---	---	---
Natural	5.8	7	2.1	2.3	9.3	7.8	5	2.9	3.1	12.5
Vehicle	6.7	8	2.5	3.2	10.1	1.8	2	1.7	0.0	4.6
Unknown	4.7	5	2.1	1.2	8.1	5.2	3	2.9	0.4	10.0

were more likely to be killed by harvest than all other *Canis* types, whereas residents (z = -2.6, *P* = 0.008, hazard ratio = 0.37[0.18, 0.78]) were less likely to die of harvest than non-residents.

DISCUSSION

Eastern wolves are the dominant canid within APP, but are extremely rare in adjacent areas (Rutledge *et al.* 2010; Chapter 2). Eastern wolves survived poorly outside of APP in the adjacent, unprotected landscapes where they were more likely to be trapped and shot than other *Canis* types. My hypotheses were supported as eastern wolf survival in harvested areas was poor relative to 1) other sympatric *Canis* types in areas adjacent to APP, and 2) eastern wolves within APP. In APP, annual survival was high ($\hat{s}$ = 0.85) for all canids and mortality risk did not differ significantly in relation to genetic ancestry or any other factors I investigated. Thus, *Canis* mortality risk and hybridization dynamics between eastern wolves and other genotypes are environmentally-mediated by variable harvest regulations across the hybrid zone. APP has played a crucial role in allowing eastern wolves to persist despite high mortality and extensive hybridization in the adjacent matrix and has exerted a powerful influence on the dynamics of this diverse *Canis* hybrid zone. Within APP, eastern wolves appear to maintain their numerical abundance despite the absence of superior survival due to the scarcity of gray wolves in adjacent areas and their apparent ability to largely exclude coyotes from APP (Rutledge *et al.* 2010*a*; Chapter 2). Given the importance of this population for eastern wolf persistence, I suggest continued study of the APP population would be prudent to monitor whether their numeric dominance is maintained within the protected area.

An intuitive explanation for the lower survival and higher harvest mortality of eastern wolves relative to other genotypes is that most eastern wolves in adjacent, unprotected landscapes originated from the population core of APP, and are therefore naïve regarding mortality risk from humans. Wolves in protected areas tend to be less fearful of humans and wolves that are less cautious are more likely to be harvested (McNay 2002; Fritts *et al.* 2003). Wolves learn skills crucial to their survival while traveling with their natal packs (Mech 1991; Packard 2003). Thus, wolves raised in APP may not learn skills necessary to reduce trapping and shooting mortality from humans, such as cautious behavior around baited trap sites. My results likely reflect associations between genotype, harvest regulations, and environmentally-mediated learned behavior, rather than an intrinsic inferiority of eastern wolves. Indeed, 4 of 5 radio-collared eastern wolves that dispersed from APP into adjacent unprotected areas during the study (but remained within the study area) were harvested before establishing residency <1 year after leaving APP (mean = 175 days, SE = 47; the fifth dispersing eastern wolf died of unknown causes 153 days after leaving APP). As both residency status and eastern wolf ancestry were important predictors of survival and harvest mortality outside of the protected area, these non-resident eastern wolves were at especially high risk of being shot or trapped outside of APP. The results with respect to residency status were consistent with many previous studies of wolf and coyote survival indicating that residents survive better than non-residents in harvested populations (e.g., Berger and Gese 2007; Smith *et al.* 2010), but that residency status does not affect survival in protected areas (e.g., Fuller *et al.* 1989).

Documentation of the variable influence of increasing road density on survival of the different *Canis* types provides insight into potential mechanisms underlying the susceptibility of eastern wolves to harvest. All residents survived poorly in response to increasing secondary road density within home ranges, but eastern wolves survived significantly worse than other *Canis* types in relation to roads. In protected areas, wolves may actually be attracted to roads to facilitate rapid movement across rugged terrain and increase predation efficiency (James & Stuart-Smith 2000; Whittington *et al.* 2005). In harvested areas, roads are used by hunters and trappers as access routes and for setting traps such that trapping and shooting mortality of canids is often associated with roads (Person & Russell 2008). Canids raised in harvested areas may adopt behavioral mechanisms to mitigate harvest risk, such as avoidance of roads during daytime when human activity is highest (Hebblewhite & Merrill 2008). These behavioral mechanisms may be absent or poorly developed in animals originating from APP where wolves are accustomed to exploiting the beneficial qualities of roads without increased risk of mortality. Thus, roads could represent an ecological trap (*sensu* Gates & Gysel 1978) or an attractive sink (*sensu* Delibes *et al*. 2001) for eastern wolves dispersing into harvested landscapes from protected areas. Interestingly, even admixed gray wolves survived better than eastern wolves in relation to roads indicating that this phenomenon was not simply the result of differences in sensitivity to human disturbance between wolf-like and coyote-like canids. Regardless, my results indicate that poor survival is an important mechanism underlying the negative association between eastern wolf ancestry and road density documented in the APP hybrid zone (Chapter 2).

Adult survival is the most important demographic parameter influencing population growth of eastern wolves within APP (Patterson & Murray 2008), consistent with most species of large carnivores. Previous concern regarding harvest mortality of wolves adjacent to APP has focused mainly on its effect on the viability of the population within APP (Forbes & Theberge 1996; Patterson & Murray 2008). In contrast to the apparent stability of the APP population within the park boundaries (Patterson & Murray 2008), my findings indicate that the unprotected areas adjacent to APP likely represent a population sink for eastern wolves. Fuller *et al.* (2003) estimated that wolf populations should stabilize (with no population growth or decline) with an annual survival rate of 0.66 which is considerably higher than the survival rate for eastern wolves documented here. Poor survival of eastern wolves negatively impacts the potential for expansion of the APP population both by limiting population growth and also by influencing hybridization dynamics. The lower density of eastern wolves outside of APP may reduce their ability to resist breeding with coyotes and other *Canis* types because of Allee effects associated with limited conspecific mating opportunities (Stephens & Sutherland 1999; Adams *et al.* 2003). Indeed, Rutledge *et al.* (2010*a*) suggested that assortative mating was responsible for the lower levels of hybridization in APP where >70% of breeding unions were between eastern wolves. Furthermore, high levels of human-caused mortality appear to have previously facilitated coyote introgression in APP during intensive wolf culls in the 1960s (Rutledge *et al.* 2011). Data on eastern wolf reproduction outside of APP is limited by the scarcity of resident eastern wolves and because some radio-collared eastern wolves in apparent breeding unions died before reproduction could be documented. Nonetheless, using telemetry and genetic data, I documented 7 cases of definitive (*n* = 5, i.e. confirmed by genetic analysis) or apparent (*n* = 2; telemetry data only) breeding unions outside of APP involving eastern wolves. Four of these unions were eastern wolves paired with hybrids (*n* = 3) or coyotes (*n* =1), supporting the contention that assortative mating is less common outside of APP.

Populations subjected to extreme rates of harvest mortality may be sustained with sufficient immigration from nearby reserves (Lariviere *et al.* 2000), which likely explains the persistence of eastern wolves in patches outside of APP. Indeed, parental-type eastern wolves make up significant proportions of the resident canids in KH and WMU47 (Chapter 2). Their poor survival in these areas relative to other genotypes suggests their presence is maintained by dispersal from the population core of APP. We found that eastern wolves dispersing from APP are usually killed quickly, as non-resident eastern wolves inhabiting unprotected landscapes were at high risk of harvest mortality. Some eastern wolves were able to establish residency, which reduced their risk of mortality, but their survival was still poor relative to other *Canis* types in these areas. Thus, the structure of the hybrid zone appears to be maintained by regular dispersal of parental types (eastern wolves) from APP into the hybrid zone, consistent with theoretical models suggesting hybrid zones are tension zones maintained by a balance between dispersal and selection against hybrids (Barton & Hewitt 1985). However, in direct contrast to the tension zone model, the structure of the *Canis* hybrid zone is also influenced strongly by environmental heterogeneity (Chapter 2), which is more consistent with environmentally-mediated mosaic hybrid zones (Ross & Harrison 2002). Furthermore, there was no evidence of selection against hybrids. Higher mortality of eastern wolves in harvested landscapes and regular dispersal from APP may also contribute to their patchy distribution as extinction-recolonization dynamics have been suggested to structure mosaic hybrid zones involving other species (Bridle *et al.* 2002).

Conclusions

I am unaware of previous research documenting a similarly strong influence of a protected area on hybridization dynamics between animal species. Given their poor survival and propensity for hybridization with coyotes in unprotected landscapes, parental-type eastern wolves in central Ontario would be greatly reduced, and could go locally extinct, without protection within APP. In the absence of changes to current harvest regulations, expansion of the APP eastern wolf population is unlikely. This finding has implications for the current federal eastern wolf status review (2013) by the Committee on the Status of Endangered Wildlife Species in Canada (COSEWIC) as estimates of effective population size are needed, but have been elusive due to uncertainty regarding the distribution and demography of eastern wolves beyond the APP boundary. My results raise at least two important management questions. First, would additional harvest protection outside of APP allow eastern wolves to increase in density in areas of suitable environmental conditions (e.g., KH)? Second, with higher densities outside APP would eastern wolves re-establish reproductive barriers (e.g., assortative mating) that appear to allow them to minimize contemporary hybridization with coyotes and gray wolves within APP (Rutledge *et al.* 2010*a*)?

My results, in combination with those in Chapter 2 show that protected areas can exert a powerful influence on hybridization dynamics between species and suggest that rare hybridizing taxa are able to maintain genetic distinctiveness within protected areas, even when reproductive barriers are few, and hybrids and other parental types are more abundant, outside the reserve. Although large protected areas similar to APP may be difficult to establish in many human-altered landscapes, my results highlight the importance of existing parks and reserves with respect to their potential to influence the structure of hybrid zones involving rare species. Thus, efforts to maintain or restore naturally regulated systems by protecting rare, hybridizing species from exploitation can help to address the challenge of conserving hybridizing species. Many hybridizing species are of taxa (e.g., birds, fish, amphibians) with modest space requirements compared to wolves, which may facilitate mitigation of negative consequences of hybridization through the influence of protected areas smaller than APP.

CHAPTER 4. INTER-SPECIFIC TERRITORIALITY IN A CANIS HYBRID ZONE: SPATIAL SEGREGATION BETWEEN WOLVES, COYOTES, AND HYBRIDS

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ABSTRACT

Gray wolves (*Canis lupus*) and coyotes (*Canis latrans*) generally exhibit intraspecific territoriality manifesting in spatial segregation between adjacent packs. However, previous studies have found a high degree of interspecific spatial overlap between sympatric wolves and coyotes. The eastern wolf (*C.lycaon*) is the most common wolf in and around Algonquin Provincial Park (APP), Ontario, Canada and hybridizes with sympatric gray wolves and coyotes. I hypothesized that all *Canis* types (wolves, coyotes, and hybrids) exhibit a high degree of spatial segregation due to greater genetic, morphologic, and ecological similarities between wolves and coyotes in this hybrid system compared with western North American ecosystems. I used GPS telemetry and probabilistic measures of spatial overlap (PHR and UDOI) to investigate spatial segregation between adjacent *Canis* packs. My hypothesis was supported as: 1) the probability of locating wolves, coyotes, and hybrids within home ranges ($\bar{x}$ PHR = 0.05) or core areas ($\bar{x}$ PHR < 0.01) of adjacent packs was low, and 2) the amount of shared space was negligible. Spatial segregation did not vary substantially in relation to genotypes of adjacent packs or local environmental conditions (i.e. harvest regulations or road densities). Herein, I provide the first telemetry-based demonstration of spatial segregation between wolves and coyotes, highlighting the novel relationships between *Canis* types in the Ontario hybrid zone relative to areas where wolves and coyotes are reproductively isolated. Territoriality among *Canis* may increase the likelihood of

eastern wolves joining coyote and hybrid packs, facilitate hybridization, and play a role in limiting expansion of the genetically distinct APP eastern wolf population.

INTRODUCTION

Territoriality has been defined in numerous ways by ecologists, but a common definition refers to defense of home ranges that are spatially segregated from other individuals or social groups (Burt 1943; Gordon 1997; Maher & Lott 1995). Territoriality allows animals to retain relatively exclusive use of space and access to the resources therein, usually food, dens or nests, and/or mates (Gese 2001). In addition to ensuring access to limited resources, spatial segregation among territorial animals may also serve to minimize encounters and direct conflict between individuals or social groups that depend on the same or similar resources for survival and reproduction (Wilson 1975).

Territoriality is most often used to describe behavior among individuals of the same species (Burt 1946; Gese 2001), as morphological and ecological similarities between conspecifics often leads to strong intraspecific competition for resources (Begon *et al.* 1996). However, territoriality also sometimes occurs between individuals of different species, often among congeners or within guilds when competition for shared resources is strong (Begon *et al.* 1996; Hoi *et al.* 1991; Tynkkynen *et al.* 2006). Logically, interspecific territoriality and spatial segregation might be predicted between parental types of hybridizing species that also exhibit intraspecific territoriality, because hybridization involves closely related species that often share morphological and ecological traits, and have similar resource requirements. Additionally, hybrid morphology is often intermediate to that of parental types (Grant & Grant 1994; Wolf &

Mort 1986) which may further intensify competition between individuals within hybrid zones.

Wolves (*Canis spp.*) and coyotes (*Canis latrans*) both exhibit intraspecific territoriality, which manifests in a high degree of spatial segregation between social units (Gese 2001; Mech & Boitani 2003). However, as noted by Berger & Gese (2007), no telemetry-based studies have documented a high degree of interspecific spatial segregation between wolves and coyotes. Fuller *et al.* (1981) suggested that wolves and coyotes had non-overlapping home ranges in Northeastern Alberta, in a study based on movements of 1 radio-collared coyote and capture location of 4 others in relation to wolf territories. Subsequent research with larger sample sizes has invariably found a high degree of overlap between sympatric wolves and coyotes. For example, coyotes in Riding Mountain National Park, Manitoba overlapped spatially and temporally with wolves and were attracted to areas of wolf activity, as scavenging wolf-killed ungulate carcasses was common by coyotes (Paquet 1991). Thurber *et al.* (1992) found that home ranges of all coyotes they studied overlapped with those of wolves in Alaska. Arjo & Pletscher (1999) showed that annual coyote home ranges overlapped with those of wolves more than seasonal home ranges, but overlap was considerable for both periods (median overlap = 100% annually, 60% seasonally). Berger & Gese (2007) documented a high degree of spatial overlap between home ranges and, in some cases, core areas of wolves and coyotes in Wyoming where coyote home ranges were completely subsumed within those of wolves in areas of highest wolf density. In areas with substantial spatial overlap between coyote and wolf home ranges and core areas, coyotes apparently reduce risk of interspecific aggression from wolves by modifying resource selection within these shared ranges in response to escalated risk of encountering wolves (Atwood & Gese 2010). Thus, virtually all studies have found that coyotes and wolves do not exhibit spatial segregation, and it appears that coyotes must balance the risks of wolf aggression with the rewards gained by scavenging from wolf-kills (Arjo & Pletscher 1999; Atwood & Gese 2008, 2010; Berger & Gese 2007; Paquet 1991; Wilmers *et al.* 2003).

Most previous investigations of spatial relationships between wolves and coyotes have involved populations of gray wolves (*C. lupus*) and coyotes in western North America, where the species are reproductively isolated (Garcia-Moreno *et al.* 1996; Kyle *et al.* 2006; Pilgrim *et al.* 1998). In these western ecosystems, wolf and coyote interactions tend to be characterized by aggression from wolves towards coyotes and scavenging of wolf food resources by coyotes (Atwood & Gese 2010; Berger & Gese 2007; Paquet 1991). In portions of eastern North America inhabited by intermediate sized wolves, eastern (*C. lycaon*, Ontario) or red (*C. rufus*, North Carolina) wolves, I would predict different relationships between wolves and coyotes and a higher degree of spatial segregation for several reasons. First, eastern and red wolves share genetic similarities with coyotes, either because they share a recent common ancestor with coyotes (Wilson *et al.* 2000) or because they represent the outcome of past hybridization between gray wolves and coyotes (von Holdt *et al.* 2011). Regardless, eastern and red wolves are intermediate in size between gray wolves and coyotes (Chapter 2; Phillips & Henry 1992), which likely increases competition for food and other resources with coyotes. Furthermore, eastern and red wolves have both hybridized extensively with coyotes where they are sympatric, hybridization appears to be ongoing, and backcrossing of hybrids with wolves and coyotes has been documented (Adams *et al.* 2007; Rutledge *et al.* 2010*a*; Wayne & Jenks 1991; Chapter 2). This means that eastern/red wolves, coyotes, and hybrids all represent potential mating opportunities to one another, suggesting potential competition for mates as well as food.

I studied wolves, coyotes, and hybrids in a hybrid zone central Ontario in Algonquin Provincial Park and vicinity to investigate spatial segregation among sympatric *Canis* types. I hypothesized that, in contrast to sympatric wolves and coyotes in western North America, packs of all *Canis* types in the study area would exhibit a high degree of both intra and inter-specific spatial segregation of home ranges and core areas. Additionally, I conducted research in 3 separate study units that were characterized by differences in genetic composition of the resident packs, habitat fragmentation (i.e. by roads), and harvest regulations (see details in Study Area, below). Therefore, my analyses allow for an assessment of whether variation in these factors influenced spatial segregation between adjacent packs and resulted in differences in spatial overlap across the 3 study units. My results provide novel documentation of interspecific spatial relationships between wolves and coyotes in an area where hybridization occurs and clarify potential differences with previously studied systems in western North America where wolves and coyotes are reproductively isolated.

METHODS

Study Area

Density (km/km^2) of primary (1°) and secondary (2°) roads in the home ranges of wolves, coyotes, and hybrids were lowest in APP (1°= 0.02, 2° = 0.09), intermediate in KH (1° = 0.05, 2° = 0.34), and highest in WMU49 (1° = 0.09, 2° = 0.57; Appendix D). Primary roads were paved roads with relatively high traffic volume classified as freeways, expressways or highways. Secondary roads were mostly paved and were classified as arterial, local/street, or collector roads, except for a few major gravel forest access roads in APP that received relatively high traffic volume and allowed speeds of > 50 km/hr. Therefore, I studied wolves, coyotes, and hybrids in 3 study units representing a gradient of human disturbance: APP (full protection for wolves/coyotes, lowest road density), KH (partial protection for wolves/coyotes, intermediate road density), and WMU49 (no protection for wolves/coyotes, highest road density).

Field Methods

I captured wolves, coyotes, and hybrids using padded foothold traps, modified neck snares, and with net-guns fired from helicopters. I immobilized animals captured in traps and snares, whereas animals captured with net-guns were restrained manually without immobilizing agents. I deployed mortality-sensitive Global Positioning System (GPS) or Very High Frequency (VHF) radio-collars on captured animals to monitor movements and survival. GPS collars were programmed to remain on the animals for approximately 1 year and collect approximately 4000 locations. I monitored collared animals ≥1 once per week using aerial and ground telemetry for survival and to determine/verify pack associations for the duration of the study.

Home Range Estimation and Spatial Overlap Analysis

I estimated fixed kernel (Börger *et al.* 2006; Worton 1989) home ranges (95%) and core areas (60%) using the plug-in estimator to determine optimal bandwidth (Sheather & Jones 1991) for focal packs using GPS telemetry data. I used 95% and 60% for home ranges and core areas, respectively, for consistency with previous studies investigating spatial segregation between wolves and coyotes (Arjo & Pletscher 1999; Atwood & Gese

2010; Berger & Gese 2007). My telemetry fix schedules were variable within and across some months for some collars such that areas used during intensive fix periods would have been overrepresented if I had used all of the data. Therefore, I systematically subsampled data from collars with variable fix schedules such that the data used to estimate each home range were collected at regular intervals throughout the year (i.e., a fix every 1.5, 2, 4, 5 or 6 hours depending on the collar). I estimated home ranges using concurrent data (i.e. from identical date ranges) for both packs in each dyad to ensure that I was comparing space use during periods when both packs were occupying their home ranges. I used data from 58-365 days to estimate home ranges for each dyad ($\bar{x}$ = 165 days, SE = 20, *n* = 26 dyads), beginning with the first day that concurrent data were collected for a given dyad. As noted by Börger *et al.* (2006) it is critical to use data sampled from an equal number of days when comparing kernel home ranges, but inferences from such comparisons are robust to variation in the number of fixes. After subsampling, I used all data to estimate home ranges in most cases ($\bar{x}$ = 729 locations, SE = 46, *n* = 52 home ranges); however, I excluded locations from one long range (>84 km) movement by 1 pack outside of their normal area of use during winter to visit a known deer yard. Some resident wolves migrate from their home ranges within APP to exploit abundant prey in deer yards in areas adjacent to APP (Forbes & Theberge 1996; Cook *et al.* 1999). Given that my objective was to compare space use of adjacent packs while they were occupying their home range, excluding extra-territorial locations from such packs was appropriate. For packs that I monitored over multiple years, I calculated separate annual home ranges for each year, thus, in 2 cases, I estimated 2 sets of annual home ranges and core areas for a given dyad. I included these home ranges as separate data points given that annual differences in resource availability or pack membership may have influenced the degree of overlap between adjacent packs in different years. In total, I estimated 52 home ranges and core areas for 26 pack dyads (11 from WMU49, 9 from APP, 6 from KH) comprising 28 different packs of wolves, coyotes, and hybrids.

To investigate spatial segregation, I estimated home ranges for pack dyads occupying adjacent 95% home ranges. I considered home ranges to be adjacent if two 95% home ranges either: 1) overlapped to some extent (89% of dyads), or 2) were separated by <1.5 km at their closest boundaries (n = 3, i.e., 1.15 km, 0.5 km, 0.06 km). Kochanny & Fieberg (2005) reviewed methods to evaluate spatial segregation and overlap and concluded that methods using the utilization distribution (UD; Worton 1989) were superior to earlier, simplistic comparisons of home range overlap. Accordingly, I compared overlap of home ranges and core areas using 2 probabilistic methods utilizing UDs of neighboring animals to investigate spatial segregation. I used the probability of home range overlap (PHR) to estimate the probability of an individual from pack j being in pack i's home range or core area ($\mathrm{PHR}_{i,j}$) and vice versa ($\mathrm{PHR}_{j,i}$) using the formula proposed by Fieberg & Kochanny (2005):

$$\mathrm{PHR}_{i,j} = \iint\limits_{\mathrm{A}_i} \hat{\mathrm{U}}\mathrm{D}_j\,(x,y)\;dxdy \tag{1}$$

Where $\hat{\mathrm{U}}\mathrm{D}_{\mathrm{j}}$ is the estimated UD for canid pack j and A_i is the area of overlap of pack i's home range with pack j's, x and y represent estimates of the UD at a set of grid points, and dxdy are the area of each grid cell. PHR should be superior to a similar, commonly used method:

$$\text{Proportion of overlap} = \text{Area of overlap}_{i,j}/\text{area of home range}_i\,, \tag{2}$$

which simply determines the proportion of an individual's home range that is overlapped by another individual because the PHR accounts for differences in the relative probability of space-use by utilizing the UD (Fieberg & Kochanny 2005). However, because PHR is similar in principal to the proportional measure of area of overlap (equation 2), it should be useful for coarse comparisons with previous studies that did not estimate UDs. Next, I estimated the Utilization Overlap Index (UDOI):

$$\mathrm{UDOI} = A_{i,j} \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \widehat{\mathrm{UD}}_{i}(x,y) \times \widehat{\mathrm{UD}}_{j}(x,y)\, dxdy, \qquad (3)$$

which provides a joint measure of overlap between 2 neighboring individuals or packs. A value of 0 indicates no overlap and a value of 1 indicates complete overlap and uniform space use (Fieberg & Kochanny 2005). UDOI >1 is also possible if 2 UD's are non-uniformly distributed and have a high degree of overlap (Fieberg & Kochanny 2005). UDOI is likely the most appropriate technique for quantifying overlap in terms of shared space-use, particularly for studies using UD-based estimates of home range (e.g., kernels) and GPS telemetry (Fieberg & Kochanny 2005; Whittington *et al.* 2011). I calculated home ranges, utilization distributions, PHR, and UDOI in R Statistical software 2.13.1 using the 'Adehabitat' package and code obtained from the lead author of Fieberg & Kochanny (2005).

Genetic Ancestry

I extracted DNA from blood or hair samples obtained from study animals at capture and amplified and scored12 microsatellite loci following previously described PCR amplification and scoring procedures (Wheeldon *et al.* 2010; Chapter 2). I determined genetic ancestry of all captured and radio-collared animals with genetic analyses described previously (see Chapter 2). Animals of known genetic ancestry in most (82%) of the packs included in the spatial overlap analyses were eastern wolves, coyotes, or eastern wolf × coyote hybrids. However, there were animals from 5 packs (3 in APP, 1 in WMU49, 1 in KH) that were eastern wolf × gray wolf hybrids (*n* = 3 packs), coyote × gray wolf hybrids (*n* = 1 pack), or eastern wolf × gray wolf × coyote hybrids (*n* = 1 pack), For simplicity, hereafter I refer to all animals of wolf origin (eastern wolves and eastern wolf × gray wolf hybrids) as wolves and all animals of mixed wolf and coyote ancestry (eastern wolf × coyote, coyote × gray wolf, or eastern wolf × gray wolf × coyote) as hybrids. Packs in which individuals of known ancestry were either all wolves or all coyotes were classified as wolf and coyote packs, respectively. However, not all packs were composed of individuals from a single *Canis* type. Any pack that contained at least 1 hybrid, or contained both wolves and coyotes, was considered to be a hybrid pack. I knew the genetic identity of both breeding animals in 75% of the packs included in overlap analyses, through genetic analysis or by inferring the genotype of an unknown breeder from the genotypes of a known breeder and their direct offspring (Chapter 2; Table 4.1). For packs where both breeders were known, I suspect that most other pack members that were not sampled were offspring of the breeding pairs because *Canis* packs in the APP region are family-based and are generally composed of a breeding pair and their direct offspring (Rutledge *et al.* 2010*b*; Chapter 2). Offspring that were not sampled would not have changed the pack-genotype classifications because their genotypes would reflect those of their parents. Therefore, although knowledge of genetic ancestry for all pack members of all focal packs was incomplete, I believe my data allowed me to reliably classify packs as wolf, coyote, or hybrid for the purposes of the analyses.

Table 4.1. Genotypes of breeding pairs in Canis packs included in analyses of spatial overlap between adjacent pack dyads in central Ontario 2007-2011. All breeding relationships were determined via genetic pedigree analysis from Chapter 2 unless otherwise noted.

Breeding Pair	*n*
Wolf-Wolf	7[a,b]
Coyote-Coyote	5[c,d]
Coyote-Hybrid	5
Wolf-Unknown	4
Hybrid-Hybrid	2
Coyote-Unknown	2
Coyote-Wolf	1[e]
Wolf-Hybrid	1
Unknown	1[f]

[a] 2 breeding genotypes inferred from wolf parent and direct wolf offspring in pack

[b] 1 breeding relationship inferred because only 2 adults (both wolves, male and female) in pack

[c] 2 breeding genotypes inferred from coyote parent and direct coyote offspring in pack

[d] 1 breeding relationship inferred because only 2 adults (both coyotes; male and female) in pack

[e] breeding genotype inferred from 1 wolf parent and 50%-50% (approximately) wolf-coyote hybrid offspring in pack

[f] Adult male coyote only captured animal in pack, breeding status unknown

Observational Data

As noted by Gese (2001), territoriality among wolves and coyotes is readily accepted, but the actual mechanisms are rarely observed. As in the present study, territoriality is often inferred via indirect means such as spatial segregation of home ranges or through evidence of scent-marking, as direct observation of territorial defense is rare for elusive carnivores (Gese 2001). I describe 3 observations from the field that provided more direct, although limited, information regarding inter- and intraspecific territoriality among wolves, coyotes, and hybrids in the study area.

