

**SPATIAL AND TEMPORAL GENETIC STRUCTURE
OF WOLVERINE POPULATIONS**

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ABSTRACT

SPATIAL AND TEMPORAL GENETIC STRUCTURE OF WOLVERINE POPULATIONS

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Habitat loss and fragmentation can disrupt population connectivity, resulting in small, isolated populations and low genetic variability. Understanding connectivity patterns in space and time is critical in conservation and management planning, especially for wide-ranging species in northern latitudes where habitats are becoming increasingly fragmented. Wolverines (*Gulo gulo*) share similar life history traits observed in large-sized carnivores, and their low resiliency to disturbances limits wolverine persistence in modified or fragmented landscapes - making them a good indicator species for habitat connectivity. In this thesis, I used neutral microsatellite and mitochondrial DNA markers to investigate genetic connectivity patterns of wolverines for different temporal and spatial scales. Population genetic analyses of individuals from North America suggested wolverines west of James Bay in Canada are structured into two contemporary genetic clusters: an extant cluster at the eastern periphery of Manitoba and Ontario, and a northwestern core cluster. Haplotypic composition, however, suggested longstanding differences between the extant eastern periphery and northwestern core clusters. Phylogeographic analyses across the wolverine's Holarctic distribution supported a postglacial expansion from a glacial refugium near Beringia. Although Approximate Bayesian computations suggested a west-to-east stepping-stone divergence pattern across North America, a mismatch distribution indicated a historic bottleneck event approximately 400 generations ago likely influenced present-day patterns of haplotype

distribution. I also used an individual-based genetic distance measure to identify landscape features potentially influencing pairwise genetic distances of wolverines in Manitoba and Ontario. Road density and mean spring snow cover were positively associated with genetic distances. Road density was associated with female genetic distance, while spring snow cover variance was associated with male genetic distance. My findings suggest that northward expanding anthropogenic disturbances have the potential to affect genetic connectivity. Overall, my findings suggest that (1) peripheral populations can harbour genetic variants not observed in core populations - increasing species genetic diversity; (2) historic bottlenecks can alter the genetic signature of glacial refugia, resulting in a disjunct distribution of unique genetic variants among contemporary populations; (3) increased temporal resolution of the individual-based genetic distance measure can help identify landscape features associated with genetic connectivity within a population, which may disrupt landscape connectivity.

KEY WORDS

Approximate Bayesian Computation, circuit theory, conservation genetics, genetic structure, *Gulo gulo*, Holarctic species, landscape genetics, landscape resistance, microsatellites, mitochondrial DNA, peripheral population, postglacial recolonization, phylogeography, wolverine

PREFACE

My dissertation has been written in manuscript format. Chapter 2 has been published in Conservation Genetics and Chapter 3 has been published in PLoS ONE. My research has been done in collaboration with others, and therefore the plural "we" and "our" have been used in my dissertation. The full citation of each publication has been included on the title page of the respective chapter.

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CHAPTER 1

GENERAL INTRODUCTION

Vulnerability of high-latitude ecosystems to disturbances and carnivore range contraction

Climate change and land-use are key drivers of environmental change and the loss of biodiversity (Lovejoy and Hannah 2005; Haines-Young 2009; Riordan and Rundel 2014). Although increases in mean global surface temperatures are widespread, the rate of temperature increase for higher northern latitudes is almost twice the global average (Intergovernmental Panel on Climate Change, IPCC 2007; Goetz et al. 2011). This has led to more pronounced impacts of climate change for arctic and subarctic regions in comparison to other global habitats (Scholze et al. 2006). Potential effects of warmer temperatures on northern, high-latitude ecosystems include increased habitat loss and fragmentation due to higher fire frequencies (Stocks 2004) and the poleward movement of lower-latitude species and associated disease or pest outbreaks (Kirschbaum and Fischlin 1995; Mills et al. 2010). Since the late 20th century, arctic and subarctic regions have also experienced a northward expansion of mineral, oil and gas extraction (Klein 2000; Anttonen et al. 2011), further contributing to habitat loss and fragmentation (Jones and Pejchar 2013). Even with increased temperature stress and anthropogenic activity, arctic tundra and boreal forest biomes remain largely intact (Ruckstuhl et al. 2008; Conservation of Arctic Flora and Fauna, CAFF 2003). However, the environmental conditions and ecological systems that characterize these polar regions make them particularly vulnerable to changing disturbance regimes.

Arctic and subarctic regions are characterized by long winters, low temperatures, short growing seasons, and low nutrient availability, which limit primary productivity (Warren-Wilson 1966; Ricketts et al. 1999; Foster and Bhatti 2006). Reduced primary production leads to lower biodiversity and simpler trophic structures (Murray et al. 1998; CAFF 2003) that have few alternative or shared food sources at each trophic level (Miller 1989). An inverse relationship has been observed for multiple carnivores between species home range size and habitat productivity (Herfindal et al. 2005; Nilsen et al. 2005). This low primary productivity may explain why home ranges of carnivores are larger at higher latitudes (e.g., wolverine *Gulo gulo*; Banci and Harestad 1990; Banci 1994), highlighting their dependence on large intact and/or connected tracts of suitable habitat. Vulnerability of carnivores to disturbances is influenced not only by wide-ranging behaviours, but also by natural history traits.

Large home range requirements of mammalian carnivores, in conjunction with intrinsic life-history characteristics such as low population densities, large body size, and slow intrinsic population growth rates make them vulnerable to habitat loss and fragmentation (Noss et al. 1996; Woodroffe and Ginsberg 1998; Crooks 2002; Davidson et al. 2009). Global comparisons of contemporary and historic species' ranges reveal many mammalian predators have experienced range contractions (Ceballos and Ehrlich 2002; Laliberte and Ripple 2004; Morrison et al. 2007; Pillay et al. 2011). Habitat loss and fragmentation can lead to reduced population abundance, the loss of genetic diversity, and increased genetic subdivision due to decreased connectivity (Flather and Bevers 2002; Rubidge et al. 2012; Ruell et al. 2012; Koen et al. 2014). Carnivores are among the most threatened terrestrial mammals (Schipper et al. 2008); approximately 27% of extant carnivores are at risk of extinction according to the International Union for Conservation

of Nature and Natural Resources (IUCN) Red List of threatened species (IUCN 2012). Predators play a central role in regulating the structure and function of ecosystems, and their absence may lead to the reconfiguration or loss of biological diversity (Estes et al. 2011).

Ecological role of large carnivores and importance of habitat connectivity

Mammalian carnivores in terrestrial ecosystems are ecologically important because of their regulatory effect in shaping community structure (Beschta and Ripple 2009; Prugh et al. 2009; Ripple et al. 2014). Typically carnivores exert top-down control in ecosystems, where predation not only influences the abundance of species at the next lower trophic level (herbivore prey) but the effects cascade down to the primary producer (plants) level. Large carnivores (>15 kg) tend to be specialists due to their selective killing of larger prey, while medium-sized generalist predators (mesocarnivores) affect a broader range of prey species (Prugh et al. 2009; Roemer et al. 2009; Khali et al. 2014). The decline and eventual extirpation of larger-bodied carnivores may result in increased ungulate densities, leading to increased browsing intensity and an overall reduction in species abundance (Ripple and Beschta 2006; Beschta and Ripple 2009). Loss of large carnivores may also result in mesopredator release (increased abundance), as medium-sized carnivores are no longer suppressed through predation and intraguild competition (Prugh et al. 2009; Ripple et al. 2014). The importance of carnivores to maintaining ecosystem function and their requirement of large, unfragmented habitats are reflected in the development of conservation and management plans to prevent further loss of these predators (e.g., Eurasian lynx *Lynx lynx*; Breitenmoser et al. 2000; snow leopard *Panthera uncia*; Dhakal et al. 2013).

Connectivity is a critical component to understand in the context of conservation and management planning, especially for large-sized carnivores that have extensive ranges in northern latitudes where habitats are becoming increasingly fragmented. Past studies are hampered by traditional field-based methods (e.g., radio telemetry, mark-recapture) that are often limited in sample size and spatial or temporal scale (Proctor et al. 2005; Moore et al. 2014). This information is important for developing conservation and management measures, and may provide insight into future connectivity patterns based on assumed habitat preferences and in the context of climate change. For example, habitat fragmentation can result in genetic sub-structuring of large terrestrial carnivores regardless of their high rate of dispersal, emphasizing the value of corridors in maintaining genetic connectivity (Dutta et al. 2013). Conversely, spatial genetic structure in the absence of fragmentation may be reflective of a climatic gradient like snow cover (Stenseth et al. 2004; Row et al. 2014), biased dispersal towards natal habitat and prey specialization (Pilot et al. 2006), or multiple glacial refugia (Ruiz-González et al. 2013). In addition, populations closer to range peripheries may display lower neutral genetic diversity but greater genetic uniqueness in comparison to core populations that inhabit interior regions of range distributions (Cheng et al. 2014). Extreme environmental conditions at range peripheries and reduced gene flow with core populations may result in genetically distinct edge populations with biologically significant differences (Santamaria et al. 2003; Cassel-Lundhagen et al. 2009; Hardie and Hutchings 2010). Under these scenarios, each population would likely be managed as a separate unit to ensure that unique, locally adapted alleles are retained, in turn increasing the species overall genetic diversity and adaptive potential (Parmesan 2006; Cheng et al. 2014; Hazlitt et al. 2014). Although neutral diversity has limited predictive ability of adaptive genetic variation

(Reed and Frankham 2001), making neutral markers unsuitable surrogates of variation in fitness-related loci (Aguilar et al. 2004) and adaptive variation (Zeisset and Beebee 2010), both neutral and adaptive markers are influenced by drift and gene flow (Koen et al. 2014). Thus, spatial variance of neutral genetic diversity across the species range may reflect patterns of adaptive genetic diversity. Lastly, information on population structure can serve as a baseline to evaluate how management actions affect wildlife populations and distributions.

Understanding spatial and temporal gene flow patterns through genetic approaches

Non-invasive genetic approaches have proven effective in sampling elusive, low-density species (Henry and Russello 2011; McCall et al. 2013), with DNA obtained from hair, feathers, feces, and skin exuvates (Miño and Del Lama 2009; Harris et al. 2010; Khedkar et al. 2014; Sugimoto et al. 2014). One drawback with non-invasive sampling is low DNA quantity (Taberlet et al. 1999; Kelly et al. 2012), which can result in genotyping errors due to allelic dropout and false alleles (Taberlet et al. 1996; Pompanon et al. 2005; Beja-Pereira et al. 2009). Ongoing advancements in technical and analytical methods have contributed to minimizing genotyping errors associated with non-invasive genetic samples (e.g., Taberlet et al. 1996; McKelvey and Schwartz 2005; Schwartz and Monfort 2008; Beja-Pereira et al. 2009). Non-invasive genetic sampling is now widely used in wildlife research.

The majority of genetic studies in ecological research have been based on neutral genetic markers (Kirk and Freeland 2011), with microsatellites and mitochondrial DNA (mtDNA) being two of the most popular (Odgen et al. 2009; Abdul-Muneer 2014). Microsatellites are widely used as genetic markers due to their bi-paternal inheritance and high polymorphism (Chambers and MacAvoy 2000), allowing for individual animal

identification (Oliveira et al. 2006) and parentage analysis (Moore et al. 2014). Nuclear markers have an estimated mutation rate of $\mu = 4.5 \times 10^{-4}$ (Whittaker et al. 2003), providing a resolution of recent and ongoing microevolutionary processes (Wang 2010). Analysis of multilocus genotype data using spatially (Guillot et al. 2005; Chen et al. 2007) and non-spatially (Pritchard et al. 2000) Bayesian clustering models provides insight into contemporary population structure and connectivity (Norén et al. 2011). More recently, the emergence of landscape genetics has allowed researchers to evaluate the influence of geographical and environmental features on genetic variation at both population and individual levels (Murphy et al. 2010; Garroway et al. 2011). In comparison, mitochondrial DNA is maternally inherited as a single locus (Avisé 1994; Brito and Edwards 2009), allowing for sex-biased dispersal to be determined when directly compared to nuclear microsatellites (Pelletier et al. 2011). Its high copy number within cells, lack of recombination, estimated mutation rate of $\mu = 6.2 \times 10^{-8}$ (Haag-Liautard et al. 2008), and associated intraspecific polymorphism have contributed to mtDNA being widely used in phylogeographic studies investigating molecular signatures of historical microevolutionary processes (Avisé 2000; Hickerson et al. 2010; Wang 2010). Analysis of phylogenetic data in the context of species' geographic distributions (i.e., phylogeography) allows for inferences to be made regarding the location of refugia during glacial maximums (Provan and Bennett 2008) and postglacial recolonization routes (Taberlet et al. 1998). Additionally, Bayesian coalescent analyses can be used to estimate time to most recent common ancestor, and demographic history can be inferred from skyline plots (Ho and Shapiro 2011; Klütsch et al. 2012).

Each of these three fields of genetic research – population genetics, landscape genetics and phylogeography – provides spatial and temporal insight into species'

distributions, population connectivity, ecology and evolutionary history. However, the knowledge that each approach provides on its own represents only a fraction of the information needed to discern the effects of environmental change on species persistence. Identifying evolutionarily distinct lineages and maximally preserving diversity rely on understanding how both historic and contemporary processes contribute to the observed geographic patterns of genetic structure (Pilgrim et al. 2012). By applying an integrative framework, information from these three fields of genetic research can be used to evaluate the effects of climate change and anthropogenic activities and, in turn, better inform conservation and management strategies. This approach is applicable across a wide range of taxonomic groups, and is particularly informative for species facing a high risk of extinction due to their large home range requirements, low population densities, and low fecundity (Cardillo et al. 2005; Davidson et al. 2009).

Study Species

Distribution and status

The wolverine is the largest terrestrial member of the Mustelidae and has a circumboreal distribution (Hash 1987; Landa et al. 2000). In Eurasia, wolverines occur north of 48°N, extending eastwards across forest-tundra and taiga ecozones from Scandinavia to Siberia (Copeland and Whitman 2003). In North America, extant wolverine populations are limited to high-elevation forest and alpine habitats in the western United States (Aubry et al. 2007), extending north along the Rocky Mountain corridor into Canada and Alaska. In Canada, the range extends eastward across the boreal forest-tundra ecozone all the way to the Atlantic coast, with the exception of Prince Edward Island, Nova Scotia and the island of Newfoundland (Figure 1.1; Banfield 1974; Copeland and Whitman 2003; Committee on the Status of Endangered Wildlife in

Canada, COSEWIC 2003). Wolverine density in Canada is highest in the northwest. The Yukon Territory is estimated to have 5.64 - 10.75 wolverines/1000 km² (Banci and Harestad 1990) and Northwest Territories has an estimated 4.6 - 17.2 wolverines/1000 km² (Mulders et al. 2007; Boulanger and Mulders 2008), where both territories encompass the Rocky Mountain and Boreal ecological regions of northwestern Canada (Figure 1.2; COSEWIC 2003). Abundance tends to decrease to the east (COSEWIC 2003).