RESULTS

Across the study area, there was a high degree of spatial segregation between wolves, coyotes, and hybrids (Fig. 4.1-4.3, Tables 4.2-4.4). For all animals, the probability of being located within the 95% home range of a specific adjacent pack was < 0.22 ($\bar{x}$ $PHR_{95\%} = 0.052$, SE = 0.007, $n = 52$). For all animals, the probability of being located within the 60% core area of a specific neighbor was <0.08 and for 65% of animals the PHR was 0 ($\bar{x}$ $PHR_{60\%} = 0.009$, SE = 0.002, $n = 52$). The UDOI also indicated that wolves, coyotes, and hybrids were spatially segregated as all dyads exhibited low overlap of 95% home ranges ($\bar{x} = 0.004$, SE = 0.001, $n = 26$) and 60% core areas ($\bar{x} < 0.001$, SE < 0.001, $n = 26$) relative to uniform space use. All UDOI values for 95% home ranges were <0.03 and all UDOI values for 60% core areas were <0.002.

Overlap between both home ranges and core areas was negligible and appeared to be similar between adjacent Wolf-Wolf, Wolf-Coyote, Wolf-Hybrid, Coyote-Hybrid, and Hybrid-Hybrid pack dyads (Tables 4.2,4.3). There also were not substantial differences in home range or core area overlap among the 3 study units (Table 4.4). Given the consistently negligible degree of overlap of 95% home ranges and near complete spatial segregation of 60% core areas in relation to genetic ancestry of adjacent packs and across study units, statistical analysis of overlap estimates did not seem appropriate or necessary to evaluate my hypothesis, or to investigate potential differences across study units. Wolves, coyotes, and hybrids were clearly spatially segregated and the degree of spatial segregation did not appear to be strongly influenced by the genetic composition of adjacent packs or by differences in environmental conditions across study units.

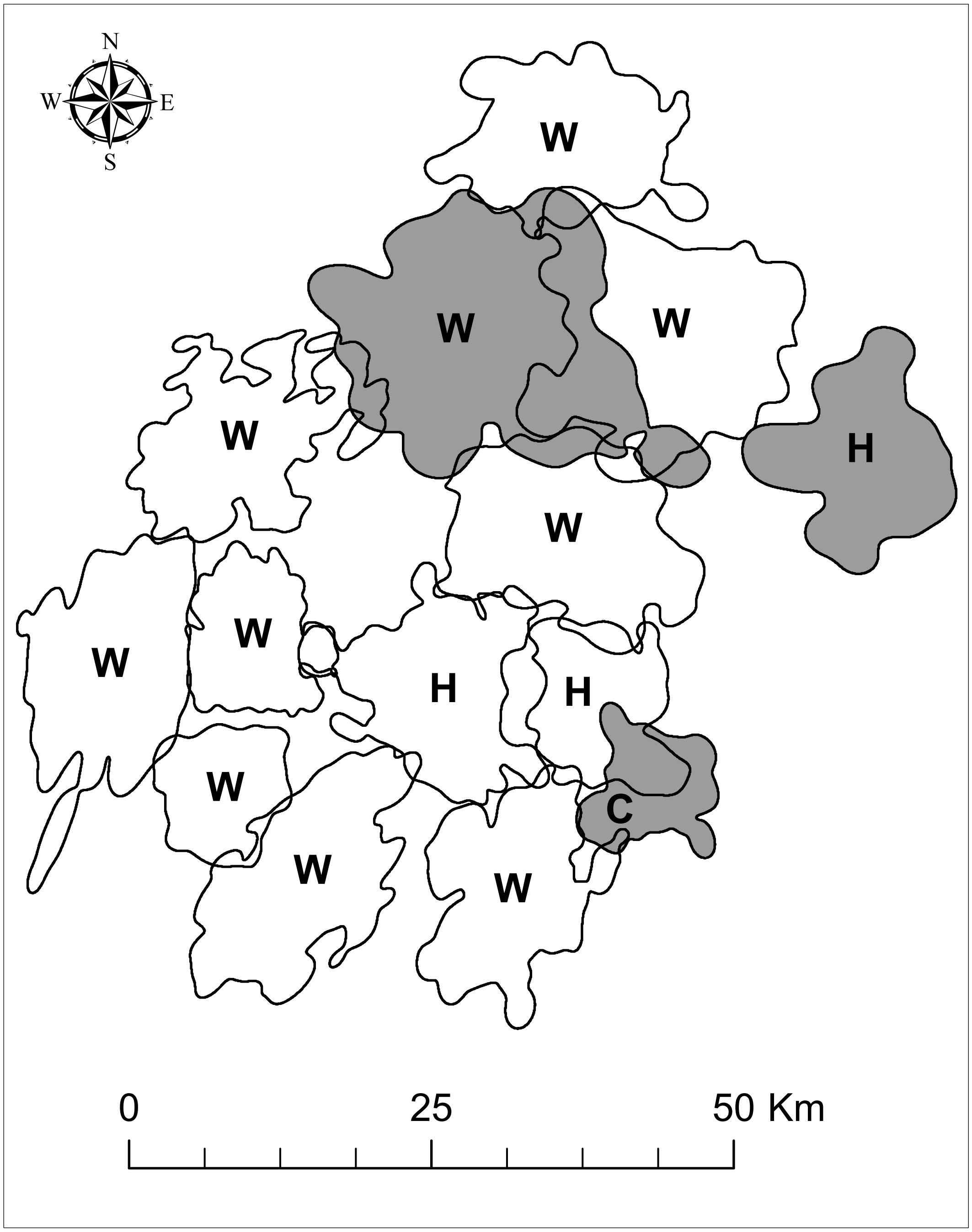

Fig. 4.1. Annual 95% fixed kernel home ranges of wolf (W), coyote (C), and hybrid (H) packs in Algonquin Provincial Park, Ontario, Canada, 2007-2011. Home ranges shown here differ slightly from those used in overlap analyses because: 1) analyses were restricted to concurrent data for all dyads; 2) small, non-contiguous portions of home ranges were removed for clarity; and 3) not all packs shown were included in overlap analyses because I restricted analyses to concurrent dyads. Packs not included in analyses are shown in gray shading.

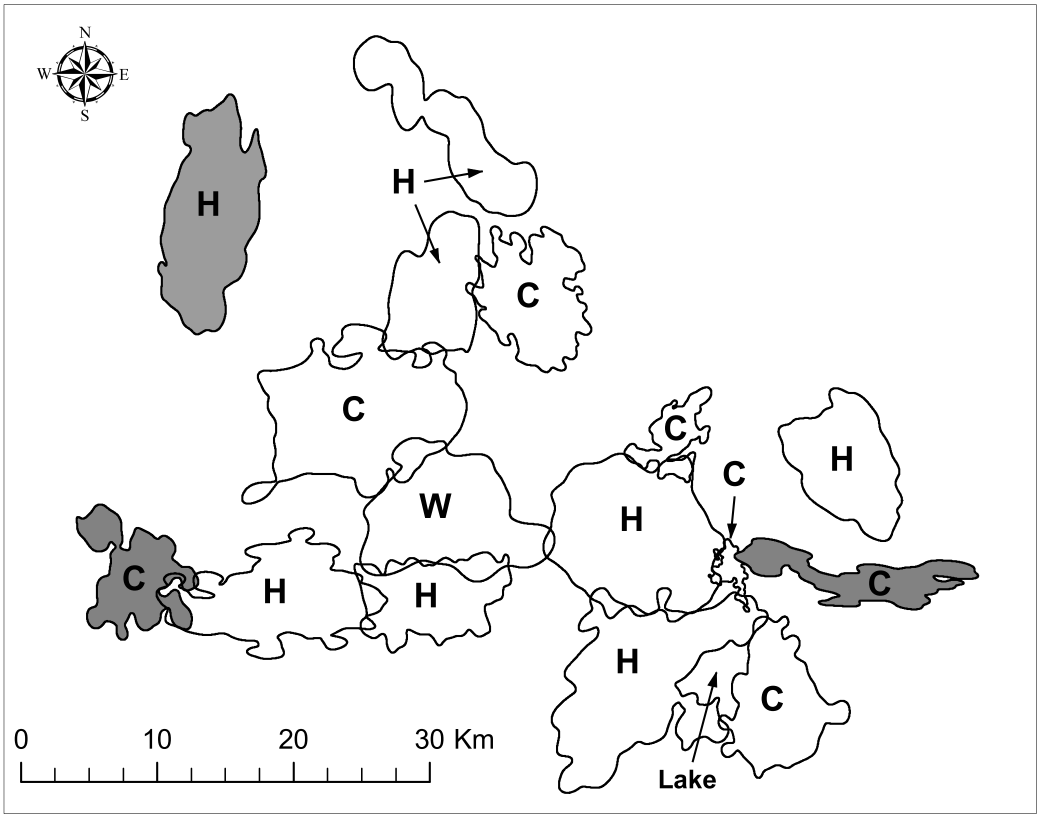

Fig. 4.2. Annual 95% fixed kernel home ranges of wolf (W), coyote (C), and hybrid (H) packs in Wildlife Management Unit 49, Ontario, Canada, 2007-2011. Home ranges shown here differ slightly from those used in overlap analyses because: 1) analyses were restricted to concurrent data for all dyads; 2) small, non-contiguous portions of home ranges were removed for clarity; and 3) not all packs shown were included in overlap analyses because I restricted analyses to concurrent dyads. Packs not included in analyses are shown in gray shading. A large lake that occupied an area between adjacent home ranges is also shown.

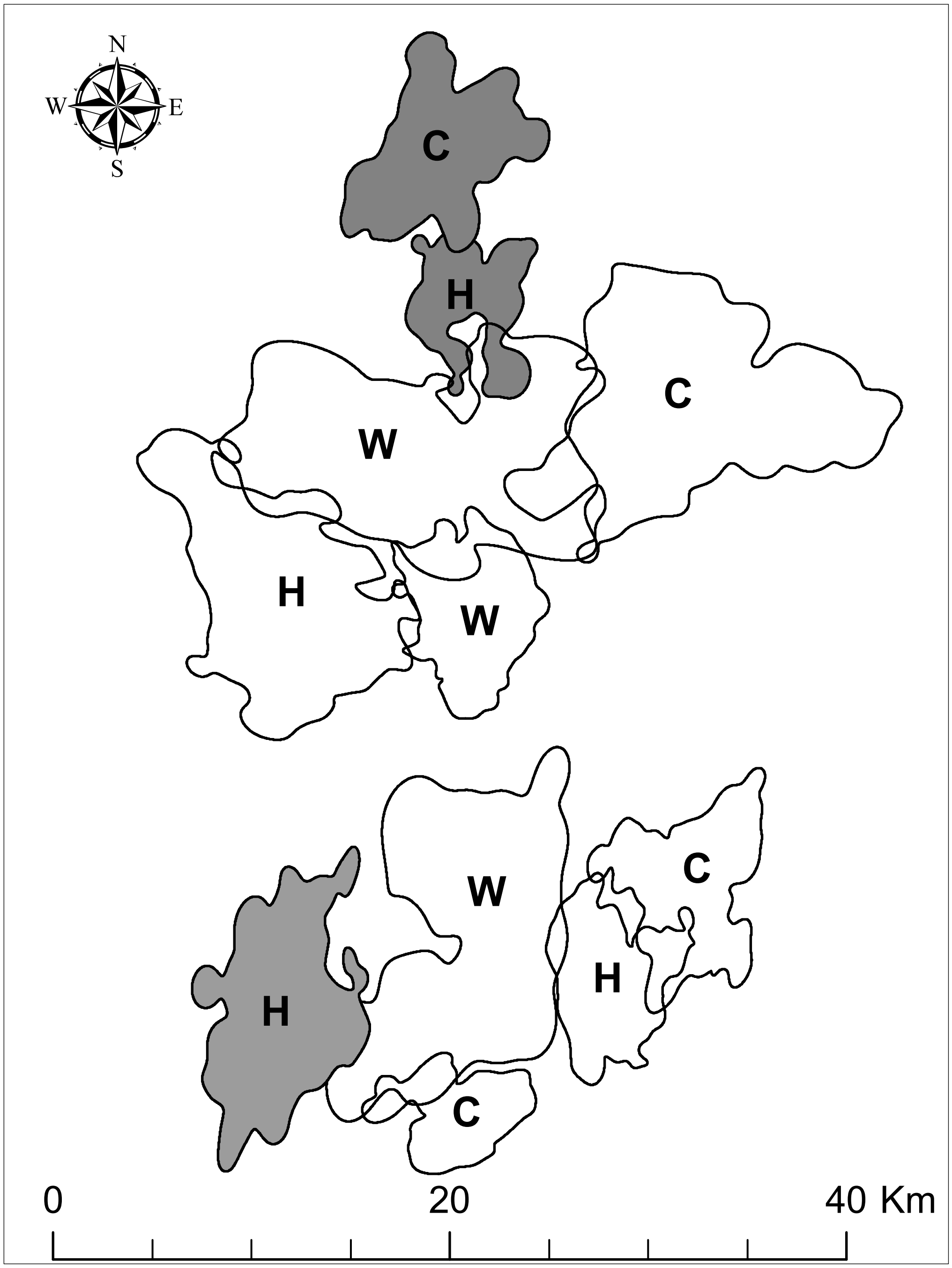

Fig. 4.3**.** Annual 95% fixed kernel home ranges of wolf (W), coyote (C), and hybrid (H) packs in Kawartha Highlands, Ontario, Canada, 2007-2011. Home ranges shown here differ slightly from those used in overlap analyses because: 1) analyses were restricted to concurrent data for all dyads; 2) small, non-contiguous portions of home ranges were removed for clarity; and 3) not all packs shown were included in overlap analyses because I restricted analyses to concurrent dyads. Packs not included in analyses are shown in gray shading.

Table 4.2. Mean probability a pack of given genotype being located in the 95% home range ($PHR_{95\%}$) and 60% core area ($PHR_{60\%}$) of an adjacent pack of a given genotype in central Ontario, Canada 2007-2011. Also shown are standard errors (SE) and number of packs (*n*).

	$PHR_{95\%}$	SE	*n*	$PHR_{60\%}$	SE	*n*
Wolf in Wolf	0.039	0.016	12	0.011	0.007	12
Hybrid in Hybrid	0.073	0.020	6	0.010	0.007	6
Wolf in Coyote	0.084	0.044	3	0.018	0.017	3
Coyote in Wolf	0.065	0.006	3	0.008	0.007	3
Wolf in Hybrid	0.020	0.010	7	0.003	0.003	7
Hybrid in Wolf	0.042	0.020	7	0.007	0.007	7
Coyote in Hybrid	0.075	0.049	7	0.011	0.007	7
Hybrid in Coyote	0.055	0.028	7	0.008	0.008	7
Overall	0.052	0.008	52	0.009	0.002	52

Table 4.3. Mean overlap of 95% home ranges ($UDOI_{95\%}$) and 60% core areas ($UDOI_{60\%}$) between neighboring dyads of wolf, coyote, and hybrid packs as estimated by the Utilization Distribution Overlap Index (UDOI). Also shown are standard errors (SE), and number of pack dyads (*n*) for each mean in central Ontario, 2007-2011.

Pack1	Pack2	$UDOI_{95\%}$	SE	*n*	$UDOI_{60\%}$	SE	*n*
Wolf	Wolf	0.002	0.002	6	<0.001	<0.000	6
Wolf	Coyote	0.006	0.003	3	<0.001	0.001	3
Wolf	Hybrid	0.002	0.001	7	<0.001	<0.000	7
Coyote	Hybrid	0.006	0.004	7	<0.001	<0.000	7
Hybrid	Hybrid	0.007	0.008	3	<0.001	<0.000	3

Table 4.4. Mean ($\bar{x}$) overlap indices for 95% home ranges and 60% core areas of adjacent *Canis* packs in 3 study units in (APP) and adjacent to (KH, WMU49) Algonquin Provincial Park (APP) and the surrounding buffer area where Canis harvest is banned in central Ontario, Canada, 2007-11. I also show standard errors (SE) and samples sizes (*n*) for each mean overlap index.

	$PHR_{95\%}$			$PHR_{60\%}$			$UDOI_{95\%}$			$UDOI_{60\%}$		
	$\bar{x}$	SE	n^a	$\bar{x}$	SE	n^a	$\bar{x}$	n^b	SE	$\bar{x}$	SE	n^b
APP	0.042	0.012	18	0.009	0.003	18	0.003	9	0.002	<0.000	<0.000	9
KH[c]	0.072	0.018	12	0.014	0.005	12	0.005	6	0.002	<0.000	<0.000	6
WMU49[d]	0.049	0.011	22	0.006	0.004	22	0.004	11	0.003	<0.000	<0.000	11

[a] number packs in dyads of adjacent packs
[b] number of dyads of adjacent packs
[c] Kawartha Highlands
[d] Wildlife Management Unit 49

Observations of Aggressive Encounters between Canids

First, I documented a hybrid (coyote × eastern wolf) breeding adult male in WMU49 (whose mate was a coyote) that was apparently killed by a neighboring pack of wolves (gray wolf × eastern wolf). Field evidence and necropsy of the carcass conclusively attributed cause of death to be aggression from wolves. I documented a GPS collared wolf from the adjacent pack at the mortality site concurrent with the estimated time of death and I found beds of wolves in the snow nearby the carcass (<100m). I also established that a second wolf from the suspected aggressor pack was present at the mortality site through genetic identification of a hair sample collected from one of the beds. This mortality occurred in an area of home range overlap between the 2 packs (included as a dyad in my analysis) approximately 0.7 and 1.6 km from the closest border of the hybrid and wolf packs' home range, respectively.

Second, while tracking a hybrid breeding female (her mate was a coyote) in a focal pack in WMU49 during a helicopter tracking session, I observed the radio-collared female hybrid running from 2 larger uncollared canids across an open sand pit. After being cornered against a pond by the larger animals, the collared female turned to face the aggressors and the 3 canids fought intensely for approximately 20 seconds. The attacking animals appeared to be uncomfortable with the hovering helicopter, turning to look at it several times during the encounter before abandoning the fight and running in the direction they came from. The hybrid female limped away in the other direction, but survived the incident and was alive at the end of the study > 1 year later. This observation occurred approximately 1 km from the closest border to her home range. Given the disparity in size between the attacking animals and the hybrid female, I suspected the 2 attacking animals were wolves, but their specific genetic identity was unknown.

Third, I heard aggressive barking, howling, and fighting noises originating from across a lake (<100m from my location) outside of my field station. I immediately confirmed that 2 radio-collared coyotes (a resident breeding male [within its home range] and a resident pup [approximately 7 months old, just outside its pack's home range] from 2 different, adjacent packs were present at the location of the encounter. I tracked the pup as it moved quickly to the east, across a road and back to its territory following the apparent fight. Based on ground telemetry, the resident breeding male did not appear to continue the chase beyond the border of its territory. I investigated the site the next day and found the remains of a deer at the approximate location where the aggressive vocalizations were heard. This encounter occurred on the periphery of one pack's home range (approximately 150m from the closest boundary) and just outside the second pack's (<200 m from closest boundary). I suspect the pup from the adjacent pack was attracted by the scent of the deer carcass and was attacked and chased off by the breeding male of the resident pack.

DISCUSSION

The hypothesis was supported as wolves, coyotes, and hybrids exhibited a high degree of spatial segregation with neighboring packs, regardless of genotype. However, I found no evidence that differences across study units in harvest regulations, fragmentation by roads, or genetic structure of the local *Canis* populations influenced spatial overlap. Spatial segregation between adjacent packs was high throughout the study area, such that any differences in relation to these environmental conditions were inconsequential. Thus, wolves, coyotes, and hybrids in my study area appear to be territorial with each other, regardless of the genotypic composition of adjacent packs. My results highlight the novelty of the relationships between genetically distinct *Canis* types present in this eastern wolf-eastern coyote hybrid zone relative to previous studies of gray wolves and coyotes.

It is generally accepted that wolves are highly territorial and that a high degree of spatial segregation exists between adjacent wolf packs (reviewed by Mech & Boitani 2003). Coyote territoriality is also well established in the literature (e.g., Barrette & Messier 1980; Camenzind 1978; Gese & Ruff 1997; Gese 2001). Differences in methodology between my study and many earlier studies in terms of home range estimators (MCP vs. kernel UD), overlap indices (proportion of overlap vs. UD-based measures), and telemetry (VHF vs. GPS) makes comparing results of overlap analyses across studies difficult. Furthermore, although many studies have conducted telemetry-based studies of wolves in North America, few, if any, have quantified the actual proportion or probability of overlap between adjacent packs. Many previous studies have provided convincing visual representations of the degree of overlap by plotting adjacent home ranges (e.g., Ballard *et al.* 1987; Fritts & Mech 1981; Fuller 1989; Peterson *et al.* 1984; Theberge & Theberge 2004; Van Ballenberghe *et al.* 1975), but did not report results of spatial overlap analyses. Methodological differences notwithstanding, mean proportion of overlap of home ranges between adjacent packs of wolves in Poland appeared to be similar to the overlap I documented between *Canis* packs in Ontario (Jędrzejewski *et al.* 2007). I suggest that wolves, coyotes, and hybrids in the Ontario hybrid zone I studied exhibit spatial segregation comparable to that observed among wolf packs studied elsewhere, even though direct comparisons of the exact degree of overlap were problematic.

Studies in the Greater Yellowstone Ecosystem in Wyoming and Montana, quantified spatial overlap of wolf and coyote home ranges and core areas using the simple, proportional overlap metric described above (equation 2; Arjo & Pletscher 1999; Atwood & Gese 2010; Berger & Gese 2007). This metric is similar, but not equivalent, to the UD-based $PHR_{i,j}$ that I used. Atwood & Gese (2010) reported a high degree of 95% home range ($\bar{x} = 0.78$) and 60% core area overlap ($\bar{x} = 0.82$) for coyotes in wolf home ranges in southwest Montana. In northwestern Montana, overlap of annual male and female coyote 94% home ranges was high ($\bar{x} = 0.74$-1.00 across years and sexes, whereas 62% core area overlap was more variable (range: 0 - 1.00; Arjo & Pletscher 1999). In Grand Teton National Park, Wyoming 4 coyote 95% home ranges were completely subsumed within wolf home ranges ($\bar{x} = 1.00$), whereas 60% core area overlap was more variable with 2 coyotes exhibiting close to complete overlap with wolf core areas (0.96 and 0.97) and 2 coyotes showing no overlap of core areas (Berger & Gese 2007). Thus, although different methods were used for estimating overlap, the differences in terms of spatial overlap between studies of western gray wolves and coyotes and spatially segregated wolves and coyotes in my study (95% home ranges, $\bar{x} = 0.065$; 60% core areas, $\bar{x} = 0.008$; Table 2) were sufficient to establish that interspecific relationships between wolves and coyotes are drastically different between the 2 systems.

Berger & Gese (2007) also calculated the UDOI for wolves and coyotes, facilitating more direct comparisons. UDOI for wolves and coyotes in Grand Teton National Park ranged from 0.03 – 0.23 for 95% home ranges ($\bar{x} = 0.13$) and 0 - 0.17 for

60% core areas ($\bar{x}$ = 0.08, [Berger & Gese 2007]). The mean UDOI for wolf-coyote dyads in my study was 0.006 for 95% home ranges and <0.001 for 60% core areas (Table 4.3), which are both more than an order of magnitude lower than mean UDOI values documented in Wyoming. Although the samples sizes of wolf-coyote dyads were low for both studies (*n* = 3, my study; *n* = 4, Berger & Gese 2007), 4 other dyads in my analyses contained wolves and coyotes in adjacent packs and were spatially segregated ($\bar{x}$ $PHR_{95\%}$ = 0.03, SE = 0.04, *n* = 4; $\bar{x}$ $UDOI_{95\%}$ < 0.001, SE < 0.001, *n* = 4). These dyads were classified as wolf-hybrid because the packs with coyotes each contained 1 hybrid animal. Regardless, when compared with previous work, my results clearly illustrate differences between western wolf-coyote systems and indicate that wolves, coyotes, and hybrids are spatially segregated in the APP region, regardless of genetic ancestry of individuals of adjacent packs.

My results have important implications, both for conservation and increasing understanding of hybridization dynamics. Eastern wolves are the dominant canid in APP and appear to have resisted hybridization more effectively within the park than in adjacent areas (Rutledge *et al.* 2010*a*; Chapter 2). Exclusion of coyotes from home ranges within APP may be one mechanism by which eastern wolves have minimized hybridization and retained their genetic distinctiveness. However, territorial aggression of resident coyotes and hybrids in response to dispersing wolves in areas adjacent to APP where coyotes are abundant (e.g., WMU49) may also make it difficult for eastern wolves to establish home ranges outside of the park. Displacement of hybrids and coyotes by pairs of endangered red wolves in North Carolina was identified as the most important parameter influencing quasi-extinction and persistence probabilities in a population viability analysis (Fredickson & Hedrick 2006). However, to my knowledge, aggression by resident coyotes and hybrids towards dispersing eastern or red wolves has not been considered as a mechanism limiting population expansion for hybridizing wolves of conservation concern in Ontario or North Carolina. A recent sampling effort adjacent to the population core of reintroduced red wolves in coastal North Carolina failed to detect any red wolves outside of the experimental population area, as all canids detected were coyotes and hybrids (Bohling & Waits 2011). Harvest mortality of red wolves in these adjacent areas likely plays a significant role in reducing dispersal success into unprotected landscapes (Bohling & Waits 2011), as it does with eastern wolves outside of APP (Chapter 3). Dispersing red and eastern wolves that are able to avoid harvest mortality may still have difficulty establishing breeding ranges beyond their core populations due to territorial aggression from resident coyotes and hybrids. Eastern wolves are larger than both coyotes and hybrids, but the differences are subtle (Chapter 2). Wolves generally disperse individually (Mech & Boitani 2003), and would be unlikely to successfully displace a pack of resident coyotes or hybrids. Eastern wolves dispersing into areas saturated with coyote and hybrid territories such as WMU49, probably have limited options for territory establishment and instead may join packs and establish breeding unions with coyotes or hybrids when possible. Thus, territoriality among *Canis* types in areas adjacent to APP may play significant roles in reducing eastern wolf dispersal success, facilitating hybridization, and limiting the expansion of the genetically distinct APP eastern wolf population.

Rich *et al.* (2012) found that human-caused mortality influenced territoriality of wolves and suggested conflict with humans can increase the cost territoriality by reducing the number of pack members available for defense and/or by causing wolves to avoid areas rich in resources where harvest risk was high. Rutledge *et al.* (2010*b*) suggested that harvest mortality increases the occurrence of unrelated animals in wolf packs, which could decrease pack cohesion. Thus, territoriality and spatial segregation between canid packs might be predicted to be greatest in unharvested areas that are relatively free of human disturbance. However, despite the fact that I studied canids in 3 study units that represented a gradient of human disturbance and harvest regulations (see Study Area), these differences did not appear to influence territoriality between wolves, coyotes, and hybrids in my study area as spatial segregation was consistently high across all study units (Figures 4.1-4.3).

I did not capture every canid in the study area; however, I am confident that the degree of spatial segregation I documented was not due to missing entire packs that overlapped with the packs I monitored. My capture efforts were intensive within each study unit during the years of the study (APP and WMU49 2007-2010, KH 2009-2010), I captured >150 canids, and the spatial configuration of their home ranges closely resembles the mosaic pattern of territorial canids found by other studies (e.g., Ballard *et al.* 1987; Fritts & Mech 1981; Fuller 1989; Peterson *et al.* 1984; Theberge & Theberge 2004; Van Ballenberghe *et al.* 1975; Fig. 4.1-4.3). There were a few noticeable gaps in this mosaic of packs (Fig. 4.1-4.3), which were due to difficultly in establishing traplines in these areas because of poor road access or private lands that were unavailable for trapping. However, there is no reason to suspect the pattern of overlap between these unknown packs and adjacent canids was any different from what I observed in the collared sample. I often captured multiple animals on the same trapline, or within the same home range of a focal pack, and animals captured within a given area always belonged to the same pack except when: 1) they were transient or dispersing (non-pack) animals; or 2) I was trapping near home range boundaries and captured animals from adjacent home ranges. Therefore, I am confident that my capture and telemetry data accurately reflect the spatial relationships between resident wolves, coyotes, and hybrids within the study area in the hybrid zone in and adjacent to APP.

Although my direct field observations represented a small number of occurrences, they were consistent with wolf-wolf (Mech 1994) and coyote-coyote (Bekoff & Wells 1986; Carmenzind 1978; Gese 2001) territoriality and support the conclusion that wolves, coyotes, and hybrids are territorial with each other in the central Ontario *Canis* hybrid zone. Specifically, the first 2 observations and supporting data reported above are notable, because 1) they were interactions between animals known or suspected to be of different genetic types, and 2) they were consistent with previous descriptions of aggressive, intraspecific wolf territorial interactions in terms of their position relative to known home range boundaries. Most cases of intraspecific killing among wolves occur in areas of overlap between adjacent packs and close to home range boundaries (Mech 1994), as in my first 2 observations. In contrast, previous descriptions of aggressive encounters between wolves and coyotes have generally involved coyotes scavenging at wolf kills (Gese 2001; Wilmers *et al.* 2003; Atwood & Gese 2008). Furthermore, Atwood & Gese (2008) reported that all interspecific mortalities of coyotes attributed to wolves were <200m from a wolf-killed carcass. I visited all clusters of GPS locations for the hybrid packs (i.e. the recipients of aggression) involved in the first 2 observations described above as part of a predation study to locate all ungulate kills. However, I failed to find any evidence of a carcass within 2 km of the aggressive encounters. Thus, the first 2 observations appear to be more consistent with reports of wolf-wolf territorial aggression, rather than wolf-coyote competition for carcasses. The third observation, likely involved a carcass, but was also consistent with previous descriptions of intraspecific territorial behavior between coyotes. Gese (2001) observed 112 instances of intraspecific territorial defense by coyotes and noted that the alpha male (i.e. breeding male) was mostly likely to confront intruding animals, that residents in their home range were usually successful at repelling the intruders, that they abandoned the chase near the home range boundary, and that fights did not end in mortality (but see Okoniewski 1982; Patterson & Messier 2001). All of these characteristics are consistent with my observation and have also been described by other researchers (Bekoff & Wells 1986; Carmenzind 1978). Thus, I conclude that my third observation likely represented both a competitive interaction over a carcass and active territorial defense between a resident and intruding coyote. Gese (2001) noted that direct observations of wild canid territorial defense are difficult to obtain due to the elusive nature of wolves and coyotes, and that few researchers have been fortunate enough to document such behavior.

CHAPTER 5. AN ADAPTIVE FUNCTIONAL RESPONSE TO ROADS BY WOLVES, COYOTES, AND HYBRIDS OUTSIDE A PROTECTED AREA

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ABSTRACT

Resource selection that varies as a function of availability is referred to as a functional response. Considering functional responses and linking behavior to fitness are important for effectively modeling resource selection. Although assumed to be adaptive, previous studies have not explicitly investigated fitness consequences of functional responses in resource selection. I modeled resource selection of wolves, coyotes, and hybrids within their home ranges in and adjacent to Algonquin Provincial Park (APP), Ontario, Canada to investigate functional responses to roads and the implications for mortality risk. Canids outside of APP selected roads more strongly during night than day similar to previous studies of wolves in other areas. Next, I modeled the relationship between differences in night and day use of secondary roads by individual canids as a function of availability of these roads and documented a significant, non-linear relationship. Specifically, canids outside APP modified their selection between night and day strongly at high road densities (selecting roads more at night), whereas they responded weakly at lower road densities (generally no selection). In APP where road densities were lower and harvest was illegal canids did not modify their use of roads between day and night or exhibit a strong functional response. Outside APP, individuals that survived exhibited a highly significant functional response, whereas those that died showed a weaker, non-significant response. My results suggest the functional response is adaptive and that canids in the unprotected landscape outside APP must balance trade-offs between

exploiting benefits associated with secondary roads and mitigating risk of human-caused mortality. Additionally, these models represent the first detailed investigation of canid resource selection in and adjacent to APP and improve understanding wolf and coyote habitat relationships in central Ontario.

INTRODUCTION

Studying resource selection of animals is a fundamental component of wildlife ecology (Rosensweig 1981; McLoughlin *et al.* 2009). Generally, the objective is to identify resources that are important to a species or population by quantifying which resources are used disproportionately to their availability to infer selection or avoidance (Johnson 1980; Beyer *et al.* 2010). However, at least 2 problems arise that can introduce uncertainty into the results of resource selection studies. First, selection of a resource may vary among individuals in the population as a function of availability, referred to as a functional response in resource selection (Mysterud & Ims 1998). Second, the assumption that resources which are selected or avoided actually influence survival or reproduction is usually not tested precluding a mechanistic understanding of the presumed link between resources and fitness (Garshelis 2000; McLoughlin *et al.* 2005). Recent studies have convincingly shown functional responses in resource selection (e.g., Hebblewhite & Merrill 2008; Godvik *et al.* 2009) and the link between resource selection and fitness or demography (McLoughlin *et al.* 2005, 2007; Dussault *et al.* 2012). However, I am unaware of studies combining these concepts to demonstrate that a functional response in resource selection directly influences fitness in a free-ranging population.

Although poorly understood, individual variation in both resource utilization and response to risk within populations can impact how individuals respond to environmental change and strongly influence fitness (Wilson *et al.* 1998; Sih *et al.* 2004). An important phenomenon underlying many functional responses in resource selection is that individuals must make trade-offs between fitness benefits provided by a given resource (e.g., access to food), and fitness consequences (e.g., increased mortality risk; Testa 2004; Nielsen *et al.* 2005; Creel & Christianson 2008). Thus, roads are a resource that can elicit functional responses because they potentially offer a variety of negative and positive fitness consequences for wildlife, and often vary in density across landscapes (Beyer *et al.* 2013). In areas of high road density where natural habitat is severely fragmented, animals with large space requirements may be unable to avoid using areas close to roads and/or may be reliant on resources associated with roads given the scarcity of undisturbed habitat (Mech 1995; Vila *et al.* 1995; Hebblewhite & Merrill 2008). Therefore, for individuals in areas of high road density trade-offs between positive and negative qualities of roads will be critical, whereas they may be relatively unimportant for animals in the same population at lower road densities. In such populations, functional responses to roads should be adaptive and failure to adopt behavioral strategies to mitigate mortality risk while exploiting beneficial qualities of roads may negatively influence fitness (Hebblewhite & Merrill 2008).

Wolves and coyotes are excellent study species for investigating functional responses to human disturbance for several reasons. Both species are persecuted in many areas meaning that direct interactions with humans represent significant mortality risk (Musiani & Paquet 2004). However, wolves and coyotes can also exploit human presence in a variety of ways such as utilizing human food source sources, and by using roads as travel routes or to facilitate increased predation rates (James & Stuart-Smith *et al.* 2000; Fedriani *et al.* 2001; Whittington *et al.* 2005). Wolves inhabiting areas subject to harvest, and other forms of human disturbance, often modify their behavior around roads to minimize dangerous encounters with humans (Ciucci *et al.* 2003; Theuerkauf *et al.* 2003; Whittington *et al.* 2005; Hebblewhite & Merrill 2008). One such behavioral modification by wolves is to use roads more at night than during daytime (Vila *et al.* 1992; Theurkauf *et al.* 2003; Hebblewhite & Merrill 2008). Hebblewhite & Merrill (2008) demonstrated that wolves in Alberta exhibited a functional response by avoiding areas close to humans during the day in areas of high human activity, whereas resource selection was independent of proximity to humans in remote areas. The underlying implication of this and other studies involving functional responses to human disturbance is that the observed behavioral modification represents adaptive behavior and influences individual fitness (Hebblewhite & Merrill 2008; Beyer *et al.* 2013). Quantifying effects of human disturbance on survival and reproduction of wildlife is difficult and time consuming such that detecting functional responses is often used as a proxy to infer fitness consequences (Beyer *et al.* 2013). However, empirical studies linking these behavioral trade-offs explicitly to survival and reproduction are needed confirm the mechanistic link between fitness and functional responses in resource selection.

The hybrid zone between eastern wolves, gray wolves, and coyotes in and adjacent to Algonquin Provincial Park (APP) is an effective study area for investigating functional responses to roads and linking behavioral modifications to human disturbance with mortality risk. Increasing secondary road density within home ranges of wolves, coyotes, and hybrids significantly increased mortality risk of resident canids in this hybrid zone (Chapter 3). Secondary road densities are variable across the hybrid zone such that some canids encounter roads frequently, whereas others live in more remote areas comprising mostly natural habitat (Chapter 4; Appendix D). Furthermore, high mortality of wolves dispersing from the protected area of APP into adjacent non-protected areas, and the greater susceptibility of eastern wolves to harvest mortality in areas outside the park, may suggest that some individuals in the population exhibit maladaptive behavior around roads and human mortality risk (Chapter 3).

I used GPS telemetry data collected during 2004-2011 to develop models of seasonal resource selection for canids separately within and adjacent to APP to investigate functional responses to human disturbance and examine the link between use of roads and mortality risk. Specifically, for areas outside of APP I hypothesized that: 1) canids would modify their behavior to minimize encounters with humans by selecting roads more at night than during the day; 2) this change in the use of roads between day and night would vary as a function of availability, consistent with a functional response; and 3) the functional response of canids to roads would be weaker for animals that died than those that survived. In contrast to unprotected areas, I hypothesized that canids in APP would not show a pronounced functional response due to lower road density and the absence of harvest mortality. I focused my investigation of functional responses on secondary roads which significantly influence mortality risk in the study area (Chapter 3). My results advance understanding of the behavioral mechanisms by which wolves and coyotes respond to spatial and temporal heterogeneity in mortality risk in human dominated landscapes and provide insight into fitness implications of functional responses in resource selection. This study will also improve basic understanding of wolf and coyote habitat relationships by quantifying seasonal and day-night selection of habitat and landscape features by canids in the central Ontario hybrid zone.

METHODS

Telemetry Data

I deployed GPS collars on wolves, coyotes, and hybrids during 2004-2010 (see General Methods). I modeled resource selection using 86, 918 locations obtained from 56 resident GPS-collared study animals (*n* = 36 outside of APP, *n* = 20 inside of APP) from 47 packs (*n* = 33 outside APP, *n* = 14 inside APP) across the study area (Table 5-1). Collars were programmed with variable fix schedules both within and across collars (see General Methods). Thus, I subsampled data when necessary such that data used in seasonal RSF models were collected at regular intervals (1-6 hours) within annual datasets for each individual. Inclusion of random effects terms for individuals (see details below) accounted for unbalanced and irregular fix intervals between different study animals. Only animals for which I obtained > 1 month of continuous telemetry data within a given season were included in the analyses. I estimated 95% fixed kernel seasonal home ranges with the plug-in estimator to determine bandwidth (Sheather & Jones 1991) for all study animals during winter (1 Dec – 31 Mar) and summer (1 Apr – 30 Nov). The winter season corresponded (approximately) to the period of continuous ice on lakes (allowing for wolf and coyote travel) and continuous snow cover throughout the study area. Fix success of GPS collars deployed on canids across the study was 89%. A concurrent study in APP tested GPS collar accuracy and fix success of stationary GPS collars within the same vegetative cover types used for my analyses, using the same make, and the same or similar models, that I used (Maxie 2009). The mean location error for all 2 and 3 dimensional fixes was 12.5 m and there was no evidence of habitat-induced GPS bias (Maxie 2009). Given these results, I assumed that my results and inferences were not strongly affected by location error or habitat-induced GPS bias.

Resource Use and Availability

GPS telemetry locations represented locations used by study animals and I systematically sampled 30 m pixels (i.e. 30 x 30m) throughout each seasonal home range (resulting in 37 evenly distributed pixels/ km^2) to estimate availability of resources for each animal (Benson 2013). I created separate distance rasters with primary, secondary, and tertiary roads layers using the ‘Euclidean Distance’ tool in the Spatial Analyst toolbox in ArcGis 10 which calculated the distance from every pixel (30m resolution) to the closest road of each type. I then intersected all used and available locations with the distance rasters using the ‘isectpolyrst’ function in Geospatial Modelling Environment to derive distance values for all locations. I also calculated distances of used and available locations to 6 broad habitat classes (hardwood forest, conifer forest, mixed forest, wetlands, open water, dry open-canopy habitats of rock or grass; Table 5.1). These distances were calculated using a GIS layer I developed with raw Ontario Forest Resource Inventory (FRI; OMNR 2008) data (updated to 2008) which I converted into habitat classes using Ontario’s Landscape Tool 3.0 (Elkie *et al.* 2009). I followed the recommendations of Maxie *et al.* (2010) for combining forested habitat classes to improve classification success based on their field validation analyses. I used continuous, distance-based variables for the habitat classes for several reasons. First, distance-based variables (as opposed to categorical, classification-based variables) allow for assessing selection and/or avoidance of habitat

Table 5.1. Resource variables included in resource selection function models for wolves, coyotes, and hybrids in and outside of Algonquin Provincial Park, 2004-2011. Units shown in parentheses.

Resource variable	Type	Description (units)
Slope	Continuous	Inclination of terrain from horizontal (degrees)
Elevation	Continuous	Vertical distance above sea level (m)
Hardwood forest†	Continuous	Maple, birch, poplar, beech, oak (distance)
Conifer forest †	Continuous	Pine, spruce, fir, cedar (distance)
Mixed forest †	Continuous	Maple, poplar, birch, ash, cedar, fir, pine (distance)
Wetlands †	Continuous	Brush and alder, treed and open muskeg (distance)
Rock/Grass	Continuous	Open rocky areas and grass meadows (distance)
Primary Roads	Continuous	Freeways, highways, expressways (distance)
Secondary Roads	Continuous	Local, connector, arterial (distance)
Tertiary Roads	Continuous	Dirt roads and trails (distance)

†Detailed descriptions of these habitat classes including stand types and species composition provided in Maxie *et al.* (2010)

classes which may contain important resources but within which the animals are rarely located (e.g., open water; Conner & Plowman 2001; Conner *et al.* 2003). Second, distance-based evaluation of habitat-use is robust to telemetry error (Conner & Plowman 2001; Conner *et al.* 2003) and likely other sources of error common in resource selection studies (e.g., some forms of GIS error). Third, continuous habitat class variables eliminated the need to subjectively assign a habitat class as the reference group in my logistic regression RSF models from which inferences on these habitat classes would be based. I excluded areas on the landscape identified as hemlock forests, buffered roads, and developed agriculture in FRI data due to unacceptable misclassification rate (i.e. 50% for hemlock forests; Maxie *et al.* 2010) or because of redundancy and correlation with the road variables included in the models (buffered roads and developed agriculture).

Topographical features such as elevation and slope can strongly influence canid resource selection patterns (Whittington *et al.* 2005; Hebblewhite & Merrill 2008). Accordingly, I estimated slope and elevation from digital elevation models (DEM) in ArcGis 10. Slope was estimated as inclination of the terrain from horizontal in degrees (°) and elevation was estimated as vertical distance (m) above sea level. DEM data were estimated at 10 m (93%) or 20 m (7%) resolution in different portions of the study area, but I averaged all data across 30 m used and available pixels for my analyses. I screened all continuous variables to assess correlation between individual predictor variables which was relatively low (r <51%). Prior to modeling, I rescaled values for all continuous variables by subtracting their mean and dividing by 2 standard deviations which allowed them to be directly interpretable along with unscaled binary predictor variables (Gelman 2008).

Seasonal and Daily Variation in Resource Selection

Canid resource selection may vary between seasons and from day to night (Theuerkaupf *et al.* 2003; Hebblewhite & Merrill 2008). Thus, I modeled 1) summer and winter and 2) day and night resource selection separately to account for and investigate seasonal and diurnal variability in resource selection. I classified all telemetry locations as either day (0800-1959) or night (2000-0759). I used the same day and night classifications during winter and summer as my intention was to distinguish between periods of higher and lower human activity rather than periods of daylight and darkness. Cutoffs for day and night periods were based on my experiences with encountering humans on roads throughout the study area. The probability of encountering humans in the study area was lower on all road types at night than during day, but varied across road types with the probability being highest on primary roads, intermediate on secondary roads, and lowest on tertiary roads (J. Benson, personal observation).

Modeling Framework

I developed models of seasonal resource selection for wolves, coyotes, and hybrids with Bayesian generalized linear mixed models (GLMMs). I investigated resource selection at Johnson's (1980) 3rd order of selection by comparing locations used by radio-collared animals to those available to them within their seasonal home ranges (design 3; Manly *et al.* 2002). I did not investigate resource selection at Johnson's (1980) 2nd order of selection as this would have been redundant to the landscape-genetic analysis in Chapter 2, at least with respect to selection of roads. I included random terms for individual (coded by ID#) and pack in each model. Including random effects for individuals accounted for the unbalanced telemetry sample sizes among animals and potential lack of independence between sequential telemetry locations due to spatial autocorrelation (Gillies *et al.* 2006). The random term for pack accounted for the social nature of wolves and coyotes in and around APP and the potential lack of independence in resource use within packs (Hebblewhite & Merrill 2008). Individuals were nested within pack. However, in 2 cases an individual included in one pack dispersed and joined another pack in a new home range. To avoid the added statistical complexity of a partially crossed design, I changed the IDs of these 2 individuals upon dispersal such that their data were modeled as if they were new individuals once they established the new home ranges. I assume this modification influenced my results very little given that it involved only 2 individuals and seemed superior to excluding these data or the uncertainty of a partially crossed GLMM. Although potentially of interest, I did not make inference on the variance component of the random effect of pack because I only obtained GPS data from a single individual in the majority of packs. The random effect of pack was simply included to avoid biasing model coefficients due to potential correlation between pack members.

Resource Selection Models

I developed multiple Bayesian RSF models separating data by: study unit (APP and outside APP), season (summer and winter), and time of day (night and day). Thus, a separate model was run for night and day within each season for both study units resulting in 8 models total. The global model for each model set included main effects for all fixed predictor variables (Table 5.1) and the resulting coefficients for fixed effects represented the population-level (marginal) response to each resource. Next, I developed a second set of 8 models that were identical except that instead of including secondary roads as a fixed effect, I allowed it to interact with the random term for individual. Thus, in these models a different intercept and slope, modeling relative use of secondary roads, was fit for each individual. This allowed me to derive individual-level (conditional) coefficients indicating the relative use of secondary roads for each canid. These models were the Bayesian equivalent of the maximum likelihood GLMMs used by Gillies *et al.* (2006) and Hebblewhite & Merrill (2008) to investigate functional responses in resource selection in which they included both a random intercept and random coefficient to model individual or group-level responses to a specific resource variable. I based inference on individual-level coefficients, rather than pack-levels coefficients, because I was interested in linking individual selection/avoidance behavior with mortality.

I selected between models of varying complexity using deviance information criteria (DIC) which is a Bayesian analogue to AIC (Spiegelhalter 2002). DIC estimates an automatic penalty for model complexity and provides a relative measure of model fit with lower DIC scores indicating reduced deviance and increased information gained from model parameters (Spielgelhater *et al.* 2002; Bolker *et al.* 2009). Hierarchical Bayesian models are adept at handling large number of predictor variables and over-fitting models is less of a concern than with likelihood methods (Gelman *et al.* 2004). However, I only included variables in the models that I believed could plausibly influence the behavior of wolves and coyotes (Table 5.1). In total I compared 4 possible models for each study unit-seasonal-day/night model sets and based inferences on top models with the best fit as indicated by lower DIC scores (Table 5.2). Additionally, I compared the top model with and without different random slopes for secondary roads

Table 5.2. Model selection results for seasonal, night and day resource selection function models outside APP and within APP in central Ontario, Canada, 2004-2011. Shown are deviance information criteria values (DIC) and difference between DIC of a given model the strongest supported model (ΔDIC) for each model considered.

Outside APP

	Winter				Summer			
	Day		Night		Day		Night	
Model	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC
Random Slope †	84226	0	85361	0	90116	0	89037	0
Habitat & Roads	84952	726	85934	573	90789	673	89566	529
Habitat‡	85074	848	85969	608	91293	1177	89814	777
Roads§	85733	1507	86685	1324	91675	1559	90371	1334
Null Model¶	85904	1678	86707	1346	92154	2038	90560	1523

APP

	Winter				Summer			
	Day		Night		Day		Night	
Model	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC	DIC	ΔDIC
Random Slope†	50668	0	51658	0	38518	0	42354	0
Habitat & Roads	50749	81	51754	96	38623	105	42483	129
Habitat‡	50806	138	51786	128	38644	126	42492	138
Roads§	52837	2169	53922	2264	39669	1151	43591	1237
Null Model¶	52908	2240	53998	2340	39727	1209	43616	1262

†Random slope for use of secondary roads for each individual

‡Habitat variables = slope, elevation, distance to each habitat class (conifer, hardwood, mixed, open water, wetlands, rock/grass)

§ Road variables: distance to primary, secondary, tertiary roads

¶ Intercept only

fitted for each individual to see if model fit was improved by accounting for individual variation in selection of this resource.

RSF Model Specification and Diagnostics

I fitted RSF models using Bayesian GLMM methods implemented in the R package 'MCMCglmm' v. 2.17 (Hadfield 2010) with a binary (0 = available, 1 = used) response variable and logit-link functions. I used priors for fixed effects that were normally distributed and diffuse

with mean = 0 and a large variance (10^8). I fixed the residual variance at 1, as recommended for binary GLMMs (Hadfield 2010). I specified an inverse-Wishart distributed prior for random effects with variance = 1 at the limit and low belief parameter (nu) = 0.002. Using a low value for nu reflected the lack of prior information provided in the models. Thus, these priors were intended to be uninformative or weakly informative, and I confirmed that the posterior distribution was robust to variation in prior specification by manipulating the priors and assessing consistency of parameter estimates (Reid 2012). 'MCMCglmm' estimates the height of the posterior distribution using Markov chain Monte Carlo (MCMC) simulation to draw samples from approximate distributions to provide robust parameter estimates for fixed and random effect terms included in the model from the marginal posterior distribution. I ran models for 200,000 iterations with a burnin period of 30,000 and thinning interval of 200. These specifications ensured that autocorrelation was low (<0.10) between thinned samples and that independent, effective sample sizes of 500-4000 were generated for each fixed and random effect included in the model. I further assessed MCMC chain convergence and healthy chain mixing properties by inspecting MCMC trace and density plots for each parameter (Hadfield 2012). I assessed relative selection or avoidance of resource variables by using 95% highest posterior density (HPD) intervals based on the MCMC simulation to identify fixed-effect beta coefficients that differed significantly from 0 (i.e. when 95% HPD interval did not overlap 0). Although I refer to my results as indicating selection or avoidance of resources for brevity, it should be understood that, due to the used-available designed employed here, my models yield estimates of relative (rather than absolute) probability of use (Lele & Keim 2006).

Modeling the Functional Response

Within each study area and season, I subtracted the conditional coefficient for night from the corresponding coefficient for day to derive an estimate of the difference between the relative use of secondary roads during day and night by each canid. Positive values indicated secondary roads were selected more during the night whereas negative values indicated that secondary roads were selected more during the day. Values close to zero indicated little difference between day and night use of roads. I used generalized additive models (GAMs; Hastie & Tibshirani 1986) to investigate functional responses of canids to human disturbance by modeling differences in use of secondary roads between night and day as a function of their availability. GAMs are flexible non-parametric or semi-parametric regression models adept at modeling non-linear relationships (Wood 2006) which were appropriate because functional responses are generally smooth, non-linear changes in preference as a function of availability (Beyer *et al.* 2010). I ran separate GAMs for summer and winter in both APP and outside APP (4 GAMs total). In each GAM, the difference between night and day coefficients was the response variable and I included a non-parametric (smooth) term for the mean distance to secondary roads in each individual's home range as a predictor variable. In GAMs non-parametric predictor variables are specified in terms of smooth functions, which in this case were thin-plate regression splines (Wood 2006). I used this basic model to investigate potential linear or non-linear relationships between differences in night-day use of secondary roads and the (distance-based) availability of these roads.

For models with data outside APP, I also included a parametric term for genotype to investigate whether differences in night-day use of secondary roads varied among *Canis* types. Levels of this categorical variable included coyote, eastern wolf, eastern wolf × gray wolf hybrid, eastern wolf × coyote hybrid, or gray wolf × coyote hybrid. I set coyote as the reference group to test the hypotheses that day-night differences in use of secondary roads differed between coyotes and any of the other *Canis* types outside of APP. I acknowledge that this test was at least partially confounded by: 1) small samples within genotype classes, and 2) the positive relationship between coyote ancestry and secondary road density within home ranges (Chapter 2). Nonetheless, I wanted to determine whether there were strong differences in night-day modification between genotype classes within the dataset that might provide an alternative explanation to a functional response. I did not consider genotype in the GAMs for APP due to insufficient diversity in ancestry as most animals in APP were eastern wolves (81% winter, 79% summer). To test the hypothesis that functional responses to secondary roads influenced mortality risk, I also created a fate variable by separating individuals into 2 categories based on whether they were survived or died during the study. As a parametric main effect, the fate variable allowed me to test whether the difference in day and night use of secondary roads varied between animals that lived and died. I also considered an interaction between the smooth term for availability of secondary roads and fate to see if animals that lived or died exhibited different relationships between night-day road selection and availability. I only considered the fate variable in the winter GAM outside APP because: 1) most (82%) of the mortality occurred during winter, and 2) mortality was low in APP (Chapter 3). I made 2 adjustments to the dataset to avoid misclassifying the fate of individuals. First, one animal was alive when it's GPS collared failed but was found dead on the side of a secondary road 9 months later. I coded this animal as "dead" even though the mortality occurred beyond the monitoring period. In a formal survival analysis (e.g., Chapter 3), this individual would be censored upon radio failure. However, given that I was specifically interested in detecting mortality risk associated with secondary roads, classifying an animal that died on a secondary road as having survived would clearly have led to misleading results. Second, I excluded data from one individual because its movement behavior was irregular and its fate was uncertain. This individual was the breeding female for a pack of 5 coyotes that used the smallest (8 km^2) home range I documented, which was centered on a municipal landfill. In January-February 2011, 3 animals from the pack were killed by trappers and during the same period I lost telemetry contact with the remaining 2 animals (including the GPS collared female). Given the circumstances I was unsure whether these 2 animals were also killed, dispersed, or their transmitters failed. As her behavior around roads was atypical, presumably due to the presence of the landfill, I excluded her to avoid influencing the results with an animal whose fate was uncertain. Most of the coyotes included in this analysis survived (i.e. 9 of 11), whereas for other *Canis* types the fate variable was more balanced (i.e. 10 died, 9 survived). Thus, I also excluded coyotes and ran a separate GAM with this reduced dataset which included only animals with some level of wolf ancestry (eastern wolves, eastern wolf × gray wolf hybrids, eastern wolf × coyote hybrids, and gray wolf × coyote hybrids). I conducted this analysis to confirm that general relationships between mortality and use of secondary roads by canids could be distinguished from genotype-specific mortality and behavior patterns which may have been confused due to high survival of coyotes.