During the 19th and 20th centuries wolverines experienced substantial range reductions along the southern edge of their distribution in Europe and North America (Hash 1987; Pasitschniak-Arts and Larivière 1995) as a result of persecution, habitat loss, and other anthropogenic activities (Wilson 1982; Schreiber et al. 1989; Landa et al. 2000). The wolverine population in Sweden is listed as vulnerable, and as endangered in Finland and Norway (The Norwegian Biodiversity Centre; Kålås et al. 2006). Currently in North America, wolverines occupy about 63% of their historic range (Laliberte and Ripple 2004). In February 2013, the United States Fish and Wildlife Service proposed listing the wolverine as threatened under the Endangered Species Act in the continental United States (Federal Register 2013), but this has not yet been finalized. Historically, wolverines in Canada have been considered as a single panmictic population given their high dispersal ability (COSEWIC 2003). For a population to be recognized as a COSEWIC Designatable Unit (DU), the population must contain attributes that make it both 'discrete' and 'evolutionary significant' (COSEWIC 2012). Recently, the eastern population of Quebec and Labrador and the extant population west of James Bay (Figure 1.2) were reassessed under these two criteria, resulting in the reclassification of wolverines across Canada into a single DU with a status of 'special concern' (COSEWIC

2014). Wolverines in Quebec and Labrador are considered to be functionally extirpated as there have been no confirmed sightings in more than 30 years (Fortin et al. 2005); they have an endangered SARA (Species at Risk Act) status (SARA 2014). Given this, Ontario represents the extant eastern range edge for wolverines in North America. Wolverines inhabiting this eastern periphery are designated as threatened by the province of Ontario (Species at Risk in Ontario, SARO 2012).

Life history

Adult male wolverines generally weigh between 11 to 18 kg, while females weigh between 6 to 12 kg (Banci 1994). Large body size (>5.5 kg) for mammals is a strong predictor of extinction risk (Davidson et al. 2009). Although wolverines are mid-sized based on weight, this carnivore has many life history traits observed in large-sized predators (discussed herein); thus wolverines are considered a large carnivore. Wolverines have very large home ranges in relation to their body size, are solitary, and exhibit intra-sexual territoriality (Banci 1994). Male wolverines have territories that range in size from 209 to 2563 km² (Banci and Harestad 1990; Dawson et al. 2010) and commonly overlap several female home ranges (76 to 428 km²; Magoun 1985; Banci and Harestad 1990; Dawson et al. 2010). The size of home ranges may be inversely correlated with resource availability, where home range size for females is inversely correlated with food availability while home range size of males is influenced by the distribution of females (Sandell 1989). Although this relationship has not been confirmed for wolverines, some of the largest home ranges (e.g., Ontario; Dawson et al. 2010) occurred in regions with lower prey diversity in comparison to areas with smaller home range sizes (e.g., Yukon; Banci and Harestad 1990). In northern Sweden, where wolverine densities are high, the reproductive status of females did not influence female

home range size (Persson et al. 2010). In North America, however, reproductive females had a smaller home range than females without young (Magoun 1985; Copeland 1996). In some areas male home range size increased in the spring and summer during the breeding season (Hornikor and Hash 1981; Magoun 1985).

The wolverine's seasonal preference for carrion and utilization of a wide range of additional prey has contributed to this mustelid being described as a facultative scavenger (van Dijk et al. 2008a; Mattisson et al. 2011a) and generalist (Myrberget and Sørungård 1979; Magoun 1987). Although wolverines are capable of hunting large prey (Magoun 1985; Landa et al. 1997), carrion is the primary source of ungulates in wolverine diets, particularly during winter (Rausch and Pearson 1972; van Zyll de Jong 1975; Hornocker and Hash 1981). For breeding females in Finland, scavenging on wolf- and human-killed carrion plays a crucial role in food acquisition where medium-sized ungulate density is low (Koskela et al. 2013a,b). In North America, woodland caribou (*Rangifer tarandus*) and moose (*Alces alces*) are the main ungulate species consumed (Rausch and Pearson 1972; Hornocker and Hash 1981; Banci 1994; Lofroth et al. 2007), presumably by scavenging. Utilization of smaller prey appears to vary both spatially and temporally, reflecting regional and seasonal variation in prey abundance (e.g., Magoun 1987; Lofroth et al. 2007). Diversity of prey species has been found to have an effect on the reproductive success of females (Landa et al. 1997; Persson 2005). Cached ungulate carcasses and summer foods – including predation on caribou calves (Gustine et al. 2006), reindeer and other ungulate neonates (Landa et al. 1997; Mattisson et al. 2011b) – appear to be essential for reproduction to be successful (Inman et al. 2012).

Parentage analysis of wolverines in Scandinavia revealed a polygamous mating system, where males produced offspring with several females in a single year (Hedmark

et al. 2007). Although female wolverines attain sexual maturity by their second year, reproduction generally does not occur before the age of three years (Banci and Harestad 1988; Persson et al. 2006). Between 62 to 92% of females ≥ 3 -years old were pregnant or post-partum in the Yukon (Banci and Harestad 1988), while only 53% of females produced offspring during any given year in Scandinavia (Persson et al. 2006). Parturition occurs between January and April (Banci and Harestad 1988), which corresponds with periods of increased carrion availability and greater snow cover for denning (Banci 1994). Observed mean annual birth rates in North America ranged between 0.43 and 0.89 young per female (Magoun 1985; Copeland 1996; Krebs and Lewis 2000), and 0.74 young per female in Scandinavia (Persson et al. 2006). Life history characteristics of wolverines are similar to those observed in large-sized predators, like low population density, low fecundity, and large home ranges. Wolverines appear to have lower lifetime productivity than grizzly bears (*Ursus arctos horribilis*), for example, reflecting the association between small and infrequent litters and the nutritional regime of this mustelid scavenger (Weaver et al. 1996). This suggests low resilience to population disturbance and a low potential for population growth (Persson et al. 2006). Additionally, Krebs et al. (2004) found a 12.2% annual decline in trapped areas, while untrapped populations were capable of increasing by 6.4% annually. Population demography and persistence can be significantly affected by harvesting, with mortality being most prevalent for subadult males (Krebs et al. 2004).