For the 4 main GAM model sets (Non-APP winter and summer, APP winter and summer), I first ran the basic model with only availability as a predictor differences between night and day road use. Then for winter and summer analyses outside APP, I included the genotype variable and assessed whether model fit improved relative to the simple model. Next, for the winter analysis outside APP, I included the fate variable both as a main effect and in the availability × fate interaction. If the interaction was significant, I fit separate smooth terms for availability for animals that lived and died. I assessed model fit and conducted model selection as described in detail for the GAMMs in Chapter 2 (see Methods, *Landscape Analysis*) except that I used Generalized Cross Validation (GCV) scores instead of AIC values following Wood & Augustin (2002) and Parra *et al.* (2011). For parametric predictor variables, I present model coefficients (β), standard errors, and *P*-values. For smooth predictor variables, I present estimated degrees of freedom (edf, a measure of non-linearity in the relationship with the response variable with 1 being no significant departure from linearity; Wood 2006), *F* statistics, and (approximate) *P*-values. The GCV score is shown for each model whereas the % deviance is provided only for the top models model sets with significant predictor variables. All GAMs were run in R with package 'mgcv' version 1.7-19.

RESULTS

Resource Selection

There was no model uncertainty for RSF models as DIC indicated that the full models were the most strongly supported models, whereas the null models were the least supported in each instance (Table 5.2). Outside of APP, the full models differed from the 2nd most supported model by ΔDIC > 600 and from the null models by ΔDIC > 2000 (Table 5.2). Inside of APP, the full models differed by ΔDIC > 10 from the next most supported models and from the null models by > 1500 ΔDIC (Table 5.2).

Population-level Resource Selection

Outside APP, primary, secondary, and tertiary roads were avoided in both seasons and during night and day, with the exception of secondary roads at night during winter which were not selected or avoided (Table 5.3). Use of primary and tertiary roads was consistent between day and night, whereas the selection coefficients for secondary roads differed substantially between day and night indicating that canids used areas closer to secondary roads more at night than during the day (Table 5.3). In APP, primary roads were selected whereas tertiary roads were avoided in both seasons and during both night and day (Table 5.4). Secondary roads were avoided during winter (both night and day) and used proportionate to availability during summer (both night and day; Table 5.4). Population-level coefficients for all resource variables are shown in Tables 5.3 and 5.4.

Table 5.3. Summary of mixed-effect Bayesian logistic regression models for resource selection of wolves, coyotes, and hybrids separated by season and by night and day adjacent to Algonquin Park, Ontario, 2004-2011. Shown are β coefficients with lower and upper 95% HPD intervals. Significant effects shown in bold.

	Winter-Day			Winter-Night			Summer-Day			Summer-Night		
Variable†	β	Lower	Upper	β	Lower	Upper	β	Lower	Upper	β	Lower	Upper
Intercept	**-2.57**	-3.11	-2.02	**-2.55**	-3.09	-2.03	**-2.51**	-2.90	-2.10	**-2.50**	-2.91	-2.11
Water	-0.04	-0.08	0.00	**-0.24**	-0.28	-0.19	**-0.08**	-0.12	-0.04	**-0.19**	-0.23	-0.15
Conifer	**-0.06**	-0.10	-0.01	0.01	-0.03	0.05	**-0.05**	-0.10	0.00	**-0.06**	-0.10	-0.01
Wetlands	**-0.62**	-0.67	-0.57	**-0.33**	-0.37	-0.28	**-0.42**	-0.47	-0.38	**-0.30**	-0.35	-0.26
Rock/Grass	-0.01	-0.06	0.03	**-0.20**	-0.25	-0.16	**-0.13**	-0.18	-0.09	**-0.27**	-0.32	-0.23
Hardwood	**-0.08**	-0.15	-0.01	-0.03	-0.11	0.05	**-0.29**	-0.37	-0.21	**-0.25**	-0.34	-0.18
Mixed	-0.01	-0.06	0.04	**-0.08**	-0.12	-0.03	-0.01	-0.05	0.04	**0.06**	0.01	0.11
Slope	**0.17**	0.13	0.21	**0.18**	0.14	0.22	**-0.28**	-0.32	-0.24	**-0.16**	-0.20	-0.12
Elevation	-0.58	-0.68	-0.47	**-0.86**	-0.97	-0.75	**-0.87**	-0.98	-0.74	**-1.00**	-1.13	-0.86
1° Roads	**0.20**	0.10	0.28	**0.23**	0.14	0.32	**0.27**	0.19	0.35	**0.34**	0.26	0.42
2° Roads	**0.30**	0.24	0.37	-0.04	-0.11	0.02	**0.52**	0.46	0.58	**0.21**	0.15	0.28
3° Roads	**0.13**	0.08	0.18	**0.12**	0.07	0.16	**0.37**	0.32	0.42	**0.32**	0.27	0.37

† See Table 1 for details of each variable

Table 5.4. Summary of mixed-effect Bayesian logistic regression models for resource selection of wolves, coyotes, and hybrids separated by season and by night and day in Algonquin Park, Ontario, 2004-2011. Shown are β coefficients with lower and upper 95% HPD intervals. Significant effects shown in bold.

	Winter-Day			Winter-Night			Summer-Day			Summer-Night		
Variable†	β	Lower	Upper	β	Lower	Upper	β	Lower	Upper	β	Lower	Upper
Intercept	**-4.08**	-4.79	-3.47	**-4.03**	-4.60	-3.39	**-2.92**	-4.63	-1.22	**-4.05**	-4.37	-3.73
Water	**-0.84**	-0.91	-0.76	**-0.95**	-1.02	-0.88	**-0.20**	-0.27	-0.13	**-0.22**	-0.28	-0.14
Conifer	-0.04	-0.10	0.01	-0.02	-0.08	0.04	**-0.11**	-0.18	-0.04	**-0.12**	-0.19	-0.05
Wetlands	**-0.87**	-0.93	-0.80	**-0.75**	-0.81	-0.69	**-1.05**	-1.15	-0.95	**-1.01**	-1.09	-0.93
Rock/Grass	**0.15**	0.07	0.23	**0.17**	0.09	0.24	0.06	-0.03	0.15	**0.09**	0.01	0.17
Hardwood	**-0.37**	-0.45	-0.29	**-0.31**	-0.38	-0.23	**-0.12**	-0.19	-0.05	**-0.09**	-0.16	-0.02
Mixed	-0.05	-0.10	0.01	**-0.12**	-0.18	-0.07	0.05	-0.02	0.11	**0.09**	0.03	0.17
Slope	-0.04	-0.09	0.01	0.01	-0.04	0.07	**-0.31**	-0.37	-0.24	**-0.27**	-0.34	-0.21
Elevation	**-0.84**	-0.91	-0.76	**-0.87**	-0.94	-0.79	**-0.45**	-0.54	-0.36	**-0.45**	-0.54	-0.36
1° Roads	**-0.15**	-0.27	-0.02	**-0.25**	-0.37	-0.12	**-0.51**	-0.69	-0.31	**-0.32**	-0.47	-0.15
2° Roads	**0.11**	0.03	0.19	**0.09**	0.02	0.17	-0.06	-0.15	0.05	-0.08	-0.16	0.01
3° Roads	**0.24**	0.19	0.30	**0.17**	0.11	0.23	**0.15**	0.08	0.23	0.04	-0.03	0.11

† See Table 1 for details of each variable

Functional Responses

Outside APP, availability of secondary roads was a significant, non-linear predictor of differences in use of secondary roads between night and day during both winter (edf = 1.9, F = 5.1, P = 0.010, GCV = 0.92, n = 31) and summer (edf = 2.0, F = 4.0, P = 0.022, GCV = 0.39, % Deviance = 26.9, n = 35; Figure 5.1). Within APP, availability of secondary roads was a weak, non-significant predictor of differences in use of secondary roads between night and day within APP during winter (edf 1.6, F = 2.2, P = 0.148, n = 16) and summer (edf = 1, F = 3.0, P = 0.101, n = 19). Outside APP, inclusion of genotype class did not improve model fit during winter (GCV = 1.11) or summer (GCV = 0.42). However, for winter outside APP, inclusion of the availability × fate interaction improved model fit (GCV = 0.88, % Deviance = 53.4) and the difference in use of secondary roads between night and day was marginally greater for animals that lived compared to those that died (β = 0.59, SE = 0.33, P = 0.091). Night-day differences in secondary road use varied significantly as a non-linear function of availability for animals that survived (edf = 3.5, F = 4.8, P = 0.005, n = 18; Figure 5.2a), whereas the relationship was not significant for animals that died (edf = 1.2, F = 2.0, P = 0.163, n = 12; Figure 5.2b). Finally, I removed all coyotes from the analysis to confirm that the difference between night-day use of roads and availability was not driven by either: 1) the smaller sample of animals that died in the overall dataset or 2) different behavior by coyotes, most of which survived (9 or 11). Even without coyotes, the model explained 50.5% of deviance and the relationship between differences in road use between night and day varied significantly as a non-linear function of availability for animals that lived (edf =

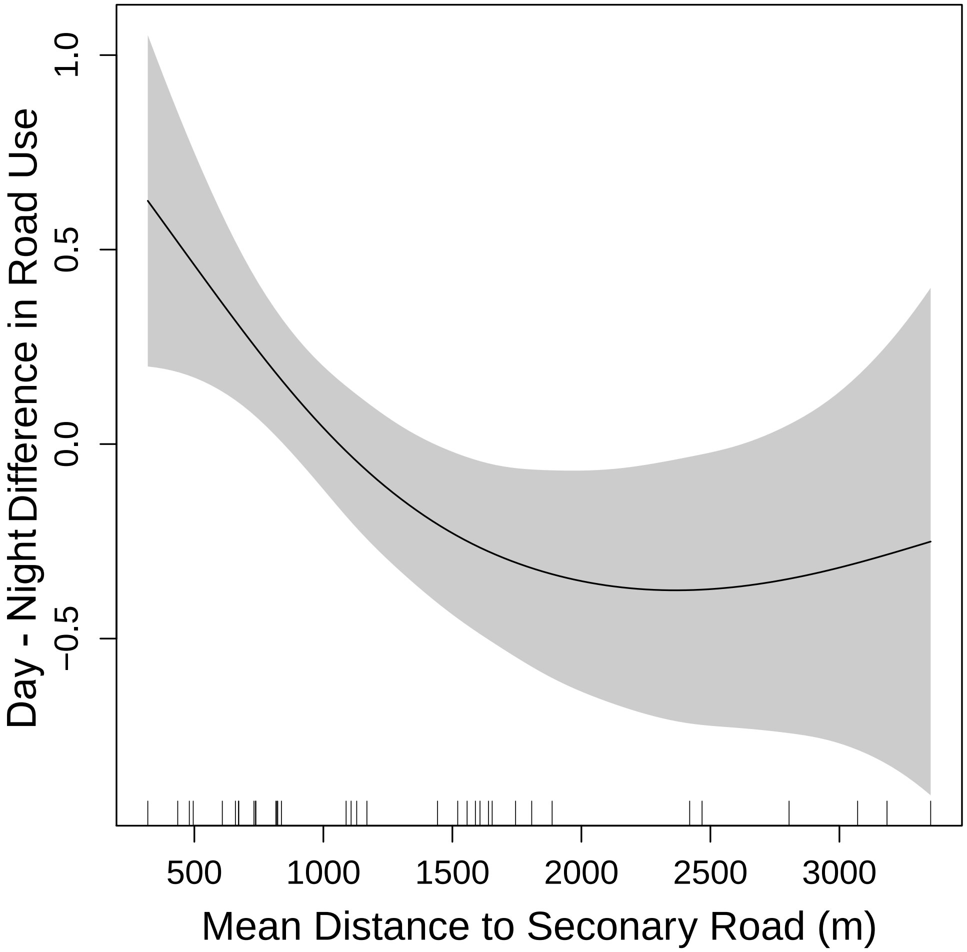

Figure 5.1. Differences between night and day individual resource selection function coefficients for secondary roads modeled as a smooth function of availability of secondary roads (mean distance to secondary roads within home ranges) for canids outside Algonquin Provincial Park (APP) in summer, 2004-2011. Y axis is centered on 0, positive values indicate greater selection during day, whereas negative values indicate smaller differences or greater selection at night. Shaded area is 95% confidence interval around predicted trend and vertical bars on x-axis indicate sample locations.

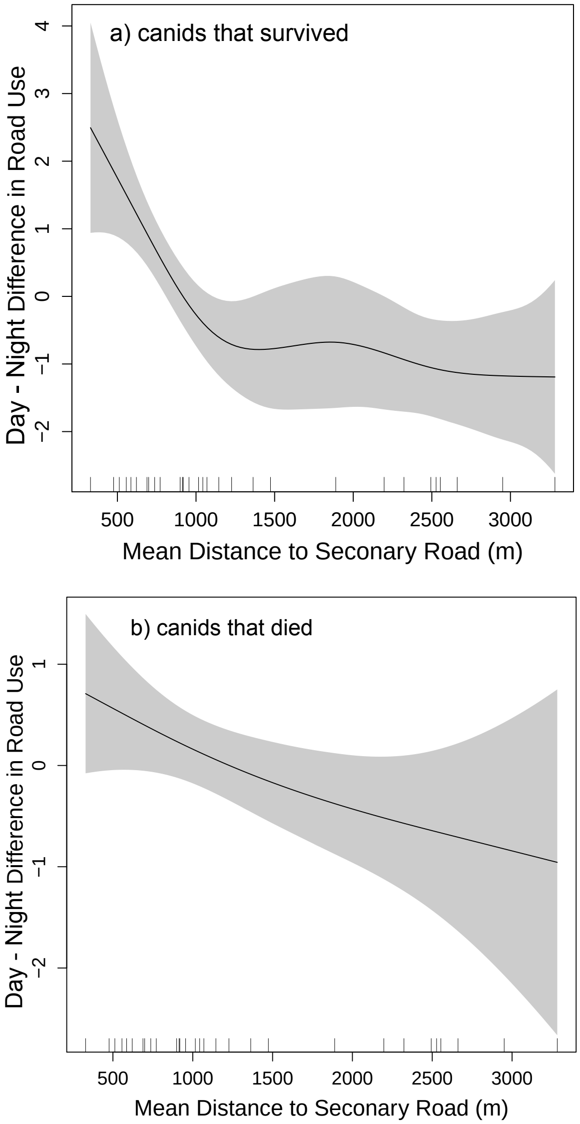

Figure 5.2a-b. Differences between night and day individual resource selection function coefficients for secondary roads modeled as a smooth function of availability of secondary roads (mean distance to secondary roads within home ranges) for canids that survived (a) or died (b) outside Algonquin Provincial Park (APP) in winter, 2004-2011. Y axis is centered on 0, positive values indicate greater selection during day, whereas negative values indicate smaller differences or greater selection at night. Shaded area is 95% confidence interval around predicted trend and vertical bars on x-axis indicate sample locations.

1.9, F = 4.1, P = 0.034, n = 9; Figure 5.3a), but not those that died (edf = 1.1, F = 2.3, P = 0.144, n = 10; Figure 5.3b).

DISCUSSION

Canids outside of APP exhibited a significant, non-linear response to human disturbance by selecting secondary roads more strongly at night than during the day in areas of higher road density. Conversely, canids at lower road densities showed a much weaker response to roads, generally using them in proportion to availability and not modifying their use between night and day. Functional responses are usually described as a non-linear change in preference as a function of availability (Mysterud & Ims 1998; Beyer et al 2010). The functional response I quantified deviates from the strict definition only in that it was the difference in preference between day and night that changed as a non-linear function of availability, rather than preference *per se*. Hebblewhite & Merrill (2008) quantified a functional response in selection for human activity by wolves that changed in relation availability and between night and day. My results are similar except that I explicitly modeled this difference in selection between night and day and showed that it varied in relation to availability.

Adaptive individual variation in resource utilization and response to risk within single populations can strongly influence survival and reproduction (Wilson *et al.* 1998). Hebblewhite & Merrill (2008) suggested that the functional response by wolves to human activity in Alberta was adaptive, but noted that they did not have enough mortality data to link it directly to survival. Thus, I build on their results by showing that a similar functional response exhibited outside APP influenced survival with canids that survived showing a stronger behavioral modification in their use of secondary roads as a function

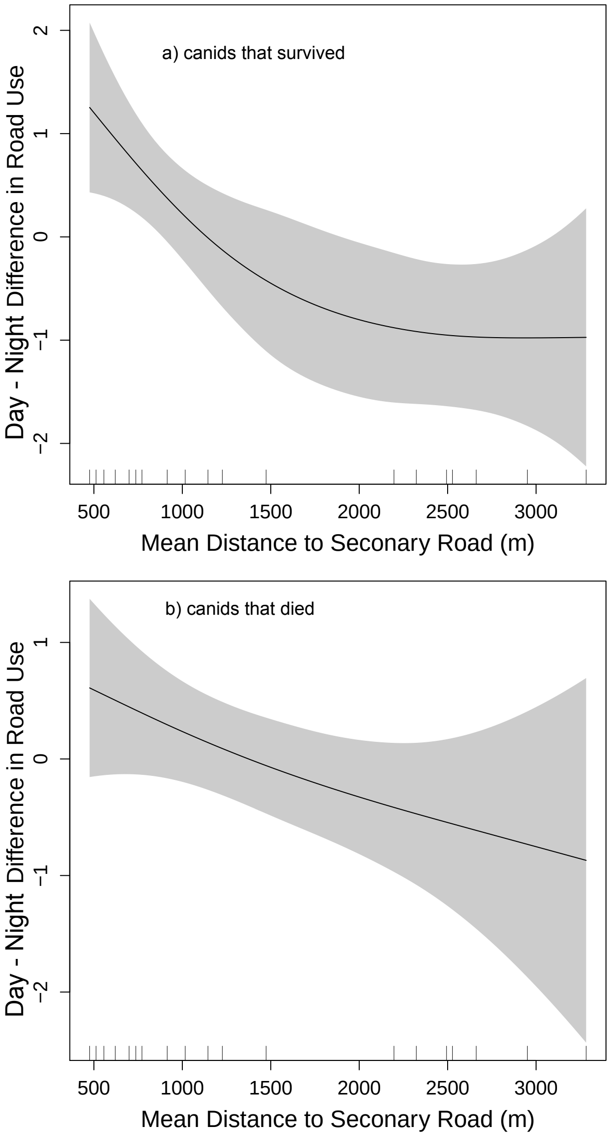

Figure 5.3a-b. Differences between night and day individual resource selection function coefficients for secondary roads modeled as a smooth function of availability of secondary roads (mean distance to secondary roads within home ranges) for canids (with coyotes excluded) that survived (a) or died (b) outside Algonquin Provincial Park (APP) in winter, 2004-2011. Y axis is centered on 0, positive values indicate greater selection during day, whereas negative values indicate smaller differences. Shaded area is 95% confidence interval; vertical bars on x-axis indicate sample locations.

of availability than canids that died. This finding has potentially important practical and evolutionary implications by suggesting that some individuals in the hybrid zone exhibit maladaptive behavior in response to human disturbance that leads to higher mortality. In addition to showing a mechanism by which the functional response may have evolved, individual differences in behavioral responses to human disturbance offer at least a partial explanation for the observed variation in survival outside APP, especially at higher road densities (Chapter 3). Mortality risk associated with roads manifested in at least 2 mortality agents outside APP: harvest and collisions with vehicles (Chapter 3). Indeed, of the mortality events comprising the fate variable used in my models, 80% (for which cause of death was known) were due to harvest (n = 7) or vehicle collisions (n = 1). Human causes were also strongly suspected for 2 additional mortalities (for which cause of death was not definitively known) as they were found dead on or in close proximity to secondary roads (<250m), respectively. Thus, my results suggest that the failure by some animals to modify their use of roads between night and day resulted in greater risk of human caused mortality.

Given the increased mortality risk for wolves and coyotes associated with roads (Chapter 3) it may be assumed that avoiding roads and humans altogether would be the most adaptive behavioral response. However, as with similar trade-offs demonstrated for herbivores between predation risk and foraging efficiency (e.g., Lima & Dill 1990; Cowlishaw *et al.* 2004), it should be recognized that mortality risk is only one component of the trade-off associated with selection of roads by wolves and coyotes. In the predation literature, direct effects of predation mortality are distinguished from risk effects associated with predation avoidance behavior, which manifest in reduced survival, growth or reproduction (reviewed by Creel & Christianson 2008). The influence of risk effects on population dynamics of prey can equal or exceed those of direct mortality (Schmitz *et al.* 1997; Nelson *et al.* 2004; Preisser *et al.* 2005; Pangle *et al.* 2007). Thus, indirect fitness costs associated with avoidance of roads should not be discounted. In human altered landscapes, natural prey base and habitat may be reduced or altered such that avoiding humans and roads completely may have unacceptably negative fitness consequences, even if doing so would reduce risk of human-caused mortality. Wolves and coyotes benefit from roads through access to human food sources (Fedriani *et al.* 2001; Fritts *et al.* 2003), greater traveling efficiency (Whittington *et al.* 2005) and perhaps increased predation rates (James & Stuart-Smith 2000). Outside of APP, I documented canids feeding on garbage at landfills, carcass piles from hunters, and highly concentrated deer that were supplementally fed in close proximity to human residences (J. Benson & B. Patterson, unpublished data). Thus, although some animals appear to have been more likely to die because they failed to mitigate mortality risk, others may also have compromised reproduction or increased risk of natural mortality by failing to exploit benefits of roads via selection at night. Only 2 of the mortalities used in the analysis were attributed to natural causes (mange and territorial aggression). Although these deaths may have had nothing to do with avoidance of secondary roads, it cannot be ruled out that failure to adopt an adaptive behavioral strategy with respect to human presence in fragmented landscapes could reduce fitness in ways beyond human-caused mortality. Although challenging, quantifying risk effects on survival and reproduction of canids in fragmented landscapes where adaptive behavioral trade-offs may be required to maximize fitness would yield a more comprehensive understanding of the cost-benefits of roads for wolves and coyotes.

Canids in APP did not select roads differently between night and day and did not exhibit a significant functional response. This may be because secondary road density was much lower in APP (Appendix D) and/or because the roads in APP did not represent substantial mortality risk. Interestingly, canids in APP in both winter and summer exhibited a similar negative (but non-significant) relationship between the change in night and day selection of secondary roads and availability. This was likely driven by a few individuals in APP with territories on the border of the protected area that were exposed to relatively high road densities and human mortality risk in those areas. The smaller sample sizes in APP also made the results more difficult to intepret. Regardless, it remains unclear whether eastern wolves survived poorly outside APP because they are naïve to harvest risk due to being raised in the protected area (as I hypothesized in Chapter 3) or for other reasons. Genotype class was a poor predictor of differences in road use between night and day outside of APP. However, my ability to investigate genotype-specific use of roads was confounded by: 1) small sample sizes within genotype classes and 2) the positive relationship between coyote ancestry and secondary road density within home ranges of resident canids across the study area (Chapter 2). I only tracked 5 eastern wolves with GPS telemetry outside APP and all but 1 had home ranges with low density (< 0.2 km/km^2) of secondary roads. Exploratory analyses (not shown) of genotype-specific resource selection indicated that, in contrast to other *Canis* types, eastern wolves did not modify their behavior to select roads more strongly at night than during the day. In light of the strong, population-level functional response, it is not clear whether eastern wolves did not modify their use of secondary roads between night and day because they are naïve or whether they were simply responding to low availability. Ironically, poor survival in relation to increased road density is likely the mechanism underlying their negative association with secondary road density, which prevented me from properly testing this hypothesis. The single eastern wolf tracked at relatively high secondary road density (0.5 km/km^2) was one of the few animals that selected roads strongly during the day and was shot 37 days after being radio-collared. As the goals of my study focused primarily on studying resident animals, I generally reserved GPS collars for adult animals believed to be residents, whereas I intentionally deployed VHF collars on younger animals that were more likely to disperse. Several of these VHF-collared animals dispersed from APP and were killed within a year of leaving the protected area (Chapter 3). Future studies should consider targeting yearlings and other animals likely to disperse from APP for GPS collaring which could yield valuable data on the use of roads outside of APP by eastern wolves and other canids raised in the protected area.

Resource Selection Patterns In and Adjacent to APP

Outside of APP canids avoided primary and tertiary roads during night and day and in both seasons. Avoidance of primary roads was likely an adaptive response to minimize vehicular collisions as most mortality from vehicles occurred on primary roads. Canids in APP did not exhibit different selection patterns for secondary roads between night and day, avoiding secondary roads during winter and selecting them in summer, regardless of time of day. Canids selected and avoided primary roads and avoided tertiary roads, respectively, in both seasons and at day and night. I am unsure why wolves within APP avoided tertiary roads during both seasons and secondary roads during winter as they would seem to provide easier travel corridors without risk of harvest mortality. Indeed, I captured wolves primarily along tertiary roads, observed abundant wolf scat and other sign on logging roads and trails, and occasionally saw them traveling on these roads. As most secondary and tertiary roads are not maintained during winter in APP, they may be of little benefit for traveling once snow is continuous as it may actually be deeper on roads relative to canopied, forested areas (Anderson 2012). Exploratory analyses (not shown) did not reveal a functional response to primary or tertiary roads outside APP and results of the basic RSF models indicated consistent use of these roads during night and day (Table 5.3). However, I did not investigate functional responses to primary or tertiary roads, or other resources, for canids in APP which leaves open the possibility that individual variation was obscured by only estimating average use (Hebblewhite & Merrill 2008; Beyer *et al.* 2013).

Outside of APP canids selected for steeper slopes during winter but avoided them in summer. These results may reflect the greater reliance of wolves, coyotes, and hybrids in my study area on ungulate predation during winter (Benson & Patterson, unpublished data) and the potential advantage of steeper slopes for increasing predation on moose and deer. Deeper snow likely accumulates on relatively steep slopes and footing may be more precarious for moose and deer on these slopes (Muntz & Patterson 2004). Although somewhat anecdotal, I observed multiple deer and moose carcasses killed by radio-collared study animals on, or at the bottom of, relatively steep slopes (30-45°). Most studies in mountainous regions where slopes are steeper have found that wolves strongly avoid areas of increased slope during all seasons (Ciucci *et al.* 2003; Whittington *et al.* 2005; Hebblewhite & Merrill 2008). Slopes in my study area are moderate compared to mountainous areas with mean slopes at 30m pixels ranging from 0 to 40° outside of APP. Thus, it appears that in areas of moderate slopes outside APP wolves select steeper slopes in winter, perhaps to increase predation success, whereas in mountainous areas wolves avoid slopes to increase traveling efficiency and because prey are unlikely to use the steepest slopes (Ciucci *et al.* 2003; Whittington et at. 2005). APP contained slightly steeper slopes (range 0 to 50°) than adjacent areas which could explain the proportional use of slopes I documented by park wolves in winter, as APP was intermediate between adjacent areas where slope were selected and mountainous regions where slopes are avoided.

Beavers are an important food resource for canids in central Ontario (Voigt *et al.*, 1976; Forbes & Theberge, 1996). I did not have fine scale data on beaver (*Castor canadensis*) abundance, but the response of wolves and coyotes to elevation and wetlands may reflect the importance this prey item. Beaver aerial surveys were conducted within wolf home ranges during a previous study in APP and preliminary analysis of these data indicated: 1) a strong (non-linear) negative relationship between beaver abundance and elevation and 2) a positive relationship between proportion of wetlands and beaver abundance (K. Loveless *et al.*, unpublished data). Thus, I interpret the strong selection for wetlands and lower elevation to be at least partially due to greater availability of beavers. Furthermore, as studies of beaver in APP indicated that >93% of foraging activity occurred <40m from water (Donkor & Fryxell 1993), the strong selection for areas closer to water in both study units was likely also partly a reflection of the importance of beavers to wolves and coyotes. Given that wolves were unlikely to actually be located in open water during summer, my use of a distance-based predictor variable for water was effective at detecting this selection. Aside from roads and slopes, the only other notable difference between canids in and out of APP was the selection for areas closer to Rock/Grass habitats outside of APP and avoidance of these areas within APP. This habitat class is composed primarily of open, rocky areas and differences in relative availability of these habitat types may explain the discrepancy in use between study units. Whereas this habitat class was very rare in APP (<1%) it was more abundant outside of the park (7%) where it was used by wolves and coyotes for denning and foraging habitat (J. Benson, personal observations). As I collected spatial data on den and kill sites, a future RSF model comparing these specific sites to available locations would be useful to confirm my interpretation of the results from seasonal RSF models with respect to activities influencing fitness of wolves and coyotes.

Wolves and coyotes are usually characterized as habitat generalists and previous studies have found that they select areas on the landscape that will maximize predation success rather than specific vegetation types or landscape features *per se* (Mech 1970; Mladenoff *et al.* 1995; Mech & Boitani 2003). However, I did not include the prey availability data for moose and deer used in previous analyses. These variables provided useful estimates of relative winter availability of prey at larger spatial scales (i.e. landscape level; see Chapter 2 & 3), but are likely much less effective at reflecting spatial variation in moose and deer abundance at finer resolution (i.e. within home ranges). Specifically, GPS data were collected at ≤15m resolution whereas moose were surveyed within 25 km^2 plots. Daily movement of individuals, annual variation in abundance and a dispersion of moose and deer, and the coarse nature of these GIS layers could preclude their use as meaningful predictors of wolf and coyote resource selection within home ranges. A recent study of wolves in northern Ontario using similarly derived Kriged moose density also found that it was not an effective predictor of space use and resource selection within home ranges, despite moose being the main winter prey for wolves in this study (Anderson 2012; Patterson *et al.*, unpublished data). The deer wintering habitat data does not reflect annual spatial variation in deer deer density within yards, inter-annual variation in yard boundaries and other wintering habitat, and also does not capture small areas where deer were supplementally fed, limiting its utility for the 3rd order resource selection analyses conducted here. The moose and deer layers were especially problematic for animals with smaller home ranges as they introduced singularities in the dataset when all used and available locations for a given animal had values for these variables that were identical or nearly identical.

Advantages of Bayesian Framework

Bayesian mixed effects RSF models provided an effective framework to test hypotheses regarding population and individual level variation in canid resource selection. These flexible regression models easily accommodated multiple random effects, unbalanced sample sizes and variable sampling intensity of telemetry datasets. Bayesian mixed effect regression models are naturally suited for understanding hierarchical processes such as resource selection (Wikle 2003; Bakian *et al.* 2012) and yield robust parameter estimates and confidence intervals based on the highest density posterior distribution which alleviate concerns regarding model-based confidence intervals derived from likelihood-based mixed effect models (Bolker *et al.* 2009; Fieberg *et al.* 2010; van Beest *et al.* 2010). However, due to the statistical and computer programming complexity associated with predicting new data with the Bayesian GLMMs I used, I was unable to perform cross-validation or other external model validation procedures. Thus, currently the inferences from my models should be restricted to the areas and animals that I studied. However, model diagnostics indicated sound MCMC mixing properties and low autocorrelation between thinned samples and DIC indicated substantial information gained from the variables included in the top models. As I obtained a relatively large and representative sample of resident canids across the study units, results and inferences from these models should be reliable and valuable for understanding resource selection of canids in central Ontario.

CHAPTER 6. GENERAL CONCLUSION

I have provided a detailed assessment of the spatial genetic, demographic (i.e. mortality), and behavioral structure of the *Canis* hybrid zone in and adjacent to APP. As expected, eastern wolves were the dominant canid within APP but were relatively rare outside of the park (Rutlege *et al.* 2010). However, resident breeding eastern wolves were identified beyond the boundaries of APP and the surrounding harvest ban area where they were associated with areas of higher moose and lower road densities. The hybrid zone outside of APP appears be a mosaic of *Canis* genotypes as higher levels of wolf ancestry were associated with favorable, heterogeneous environmental conditions. On one extreme the relatively high road densities and other forms of human disturbance in WMU49 appear to have facilitated almost complete replacement of wolves with eastern coyotes and hybrids, while in areas like KH and WMU47 the lower road densities and patches of high moose density create suitable habitat conditions for resident eastern wolves to establish residency. However, eastern wolves in these unprotected landscapes survived poorly ($\hat{s}$ = 0.39) and were more susceptible to shooting and trapping mortality than other *Canis* types inhabiting unprotected landscapes. Poor survival and low density of eastern wolves in areas like KH and WMU47, where other *Canis* types are abundant, exacerbates hybridization and limits the potential for the distinct eastern wolf population in APP to expand numerically and geographically.

My analysis of home range overlap revealed the novel social structure of this *Canis* hybrid zone which differs from all previously studied wolf-coyote systems. The relatively subtle differences in body size among the different *Canis* types likely manifest in intense competition for space and food resources, and appear to have led to territoriality between all *Canis* types regardless of genetic ancestry. That individuals of all *Canis* types may represent mating opportunities to one another probably further intensifies territoriality and spatial segregation between packs such that the mosaic pattern of adjacent home ranges is more similar to a homogenous wolf population than traditional wolf-coyote systems. The territoriality between wolves and coyotes may further reduce the probability of eastern wolf population expansion as the saturation of coyotes, gray wolves, and hybrids on the landscape outside of APP, coupled with poor survival of dispersing eastern wolves, likely limits successful establishment of breeding pairs of eastern wolves outside the park. Although the sample size of GPS collared eastern wolves outside of APP was relatively low ($n = 5$) I also identified a behavioral mechanism underlying the poor survival of eastern wolves in relation to secondary roads. Whereas all other *Canis* types modified their behavior by selecting areas farther from secondary roads during day, eastern wolves did not exhibit this behavior and instead selected areas closer to roads during winter when most of the shooting and trapping of canids occurred.

Conservation Implications

Despite the poor survival rates of eastern wolves outside of APP, and the pessimistic prognosis for expansion of the APP population under the current environmental conditions and harvest regulations, I have gained important information that may allow for improved conservation efforts for eastern wolves. The first question that must be addressed is whether conserving eastern wolves is a priority for Canada and Ontario. COSEWIC is currently (2013) reviewing the status of eastern wolves and their status could be elevated to threatened due to their extremely restricted distribution, the low number of individuals identified on the contemporary landscape, and their propensity to hybridize with coyotes and gray wolves outside of APP. If eastern wolves are elevated to a federally threatened species, it would seem prudent to undertake management actions that would facilitate the expansion of the distinct eastern wolf population of APP beyond the boundaries of the current protected area. The population within APP appears to be demographically stable (Patterson & Murray 2008), but sampling efforts have thus far failed to identify other areas inhabited by significant numbers of highly assigned breeding eastern wolves. A fundamental principle of conservation biology is to avoid scenarios where a species is limited to a single, small population (Meffe *et al.* 1997). Furthermore, even large reserves such as APP may be too small to maintain viable populations of large mammals indefinitely if they are isolated (Allendorf 1997; Woodruffe & Ginsberg 1998). Several recommendations that follow logically from my research may increase the probability of eastern wolf population expansion beyond APP.

Three parameters influence population growth: 1) survival, 2) reproduction, and 3) dispersal (i.e. immigration/emigration). As clearly documented in chapter 3, survival of both resident and non-resident eastern wolves is poor outside of APP where they are particularly susceptible to harvest. This poor survival, even accounting for potential spatial and/or temporal fluctuation, is not sufficient to facilitate population growth that would be necessary for population expansion. Fuller *et al.* (2003) estimated that wolf populations should stabilize (with no population growth or decline) with an annual survival rate of 0.66 which is far above the survival rate of 0.39 that I documented. Populations subjected to extreme rates of harvest mortality may be sustained with sufficient immigration from nearby reserves (Lariviere *et al.* 2000), which likely explains the persistence of eastern wolves in patches outside of APP. Given that harvest is by far the leading cause of death for eastern wolves (and indeed all canids) outside of APP, increased harvest protection in some areas outside of the current protected area may be warranted within the framework discussed below. Although harvest protection should be effective at increasing survival, whether this allows for eastern wolf population expansion within the dynamic central Ontario hybrid zone remains an open question. In terms of reproduction, I have documented eastern wolves breeding outside of the protected area, but reproductive barriers to hybridization at low density appear to be minimal. Thus, poor survival, low density, and a propensity for hybridizing with the other *Canis* types suggests successful production of highly assigned eastern wolves outside of APP will be rare. Dispersal from the protected population core will be of critical importance to eastern wolf population expansion. However, at least 3 known factors limit the efficacy of APP as source of dispersing eastern wolves to adjacent areas. First, annual dispersal rates of wolves from APP were modest compared to previous studies (reviewed by Fuller *et al.* 2003) and seem unlikely to contribute significantly to population expansion (Patterson *et al.* unpublished data). Second, given the especially poor survival of non-resident, dispersing eastern wolves outside of APP (Chapter 3), dispersal from APP is often not successful. Third, even those eastern wolves that survive will likely have difficulty establishing breeding ranges in a landscape saturated by other *Canis* types, all of which exhibit territoriality with eastern wolves (Chapter 4). Successful establishment outside of APP by eastern wolves is probably most likely when eastern wolves join existing packs composed of coyotes, gray wolves, and/or hybrids meaning that subsequent reproduction by dispersing eastern wolves will often result in hybrid offspring.

I would suggest that conservation efforts for eastern wolves outside of APP should focus on increasing adult survival rates. Adult survival is the most important demographic parameter influencing population growth for most large carnivores (e.g., Robinson *et al.* 2002; Hebblewhite *et al.* 2003; Carroll & Miquelle 2006) including eastern wolves (Patterson & Murray 2008). Wolves differ from many large carnivores because they have high reproductive potential which allows them to tolerate high mortality in some situations (Fuller *et al.* 2003). However, the low density of eastern wolves outside of APP means that much of their reproduction will be achieved through hybrid matings (Benson & Patterson, unpublished data) and will not contribute to eastern wolf population growth. Higher survival has the potential to increase densities of eastern wolves in areas of suitable environmental conditions such as KH and WMU47. With increased density there would be increased probability of: a) dispersing eastern wolves joining packs containing conspecifics and b) successful breeding between highly assigned eastern wolves. Currently, even when breeding packs of eastern wolves are established outside of APP, their reproductive success will be limited given the high probability that one or both individuals in the breeding pair will be harvested. Thus, increasing survival of eastern wolves should also increase successful dispersal and reproduction outside of APP. Another possibility that is worth considering is whether dispersal rates from APP could be increased. Offspring survival is spatially variable within APP and a pending analysis of factors influencing pup survival may provide insight into management strategies to increase pup survival within portions of the park (Benson *et al.*, unpublished data). Increased pup survival could increase the number of eastern wolf pups eligible to disperse into adjacent areas each year.

As noted above, additional harvest protection outside of APP would seem to be the only logical management action that could increase eastern wolf survival. The relatively subtle morphologic differences between *Canis* types, indiscriminate nature of canid trapping, and difficulty of distinguishing eastern wolves from other sympatric canids (especially through the sight of a gun) mean that any effective harvest protection adjacent to APP would need to be applied to both wolves and coyotes (Wheeldon & Patterson 2012). This would likely be an unpopular policy among many in the hunting and trapping community and restrictive management should not be undertaken in the absence of sound scientific evidence (Patterson & Murray 2008). Thus, I would recommend the following framework within which to evaluate the utility of additional harvest protection. First, although the results reported in this dissertation clearly demonstrate that eastern wolves are rare outside of the protected area, and that poor survival is an important demographic mechanism underlying their distribution, I did not estimate population growth. Thus, results from the genetic, demographic, and behavioral analyses contained here should be synthesized into population models. In the short-term, a traditional Leslie matrix population model could be constructed with existing data to provide reliable estimates of eastern wolf population growth outside of APP. Ultimately, a more sophisticated approach (also possible with existing data), would be to create a spatially-explicit individually-based model of eastern wolf population dynamics in and around APP. Such a model could be used to: 1) estimate eastern wolf population growth outside of APP while accounting for genotype-specific behavior and demography in relation the heterogeneous environmental conditions; and 2) facilitate testing of potential harvest scenarios in a modeling environment to make data-based predictions on the effect of hypothetical management policies. The models I suggest would allow for the most informed conservation decisions possible with the best scientific evidence available.

If additional harvest protection for wolves is deemed necessary and desirable, I would suggest initiating a detailed long-term study of demography and hybridization dynamics within areas where protection is enacted. Such a study would be critical to document the precise effects of this conservation action on the demographic and genetic structure of the targeted areas, as well as to evaluate the success of the overall objective of eastern wolf population expansion. The models discussed above should provide explicit predictions on desired outcomes under different harvest management strategies that could be rigorously tested with data collected during the proposed monitoring efforts. In addition to evaluating the success of the management policies specifically for eastern wolves, results from such a study would provide invaluable documentation of the potential for rare hybridizing species to increase in abundance and re-establish reproductive barriers to hybridization following implementation of sufficient harvest protection. Given the paucity of reliable and sustainable management strategies for conserving small, hybridizing populations, the proposed study would contribute broadly to the field of conservation biology.

LITERATURE CITED

Ackermann, R.R., Rogers, J., Cheverud, J.M. (2006) Identifying the morphological signatures of hybridization in primate and human evolution. *Journal of Human Evolution*, **51**, 632-645.

Adams, J.R., Leonard, J.A. & Waits, L.P. (2003). Widespread occurrence of a domestic dog mitochondrial DNA haplotype in southeastern coyotes. *Molecular Ecology*, **12**, 541-546.

Adams, J.R., Lucash, C., Schutte, L.,Waits, L.P. (2007) Locating hybrid individuals in the red wolf (*Canis rufus*) experimental population area using a targeted sampling strategy and faecal DNA genotyping. *Molecular Ecology*,**16**, 1823-1834.

Allendorf, F.W. (1997) Genetically effective sizes of grizzly bear populations. In: *Principles of Conservation Biology*. (eds Meffe,G.K., Carroll,C.R.), pp 174-175. Sunderland Associates Inc. Publishers, Sunderland, MA, USA.

Allendorf, F.W., Leary, R.F., Spruell, P., Wendburg, J.K. (2001) The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution*, **16**, 613-622.

Anderson, E. (1948) Hybridization of the habitat. *Evolution*, **2**, 1-9.

Anderson, M.L. (2012) Wolf responses to spatial variation in moose density in northern Ontario. M.S. Thesis, University of Guelph, Ontario, Canada.

Argue, A.M., Mills, K.J., Patterson, B.R. (2008) Behavioural response of eastern wolves (*Canis lycaon*) to disturbance at homesites and its effects on pup survival. *Canadian Journal of Zoology*, **86**, 400-406.

Arjo W.M., Pletscher, D.H. (1999) Behavioral responses of coyotes to wolf recolonization in northwestern Montana. *Canadian Journal of Zoology*,**77**,1919-1927.

Arnold, M. L., and S.A. Hodges. 1995. Are natural hybrids fit or unfit relative to their parents? *Trends in Ecology and Evolution*, **10**,67-71.

Atwood, T.C., Gese, E.M. (2008) Coyotes and recolonizing wolves: social rank mediates risk-conditional behavior at ungulate carcasses. *Animal Behaviour*, **75**, 753-762.

Atwood, T.C., Gese E.M. (2010) Importance of resource selection and social behavior to partitioning of hostile space by sympatric canids. *Journal of Mammalogy*, **91**,490-499.

Bakian, A.V., Sullivan, K.A., & Paxton, E.H. (2012) Elucidating spatially explicit behavioral landscapes in the willow flycatcher. *Ecological Modelling*, **232**, 119-132.

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

Barrette, C., Messier, F. (1980) Scent-marking in free-ranging coyotes, *Canis latrans*. *Animal Behaviour*, **28**, 814-819.

Barton N.H., Hewitt, G.M. (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, **16**,113-48.

Barton, N.H. (2001) The role of hybridization in evolution. *Molecular Ecology*, **10**, 551-568.

Beale, C.M., Lennon, J.J., Yearsley, J.M., Brewer, M.J., Elston, D.A. (2010) Regression analysis of spatial data. *Ecology Letters*, **13**, 246-264.

Bekoff, M.,Wells, M.C. (1986) Social ecology and behavior of coyotes. Advances in the *Study of Behavior* **16**, 251-338.

Benson, J.F. (2013) Improving rigour and efficiency of use-availability habitat selection analyses with systematic estimation of habitat availability. *Methods in Ecology and Evolution*, **4**, 244-251.

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

Berger, K.M., Gese, E.M. (2007) Does interference competition with wolves limit the distribution and abundance of coyotes? *Journal of Animal of Ecology,* **76**, 1075-1085.

Bergman, E.J., Garrott, R.A., Creel, S., Borkowski, J.J., Jaffe, R., Watson, F.G.R. (2006) Assessment of prey vulnerability through analysis of wolf movements and kill sites. *Ecological Applications*, **16**, 273-284.

Beyer, H.L., Haydon, D.T., Morales, J.M., Frair, J.L., Hebblewhite, Mitchell, M., Matthiopoulos, J. (2010) The interpretation of habitat preference metrics under use-availability designs. *Philosophical Transactions of the Royal Society, B,* **365**, 2245-2254.

Beyer, H.L., Ung, R, Murray, D.L., Fortin, M-J. (2013) Functional responses, seasonal variation and thresholds in behavioural responses of moose to road density. *Journal of Applied Ecology,* **50**, 286-294.

Bohling, J.H., Waits, L.P. (2011) Assessing the prevalence of hybridization between sympatric *Canis* species surrounding the red wolf (*Canis rufus*) recovery area in North Carolina. *Molecular Ecology,* **20**, 2142-2156.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J-S.S. (2009) Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution,* **24**, 127-135.

Boisjoly, D., Ouellet, J-P., Courtois, R. (2010) Coyote habitat selection and management implications for the Gaspésie Caribou. *Journal of Wildlife Management*, **74**, 3-11.

Börger, L., Franconi, N., De Michele, G., Gantz, A., Meschi, F., Manicia, A., Lovari, S., Coulson, T. (2006) Effects of sampling regime on the mean and variance of home range size estimates. *Journal of Animal Ecology*, **75**, 1393-1405.

Borges, T., Lindroos,K, Nadvornik, P, Syvanen A.C., and Saetre, G.P. (2005) Amount of introgression in flycatcher hybrid zones reflects regional differences in pre and post-zygotic barriers to gene exchange. *Journal of Evolutionary Biology*, **18**, 1416-1424.

Burt, W.H. (1943) Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy,* **24**, 346-352.

Bridle, J.R., Vass-De-Zomba, J., Butlin, R.K. (2002) Fine-scale ecological and genetic variation in a *Chorthippus* grasshopper hybrid zone. *Ecological Entomology*, **27**, 499-504.

Britch, S.C., Cain, M.L., Howard, D.J. (2001) Spatio-temporal dynamics of the *Allenomobius fasciatus-A. socius* mosaic hybrid zone: a 14-year perspective. *Molecular Ecology,* **10**, 627-638.

Burke, J.M, Arnold, M.L. (2001) Genetics and the fitness of hybrids. *Annual Review of Genetics*, **35**, 31-51.

Burnham, K.P, Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York.

Camus-Kulandaivelu, L., Veyrieras, J-B., Gouesnard, B., Charcosset, A., Manicacci, D.

(2007) Evaluating the reliability of Structure outputs in case of relatedness between individuals. *Crop Science*, **47**, 887-892.

Carmenzind, F.J. (1978) Behavioral ecology of coyotes on the National Elk Refuge, Jackson, Wyoming. In: Bekoff M (eds) Coyotes: biology, behavior, and management. Academic Press, New York, pp 267-294.

Carroll, C., Miquelle, D.G. (2006) Spatial viability of Amur tiger *Panthera tigris altaica* in the Russian far east: the role of protected areas and landscape matrix in population persistence. *Journal of Applied Ecology*, **43**, 1056-1068.

Cegelski, C.C., Waits, L.P., Anderson, N.J. (2003) Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. Molecular Ecology, **12**, 2907-2918.

Ciucci, P., Masi, M., & Boitani, L. (2003) Winter habitat and travel route selection by wolves in the northern Apennines, Italy. *Oikos*, **26**, 223-235.

Conner, L.M. & Plowman, B.W. (2001) Using Euclidean distances to assess nonrandom habitat use. *Radio Tracking and Animal Populations* (eds J.J. Millspaugh and J.M. Marzluff), pp. 276-288. Academic Press, San Diego.

Conner, L.M., Smith, M.D. & Burger, L. (2003) A comparison of distance-based and classification-based analyses of habitat use. *Ecology*, **84**, 526-531.

Cook, S.J., Norris, D.R., Theberge, J.B. (1999) Spatial dynamics of a migratory wolf population in winter, south-central Ontario (1990-1995). *Canadian Journal of Zoology*, **77**, 1740-1750.

COSSARO, Committee on the Status of Species at Risk in Ontario (2004) Summary of

Species at Risk in Ontario: eastern wolf. http://www.rom.on.ca/ontario/risk.php?doc_type=fact&id=287&lang=en Accessed March 2012.

COSEWIC, Committee on the Status of Endangered Wildlife in Canada (2001) COSEWIC species database: eastern wolf status report. http://www.cosewic.gc.ca/eng/sct1/searchform_e.cfm Accessed March 2012.

Costedoat, C., Pech, N, Salducci, M-D., Chappaz, R, Gilles, A. (2005) Evolution of mosaic hybrid zone between invasive and endemic species of Cyprinidae through space and time. *Biological Journal of the Linnean Society*, **85**,135-155.

Cowlishaw, G., Lawes, M.J., Lightbody, M., Martin, A., Pettifor, R., Rowcliffe, J.M. (2004) A simple rule for the costs of vigilance: empirical evidence from a social forager. *Proceedings of the Royal Society, B.*, **271**, 27-33.

Creel, S., Chistianson, D. (2008) Relationships between direct predation and risk effects. *Trends in Ecology and Evolution*, **23**, 194-201.

Cronin, M.A, Mech, L.D. (2009) Problems with claim of ecotype and taxon status of the wolf in the Great Lakes region. *Molecular Ecology*, **18**, 4991-4993.

Diamond, J. (1975) The island dilemma: lessons of modern biogeographic studies for the design of natural reserves. *Biological Conservation*, **7**, 129-145.

Delibes, M., Ferreras, P., Ganoa, P. (2001) Attractive sinks, or how individual behavioural decisions determine source-sink dynamics. *Ecology Letters*, **4**, 401-403.

Delport, W., Kemp, D.C., Ferguson, J.W. (2004) Structure of an African red-billed hornbill (*Tockus erythrorynchus rufirostris and T.E. Damarensis*) hybrid zone as revealed by morphology, behavior, and breeding biology. *Auk*, **121**, 565-586.

Donkor, N.T., Fryxell, J.M. (1999) Impact of beaver foraging on structure of lowland boreal forests of Algonquin Provincial Park, Ontario. *Forest Ecology and Management*, **118**, 83-92.

Dowling, T.E., Secor, C.L. (1997) The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics*, **28**, 598-619.

Dowling, T.E., DeMarais, B.D. (1993) Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature,* **362,** 444-446.

Dussault, C., Pinard, V., Ouellet, J-P., Courtois, R., Fortin, D. (2012) Avoidance of roads and selection for recent cutovers by threatened caribou: fitness rewarding or maladaptive behavior? *Proceeding of the Royal Society, B*, **279**, 4481-4488.

Elkie, P., Smiegielski, A., Gluck, M. *et al.* (2009) Ontario's Landscape Tool. Ontario Ministry of Natural Resources. Forest Policy Section. Sault Ste. Marie, Ontario, Canada.

Endler, J.A. (1977) *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton, NJ.

Evanno, G., Regnaut, S., Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Fain, S.R., Straughan, D.J., Taylor, B.F. (2010) Genetic outcomes of wolf recovery in the western Great Lake states. *Conservation Genetics*, **11**, 1747-1765.

Falush, D., Stephens, M., Pritchard, J. (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567-1587.

Farraway, J.J. (2002) Practical Regression and Anova using R. http://cran.r-project.org/doc/contrib/Faraway-PRA.pdf Accessed March 2012.

Fedriani, J.M., Fuller, T.K., Sauvajot, R.M. (2001) Does availability of Anthropogenic food enhance densities of omnivorous mammals? An example with Coyotes in southern California. *Ecography*, **24**, 325-331.

Fieberg, J., Kochanny, C.O. (2005) Quantifying home-range overlap: the importance of the utilization distribution. *Journal of Wildlife Management*, **69**, 1346-1359.

Fieberg, J, DelGiudice, G.D. (2009) What time is it? Choice of time origin and scale in extended proportional hazards models. *Ecology*, **90**, 1687-97.

Fieberg, J., Matthiopoulos, J., Hebblewhite, M., Boyce, M.S., Frair, J.L. (2010) Correlation and studies of habitat selection: problem, red herring or opportunity? *Proceeding of the Royal Society, B*, **265**, 2233-2244.

Forbes, G.J., Theberge, J.B. (1996) Cross-boundary management of Algonquin park wolves. *Conservation Biology*, **10**, 1091-1097.

Forbes, G.J., Theberge, J.B. (1996) Response by wolves to prey variation in central Ontario. *Canadian Journal of Zoology*, **74**, 1511-1520.

Fortin, M-J, Dale, M. (2005) Spatial Analysis: A Guide for Ecologists. Cambridge University Press, Cambridge, UK.