Dispersal of young is hypothesized to occur between January and May, with females generally establishing residency adjacent to or within their natal home range (Magoun 1985). Males disperse either as young-of-the-year or as subadults (Magoun 1985; Banci 1987). Dispersal distances of juvenile males averaged 51 km and 60 km for

females in Scandinavia. Most dispersal events had a distance less than five home range diameters of the denning female (77 to 91 km²), with dispersal distances up to 178 km (Vangen et al. 2001). In Idaho, Copeland (1996) reported dispersal distances of 168 and 199 km for two males. Wolverines are also capable of extensive, long-range movements. Over a 21 month period, a non-resident, sexually mature male travelled a straight line distance of 378 km from south-central Alaska to the Yukon Territory (Gardner et al. 1986). Juveniles have also been observed making exploratory movements around the time of dispersal, and these could reflect failed dispersal events (Vangen et al. 2001). Inman et al. (2004) found exploratory movements of a young male to exceed 100 km and exploratory periods ranged from several days to several months, indicating the male had begun the dispersal process but had not established a resident area. In comparison, forays to range perimeters by a territory-holding male were short term (e.g., two hours) and within 10 km (Murphy et al. 2011), likely reflecting home range boundary maintenance. Exploratory events appear to precede dispersal; however, extensive monitoring of individuals seems necessary to confirm that dispersal results in the permanent movement away from the natal site. Non-invasive genetic sampling and neutral genetic markers (e.g., microsatellites and mtDNA) can provide insight on dispersal patterns in addition to estimating dispersal rates (e.g., Vonholdt et al. 2008).

Overall, both males and females are capable of long-distance dispersal, with males dispersing at higher frequencies (Banci 1994; Vangen et al. 2001) and over greater distances than females. Home ranges of yearling females are typically established closer to their natal range in comparison to yearling males (Magoun 1985; Copeland 1996; Mulders 2000). Male-biased dispersal is common in mammals (Greenwood 1980) and has been suggested to reflect resource competition between females and mate competition

between males, with dispersal frequency for females likely being dependent upon unoccupied territory availability (Vangen et al. 2001).

Habitat associations

Radio telemetry tracking of individual movements and use of space can provide a comprehensive assessment of habitat selection, particularly for low-density species inhabiting inaccessible environments (Silva-Opps and Opps 2011). Radio-collared wolverines in northeastern Montana were located primarily (70%) in large areas of medium or scattered timber and were rarely located in young dense stands, wet meadow areas, or recent burns (Hornocker and Hash 1981). During the winter, male wolverines in the Kluane Game Sanctuary, Yukon, used subalpine coniferous habitat more frequently than expected, while female use of habitat types did not differ from availability (Banci and Harestad 1990). Older conifer stands were also selected by wolverines in northwestern Alberta and northern British Columbia (Lofroth 2001; Wright and Ernst 2004a,b). In British Columbia (Omineca and Columbian Mountains), both male and female winter ranges were positively associated with moose winter ranges. In the summer, females were associated with alpine and avalanche environments where small mammal prey were present (Krebs et al. 2007). While informative, these field-based studies are constrained by small data sets (Miller et al. 2010; Kelly et al. 2012). Additionally, telemetry studies are able only to provide insight on habitat associations reflective of current spatial configurations.

In north-central British Columbia, females used subalpine and alpine habitat considerably more during denning. Den sites were in close proximity to alpine meadows where marmot (*Marmota caligata*) burrows occurred and at elevations where caribou were present (Lofroth 2001). Dens in the northern Columbia Mountains of British

Columbia occurred in non-forested areas for stand and patch scales (Krebs and Lewis 2000), similar to Magoun and Copeland (1998). However, landscape-scale analyses suggested upper-elevation forests and not alpine/parkland habitats were generally used for denning, possibly reflecting prey/carrion distributions and the avoidance of predators and anthropogenic disturbance (Krebs and Lewis 2000). Dens were characterized by a snow depth of at least one metre from the snow's surface to the deepest point of the tunnel system (Magoun and Copeland 1998). Deep snow provides dens a thermoregulatory advantage, and increases the likelihood of den persistence till early spring when den abandonment occurs. Deep snow also protects kits from predation, where deep, long and complex snow tunnels discourage predation attempts (Pulliainen 1968; Magoun and Copeland 1998). In Ontario, dens consisted of snow-covered boulder piles or fallen trees (Dawson et al. 2010), similar to elsewhere in North America (Magoun and Copeland 1998; Krebs and Lewis 2000; Lofroth 2001). Dens were generally established in secluded, undeveloped areas (Copeland 1996; Krebs et al. 2007; May 2007). Suitability of the denning area may be affected by human activity, prey/carrion distribution and presence of other predators (Krebs and Lewis 2000).

Global wolverine distribution correlates strongly with persistent spring snow cover (24 April - 15 May; during the reproductive denning period) and to a lesser degree with an upper limit of thermoneutrality (average maximum August temperature $\leq 22^{\circ}\text{C}$; Copeland et al. 2010). In the northern US Rockies, spring snow cover also correlated with gene flow (Schwartz et al. 2009). Generally, mean snow depth is positively correlated with elevation (Grünwald et al. 2014). Wolverines selected higher elevations in central Idaho, where vertical movement reflected prey selection (Copeland et al. 2007). However, increased anthropogenic disturbances at lower elevations may also have

influenced wolverine connectivity. In Norway, wolverine home range location was influenced by anthropogenic development, with infrastructure mainly occurring at lower elevations (May et al. 2006). It may be misleading to extrapolate these inferences to the lower-relief topography characteristic of much of the wolverine range elsewhere on the continent (Short Bull et al. 2011). As mammals typically display male-biased dispersal and female philopatry (Greenwood 1980; Lawson Hadley and Perrin 2007), it is important to take into account sex-specific patterns of dispersal when identifying landscape variables influencing movement patterns and population connectivity. Sex-biased dispersal may result in a differential effect of a limiting factor between males and females (e.g., Talbot et al. 2012).

Response to disturbed landscapes and genetic structure

The adverse effect of human landscape disturbances on this species is well documented. For instance, landscape models for wolverines in northwestern United States predicted greater wolverine occurrence in areas with low road and human population densities, and high proportion of subalpine forest and alpine tundra habitat (Rowland et al. 2003). Although tracks crossing clear-cuts were periodically observed in Montana, no wolverines were observed directly in clear-cuts (<15-yrs-old) of any size (Hornocker and Hash 1981). Wolverine track distributions in Ontario were most commonly associated with unlogged northern regions, supporting the hypothesis of wolverines being limited by anthropogenic activities like road construction and logging (Bowman et al. 2010). Deciduous forests appeared to be avoided by wolverines, as these environments likely reflect diverse and abundant predator and scavenger communities where interspecific competition would be limiting (Bowman et al. 2010). Forested areas disturbed by fire or timber harvesting are characterized by young deciduous forests, with

population densities decreasing for woodland caribou, and increasing for moose, white-tailed deer (*Odocoileus virginianus*), and wolves (*Canis lupus*; Vors et al. 2007; Bowman et al. 2010). Low to moderate increases in wolf densities may benefit wolverines in some areas due to increased carrion (e.g., van Dijk et al. 2008a); however, there is also an increase in predation risk. In Norway, primary use of high elevations by wolverines with short-term, lower elevation excursions for carrion from wolf kills suggests avoidance by wolverines (van Dijk et al. 2008b). Although ungulate biomass is higher in disturbed areas in comparison to intact, mature forests, the increased predator and human access (i.e., leading to harvesting) may result in higher mortality risk for wolverines, and in turn influence the relative availability of ungulate prey to wolverines (Ontario Wolverine Recovery Team 2013). While invaluable insights have been gained from studies investigating the response of wolverines to direct and indirect effects of anthropogenic activities, these studies are still constrained spatially to a small portion of the range. For broadly distributed species, like the wolverine, it is important to investigate the effect of disturbances at different locations and spatial scales.