Fredickson, R.J., Hedrick, P.W. (2006) Dynamics of hybridization and introgression in red wolves and coyotes. *Conservation Biology*, **20**, 1272-1283.

Fritsche, F., Kaltz, O. (2000) Is the *Prunella* (Lamiaceae) hybrid zone structured by an environmental gradient? Evidence from a reciprocal transplant experiment. *American Journal of Botany*, **87**, 995-1003.

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

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

Fuller, T.K., Keith, L.B. (1981) Non-overlapping ranges of coyotes and wolves in northeastern Alberta. *Journal of Mammalogy*, **62**, 403-405.

Fuller, T.K., Mech, L.D., & Cochrane, J.F. (2003) Wolf population dynamics. In: *Wolves: Ecology,Behavior, and Conservation*. (eds Mech LD, Boitani L), pp 161-191. University of Chicago Press, Chicago, IL.

Fritts S.H., Stephenson R.O., Hayes R.D. & Boitani L. (2003) Wolves and humans. In: *Wolves: Ecology, Behavior, and Conservation*. (eds L.D. Mech & L. Boitani), pp. 289-316. University of Chicago Press, Chicago, IL.

Garshelis, D.L. (2000) Delusions in habitat evaluation: measuring use, selection, and importance. In: *Research Techniques in Animal Ecology: Controversies and Consequences*. (eds L. Boitani & T.K. Fuller), pp. 111-164. Columbia University Press, New York.

García-Moreno, J., Matocq, M.D., Roy, M.S., Geffen, E., Wayne, R.K. (2002) Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. *Conservation Biology*, **10**, 376-389.

Gates, J.E., Gysel, LW. (1978) Avian nest dispersion and fledgling success in field-forest ecotones. *Ecology*, **59**, 871-883.

Gehring, T.M., Swihart, R.K. (2003) Body size, niche breadth, and ecologically scaled responses to habitat fragmentation: mammalian predators in an agricultural landscape. *Biological Conservation*, **109**, 283-295.

Gelman, A., Carlin, J.B., Stern, H.S., & Rubin, D.B. (2004) Bayesian Data Analysis. Chapman and Hall/CRC, Boca Raton, FL, USA.

Gelman, A. (2008) Scaling regression inputs by dividing by two standard deviations. *Statistics in Medicine*, **27**, 2865-2873.

Gese E.M., Ruff, R.L. (1997) Scent-marking by coyotes, *Canis latrans*: the influence of social and ecological factors. *Animal Behaviour*, **54**,1155-1166.

Gese, E.M. (2001) Territorial defense by coyote (*Canis latrans*) in Yellowstone National Park, Wyoming: who, how, where, when, and why. *Canadian Journal of Zoology*, **79**, 980-987.

Gillies, C.S., Hebblewhite, M., Nielsen, S.E. *et al.* (2006) Application of random effects to the study of resource selection by animals. *Journal of Animal Ecology*, **75**, 887-898.

Gipson, P.S., Ballard, W.B., Nowak, R.M., Mech, L.D. (2000) Accuracy and precision of estimating age of gray wolves by tooth wear. *Journal of Wildlife Management*, **64**, 752-758.

Godvik, I.M.R., Loe, L.E., Vik, J.O., Veiberg, V., Langvatn, R., Mysterud, A. (2009) Temporal scales, trade-offs, and functional response in red deer habitat selection. *Ecology*, **90**, 699-710.

Good, T.P., Ellis, J.C., Annett, C.A., Pierotti, R. (2000) Bounded hybrid superiority in an avian hybrid zone: effects of mate, diet, and habitat choice. *Evolution*, **54**,1774-1783.

Gordon, D.M. (1997) The population consequences of territorial behavior. *Trends in Ecology and Evolution*, **12**,63-66.

Grant, P.R., Grant, B.R. (1994) Phenotypic and genetic effects of hybridization in Darwin’s finches. *Evolution*, **48**, 297-316.

Grewal SK, Wilson PJ, Kung TK, *et al.* (2004) A genetic assessment of the eastern wolf (*Canis lycaon* in Algonquin Provincial Park. *Journal of Mammalogy*, **85**, 625-632.

Hadfield, J. (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software*, **33**, 1-22.

Hadfield, J. (2012) MCMCglmm course notes. http://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf. Accessed 14 March 2013.

Hastie, T., Tibshirani, R. (1986) Generalized Additive Models. *Statistical Science*, **1**, 297-318.

Harrison R.G., Bogdanowicz, S.M. (1997) Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. *Evolution*, **51**, 493-505.

Hatfield, T., Schluter, D. (1999) Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution*, **53**, 866-873.

Hebblewhite, M., Percy, M. Serrouya R. (2003) Black bear (*Ursus americanus*) survival and demography in the Bow Valley of Banff National Park, Alberta. *Biological Conservation*, **112**, 415-425.

Hebblewhite, M., Merrill, E. (2008) Modelling wildlife-human relationships for social species with mixed-effects resource selection. *Journal of Applied Ecology,* **45**, 834-844.

Heisey, D.M., Patterson, B.R. (2006) A review of methods to estimate cause-specific mortality in the presence of competing risks. *Journal of Wildlife Management*, **70**, 1544-1555.

Helyar, S.J., Hemmer-Hansen J, Bekkevold D *et al.* (2011) Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources*, **11**, 123-136.

Hoi, H., Eichler, T., Dittami, J. (1991) Territorial spacing and interspecific competition in three species of reed warblers. *Oecologia*, **87**, 443-448.

Houlding, S. (2000). Practical Geostatistics: Modeling and Spatial Analysis. Springer, New York,USA.

Huxel. G.R. (1999) Rapid displacement of native species by invasive species: effects of hybridization. *Biological Conservation*, **89**,143-152.

James, A.R.C., Stuart-Smith, A.K. (2000) Distribution of caribou and wolves in relation to linear corridors. *Journal of Wildlife Management*, **64**,154-159.

Jędrzejewski, W., Schmidt, K., Theuerkauf, J., Jędrzejewski, B., Kowalczyk, R. (2007) Territory size of wolves *Canis lupus*: linking local (Bialowiea Primeval Forest, Poland) and Holarctic-scale patterns. *Ecography*, **30**, 66-76.

Jiggins, C.D., Mallet, J. (2000) Bimodal hybrid zones and speciation. *Trends in Ecology* *and Evolution*, **15**, 250-255.

Johnson, D.H. (1980) The comparison of usage and availability measurements for evaluating resource preference. *Ecology*, **61**, 65-71.

Jombart, T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403-1405.

Jombart, T., Devillard, S., Dufour, A-B., Pontier, D. (2008) Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity*, **101**, 92-103.

Jombart, T., Devillard, S., Balloux, F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94.

Jones, A.G. (2005) GERUD2.0: a computer program for the reconstruction of parental genotypes from progeny arrays with known or unknown parents. *Molecular Ecology Notes*, **5**, 708-711.

Jones, A.G., Small, C.M., Paczolt, K.A., Ratterman, N.L. (2010) A practical guide to methods of parentage analysis. *Molecular Ecology Resources*, **10**, 6-30.

Kalinowski, S.T., Wagner, A.P., Taper, J.L. (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**, 576-579.

Kalinowksi, S.T., Taper, M.L., Marshall, T.C. (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099-1106.

Kitchen, A.M., Gese, E.M., Schauster, E.R. (2000) Changes in coyote activity patterns due to reduced exposure to human persecution. *Canadian Journal of Zoology*, **78**, 853-857.

Knight, R.R., Eberhardt, L.L. (1985) Population dynamics of Yellowstone grizzly bears. *Ecology*, **66**, 323-334.

Koblmüller, S., Nord, M., Wayne, R.K., Leonard, J.A. (2009) Origin and status of the Great Lakes wolf. *Molecular Ecology*, **18**, 2313-2326.

Kolenosky, G.B., Standfield, R. (1975) Morphological and ecological variation among gray wolves (*Canis lupus*) of Ontario, Canada. In: The Wild Canids: Their Systematics, Behavioural Ecology and Evolution (ed. Fox MW), pp. 62-72. Van Nostrand Reinhold, New York.

Kreeger, T.J. (2003) The internal wolf: physiology, pathology, and pharmacology. In: *Wolves: Ecology, Behavior, and Conservation*. (eds Mech LD, Boitani L) pp. 192-217. University of Chicago Press, Chicago.

Kyle, C.J., Johnson, A.R., Patterson, B.R., *et al.* (2006) Genetic nature of eastern wolves: past, present, and future. *Conservation Genetics*, **7**, 273-287.

Lariviere, S., Jollicoeur, H., Crête, M. (2000) Status and conservation of the gray wolf (*Canis lupus*) in wildlife reserves in Quebec. *Biological Conservation*, **94**, 143-151.

Laliberte, A.S, Ripple, W.J. (2004) Range contractions of North American carnivores and ungulates. *Bioscience*, **54**, 123-138.

Lamb, T., Avise, J.C. (1986) Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proceedings of the National Academy of Science, USA*, **83**, 2526-2530.

Lauck, T., Clark, C.W., Mangel, M., Munro, G.R. (1998) Implementing the precaution principle in fisheries management through marine reserves. *Ecological Applications*, **8**, S72-S78.

Lee, C., Abdool, A., Huang, C-H. (2009) PCA-based population structure inference with generic clustering algorithms. *BMC Bioinformatics,* **10**, S73

Legendre, P., Legendre, L. (1998) Numerical Ecology. Elsevier, Amsterdam.

Lele, S.R., Keim, J.L. (2006) Weighted distributions and estimation of resource selection probability functions. *Ecology,* **87**, 3021-3028.

Lima, S.L., Dill, L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619-640.

Lin, X., Zhang, D. (1999) Inference in generalized additive mixed models by using smoothing splines. *Journal of the Royal Statistical Society*, B, **61**, 381-400.

Liu, N., Zhao, H. (2006) A non-parametric approach to population structure inference using multilocus genotypes. *Human Genomics*, **2**, 353-364.

Loveless, K. (2010) Foraging strategies of eastern wolves in relation to migratory prey and hybridization. M.Sc. thesis, Trent University, Peterborough, 73pp.

Lunn, M. & McNeil, D. (1995) Applying cox regression to competing risks. *Biometrics*, **51**, 524-532.

Maher, C.R., Lott, D.R. (1995) Definitions of territoriality used in the study of variation of vertebrate spacing systems. *Animal Behaviour*, **49**, 1581-1597.

Mallet, J. (2005) Hybridization as an invasion of the genome. *Trends in Ecology and Evolution*, **20**, 229-237.

Manel, S., Gaggiotti, O.E., Waples, R.S. (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology and Evolution*, **20**, 136-142.

Manly, B.F.J., McDonald, L.L., Thomas, D.L., McDonald, T.L., Erickson, W.P. (2002) Resource Selection by Animals. Kluwer Academic Publishers, Dordrecht, Netherlands.

Mavaréz, J., Salzar, C.A., Bermingham, E., *et al.* (2006) Speciation by hybridization in *Heliconius* butterflies. *Nature*, **441**, 868-871.

Maxie, A.J. (2009) Effects of roads and trails on landscape use by female American black bears (*Ursus americanus*) in Algonquin Provincial Park, Ontario. M.Sc. Thesis, Trent University, Canada.

Maxie, A.J., Hussey, K.F., Lowe, S.J., Middel, K.R., Pond, B.A., Obbard, M.E., Patterson, B.R. (2010) A comparison of forest inventory, provincial land cover maps and field surveys for wildlife surveys for wildlife habitat analysis in the Great Lakes-St. Lawrence forest. *The Forestry Chronicle*, **86**, 77-86.

Mayr, E. (1963) *Animal Species and Evolution*. Harvard University Press, Cambridge.

McLoughlin, P.D., Dunford, J.S., Boutin S. (2005) Relating predation mortality to broad-scale habitat selection. *Journal of Animal Ecology*, **74**,701-707.

McLoughlin, P.D., Gaillard, J.M., Boyce, M.S., *et al.* (2007) Lifetime reproductive success and composition of the home range in a large herbivore. *Ecology*, **88**, 3192-3201.

McLoughlin, P.D., Morris, D.W. Fortin, D., Wal, E.V., Contasti, A.L. (2009) Considering ecological dynamics in resource selection functions. *Journal of Animal Ecology*, **79**, 4-12.

McLoughlin, P.D., Wal, E.V., Lowe, S.J., Patterson, B.R., Murray, D.L. (2011) Seasonal shifts in habitat selection of a large herbivore and the influence of human activity. *Basic and Applied Ecology*, **12**, 654-663.

McNay, M.E. (2002) Wolf-human interactions in Alaska and Canada: a review of the case history. *Wildlife Society Bulletin*, **30**, 831-843.

Mech. L.D. (1970) The Wolf. University of Minnesota Press, Minneapolis, MN, USA.

Mech, L.D. (1991) The Way of the Wolf. Voyageur Press, Stillwater, MN, USA.

Mech L.D. (1994) Buffer zones of territories of gray wolves as regions of intraspecific strife. *Journal of Mammalogy*, **75**, 199-202.

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

Mech, L.D., Fritts, S.H., Paul W.J. (1988) Wolf distribution and road density in Minnesota. *Wildlife Society Bulletin*, **16**, 269-272.

Mech L.D., Meier T.J., Seal U.S. (1993) Wolf nipple measurements as indices of age and breeding status. *American Midland Naturalist*, **129**, 266-271.

Conservation, **141**, 669-680.

Mech L.D., Boitani, L. (2003) Wolf Social Ecology. In: *Wolves: Ecology, Behavior, and Conservation*. (eds Mech LD, Boitani L) pp. 1-34. University of Chicago Press, Chicago.

Mech, L.D., Peterson, R.O. (2003) Wolf-Prey Relations. In: *Wolves: Ecology, Behavior, and Conservation*. (eds Mech LD, Boitani L) pp. 131-160. University of Chicago Press, Chicago.

Mech, L.D. (2011) Non-genetic data supporting genetic evidence for the eastern wolf.

Northeastern Naturalist, **18**, 521-526.

Meffe, G.K., Carroll, C.R. Principles of Conservation Biology, 2nd Edition. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts, USA.

Messier, F., Barrette, C. (1985) The efficiency of yarding behavior by white-tailed deer as an antipredator strategy. *Canadian Journal of Zoology*, **63**, 785-789.

Messier, F., Barrette, C., Huot, J. (1986) Coyote predation on a white-tailed deer population in southern Quebec. *Canadian Journal of Zoology*, **64**, 1134-1136.

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

Mills, K.M., Patterson, B.R., Murray, D.L. (2008) Direct estimation of early survival and movements in eastern wolf pups. *Journal of Wildlife Management*, **72**, 949-954.

Moore, W.S. (1977) An evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology*, **52**, 263-277.

Moore, W.S., Price, J.T. (1993) Nature of selection in the northern flicker hybrid zone and its implications for speciation theory. Pages 196-225 in R.G. Harrison, editor. Hybrid Zones and the Evolutionary Process, Oxford University Press, New York, USA.

Morin P.A., Luikart, G., Wayne, R.K. (2004) SNPs in ecology, evolution, and conservation. *Trends in Ecology and Evolution*, **19**, 208-216.

Muntz, E.M., Patterson, B.R. (2004) Evidence for the use of vocalization to coordinate the killing of a white-tailed deer by eastern coyotes. *Canadian Field Naturalist*, **118**, 278-280.

Musiani, M., Okarma, H., Jedrzejewski, W. (1998) Speed and actual distances travelled by radiocollared wolves in Bialowieza Primeval Forest (Poland). *Acta Theriologica*, **43**, 409-416.

Musiani, M., Paquet, P.C. (2004) The practices of wolf persecution, protection, and restoration in Canada and the United States. *Bioscience*, **54**, 50-61.

Mysterud, A., Ims, R.A. (1998) Functional response in habitat use: availability influences relative use in trade-off situations. *Ecology*, **79**, 1435-1441.

Nelson, E.H., Matthews, C.E., Rosenheim, J.A. (2004) Predators reduce prey population growth by inducing changes in prey behavior. *Ecology*, **85**, 1853-1858.

Nielsen, S.E., McDermid, G., Stenhouse, G.B., Boyce, M.S. (2005) Dynamic wildlife habitat models: seasonal foods and mortality risk predict occupancy-abundance and habitat selection of grizzly bears. *Biological Conservation*, **143**, 1623-1634.

Okoniewski, J.C. (1992) A fatal encounter between an adult coyote and three conspecifics. *Journal of Mammalogy*, **63**, 679-680.

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

Ostrander, E.A., Mapa, F.A., Yee, M., Rine, J. (1995) One hundred and one new simple sequence repeat-based markers for the canine genome. *Mammalian Genome*, **6**, 192-195.

Packard, J.M. (2003) Wolf behavior: reproductive, social, and intelligent. In: *Wolves: Ecology, Behavior, and Conservation*. (eds Mech LD, Boitani L) pp. 35-65. University of Chicago Press, Chicago.

Pangle, K.L., Peacor, S.D., Johannsson, O.E. (2007) Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology*, **88**, 402-412.

Parra, J.L., Rahbek, C., McGuire, J.A., Graham, C.H. (2011) Contrasting patterns of phylogenetic assemblage structure along the elevational gradient for major hummingbird clades. *Journal of Biogeography*, **38**, 2350-2361.

Paschou, P,, Ziv, E., Burchard, E.G., *et al.* (2007) PCA-Correlated SNPs for structure identification in worldwide human populations. *PloS Genetics*, **3**, 1672-1686.

Paquet, P.C. (1991) Winter spatial relationships of wolves and coyotes in Riding Mountain National Park, Manitoba. *Journal of Mammalogy* **72**, 397-401.

Patterson, B.R., Messier, F. (2001) Social organization and space use of coyotes in eastern Canada relative to prey distribution and abundance. *Journal of Mammalogy*, **82**, 463-477.

Patterson, B.R., Messier, F. (2003) Age and condition of deer killed by coyotes in Nova Scotia. *Canadian Journal of Zoology*, **81**, 1894-1898.

Patterson, B.R., Murray, D.L. (2008) Flawed population viability analysis can result in misleading population assessment: a case study for wolves in Algonquin park, Canada. *Biological Conservation*, **141**, 669-680.

Patterson, N., Price, A.L., Reich, D. (2006) Population structure and eigenananalysis. *PloS Genetics*, **2**, 2074-2093.

Person, D.K., Russell, A.L. (2008) Correlates of mortality in an exploited wolf population. *Journal of Wildlife Management*, **72**, 1540-1549.

Peterson RO, Woolington JD, Bailey TN (1984) Wolves of the Kenai Peninsula. Wildlife

Monographs 88:1-52.

Phillips, M.K., Henry, V.G. (1992) Comments on red wolf taxonomy. *Conservation Biology*, **6**, 596-599.

Pilgrim, K.L., Boyd, D.K., Forbes, S.H. (1998) Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. *Journal of Wildlife Management*, 62,683-689.

Pollock, K.H., Winterstein, S.R., Bunck, C.M., Curtis, P.D. (1989) Survival analysis in telemetry studies: the staggered entry design. *Journal of Wildlife Management*, **53**, 7-15.

Poulle, M-L, Crête, M., Huot, J. (1995) Seasonal variation in body mass and composition of eastern coyotes. *Canadian Journal of Zoology*, **73**, 1625-1633.

Preisser, E.L., Bolnick, D.I., Benard, M.F. (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501-509.

Pritchard, J.K., Stephens, M., Donnelly, P. (2000) Inference of population structure from multilocus genotype data. *Genetics*, **155**, 945-959.

R Development Core Team (2011) R: a language and environment for statistical computing. *R Foundation for Statisical Computing*, Vienna, Austria.

Rand D.M., Harrison R.G. (1989) Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution*, **43**, 432-449.

Randi, E. (2008) Detecting hybridization between wild species and their domesticated relatives. *Molecular Ecology*, **17**, 285-293.

Reid, J.M. (2012) Predicting evolutionary response to selection on polyandry in the wild: additive genetic covariances with female extra-pair reproduction. *Proceedings of the Royal Society, B*, **279**, 4652-4660.

Rhymer, J.M., Simberloff, D. (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83-109.

Rich, L.N., Mitchell, M.S., Gude, J.A., Sime, C.A. (2012) Anthropogenic mortality, intraspecific competition, and prey availability influence territory sizes of wolves in Montana. *Journal of Mammalogy* 93:722-731.

Rieseberg, L.H. (1997) Hybrid origins of plant species. *Annual Review of Ecology and Systematics,* **28**, 359-389

Riley, S.P.D., Sauvajot, R.M., Fuller, T.K., York, E.C., Kamradt, D.A., Bromley, C., Wayne, R.K. (2003) Effects of urbanization and habitat fragmentation of bobcats and coyotes in southern California. *Conservation Biology*, **17**, 566-576.

Robinson, H.S., Wielgus, R.B., Gwilliam, J.C. (2002) Cougar predation and population growth of sympatric mule deer and white-tailed deer. *Canadian Journal of Zoology*, **80**, 556-568.

Rosenzweig, M.L. (1981) A theory of habitat selection. *Ecology*, **62**, 327-335.

Ross C.L., Harrison R.G. (2002) A fine-scale spatial analysis of the mosaic hybrid zone between *Gryllus firmus* and *Gryllus pennsylvanicus*. *Evolution*, **56**, 2296-2312.

Rubidge, E.M., Taylor, E.B. (2004) Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). *Molecular Ecology*, **13**, 3735-3749.

Rutledge, L.Y., Holloway, J.J., Patterson, B.R., White, B.N. (2009) An improved field method to obtain DNA for individual identification from wolf scat. *Journal of Wildlife Management*, **73**, 1430-1435.

Rutledge L.Y., Garroway C.J., Loveless K.M., Patterson B.R. (2010*a*) Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves, *Heredity*, **105**, 520-531.

Rutledge, L.Y., Patterson, B.R., Mills, K.J., Loveless, K.M., Murray, D.M., White, B.N. (2010*b*) Protection from harvesting restores the natural social structure of eastern wolf packs. *Biological Conservation*, **143**, 332-339.

Rutledge L.Y., White B.N., Row J.R., Patterson BR (2011) Intense harvesting of eastern wolves facilitated hybridization with coyotes. *Ecology and Evolution,* **2**, 19-33.

Rutledge, L.Y., Wilson, P.J., Klütsch, F.C., Patterson, B.R., White, B.N. (2012) Conservation genomics in perspective: a holistic approach to understanding *Canis* evolution in North America. *Biological Conservation*, **155**, 186-192.

Schmitz, O.J., Beckerman, A.P., O'Brien, K.M. (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*, **78**, 1388-1399.

Schtickzelle, N., Baguette, M. (2003) Behavioural responses to habitat patch boundaries restrict dispersal and generate emigration-patch area relationships in fragmented landscapes. *Journal of Animal Ecology*, **72**, 533-545.

Scribner, K.T. (1993) Hybrid zone dynamics are influenced by genotype-specific variation in life-history traits: experimental evidence from hybridizing Gambusia species. *Evolution,* **47**, 632-646.

Sears, H.J., Theberge, J.B., Theberge, M.T., Thornton, I., Campbell, G.D. (2003) Landscape influence of *Canis* morphological and ecological variation in a coyote-wolf *C. lupus* × *latrans* hybrid zone, southeastern Ontario. *Canadian Field Naturalist*, **117**, 591-600.

Seehausen, O. (2004) Hybridization and adaptive radiation. *Trends in Ecology and Evolution*, **19**, 198-207.

Shears, N.T., Babcock, R.C. (2002) Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia*, **132**, 131-142.

Sheather, S.J., Jones, M.C. (1991) A reliable data-based bandwidth selection method for kernel density estimation. *Journal of the Royal Statistical Society, Series B*, **53**, 683-690.

Sih, A., Bell, A, Johnson, J.C. (2004) Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution*, **18**, 372-378.

Snäll, T., Fogelqvist, J., Ribeiro, J., Lascoux, M. (2004) Spatial genetic structure in two congeneric epiphytes with different dispersal strategies analyzed by three different methods. *Molecular Ecology*, **13**, 2109-2119.

Smith, D.W., Bangs, E.E., Oakleaf, J.K. *et al.* (2010) Survival of colonizing wolves in the northern Rocky Mountains of the United States, 1982-2004. *Journal of Wildlife Management*, **74**, 620-634.

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

Spiegelhater, D.J., Best, N.G., Carlin, B.P., van der Linde, A. (2002) Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society, B*, **64**, 583-639.

Stephens, P.A., Sutherland, W.J. (1999) Consequences of the Allee effect for behavior, ecology, and conservation. *Trends in Ecology and Evolution*, **14**, 401-405.

Sterling, B., Conley, W., Conley, M.R. (1983) Simulations of demographic compensation in coyote populations. *Journal of Wildlife Management*, **47**, 1177-1181.

Sundqvist, A.K., Ellegren, H., Olivier, M., Vilá, C. (2001) Y-chromosome haplotyping in Scandinavian wolves (*Canis lupus*) based on microsatellite makers. *Molecular Ecology*, **10**, 1959-1966.

Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596-1599.

Testa, J.W. (2004) Population dynamics and life history trade-offs of moose (*Alces alces*) in south-central Alaska. *Ecology*, **85**, 1439-1452.

Theberge, J.B., Theberge, M.T. (2004) The wolves of Algonquin Park, a 12 year ecological study. Department of Geography Publication Series 56:1-215. University of Waterloo, Ontario.

Theberge, J.B., Theberge, M.T, Vucetich, J.A., Paquet, P.C. (2006) Pitfalls of applying adaptive management to a wolf population in Algonquin Provincial Park, Ontario. *Environmental Management*, **37**, 451-460.

Therneau, T.M., Grambsch, P.M. (2000) *Modeling Survival Data: Extending the Cox Model*. Springer Verlag, New York.

Theuerkauf, J., Jedrzejewski, W., Schmidt, K., Gula, R. (2003) Spatiotemporal segregation of wolves from humans in the Bialowieza Forest (Poland). *Journal of Wildlife Management*, **67**, 706-716.

Thiel, R.P. (1985) Relationship between road densities and wolf habitat suitability in Wisconsin. *American Midland Naturalist*, **113**, 404-407.

Thurber, J.M., Peterson, R.O., Woolington, J.D., Vucetich, J.A. (1992) Coyote coexistence with wolves on the Kenai Peninsula, Alaska. *Canadian Journal of Zoology*, 70, 2494-2498.

Turelli, M.,Orr, H.A. (1995) The dominance theory of Haldane's rule. *Genetics*, **140**, 389-402.

Tynkkynen, K., Kotiaho, J.S., Luojumäki, M., Suhonen, J. (2006) Interspecific territoriality in Calopteryx damselflies: the role of secondary sexual characters. *Animal Behaviour*, **71**,299-306.

Vähä, J.P., Primmer, C.R. (2006) Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology*, **15**, 63-72.

Van Ballenberghe, V., Erickson, A.W., Byman, D. (1975) Ecology of the timber wolf in northwestern Minnesota. *Wildlife Monographs*, **43**, 1-43.

van Beest, F.M., Mysterud, A., Loe, L.E., Milner, J.M. (2010) Forage quantity, quality and depletion as scale dependent mechanisms driving habitat selection of a large browsing herbivore. *Journal of Animal Ecology*, **79**, 910-922.

Verardi, A., Lucchini, V., Randi, E. (2006) Detecting introgressive hybridization between free-ranging domestic dogs and wild wolves (*Canis lupus*) by admixture linkage disequilibrium anlaysis. *Molecular Ecology*, **15**, 2845-2855.

Vines, T.H., Köhler, S.C., Thiel, M. *et al.* (2003) The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*. *Evolution*, **57**, 1876-1888.

Vila, C., Urios, V., Castroviejo, J. (1995) Observations on the daily activity patterns of the Iberian Wolf. In: Ecology and Conservation of Wolves in a Changing World. (eds Carbyn, L.N., Fritts, S.H., Seip, D.R.), pp. 335-340, Canadian Circumpolar Institute, Edmonton, Alberta.

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

von Holdt, B.M., Pollinger, J.P., Earl, D.A., *et al.* (2011) A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Research,* **21**, 1294-1305.

Warton, D.I., Hui, F.K. (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, **92**, 3-10.

Way, J.G., Rutledge, L., Wheeldon, T., White, B.N. (2010) Genetic characterization of eastern "coyotes" in eastern Massachusetts. *Northeastern Naturalist*, **17**, 189-204.

Wayne, R.K., Jenks, S.M. (1991) Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. *Nature*, **351**, 565-568.

Wheeldon, T.J. (2009) Genetic characterization of *Canis* populations in the western Great Lakes Region. MSc. Thesis, Trent University, Peterborough, ON.

Wheeldon, T.J., Patterson, B.R., White, B.N. (2010) Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. *Molecular Ecology*, **19**, 4428-4440.

Wheeldon, T.J., Patterson, B.R. (2012) Genetic and morphological differentiation of wolves (*Canis lupus*) and coyotes (*Canis latrans*) northeastern Ontario. *Canadian Journal of Zoology*, **90**, 1221-1230.

Whittington, J., St. Clair, C.C., Mercer, G. (2005) Spatial responses of wolves to roads and trails in mountain valleys. *Ecological Applications*, **15**, 543-553.

Wikle, C.K. (2003) Hierarchical models in environmental science. *International Statistical Review*, **71**, 181-199.

Wilmers, C.C., Stahler, D.R., Crabtree, R.L., Smith, D.W., Getz, W.M. (2003) Resource dispersion and consumer dominance: scavenging at wolf- and hunter-killed carcasses in Greater Yellowstone, U.S.A. *Ecology Letters*, **6**, 996-1003.

Wilson, E.O. (1975) Sociobiology: the new synthesis. Harvard University Press, Cambridge, Massachusetts.

Wilson, P.J., Grewal, S., Lawfor, I.D., *et al.* (2000) DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Canadian Journal of Zoology*, **78**, 2156-2166.

Wilson, P.J., Grewal, S., Mallory, F., White, B. (2009) Genetic characterization of hybrid wolves across Ontario. *Journal of Heredity*, **100**, S80-S89.

Wilson, S.D. (1998) Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society, B*, **353**, 199-205.

Wolf, H.G., Mort, M.A. (1986) Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. *Oecologia*, **68**, 507-511.

Wolf, D.E., Takebayashi, N., Riseberg, L.H. (2001) Predicting the risk of extinction through hybridization. *Conservation Biology*, **15**, 1039-1053.

Wood, S.N., Augustin, N.H. (2002) GAMs with integrated model selection using penalized regression splines and applications to environmental modeling. *Ecological Modelling*, **157**, 157-177.

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

Wood, S.N. (2006) Generalized Additive Models: An Introduction with R. Chapman and Hall, Boca Raton, FL, USA.

Worton, B.J. (1989) Kernel methods for estimating the utilization distribution in home-range studies. *Ecology*, **70**,164-168.

APPENDICES

APPENDIX A. Summary data from Structure and K-Means procedures for evaluating support for the number of *Canis* genetic clusters in central Ontario in and adjacent to Algonquin Provincial Park, 2004-2011. Shown are mean posterior probabilities (Ln P[D]) and Δ K from Structure (for K = 1-7; see Pritchard *et al.* 2000, Evanno *et al.* 2005) and Bayesian Information Criteria (BIC) scores from K-Means procedure (for K =1-10; see Jombart *et al.* 2010).

K	Ln P(D)	ΔK	BIC
1	-6593.26	NA	261.07
2	-6190.58	1080.56	251.82
3	-6045.88	319.27	250.09
4	-6014.96	13.06	250.20
5	-5966.62	40.19	250.95
6	-6036.16	3.68	251.28
7	-6016.74	NA	252.08
8	NA	NA	253.48
9	NA	NA	254.90
10	NA	NA	256.53

APPENDIX B. Q-scores from program Structure at *K* = 3 for each individual (*n* = 161) in main analysis. ID for each individual indicates study unit of residency (AP = Algonquin Provincial Park, KH = Kawartha Highlands, 47 = WMU47, 49 = WMU49, NE = Northeast Ontario [outgroup]). Q-scores are mean values across 10 runs for AP, KH, 47, 49 with standard deviation (SD). Q-scores for the NE outgroup are the scores from the run with the lowest variance, thus no SD is provided. Also shown are 90% credible regions for each cluster, my original assignment based on Q-scores and threshold criteria (see methods), and my final assignment after using PCA to corroborate or adjust original assignments (see methods and results). Individuals with possible 3-way admixture (*n* = 4) were not assigned (NA) initially and I used PCA and Q-scores to determine final assignment. For assignments, EW = eastern wolf, GW = gray wolf, G × E = gray × eastern wolf hybrid, C × E = coyote × eastern wolf hybrid, and G × C = gray wolf × coyote hybrid.

	$\bar{x}$			SD			Credible Regions			Assignments	
ID	Coyote	Gray	Eastern	Coyote	Gray	Eastern	90% Coyote	90% Gray	90% Eastern	Original	Final
AP-1	0.027	0.010	0.964	0.001	0.001	0.000	(0.000,0.163)	(0.000,0.060)	(0.803,1.000)	EW	EW
AP-2	0.068	0.454	0.478	0.000	0.001	0.002	(0.000,0.453)	(0.002,0.831)	(0.000,0.913)	G × E	G × E
AP-3	0.015	0.024	0.962	0.001	0.001	0.001	(0.000,0.087)	(0.000,0.156)	(0.784,1.000)	EW	EW
AP-4	0.012	0.023	0.965	0.000	0.001	0.000	(0.000,0.075)	(0.000,0.156)	(0.801,1.000)	EW	EW
AP-5	0.064	0.009	0.927	0.001	0.001	0.000	(0.000,0.391)	(0.000,0.055)	(0.597,1.000)	EW	EW
AP-6	0.049	0.013	0.938	0.001	0.001	0.000	(0.000,0.315)	(0.000,0.084)	(0.654,1.000)	EW	EW
AP-7	0.104	0.129	0.767	0.002	0.002	0.001	(0.000,0.688)	(0.000,0.476)	(0.173,1.000)	NA	EW
AP-8	0.017	0.049	0.934	0.001	0.001	0.001	(0.000,0.100)	(0.000,0.302)	(0.658,1.000)	EW	EW
AP-9	0.126	0.009	0.866	0.002	0.003	0.000	(0.000,0.957)	(0.000,0.054)	(0.018,1.000)	EW	EW
AP-10	0.062	0.018	0.920	0.001	0.002	0.000	(0.000,0.387)	(0.000,0.119)	(0.578,1.000)	EW	EW
AP-11	0.019	0.008	0.974	0.000	0.000	0.001	(0.000,0.117)	(0.000,0.046)	(0.854,1.000)	EW	EW
AP-12	0.023	0.011	0.966	0.000	0.000	0.000	(0.000,0.133)	(0.000,0.068)	(0.818,1.000)	EW	EW
AP-13	0.033	0.007	0.960	0.001	0.000	0.000	(0.000,0.207)	(0.000,0.041)	(0.777,1.000)	EW	EW
AP-14	0.124	0.143	0.733	0.002	0.003	0.002	(0.000,0.838)	(0.000,0.625)	(0.000,1.000)	NA	G × E
AP-15	0.032	0.708	0.260	0.000	0.002	0.002	(0.000,0.212)	(0.291,1.000)	(0.000,0.690)	G × E	G × E
AP-16	0.030	0.012	0.958	0.000	0.000	0.001	(0.000,0.190)	(0.000,0.079)	(0.767,1.000)	EW	EW
AP-17	0.231	0.010	0.759	0.002	0.002	0.000	(0.000,1.000)	(0.000,0.062)	(0.000,1.000)	C × E	C × E
AP-18	0.012	0.019	0.968	0.000	0.001	0.000	(0.000,0.074)	(0.000,0.126)	(0.824,1.000)	EW	EW
AP-19	0.971	0.007	0.022	0.000	0.000	0.000	(0.835,1.000)	(0.000,0.045)	(0.000,0.142)	Coyote	Coyote
AP-20	0.014	0.007	0.979	0.000	0.001	0.000	(0.000,0.085)	(0.000,0.045)	(0.881,1.000)	EW	EW
AP-21	0.314	0.010	0.677	0.003	0.003	0.000	(0.000,1.000)	(0.000,0.059)	(0.000,1.000)	C × E	C × E

AP-22	0.028	0.009	0.963	0.001	0.001	0.000	(0.000,0.181)	(0.000,0.054)	(0.792,1.000)	EW	EW
AP-23	0.974	0.014	0.012	0.000	0.000	0.001	(0.855,1.000)	(0.000,0.085)	(0.000,0.075)	Coyote	Coyote
AP-24	0.770	0.063	0.166	0.002	0.002	0.001	(0.143,1.000)	(0.000,0.342)	(0.000,0.808)	C × E	C × E
AP-25	0.022	0.006	0.972	0.000	0.000	0.001	(0.000,0.134)	(0.000,0.039)	(0.846,1.000)	EW	EW
AP-26	0.020	0.035	0.945	0.001	0.001	0.000	(0.000,0.121)	(0.000,0.226)	(0.705,1.000)	EW	EW
AP-27	0.015	0.014	0.971	0.000	0.000	0.001	(0.000,0.094)	(0.000,0.084)	(0.839,1.000)	EW	EW
AP-28	0.048	0.117	0.835	0.001	0.002	0.001	(0.000,0.313)	(0.000,0.466)	(0.440,1.000)	EW	EW
AP-29	0.031	0.454	0.515	0.001	0.001	0.001	(0.000,0.216)	(0.098,0.808)	(0.062,0.882)	G × E	G × E
AP-30	0.086	0.010	0.904	0.001	0.001	0.000	(0.000,0.540)	(0.000,0.058)	(0.448,1.000)	EW	EW
AP-31	0.016	0.073	0.912	0.001	0.001	0.001	(0.000,0.094)	(0.000,0.380)	(0.590,1.000)	EW	EW
AP-32	0.069	0.567	0.364	0.001	0.002	0.002	(0.000,0.444)	(0.135,0.994)	(0.000,0.804)	G × E	G × E
AP-33	0.031	0.041	0.928	0.000	0.001	0.001	(0.000,0.205)	(0.000,0.244)	(0.663,1.000)	EW	EW
AP-34	0.017	0.020	0.964	0.000	0.000	0.001	(0.000,0.106)	(0.000,0.126)	(0.800,1.000)	EW	EW
AP-35	0.120	0.046	0.834	0.002	0.003	0.001	(0.000,0.736)	(0.000,0.295)	(0.097,1.000)	EW	EW
AP-36	0.213	0.509	0.278	0.002	0.001	0.002	(0.000,0.898)	(0.000,0.968)	(0.000,0.820)	G × C	G × C
AP-37	0.026	0.187	0.788	0.000	0.002	0.002	(0.000,0.162)	(0.000,0.693)	(0.232,1.000)	G × E	G × E
AP-38	0.068	0.335	0.597	0.001	0.004	0.003	(0.000,0.455)	(0.000,0.886)	(0.000,1.000)	G × E	G × E
AP-39	0.335	0.370	0.295	0.002	0.003	0.002	(0.000,0.980)	(0.000,0.803)	(0.000,0.918)	NA	G × C
AP-40	0.654	0.297	0.049	0.002	0.001	0.002	(0.256,1.000)	(0.000,0.676)	(0.000,0.313)	G × C	G × C
KH-1	0.018	0.014	0.969	0.000	0.000	0.001	(0.000,0.107)	(0.000,0.085)	(0.829,1.000)	EW	EW
KH-2	0.428	0.149	0.423	0.005	0.005	0.001	(0.000,1.000)	(0.000,0.522)	(0.000,0.969)	C × E	C × E
KH-3	0.827	0.037	0.136	0.002	0.002	0.001	(0.323,1.000)	(0.000,0.230)	(0.000,0.614)	Coyote	Coyote
KH-4	0.895	0.097	0.009	0.002	0.000	0.002	(0.623,1.000)	(0.000,0.365)	(0.000,0.050)	Coyote	Coyote
KH-5	0.977	0.006	0.017	0.000	0.000	0.000	(0.870,1.000)	(0.000,0.036)	(0.000,0.109)	Coyote	Coyote
KH-6	0.016	0.007	0.977	0.000	0.000	0.000	(0.000,0.103)	(0.000,0.041)	(0.870,1.000)	EW	EW
KH-7	0.858	0.077	0.065	0.002	0.001	0.001	(0.409,1.000)	(0.000,0.411)	(0.000,0.414)	Coyote	G × C
KH-8	0.872	0.014	0.114	0.001	0.001	0.000	(0.357,1.000)	(0.000,0.088)	(0.000,0.629)	Coyote	Coyote
KH-9	0.412	0.055	0.533	0.003	0.004	0.001	(0.000,1.000)	(0.000,0.293)	(0.000,1.000)	C × E	C × E
KH-10	0.119	0.009	0.871	0.002	0.002	0.000	(0.000,0.694)	(0.000,0.057)	(0.293,1.000)	EW	EW
KH-11	0.067	0.059	0.874	0.001	0.002	0.001	(0.000,0.447)	(0.000,0.320)	(0.445,1.000)	EW	EW
KH-12	0.363	0.007	0.631	0.002	0.002	0.000	(0.000,1.000)	(0.000,0.040)	(0.000,1.000)	C × E	C × E

KH-13	0.475	0.042	0.484	0.003	0.003	0.001	(0.000,1.000)	(0.000,0.252)	(0.000,1.000)	C × E	C × E
KH-14	0.868	0.034	0.098	0.001	0.001	0.000	(0.390,1.000)	(0.000,0.218)	(0.000,0.577)	Coyote	Coyote
KH-15	0.972	0.012	0.016	0.000	0.000	0.001	(0.847,1.000)	(0.000,0.072)	(0.000,0.099)	Coyote	Coyote
KH-16	0.926	0.009	0.065	0.001	0.001	0.000	(0.580,1.000)	(0.000,0.054)	(0.000,0.409)	Coyote	Coyote
KH-17	0.045	0.014	0.940	0.001	0.001	0.001	(0.000,0.281)	(0.000,0.089)	(0.682,1.000)	EW	EW
KH-18	0.013	0.011	0.977	0.000	0.001	0.000	(0.000,0.077)	(0.000,0.067)	(0.869,1.000)	EW	EW
KH-19	0.014	0.010	0.976	0.000	0.001	0.001	(0.000,0.085)	(0.000,0.058)	(0.869,1.000)	EW	EW
KH-20	0.036	0.008	0.957	0.001	0.000	0.000	(0.000,0.228)	(0.000,0.047)	(0.757,1.000)	EW	EW
KH-21	0.759	0.218	0.024	0.002	0.001	0.002	(0.433,1.000)	(0.000,0.526)	(0.000,0.149)	G × C	G × C
47-1	0.063	0.187	0.751	0.001	0.003	0.002	(0.000,0.409)	(0.000,0.674)	(0.044,1.000)	G × E	G × E
47-2	0.193	0.014	0.793	0.002	0.002	0.001	(0.000,0.988)	(0.000,0.089)	(0.001,1.000)	C × E	C × E
47-3	0.198	0.691	0.111	0.001	0.001	0.001	(0.000,0.555)	(0.317,1.000)	(0.000,0.536)	G × C	G × C
47-4	0.038	0.854	0.109	0.000	0.001	0.001	(0.000,0.223)	(0.507,1.000)	(0.000,0.468)	GW	GW
47-5	0.685	0.031	0.284	0.002	0.002	0.000	(0.073,1.000)	(0.000,0.192)	(0.000,0.874)	C × E	C × E
47-6	0.012	0.018	0.969	0.001	0.001	0.000	(0.000,0.074)	(0.000,0.116)	(0.828,1.000)	EW	EW
47-7	0.932	0.021	0.047	0.001	0.001	0.000	(0.638,1.000)	(0.000,0.136)	(0.000,0.303)	Coyote	Coyote
47-8	0.023	0.872	0.105	0.000	0.001	0.001	(0.000,0.149)	(0.554,1.000)	(0.000,0.421)	GW	GW
47-9	0.185	0.028	0.788	0.003	0.003	0.000	(0.000,0.719)	(0.000,0.181)	(0.238,1.000)	C × E	C × E
47-10	0.022	0.377	0.601	0.001	0.001	0.001	(0.000,0.133)	(0.092,0.664)	(0.292,0.892)	G × E	G × E
47-11	0.014	0.971	0.015	0.000	0.000	0.000	(0.000,0.091)	(0.837,1.000)	(0.000,0.095)	GW	GW
47-12	0.928	0.027	0.045	0.001	0.001	0.000	(0.624,1.000)	(0.000,0.177)	(0.000,0.302)	Coyote	Coyote
47-13	0.319	0.152	0.528	0.003	0.004	0.002	(0.000,0.999)	(0.000,0.556)	(0.000,1.000)	C × E	3Way
47-14	0.345	0.192	0.463	0.003	0.004	0.002	(0.000,1.000)	(0.000,0.627)	(0.000,1.000)	C × E	C × E
47-15	0.024	0.021	0.956	0.000	0.000	0.001	(0.000,0.149)	(0.000,0.133)	(0.760,1.000)	EW	EW
47-16	0.209	0.122	0.669	0.002	0.003	0.001	(0.000,0.847)	(0.000,0.556)	(0.000,1.000)	C × E	C × E
47-17	0.017	0.022	0.962	0.001	0.000	0.000	(0.000,0.100)	(0.000,0.142)	(0.786,1.000)	EW	EW
47-18	0.550	0.254	0.197	0.004	0.002	0.002	(0.000,1.000)	(0.000,0.710)	(0.000,0.720)	G × C	G × C
49-1	0.817	0.133	0.050	0.002	0.001	0.002	(0.414,1.000)	(0.000,0.499)	(0.000,0.319)	Coyote	G × C
49-2	0.303	0.074	0.624	0.004	0.004	0.001	(0.000,1.000)	(0.000,0.368)	(0.000,1.000)	C × E	C × E
49-3	0.083	0.020	0.898	0.001	0.001	0.001	(0.000,0.403)	(0.000,0.126)	(0.559,1.000)	EW	EW
49-4	0.020	0.101	0.880	0.000	0.002	0.001	(0.000,0.121)	(0.000,0.489)	(0.473,1.000)	EW	G × E

49-5	0.946	0.017	0.037	0.001	0.001	0.000	(0.700,1.000)	(0.000,0.106)	(0.000,0.249)	Coyote	Coyote
49-6	0.939	0.035	0.026	0.001	0.001	0.001	(0.686,1.000)	(0.000,0.226)	(0.000,0.162)	Coyote	Coyote
49-7	0.063	0.516	0.422	0.001	0.002	0.001	(0.000,0.422)	(0.174,0.873)	(0.000,0.800)	G × E	G × E
49-8	0.464	0.013	0.523	0.005	0.005	0.000	(0.000,1.000)	(0.000,0.084)	(0.000,1.000)	C × E	C × E
49-9	0.893	0.044	0.063	0.001	0.001	0.000	(0.480,1.000)	(0.000,0.290)	(0.000,0.417)	Coyote	C × E
49-10	0.963	0.010	0.027	0.001	0.001	0.000	(0.791,1.000)	(0.000,0.062)	(0.000,0.176)	Coyote	Coyote
49-11	0.789	0.026	0.185	0.002	0.002	0.000	(0.143,1.000)	(0.000,0.169)	(0.000,0.814)	C × E	Coyote
49-12	0.942	0.033	0.025	0.001	0.001	0.001	(0.698,1.000)	(0.000,0.214)	(0.000,0.154)	Coyote	Coyote
49-13	0.813	0.058	0.129	0.002	0.001	0.001	(0.130,1.000)	(0.000,0.346)	(0.000,0.717)	Coyote	Coyote
49-14	0.904	0.024	0.072	0.001	0.001	0.000	(0.506,1.000)	(0.000,0.157)	(0.000,0.458)	Coyote	Coyote
49-15	0.822	0.122	0.057	0.002	0.001	0.002	(0.370,1.000)	(0.000,0.510)	(0.000,0.351)	Coyote	G × C
49-16	0.977	0.009	0.014	0.000	0.000	0.000	(0.869,1.000)	(0.000,0.055)	(0.000,0.090)	Coyote	Coyote
49-17	0.676	0.031	0.293	0.002	0.002	0.001	(0.154,1.000)	(0.000,0.199)	(0.000,0.825)	C × E	Coyote
49-18	0.430	0.015	0.556	0.005	0.005	0.001	(0.000,1.000)	(0.000,0.093)	(0.000,1.000)	C × E	C × E
49-19	0.878	0.051	0.072	0.001	0.001	0.001	(0.463,1.000)	(0.000,0.302)	(0.000,0.449)	Coyote	Coyote
49-20	0.929	0.046	0.025	0.001	0.001	0.001	(0.639,1.000)	(0.000,0.286)	(0.000,0.164)	Coyote	Coyote
49-21	0.978	0.008	0.014	0.000	0.000	0.000	(0.879,1.000)	(0.000,0.050)	(0.000,0.083)	Coyote	Coyote
49-22	0.866	0.039	0.095	0.001	0.002	0.001	(0.302,1.000)	(0.000,0.254)	(0.000,0.637)	Coyote	C × E
49-23	0.975	0.013	0.013	0.001	0.001	0.000	(0.858,1.000)	(0.000,0.081)	(0.000,0.076)	Coyote	Coyote
49-24	0.966	0.008	0.026	0.001	0.000	0.000	(0.810,1.000)	(0.000,0.047)	(0.000,0.166)	Coyote	Coyote
49-25	0.896	0.018	0.087	0.002	0.002	0.001	(0.439,1.000)	(0.000,0.112)	(0.000,0.533)	Coyote	C × E
49-26	0.966	0.016	0.019	0.000	0.000	0.000	(0.810,1.000)	(0.000,0.100)	(0.000,0.115)	Coyote	Coyote
49-27	0.971	0.011	0.018	0.000	0.000	0.000	(0.835,1.000)	(0.000,0.072)	(0.000,0.113)	Coyote	Coyote
49-28	0.979	0.011	0.011	0.000	0.000	0.001	(0.883,1.000)	(0.000,0.065)	(0.000,0.064)	Coyote	Coyote
49-29	0.964	0.011	0.025	0.000	0.001	0.000	(0.804,1.000)	(0.000,0.071)	(0.000,0.156)	Coyote	Coyote
49-30	0.938	0.018	0.044	0.001	0.001	0.000	(0.664,1.000)	(0.000,0.116)	(0.000,0.293)	Coyote	Coyote
49-31	0.976	0.012	0.013	0.000	0.001	0.000	(0.865,1.000)	(0.000,0.076)	(0.000,0.078)	Coyote	Coyote
49-32	0.960	0.015	0.026	0.000	0.001	0.001	(0.778,1.000)	(0.000,0.093)	(0.000,0.161)	Coyote	Coyote
49-33	0.917	0.029	0.054	0.001	0.001	0.001	(0.599,1.000)	(0.000,0.187)	(0.000,0.343)	Coyote	Coyote
49-34	0.502	0.022	0.476	0.003	0.003	0.000	(0.000,1.000)	(0.000,0.144)	(0.000,1.000)	C × E	C × E
49-35	0.914	0.018	0.068	0.001	0.001	0.000	(0.571,1.000)	(0.000,0.118)	(0.000,0.397)	Coyote	Coyote

49-36	0.407	0.039	0.555	0.005	0.004	0.001	(0.000,1.000)	(0.000,0.247)	(0.000,1.000)	C × E	C × E
49-37	0.978	0.007	0.015	0.000	0.000	0.000	(0.874,1.000)	(0.000,0.045)	(0.000,0.094)	Coyote	Coyote
49-38	0.961	0.009	0.031	0.000	0.000	0.000	(0.778,1.000)	(0.000,0.052)	(0.000,0.201)	Coyote	Coyote
49-39	0.939	0.017	0.044	0.001	0.001	0.000	(0.664,1.000)	(0.000,0.112)	(0.000,0.292)	Coyote	Coyote
49-40	0.972	0.007	0.021	0.000	0.000	0.000	(0.842,1.000)	(0.000,0.045)	(0.000,0.133)	Coyote	Coyote
49-41	0.892	0.015	0.093	0.002	0.002	0.000	(0.241,1.000)	(0.000,0.093)	(0.000,0.715)	Coyote	C × E
49-42	0.070	0.017	0.913	0.001	0.001	0.000	(0.000,0.409)	(0.000,0.113)	(0.561,1.000)	EW	C × E
NE-1	0.008	0.985	0.007	---	---	---	(0.000,0.049)	(0.914,1.000)	(0.000,0.044)	GW	GW
NE-2	0.093	0.866	0.041	---	---	---	(0.000,0.362)	(0.598,1.000)	(0.000,0.242)	GW	GW
NE-3	0.029	0.913	0.058	---	---	---	(0.000,0.189)	(0.614,1.000)	(0.000,0.333)	GW	GW
NE-4	0.020	0.963	0.018	---	---	---	(0.000,0.128)	(0.793,1.000)	(0.000,0.113)	GW	GW
NE-5	0.012	0.973	0.015	---	---	---	(0.000,0.074)	(0.847,1.000)	(0.000,0.094)	GW	GW
NE-6	0.011	0.981	0.009	---	---	---	(0.000,0.065)	(0.890,1.000)	(0.000,0.053)	GW	GW
NE-7	0.010	0.973	0.017	---	---	---	(0.000,0.060)	(0.849,1.000)	(0.000,0.108)	GW	GW
NE-8	0.018	0.966	0.016	---	---	---	(0.000,0.116)	(0.810,1.000)	(0.000,0.103)	GW	GW
NE-9	0.016	0.955	0.029	---	---	---	(0.000,0.102)	(0.779,1.000)	(0.000,0.172)	GW	GW
NE-10	0.013	0.972	0.015	---	---	---	(0.000,0.081)	(0.845,1.000)	(0.000,0.093)	GW	GW
NE-11	0.023	0.952	0.026	---	---	---	(0.000,0.149)	(0.743,1.000)	(0.000,0.170)	GW	GW
NE-12	0.010	0.982	0.008	---	---	---	(0.000,0.060)	(0.902,1.000)	(0.000,0.046)	GW	GW
NE-13	0.006	0.985	0.009	---	---	---	(0.000,0.038)	(0.916,1.000)	(0.000,0.052)	GW	GW
NE-14	0.007	0.985	0.008	---	---	---	(0.000,0.042)	(0.915,1.000)	(0.000,0.050)	GW	GW
NE-15	0.006	0.988	0.006	---	---	---	(0.000,0.038)	(0.931,1.000)	(0.000,0.036)	GW	GW
NE-16	0.014	0.972	0.014	---	---	---	(0.000,0.089)	(0.845,1.000)	(0.000,0.086)	GW	GW
NE-17	0.006	0.986	0.008	---	---	---	(0.000,0.038)	(0.920,1.000)	(0.000,0.047)	GW	GW
NE-18	0.014	0.975	0.011	---	---	---	(0.000,0.089)	(0.858,1.000)	(0.000,0.069)	GW	GW
NE-19	0.007	0.982	0.011	---	---	---	(0.000,0.044)	(0.898,1.000)	(0.000,0.067)	GW	GW
NE-20	0.006	0.987	0.007	---	---	---	(0.000,0.037)	(0.929,1.000)	(0.000,0.039)	GW	GW
NE-21	0.017	0.969	0.014	---	---	---	(0.000,0.114)	(0.827,1.000)	(0.000,0.091)	GW	GW
NE-22	0.035	0.946	0.019	---	---	---	(0.000,0.233)	(0.707,1.000)	(0.000,0.119)	GW	GW
NE-23	0.009	0.974	0.017	---	---	---	(0.000,0.057)	(0.854,1.000)	(0.000,0.109)	GW	GW
NE-24	0.060	0.885	0.055	---	---	---	(0.000,0.345)	(0.551,1.000)	(0.000,0.330)	GW	GW

NE-25	0.024	0.920	0.056	---	---	---	(0.000,0.155)	(0.639,1.000)	(0.000,0.316)	GW	GW
NE-26	0.009	0.976	0.015	---	---	---	(0.000,0.055)	(0.866,1.000)	(0.000,0.093)	GW	GW
NE-27	0.015	0.959	0.025	---	---	---	(0.000,0.098)	(0.772,1.000)	(0.000,0.169)	GW	GW
NE-28	0.014	0.962	0.024	---	---	---	(0.000,0.089)	(0.787,1.000)	(0.000,0.159)	GW	GW
NE-29	0.015	0.970	0.015	---	---	---	(0.000,0.096)	(0.829,1.000)	(0.000,0.094)	GW	GW
NE-30	0.012	0.975	0.013	---	---	---	(0.000,0.075)	(0.857,1.000)	(0.000,0.084)	GW	GW
NE-31	0.009	0.978	0.012	---	---	---	(0.000,0.057)	(0.877,1.000)	(0.000,0.079)	GW	GW
NE-32	0.011	0.973	0.015	---	---	---	(0.000,0.070)	(0.849,1.000)	(0.000,0.099)	GW	GW
NE-33	0.014	0.909	0.078	---	---	---	(0.000,0.087)	(0.586,1.000)	(0.000,0.398)	GW	GW
NE-34	0.029	0.952	0.018	---	---	---	(0.000,0.183)	(0.766,1.000)	(0.000,0.120)	GW	GW
NE-35	0.009	0.981	0.010	---	---	---	(0.000,0.052)	(0.895,1.000)	(0.000,0.063)	GW	GW
NE-36	0.008	0.985	0.007	---	---	---	(0.000,0.046)	(0.916,1.000)	(0.000,0.043)	GW	GW
NE-37	0.012	0.965	0.022	---	---	---	(0.000,0.075)	(0.805,1.000)	(0.000,0.149)	GW	GW
NE-38	0.010	0.984	0.007	---	---	---	(0.000,0.058)	(0.909,1.000)	(0.000,0.040)	GW	GW
NE-39	0.013	0.972	0.015	---	---	---	(0.000,0.082)	(0.847,1.000)	(0.000,0.096)	GW	GW
NE-40	0.012	0.977	0.011	---	---	---	(0.000,0.076)	(0.869,1.000)	(0.000,0.069)	GW	GW

APPENDIX C. Q-scores from program Structure inferred at K = 2 for each individual (*n* = 161) in main analysis. ID for each individual indicates study unit of residency (AP = Algonquin Provincial Park, KH = Kawartha Highlands, 47 = WMU47, 49 = WMU49, NE = Northeast Ontario [outgroup]). Q-scores are from the run with the lowest variance. Also shown are 90% credible regions for each cluster and assignment as either coyote/eastern wolf (C/E), gray wolf (Gray), or hybrid.