Roads have a negative effect on wolverines. In British Columbia, wolverines preferred areas >1100 m from the Trans-Canada Highway, and avoided areas within 100 m of the highway (Austin 1998). Crossing of highways by wolverines occurred at narrower rights-of-way (68 m) in comparison to approaches without crossings (165 m; Austin 1998). Female summer locations in British Columbia negatively correlated with recently logged areas and roads (Krebs et al. 2007). In Ontario, core home range (50% Minimum Convex Polygon, MCP) had lower mean road density (0.33 km/km²) than the overall home range (95% MCP, 0.43 km/km²). The two wolverines with the highest road densities in their home range were incidentally harvested (Dawson et al. 2010).

Accessibility to remote areas increases with the development of all season and winter roads, increasing the vulnerability of wolverine populations to overharvest (Mulders 2000). In Norway, dens had a mean distance of 3.06 km to the nearest private road and 7.46 km to the nearest public road (May 2007). Similarly, a single denning site observed in Ontario was 5 km to the closest human access trail and 7 km from the nearest active logging road (Dawson et al. 2010). These studies are predominately located along the southern range edge, where wolverine distribution encounters increasing human population density. Equally important may be the effects associated with winter roads, which transverse the large expanses of relatively undisturbed northern habitats of wolverines. Although winter roads are seasonal, they increase human access to remote areas resulting in increased wildlife mortality (Beazley et al. 2004).

Reduced habitat connectivity may disrupt animal movements and dispersal, in turn affecting population dynamics and spatial genetic structure. Low genetic structure among wolverine populations inhabiting the northern regions of North America has been reported in several genetic studies using neutral microsatellite markers (Wilson et al. 2000; Kyle and Strobeck 2001, 2002; Chappell et al. 2004), signifying high levels of gene flow. Genetic structure, however, has been found to progressively increase near the eastern and southern range margins (Kyle and Strobeck 2002; Cegelski et al. 2003, 2006). Populations at the southwestern periphery comprise only a subset of the genetic diversity detected in more northern populations (Kyle and Strobeck 2001, 2002; Cegelski et al. 2006) and likely reflects irregular population distributions (Banci 1994) due to population bottlenecks resulting from range contractions (IUCN 2011). Conversely, strong genetic structure over narrow geographic extents has been noted by researchers utilizing maternally inherited mitochondrial markers (Wilson et al. 2000; Tomasik and Cook 2005;

Cegelski et al. 2006; Schwartz et al. 2007; Frances 2008). These opposing patterns of genetic differentiation between nuclear and mitochondrial markers suggest that dispersal is biased towards males and that females are more philopatric (Wilson et al. 2000; Tomasik and Cook 2005; Cegelski et al. 2006; Schwartz et al. 2007; Frances 2008). Wolverines have also undergone range contraction at the eastern range edge of their North American distribution, with limited data from this region suggesting reduced gene flow with the larger northwestern continuum (Kyle and Strobeck 2002; Frances 2008). Underlying conditions contributing to range contraction may differ between southwestern and northeastern peripheries, possibly resulting in differing patterns of genetic diversity.

The wolverine as a model species

Wolverines are a good model organism for investigating genetic connectivity patterns of large carnivores in northern latitudes. Wolverines are wide-ranging, continuously distributed across the Northern Hemisphere, and occupy a diverse variety of habitats (Banfield 1974; Copeland and Whitman 2003; COSEWIC 2003). Like most large carnivores, wolverines have experienced extensive reductions in abundance and range (Laliberte and Ripple 2004) over the past two centuries, with the primary factors being analogous to those affecting carnivore species globally (i.e., human persecution and habitat loss; Wilcove et al. 1998; Treves and Karanth 2003). The introduction of favourable legislation during the second half of the 20th century has allowed for populations of large carnivores to increase (Linnell et al. 2001), which may explain the recolonization by large carnivores of their former ranges in Europe and North America (Hoffman and Genoways 2005; Falcucci et al. 2013). Similarly, wolverines have also demonstrated recent range expansions into regions previously occupied (Landa et al. 2000; Ontario Wolverine Recovery Team 2013). Although recently recolonized areas in

northern latitudes are characterized by largely intact landscapes (Ruckstuhl et al. 2008; CAFF 2003), these carnivore expansions will inevitably encounter the northward movement of anthropogenic activities. A species resilience to landscape change defines how well it is able to adapt to anthropogenic disturbances (Weaver et al. 1996).

Carnivores with high resilience will be able to withstand and even adapt to changing landscapes (e.g., coyote *Canis latrans*; Poessel et al. 2014). However, species with low resilience (e.g., grizzly bears; Weaver et al. 1996) to human activities will have difficulty persisting in modified or fragmented landscapes. This lower resiliency to disturbances can constitute an early warning mechanism of habitat connectivity. Wolverines can be considered an indicator species due to their dependence on large, intact and connected ecosystems (COSEWIC 2003) and low resiliency to anthropogenic disturbances (Weaver et al. 1996). Additionally, many life history traits of wolverines parallel those observed in large-sized predators, like large home ranges, and low population density and fecundity (Weaver et al. 1996). Based on these characteristics, wolverines likely have a low potential for population growth (Persson et al. 2006), further supporting their importance in studying connectivity patterns in the context of anthropogenic disturbance and climate change.

THESIS OBJECTIVES

My thesis examined historical and contemporary influences on the genetic diversity and structure of wolverines across their North American and circumpolar distribution. In Chapter 2, I took a population genetics approach using microsatellites to quantify contemporary genetic patterns between the eastern periphery of the wolverine's extant range and the larger continuum of populations in North America. I also included mtDNA data to test for sex-biased dispersal. Given previous mtDNA findings (Wilson et

al. 2000; Tomasik and Cook 2005; Cegelski et al. 2006; Schwartz et al. 2007), I predicted that individuals from the periphery would display genetic structure over small geographic ranges due to female philopatry. Based on increasing genetic structure towards the eastern periphery (Kyle and Strobeck. 2002), I predicted diminished levels of gene flow between the eastern periphery and core populations. Sampling along the extant eastern periphery was increased with non-invasive hair snare surveys and opportunistic sampling from road kills and trapper harvests. Samples were combined with existing data from the periphery and adjacent regions of the larger northwestern continuum (Kyle and Strobeck 2002). In Chapter 3, I used conventional Bayesian approaches to investigate the phylogeographic structure of wolverines across the full breadth of their Holarctic range, to determine how their evolutionary history has influenced patterns of present-day genetic structure. Based on the phylogeographic structure of other wide-ranging, cold-adapted mammals (e.g., woodland caribou; Klütsch et al. 2012) and initial phylogenetic patterns observed in Chapter 2, I expected wolverines in North America to be comprised of multiple lineages – each one reflective of a separate refugium. Understanding the influence of past climatic fluctuations on contemporary patterns of genetic structure could help identify evolutionary significant units (Cossíos et al. 2009) and improve predictions of the effects of climate change on arctic wildlife (Provan and Bennett 2008). Sampling was expanded across the wolverine's Holarctic distribution, and included historic samples from extirpated populations of Quebec-Labrador. To ensure a more comprehensive and representative set of samples, all new individuals were combined with existing data. Additionally, I used a coalescent-based approximate Bayesian computation (ABC) method to test among four competing hypotheses to determine the most likely postglacial recolonization pathway influencing population divergence. In Chapter 4, I used an