	Q-Scores		Credible Regions		
ID	C/E	Gray	90% C/E	90% Gray	Assignment
AP-1	0.984	0.016	(0.903,1.000)	(0.000,0.097)	C/E
AP-2	0.518	0.482	(0.062,1.000)	(0.000,0.938)	Hybrid
AP-3	0.921	0.079	(0.581,1.000)	(0.000,0.419)	C/E
AP-4	0.922	0.078	(0.642,1.000)	(0.000,0.358)	C/E
AP-5	0.987	0.013	(0.921,1.000)	(0.000,0.079)	C/E
AP-6	0.978	0.022	(0.865,1.000)	(0.000,0.135)	C/E
AP-7	0.895	0.105	(0.540,1.000)	(0.000,0.460)	C/E
AP-8	0.896	0.104	(0.520,1.000)	(0.000,0.480)	C/E
AP-9	0.986	0.014	(0.919,1.000)	(0.000,0.081)	C/E
AP-10	0.97	0.030	(0.814,1.000)	(0.000,0.186)	C/E
AP-11	0.987	0.013	(0.923,1.000)	(0.000,0.077)	C/E
AP-12	0.979	0.021	(0.869,1.000)	(0.000,0.131)	C/E
AP-13	0.988	0.012	(0.930,1.000)	(0.000,0.070)	C/E
AP-14	0.801	0.199	(0.233,1.000)	(0.000,0.767)	C/E
AP-15	0.167	0.833	(0.000,0.559)	(0.441,1.000)	Gray
AP-16	0.982	0.018	(0.891,1.000)	(0.000,0.109)	C/E
AP-17	0.985	0.015	(0.907,1.000)	(0.000,0.093)	C/E
AP-18	0.907	0.093	(0.620,1.000)	(0.000,0.380)	C/E
AP-19	0.989	0.011	(0.935,1.000)	(0.000,0.065)	C/E
AP-20	0.989	0.011	(0.933,1.000)	(0.000,0.067)	C/E
AP-21	0.986	0.014	(0.914,1.000)	(0.000,0.086)	C/E
AP-22	0.981	0.019	(0.887,1.000)	(0.000,0.113)	C/E
AP-23	0.97	0.030	(0.814,1.000)	(0.000,0.186)	C/E
AP-24	0.939	0.061	(0.673,1.000)	(0.000,0.327)	C/E
AP-25	0.99	0.010	(0.938,1.000)	(0.000,0.062)	C/E
AP-26	0.916	0.084	(0.588,1.000)	(0.000,0.412)	C/E
AP-27	0.974	0.026	(0.844,1.000)	(0.000,0.156)	C/E
AP-28	0.886	0.114	(0.511,1.000)	(0.000,0.489)	C/E
AP-29	0.445	0.555	(0.002,0.819)	(0.181,0.998)	Hybrid
AP-30	0.987	0.013	(0.920,1.000)	(0.000,0.080)	C/E
AP-31	0.721	0.279	(0.326,1.000)	(0.000,0.674)	Hybrid
AP-32	0.336	0.664	(0.000,0.795)	(0.205,1.000)	Hybrid
AP-33	0.926	0.074	(0.637,1.000)	(0.000,0.363)	C/E
AP-34	0.941	0.059	(0.695,1.000)	(0.000,0.305)	C/E
AP-35	0.928	0.072	(0.638,1.000)	(0.000,0.362)	C/E

AP-36	0.487	0.513	(0.004,1.000)	(0.000,0.996)	Hybrid
AP-37	0.532	0.468	(0.012,1.000)	(0.000,0.988)	Hybrid
AP-38	0.47	0.530	(0.000,1.000)	(0.000,1.000)	Hybrid
AP-39	0.632	0.368	(0.178,1.000)	(0.000,0.822)	Hybrid
AP-40	0.681	0.319	(0.296,1.000)	(0.000,0.704)	Hybrid
KH-1	0.975	0.025	(0.846,1.000)	(0.000,0.154)	C/E
KH-2	0.866	0.134	(0.509,1.000)	(0.000,0.491)	C/E
KH-3	0.938	0.062	(0.695,1.000)	(0.000,0.305)	C/E
KH-4	0.816	0.184	(0.536,1.000)	(0.000,0.464)	C/E
KH-5	0.991	0.009	(0.945,1.000)	(0.000,0.055)	C/E
KH-6	0.988	0.012	(0.928,1.000)	(0.000,0.072)	C/E
KH-7	0.886	0.114	(0.525,1.000)	(0.000,0.475)	C/E
KH-8	0.982	0.018	(0.890,1.000)	(0.000,0.110)	C/E
KH-9	0.943	0.057	(0.710,1.000)	(0.000,0.290)	C/E
KH-10	0.986	0.014	(0.916,1.000)	(0.000,0.084)	C/E
KH-11	0.928	0.072	(0.642,1.000)	(0.000,0.358)	C/E
KH-12	0.991	0.009	(0.945,1.000)	(0.000,0.055)	C/E
KH-13	0.952	0.048	(0.736,1.000)	(0.000,0.264)	C/E
KH-14	0.958	0.042	(0.759,1.000)	(0.000,0.241)	C/E
KH-15	0.984	0.016	(0.900,1.000)	(0.000,0.100)	C/E
KH-16	0.988	0.012	(0.927,1.000)	(0.000,0.073)	C/E
KH-17	0.967	0.033	(0.795,1.000)	(0.000,0.205)	C/E
KH-18	0.978	0.022	(0.866,1.000)	(0.000,0.134)	C/E
KH-19	0.983	0.017	(0.900,1.000)	(0.000,0.100)	C/E
KH-20	0.987	0.013	(0.924,1.000)	(0.000,0.076)	C/E
KH-21	0.694	0.306	(0.424,0.999)	(0.001,0.576)	Hybrid
47-1	0.671	0.329	(0.062,1.000)	(0.000,0.938)	Hybrid
47-2	0.985	0.015	(0.909,1.000)	(0.000,0.091)	C/E
47-3	0.303	0.697	(0.000,0.696)	(0.304,1.000)	Hybrid
47-4	0.100	0.900	(0.000,0.398)	(0.602,1.000)	Gray
47-5	0.965	0.035	(0.796,1.000)	(0.000,0.204)	C/E
47-6	0.937	0.063	(0.654,1.000)	(0.000,0.346)	C/E
47-7	0.971	0.029	(0.821,1.000)	(0.000,0.179)	C/E
47-8	0.077	0.923	(0.000,0.351)	(0.649,1.000)	Gray
47-9	0.962	0.038	(0.778,1.000)	(0.000,0.222)	C/E
47-10	0.574	0.426	(0.252,0.887)	(0.113,0.748)	Hybrid
47-11	0.020	0.980	(0.000,0.121)	(0.879,1.000)	Gray
47-12	0.962	0.038	(0.774,1.000)	(0.000,0.226)	C/E
47-13	0.789	0.211	(0.373,1.000)	(0.000,0.627)	Hybrid
47-14	0.825	0.175	(0.396,1.000)	(0.000,0.604)	C/E
47-15	0.922	0.078	(0.596,1.000)	(0.000,0.404)	C/E
47-16	0.804	0.196	(0.386,1.000)	(0.000,0.614)	C/E
47-17	0.936	0.064	(0.641,1.000)	(0.000,0.359)	C/E

47-18	0.727	0.273	(0.285,1.000)	(0.000,0.715)	Hybrid
49-1	0.829	0.171	(0.473,1.000)	(0.000,0.527)	C/E
49-2	0.890	0.110	(0.562,1.000)	(0.000,0.438)	C/E
49-3	0.945	0.055	(0.715,1.000)	(0.000,0.285)	C/E
49-4	0.682	0.318	(0.261,1.000)	(0.000,0.739)	Hybrid
49-5	0.975	0.025	(0.848,1.000)	(0.000,0.152)	C/E
49-6	0.935	0.065	(0.668,1.000)	(0.000,0.332)	C/E
49-7	0.428	0.572	(0.043,0.789)	(0.211,0.957)	Hybrid
49-8	0.980	0.020	(0.879,1.000)	(0.000,0.121)	C/E
49-9	0.937	0.063	(0.643,1.000)	(0.000,0.357)	C/E
49-10	0.981	0.019	(0.883,1.000)	(0.000,0.117)	C/E
49-11	0.970	0.030	(0.817,1.000)	(0.000,0.183)	C/E
49-12	0.920	0.080	(0.622,1.000)	(0.000,0.378)	C/E
49-13	0.918	0.082	(0.586,1.000)	(0.000,0.414)	C/E
49-14	0.972	0.028	(0.828,1.000)	(0.000,0.172)	C/E
49-15	0.800	0.200	(0.408,1.000)	(0.000,0.592)	C/E
49-16	0.985	0.015	(0.909,1.000)	(0.000,0.091)	C/E
49-17	0.970	0.030	(0.820,1.000)	(0.000,0.180)	C/E
49-18	0.979	0.021	(0.870,1.000)	(0.000,0.130)	C/E
49-19	0.937	0.063	(0.665,1.000)	(0.000,0.335)	C/E
49-20	0.904	0.096	(0.564,1.000)	(0.000,0.436)	C/E
49-21	0.986	0.014	(0.916,1.000)	(0.000,0.084)	C/E
49-22	0.951	0.049	(0.707,1.000)	(0.000,0.293)	C/E
49-23	0.976	0.024	(0.851,1.000)	(0.000,0.149)	C/E
49-24	0.988	0.012	(0.928,1.000)	(0.000,0.072)	C/E
49-25	0.973	0.027	(0.833,1.000)	(0.000,0.167)	C/E
49-26	0.970	0.030	(0.817,1.000)	(0.000,0.183)	C/E
49-27	0.984	0.016	(0.899,1.000)	(0.000,0.101)	C/E
49-28	0.981	0.019	(0.881,1.000)	(0.000,0.119)	C/E
49-29	0.980	0.020	(0.879,1.000)	(0.000,0.121)	C/E
49-30	0.974	0.026	(0.839,1.000)	(0.000,0.161)	C/E
49-31	0.976	0.024	(0.857,1.000)	(0.000,0.143)	C/E
49-32	0.973	0.027	(0.835,1.000)	(0.000,0.165)	C/E
49-33	0.965	0.035	(0.791,1.000)	(0.000,0.209)	C/E
49-34	0.975	0.025	(0.846,1.000)	(0.000,0.154)	C/E
49-35	0.973	0.027	(0.833,1.000)	(0.000,0.167)	C/E
49-36	0.963	0.037	(0.774,1.000)	(0.000,0.226)	C/E
49-37	0.988	0.012	(0.929,1.000)	(0.000,0.071)	C/E
49-38	0.986	0.014	(0.915,1.000)	(0.000,0.085)	C/E
49-39	0.975	0.025	(0.846,1.000)	(0.000,0.154)	C/E
49-40	0.987	0.013	(0.923,1.000)	(0.000,0.077)	C/E
49-41	0.975	0.025	(0.848,1.000)	(0.000,0.152)	C/E
49-42	0.966	0.034	(0.799,1.000)	(0.000,0.201)	C/E

NE-1	0.011	0.989	(0.000,0.065)	(0.935,1.000)	Gray
NE-2	0.107	0.893	(0.000,0.362)	(0.638,1.000)	Gray
NE-3	0.058	0.942	(0.000,0.314)	(0.686,1.000)	Gray
NE-4	0.025	0.975	(0.000,0.153)	(0.847,1.000)	Gray
NE-5	0.019	0.981	(0.000,0.114)	(0.886,1.000)	Gray
NE-6	0.014	0.986	(0.000,0.084)	(0.916,1.000)	Gray
NE-7	0.018	0.982	(0.000,0.107)	(0.893,1.000)	Gray
NE-8	0.025	0.975	(0.000,0.150)	(0.850,1.000)	Gray
NE-9	0.029	0.971	(0.000,0.171)	(0.829,1.000)	Gray
NE-10	0.019	0.981	(0.000,0.115)	(0.885,1.000)	Gray
NE-11	0.033	0.967	(0.000,0.200)	(0.800,1.000)	Gray
NE-12	0.013	0.987	(0.000,0.075)	(0.925,1.000)	Gray
NE-13	0.010	0.990	(0.000,0.060)	(0.940,1.000)	Gray
NE-14	0.011	0.989	(0.000,0.063)	(0.937,1.000)	Gray
NE-15	0.009	0.991	(0.000,0.052)	(0.948,1.000)	Gray
NE-16	0.020	0.980	(0.000,0.121)	(0.879,1.000)	Gray
NE-17	0.010	0.990	(0.000,0.059)	(0.941,1.000)	Gray
NE-18	0.018	0.982	(0.000,0.106)	(0.894,1.000)	Gray
NE-19	0.012	0.988	(0.000,0.071)	(0.929,1.000)	Gray
NE-20	0.009	0.991	(0.000,0.054)	(0.946,1.000)	Gray
NE-21	0.022	0.978	(0.000,0.131)	(0.869,1.000)	Gray
NE-22	0.038	0.962	(0.000,0.227)	(0.773,1.000)	Gray
NE-23	0.016	0.984	(0.000,0.096)	(0.904,1.000)	Gray
NE-24	0.082	0.918	(0.000,0.394)	(0.606,1.000)	Gray
NE-25	0.052	0.948	(0.000,0.285)	(0.715,1.000)	Gray
NE-26	0.016	0.984	(0.000,0.096)	(0.904,1.000)	Gray
NE-27	0.027	0.973	(0.000,0.165)	(0.835,1.000)	Gray
NE-28	0.026	0.974	(0.000,0.162)	(0.838,1.000)	Gray
NE-29	0.022	0.978	(0.000,0.131)	(0.869,1.000)	Gray
NE-30	0.017	0.983	(0.000,0.105)	(0.895,1.000)	Gray
NE-31	0.016	0.984	(0.000,0.094)	(0.906,1.000)	Gray
NE-32	0.019	0.981	(0.000,0.116)	(0.884,1.000)	Gray
NE-33	0.045	0.955	(0.000,0.263)	(0.737,1.000)	Gray
NE-34	0.036	0.964	(0.000,0.199)	(0.801,1.000)	Gray
NE-35	0.013	0.987	(0.000,0.079)	(0.921,1.000)	Gray
NE-36	0.011	0.989	(0.000,0.063)	(0.937,1.000)	Gray
NE-37	0.021	0.979	(0.000,0.129)	(0.871,1.000)	Gray
NE-38	0.011	0.989	(0.000,0.068)	(0.932,1.000)	Gray
NE-39	0.019	0.981	(0.000,0.115)	(0.885,1.000)	Gray
NE-40	0.015	0.985	(0.000,0.091)	(0.909,1.000)	Gray

APPENDIX D. Mean prey availability and road densities within home ranges (95% fixed kernels) of study animals across the 4 study units of my study area in and adjacent to Algonquin Provincial Park (2004-2011). Shown are primary (1°), secondary (2°), and tertiary (3°) road densities (km/km^2), mean estimated moose density (#/km^2), and proportion of deer wintering habitat in each home range, and number of home ranges (*n*).

	1° Rd	SE	2° Rd	SE	3° Rd	SE	Deer	SE	Moose	SE	*n*
APP	0.02	0.01	0.09	0.02	0.59	0.04	0.03	0.01	0.30	0.02	34
WMU49	0.09	0.03	0.57	0.06	0.25	0.02	0.08	0.02	0.20	0.02	32
KH	0.05	0.02	0.34	0.07	0.41	0.08	0.26	0.09	0.23	0.03	17
WMU47	0.08	0.08	0.24	0.05	0.36	0.24	0.00	0.00	0.25	0.04	2

APPENDIX E. Mitochondrial DNA haplotypes from individuals included in my main analysis of resident wolves, coyotes, and hybrids from central Ontario inhabiting 4 study units (Algonquin Provincial Park [APP], WMU49, Kawartha Highlands [KH], and WMU47), 2004-2011. EW= eastern wolf, GW = gray wolf, C = coyotes, 3-type = EW × C × GW hybrid. I had no mtDNA haplotype data (No Data) for 6 males whose genotypes were reconstructed (see Materials and Methods in main text and Supporting Methods).

Haplotype	APP	WMU49	KH	WMU47	EW	Coyote	GW	EW × C	EW × GW	GW × C	3-type
C1	0	1	3	2	0	3	1	0	2	1	0
C13	1	0	3	0	4	0	0	0	0	0	0
C14	22	6	0	4	17	6	1	3	4	0	0
C19	7	14	5	4	9	4	0	9	4	3	0
C22	0	0	0	1	0	0	1	0	0	0	0
C9	8	17	9	8	6	22	0	8	2	4	1
No Data	2	4	0	0	1	3	0	1	0	1	0
Totals	40	42	20	19	37	38	3	21	12	9	1

APPENDIX F. Y-Chromosome haplotypes based on 4 microsatellites loci (see Supporting Methods) from individuals included in my main analysis of resident wolves, coyotes, and hybrids from central Ontario inhabiting 4 study units (Algonquin Provincial Park [APP], WMU49, Kawartha Highlands [KH], and WMU47), 2004-2011. EW= eastern wolf, GW = gray wolf, C = coyotes, 3-type = EW × C × GW hybrid. Details on specific microsatellite alleles corresponding to haplotypes can be found in Rutledge *et al.* (2010), Wheeldon *et al.* (2010).

Haplotype	APP	WMU49	KH	WMU47	EW	Coyote	GW	EW × C	EW × GW	GW × C
AA	4	9	7	7	7	7	2	10	1	1
AF	0	0	1	3	2	0	0	2	0	0
BB	9	1	0	1	6	1	0	0	2	2
CD	2	3	1	1	0	5	0	3	0	0
CE	1	0	0	1	0	0	1	1	0	0
CM	0	5	0	0	0	2	0	0	1	1
CS	1	1	0	0	0	0	0	1	1	0
GP	1	3	1	0	1	4	0	0	0	0
No Data	1	1	1	0	1	1	0	0	0	1
Totals	19	23	11	13	17	19	3	17	5	5

APPENDIX G. Results of z-tests and parameter estimates for variables in all models with $\Delta AIC_c < 2$ in the overall analysis outside APP. These results are from models 2-8 in Table 3.3 whereas results from model 1 is in Ch. 3 (see Results). Hazard ratios provided for dummy coded variables in relation to the reference group which is listed for each variable in Table 3.1.

Model 2	z	*P*	Hazard	Lower	Upper
Resident	-3.9	<0.001	0.36	0.30	0.75
Eastern Wolf	2.9	0.004	2.04	1.26	3.31
Male	1.2	0.214	1.42	0.82	2.37

Model 3	z	*P*	Hazard	Lower	Upper
Resident	-3.6	<0.001	0.45	0.22	0.64
Eastern Wolf	3.1	0.002	2.15	1.32	3.51
Adult	-1.1	0.252	0.71	0.39	1.28

Model 4	z	*P*	Hazard	Lower	Upper
Resident	-4.0	<0.001	0.34	0.20	0.58

Model 5	z	*P*	Hazard	Lower	Upper
Resident	-4.1	<0.001	0.35	0.21	0.58
Eastern Wolf	3.1	0.002	2.32	1.36	3.97
Hybrid†	0.8	0.417	1.30	0.69	2.47

Model 6	z	*P*	Hazard	Lower	Upper
Resident	-4.0	<0.001	0.35	0.21	0.58
Eastern Wolf	2.9	0.003	2.01	1.26	3.27
2010	0.8	0.426	1.29	0.69	2.43

Model 7	z	*P*	Hazard	Lower	Upper
Resident	-4.1	<0.001	0.35	0.21	0.58
Eastern Wolf	2.5	0.012	1.96	1.16	3.30
Coyote	-0.58	0.562	0.82	0.43	1.58

Model 8	z	*P*	Hazard	Lower	Upper
Resident	-3.2	0.001	0.40	0.24	0.70
Eastern Wolf	2.9	0.004	2.07	1.26	3.42
Male	1.3	0.192	1.44	0.83	2.51
Adult	0.2	0.234	0.69	0.38	1.27

† Eastern wolf × coyote hybrid

APPENDIX H. Eastern wolf × secondary road density supporting analysis

To ensure that the importance of the eastern wolf × secondary roads interaction was not unduly influenced by data from any single individual mortality event, I sequentially removed data from each resident eastern wolf that died during the study (*n* = 10). For these supporting analyses, I included all variables retained in the top model (i.e., deer availability, secondary road density, 2010, and the eastern wolf × secondary road density interaction) and ran 10 separate models, each with data from a different eastern wolf mortality excluded and assessed the significance of the eastern wolf × secondary road density interaction. Next, for each of these 10 models, I changed the reference group from all other (non-eastern wolf) genotypes to eastern wolf to examine significance tests between eastern wolves and each of the other genotypes individually (i.e. coyotes, eastern wolf × coyote hybrids, and admixed gray wolves).

The results did not change appreciably except when I removed the eastern wolf whose home range had the highest secondary road density (EW10, Table G). Data from this individual clearly had a strong influence on my results. That the most extreme data-point would influence the results of an analysis with a relatively small number of mortalities was not surprising. However, even with data from this individual excluded, eastern wolves still survived marginally significantly worse than all other *Canis* types combined ($P = 0.060$) in relation to increased secondary road density. Specifically, eastern wolves survived marginally significantly worse than coyotes ($P = 0.053$), eastern wolf × coyote hybrids ($P = 0.091$), and admixed gray wolves ($P = 0.091$) in relation to increasing secondary road density (Table G). Thus, even though the importance and significance of this interaction was strongly influenced by data from EW10, the importance of the interaction remained, and a marginally significant trend was still evident.

Studying survival of rare species often results in smaller sample sizes and lower statistical power than desirable under optimal circumstances. However, despite the relatively low sample size, I have clearly sampled a high proportion of resident wolves and coyotes in my study units . Thus, the sample size may be low from the perspective of optimal statistical power, but my sample should be representative of the survival capabilities of the different *Canis* types in and adjacent to APP. Therefore, my results represent valuable information for understanding hybridization dynamics in the APP hybrid zone and for developing conservation strategies for eastern wolves.

Table H. Significance of genotype × secondary road density interactions when survival data from individual eastern wolves that died (n = 10) were excluded from analyses. For each eastern wolf mortality excluded I show: road densities within the home range of the excluded wolf and P-values from comparison of eastern wolf survival with: all other genotypes combined (All), coyotes (vs. Coyotes), coyotes × eastern wolves (vs. Coyote x EW), and admixed gray wolves (vs. Admixed GW). In all cases eastern wolf survival was lower than other *Canis* types, either significantly ($P < 0.05$) or marginally significantly ($0.05 < P < 0.10$).

Wolf	2° Rd Density	All	vs. Coyotes	vs. Coyote × EW	vs. Admixed GW
EW1	0.00	<0.001	<0.001	<0.001	0.011
EW2	<0.01	<0.001	<0.001	<0.001	0.012
EW3	0.04	<0.001	<0.001	<0.001	0.015
EW4	0.07	<0.001	<0.001	<0.001	0.024
EW5	0.10	<0.001	<0.001	<0.001	0.011
EW6	0.13	<0.001	<0.001	0.002	0.017
EW7	0.14	<0.001	<0.001	0.001	0.018
EW8	0.23	<0.001	<0.001	0.003	0.029
EW9	0.29	<0.001	<0.001	0.004	0.029
EW10	0.53	0.060	0.053	0.091	0.091