individual-based landscape genetics approach to evaluate the influence of landscape features on the genetic connectivity of wolverines comprising the extant eastern genetic cluster. Based on current understanding of wolverine habitat associations and responses to anthropogenic disturbances, the influence of five landscape features (forest age, road density, land cover, spring snow cover mean and variance) on genetic connectivity were investigated. Unsuitable landscape features like young forests, low mean snow cover, and high road density were associated with high movement costs. I hypothesized that low road density, increased forest age, more favourable land cover, and increased snow cover would be positively correlated with increased genetic connectivity among individuals. Based on mortality costs associated with roads, I predicted this variable to be associated with wolverine genetic distance. Given the low topographic relief for the study area (500 m, Natural Resources Canada 2004) in comparison to mountain ranges (e.g., 2500 m; Schwartz et al. 2009), I predicted spring snow cover not to be associated with genetic distance in our study. Analyses were also conducted by sex to determine the presence of sex-biased effects on connectivity, due to wolverines displaying male-biased dispersal (Cegelski et al. 2006). Individual-based genetic distance measures increase temporal resolution (Landguth et al. 2010), increasing the likelihood of associating landscape features to genetic distances within a population. Additionally, this approach highlights the importance of identifying sex-specific influences of anthropogenic effects, as these could differ from overall findings for the species as a whole.

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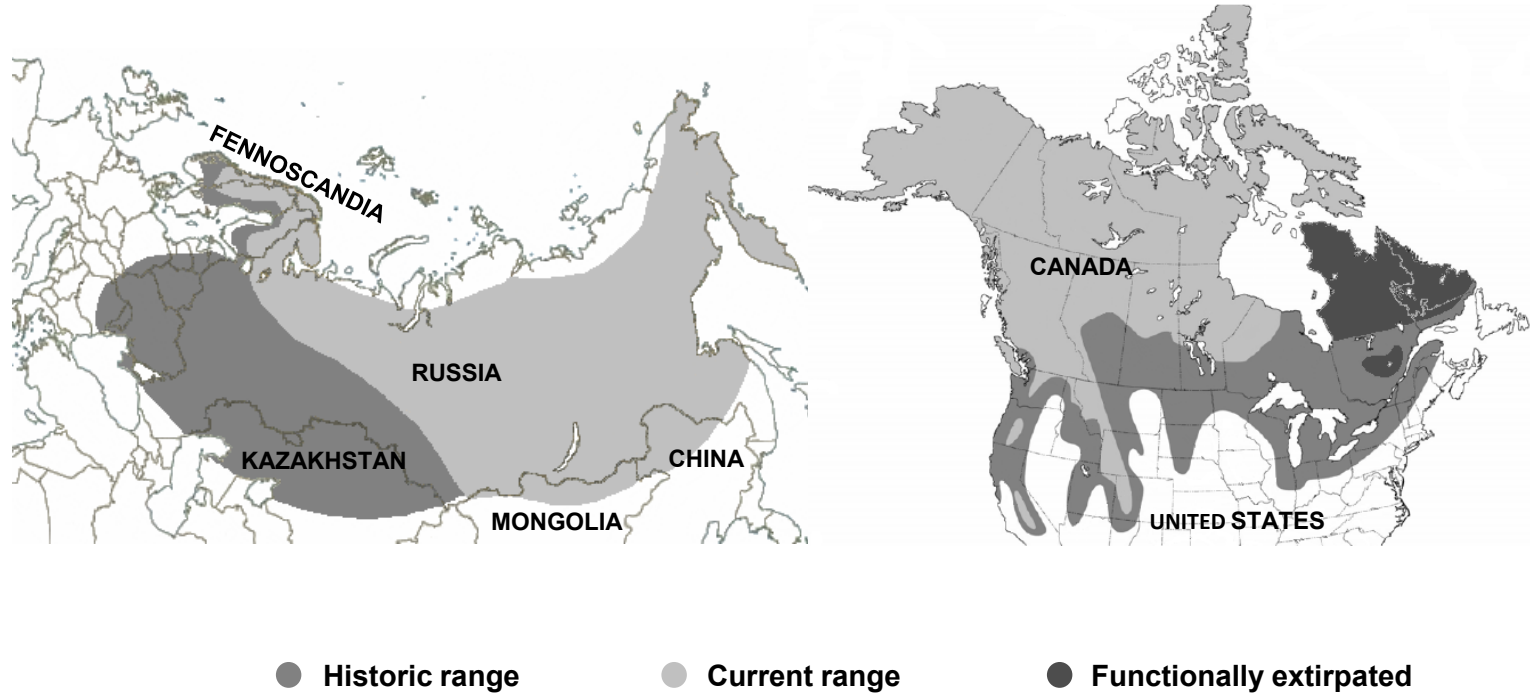


Figure 1.1 Historic and current distribution of wolverines in Eurasia (adapted from National Geographic Society 2002) and North America (adapted from COSEWIC 2003).

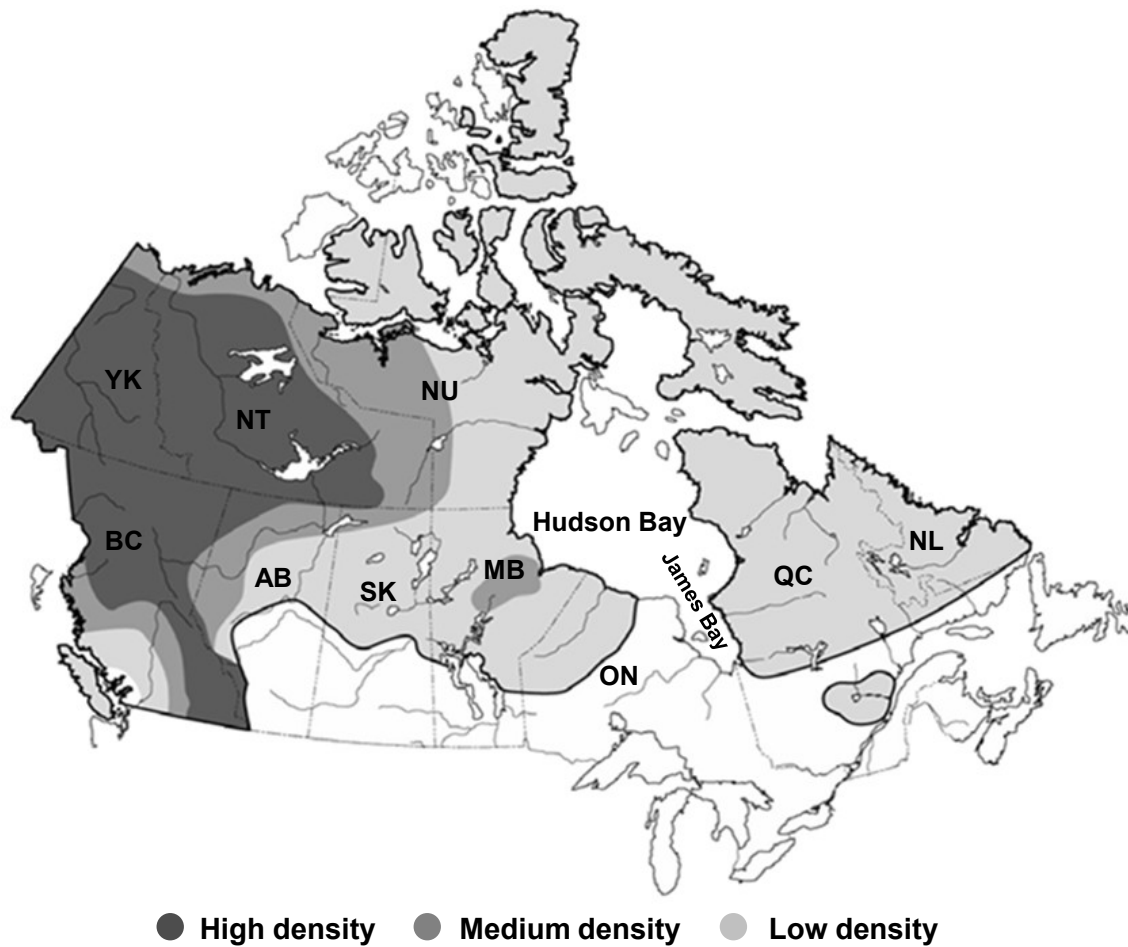


Figure 1.2 Map depicting contemporary distribution and density of wolverines in Canada (COSEWIC 2003).

CHAPTER 2

GENETIC ISOLATION OF WOLVERINE (*GULO GULO*) POPULATIONS AT THE EASTERN PERIPHERY OF THEIR NORTH AMERICAN DISTRIBUTION

A version of this chapter has been published

Zigouris J, Dawson N, Bowman J, Gillett RM, Schaefer JA, Kyle CJ (2012) Genetic isolation of wolverine (*Gulo gulo*) populations at the eastern periphery of their North American Distribution. *Conservation Genetics* 13:1543-1559, *with kind permission from Springer Science and Business Media.*

Abstract

Conservation strategies have a tendency to discount range peripheries, but recent evidence suggests that range edges may be important to species persistence by harboring genetic variants not found in the core distributions. Wolverines in Canada are recognized as existing in two populations – an endangered eastern population and an extant western population thought to be largely panmictic. Studies from western North America identified strong patterns of female philopatry and increased genetic structure at the current southwestern periphery. Due to the paucity of data from the contemporary eastern periphery, however, it remains unclear if similar patterns exist at this range edge. Using neutral microsatellite and mitochondrial DNA markers from a broad geographic extent (>2500 km), we found that wolverines at the eastern periphery displayed strong patterns of genetic distinctiveness from northwestern populations. While the microsatellite data suggest contemporary genetic structure exists, the haplotypic composition of the eastern periphery drastically differed from the core, indicating longstanding differences between regions. Further research is needed to determine if wolverines from the eastern periphery show evidence of interactions with the functionally extirpated eastern population, if functional markers display similar patterns of genetic diversity, and what relevance these may have in their evolutionary potential. Pronounced environmental fluctuations at range boundaries likely contribute to peripheral populations having genotypes with a greater capacity to respond to future selection pressures like climate change and may become a vital source of genetic diversity should core regions become replaced by edge habitats, and thus warrant separate conservation consideration.

Introduction

During a population decline, contraction of the geographical range is often predicted, with persistence occurring at the centre of the species' range (Lawton 1995). Lower density, ecologically marginal habitats, isolation and higher likelihood of extinction have contributed to the tendency of conservation strategies to discount range peripheries for species persistence (Brown 1984; Griffith et al. 1989; Gaston 1990; Lesica and Allendorf 1995; Garner et al. 2004). In spite of this, there is increasing evidence that persistence along the edge of a species' historical range is more common than expected (e.g., Channell and Lomolino 2000; Antunes et al. 2006) and important to overall population viability. Peripheral populations have a higher probability of experiencing more pronounced selection pressures due to greater environmental fluctuations (Cassel-Lundhagen et al. 2009) in addition to reduced gene flow, founder effects and small population size (Lesica and Allendorf 1995). Under these conditions, the genetic distinctiveness of edge populations may increase (Lesica and Allendorf 1995; Santamaria et al. 2003), such that they could play a role in maintaining and generating biological diversity for the species (Channell and Lomolino 2000). Genetic diversity is necessary for species to locally adapt to environmental stressors like new diseases (Kramer et al. 2010; Radwan et al. 2010), to facilitate range shifts in response to climate change (Etterson and Shaw 2001; Parmesan 2006), and to persist through time (Stockwell et al. 2003; Willi et al. 2006). Although the asymmetrical effects of contemporary processes across a species range influences patterns of genetic structure, present-day spatial configurations of genetic diversity are also reflective of past historic events.

Historical changes to population size and gene flow shape the geographical patterns of population genetic diversity and differentiation (Vucetich and Waite 2003).

Glacial cycles over the past 2.4 million years have strongly influenced the genetic structure and distribution of biota in North America. Unlike temperate taxa, Holarctic species such as caribou (*Rangifer tarandus*) and arctic fox (*Alopex lagopus*) are presumed to have had greater range distributions as ice sheets expanded (Flagstad and Røed 2003; Dalén et al. 2005). The onset of the Holocene ushered in rising temperatures and retreating glaciers, resulting in the northward displacement of taiga and tundra by temperate and mixed hardwood forests (Adams and Faure 1997). Distributions of cold-adapted species became restricted to the more northern latitudes of contracting arctic ecosystems (e.g., Dalén et al. 2005), where present-day ranges can be thought of as contemporary arctic refugia.

The wolverine (*Gulo gulo*) has a Holarctic distribution largely congruent with the taiga and boreal-tundra zones of North America and Eurasia (Landa et al. 2000; Copeland and Whitman 2003; Aubry et al. 2007). The main threats to this species have included habitat loss and fragmentation, fur harvesting and lethal predator control programs, which have resulted in extensive range contractions (Wilson 1982; Schreiber et al. 1989). The high dispersal ability of wolverines has led to the assumption that this species was historically a single panmictic population in North America (Committee on the Status of Endangered Wildlife in Canada, COSEWIC 2003); however, wolverines in Canada are now categorized as existing in two populations. A functionally extirpated population occupied northern Quebec and Labrador, and although currently designated as endangered (Dauphiné 1989; COSEWIC 2003), no sightings have been confirmed over the past 30 years (Figure 2.1; Fortin et al. 2005). An extant population occurs west of James Bay (Figure 2.1; Slough 2007) and is nationally listed as a population of special concern (COSEWIC 2003). At the eastern periphery of the extant population, wolverines

are considered threatened by the province of Ontario (Species at Risk in Ontario, SARO 2012).

Wolverines occur at very low densities (0.3 - 6.2 wolverines/1000 km²; Lofroth and Krebs 2007), have extensive home ranges (100 - 1400 km²; Magoun 1985; Dawson et al. 2010), and are highly vagile (yearling female >300 km over a 5 month period; Magoun 1985; male straight-line distance \geq 300 km for a 20 month period; Gardner et al. 1986). High levels of gene flow among wolverine populations across northern regions of North America have been noted by several studies using nuclear DNA markers (Wilson et al. 2000; Kyle and Strobeck 2001, 2002; Chappell et al. 2004), with genetic structure progressively increasing towards the southern and eastern peripheries of their distribution (Kyle and Strobeck 2002; Cegelski et al. 2003, 2006). Conversely, investigations based on maternally inherited mitochondrial markers (Wilson et al. 2000; Tomasik and Cook 2005; Cegelski et al. 2006; Schwartz et al. 2007; Frances 2008) have reported genetic structure over relatively small geographic scales despite the long-range dispersal capacity of both males and females (Magoun 1985; Gardner et al. 1986; Flagstad et al. 2004). This contrasting pattern of genetic differentiation based on nuclear and mitochondrial markers suggests that wolverines, similar to other mid- to large-sized carnivores, display male-biased dispersal and strong female philopatry (Magoun 1985; Wilson et al. 2000; Tomasik and Cook 2005; Cegelski et al. 2003, 2006; Schwartz et al. 2007).

Previous studies of wolverine genetic structure in North America have primarily focused on the range core from Hudson Bay, Canada to Alaska in the United States (Wilson et al. 2000; Kyle and Strobeck 2001, 2002; Chappell et al. 2004; Tomasik and Cook 2005). Considerable range contractions across the wolverine's circumpolar distribution (Abramov et al. 2009) have resulted in the irregular distribution of

populations along the southwestern periphery of their North American range (Banci 1994). The genetic diversity reported for the southwestern periphery (Kyle and Strobeck 2001, 2002; Cegelski et al. 2006) represents only a subset of the genetic diversity found for more northern populations and is likely reflective of population bottlenecks resulting from range contractions. Southwestern populations were likely continuous with the panmictic northern core about 150 years ago, with wolverines at the southern edge inhabiting landscapes similar to those in core areas and therefore making this periphery unlikely to harbour locally adapted genetic variants that could contribute to the future viability of the species. In comparison, information from the eastern periphery of the wolverine's distribution, where range contraction has also occurred, remains limited (e.g., for Ontario [ON] $n = 8$; Frances 2008; and $n = 12$; Kyle and Strobeck 2002) but is suggestive of restricted gene flow with the larger northwestern core (Kyle and Strobeck 2002). Wolverines occupying the eastern portion of their extant range in North America also inhabit a landscape characterized by the Hudson Plains Ecozone, a region dominated by extensive wetlands, which differs from the Taiga and Boreal Ecozones (Natural Resources Canada 2007) of core populations. Assessing the genetic diversity along the eastern periphery, and how it differs from the core, is key to recognizing any longstanding genetic divisions and the potential for peripheral populations in maintaining and generating biological diversity for wolverines. Furthermore, anecdotal evidence suggests that wolverines may be recolonizing Ontario (Dawson 2000), yet it remains unclear whether this represents ingress from adjacent regions, or growth of a resident population.

Using neutral nuclear and mitochondrial markers, we explored the genetic diversity and structure of wolverines at the eastern periphery of their extant range to examine their connectivity to the larger continuum of populations and determine if this

region harbours genetic variants not found in the core population. Similar to previous mitochondrial DNA studies, we predicted that the eastern periphery would display genetic structure over relatively small geographic ranges for the maternally inherited marker due to female philopatry. However, diminished levels of gene flow between the eastern periphery and core populations will be reflective of either recent or more historical subdivisions. The presence of an eastern peripheral genetic cluster would have important conservation implications for this listed species.

Methods

Sample collection

This study combines microsatellite data from Kyle and Strobeck (2001, 2002) for Nunavut (NU, $n = 106$), the Northwest Territories (NT, $n = 42$), Saskatchewan (SK, $n = 15$), Manitoba (MB, $n = 28$) and ON ($n = 12$), with new data from SK ($n = 1$), MB ($n = 9$) and ON ($n = 61$) (Figure 2.1). Our study focused on regions adjacent to and including the eastern periphery, as we were largely interested in detecting patterns of genetic structure along the contemporary eastern range edge of wolverines in North America.

In addition to the microsatellite data, we sequenced a 360 bp fragment of the mitochondrial DNA control region and present all new sequence data for samples used from Kyle and Strobeck (2001, 2002) and new individuals for this study. Although a multi-generation sampling scheme encompassing 20 years was used to ensure an adequate sample size for analysis, the vast majority of the samples were collected from within three generations. This sampling approach likely did not influence population genetic diversity and structure results given the life history characteristics of this species (Hedmark et al. 2007). Ninety-eight percent of our samples were collected post 1990; however, we included four samples (2%) pre 1990 in order to increase sample size along

the eastern periphery. Tissue and pelt samples were obtained opportunistically from incidental deaths and trapper harvests, respectively. All hair samples were collected with hair snares in the Boreal Shield Ecozone near Red Lake, Ontario, Canada (51°N, 93°W), using a protocol approved by the Ontario Ministry of Natural Resources animal care committee (e.g., Koen et al. 2008). Hair snares were modified from Mulders et al. (2007), and were set between November and May from 2003 to 2009. Hairs collected from each barb were stored in paper envelopes at room temperature until DNA could be extracted.

DNA extraction, sex identification, and microsatellite genotyping

Genomic DNA was isolated using a Qiagen DNeasy® Tissue Extraction Kit following the manufacturer's instructions. Sex was determined by amplification of the Zfx/Sry primer pairs P1-5EZ/P2-3EZ (Aasen and Medrano 1990) and Y53-3C/Y53-3D (Fain and LeMay 1995). Amplifications were conducted in 20 µl volumes containing 5-10 ng of DNA, 0.7 µM of each primer, 1X Mg free PCR buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs, 0.15 µM BSA, and 0.05 U/µl of *Taq* DNA polymerase. Cycling conditions consisted of 95°C for 60 s followed by 35 cycles of 94°C for 45 s, 58°C for 45 sec., and 72°C for 60 s. Fragment patterns were visualized in 1.0% agarose gels stained with ethidium bromide.

Eleven microsatellite loci developed in badgers *Taxidea taxus* (Tt-1, Tt-4; Davis and Strobeck 1998), Eurasian otters *Lutra lutra* (Lut-604; Dallas and Piertney 1998), mink *Neovison vison* (Mvis-75; Fleming et al. 1999), and wolverines (Gg-3, Gg-4, Gg-7, Gg-14; Davis and Strobeck 1998; and Ggu-101, Ggu-216, Ggu-234; Duffy et al. 1998) were amplified following Davis and Strobeck (1998). Fragments were visualized using

an ABI™ 3730 DNA Analyzer (Applied Biosystems), and bands were scored with GeneMarker® (SoftGenetics).

All hair samples ($n = 102$) were obtained in ON. We compared all genotypes for ON using Cervus 3.0.3 (Kalinowski et al. 2007) to identify resampled individuals and estimate the probability of identity for two randomly drawn individuals (PI) and full sibs (PI_{SIB}) (Waits et al. 2001). Ggu-101 was omitted from identity analyses due to a large positive estimate of null allele frequencies indicating excess homozygotes found at this locus for ON animals. However, no evidence of excess homozygotes for Ggu-101 was observed for the final data set, which included the consensus profiles (combined genotypes) of repeatedly sampled individuals. Ggu-101 was included in population genetic diversity and structure analyses as no excess homozygotes were identified for the whole data set or the two genetic clusters. To increase the resolution of identifying repeated sampling nine additional loci developed in wolverines (Gg-10, Gg-25, Gg-37, Gg-42, Gg-443, Gg-452, Gg-454, Gg-465; Walker et al. 2001) and marten *Martes americana* (Ma-19; Davis and Strobeck 1998) were amplified for ON samples following Davis and Strobeck (1998), and included in the identity analyses. Identity analyses were done separately by sex and repeat sampling was accepted if genotypes matched for ≥ 9 loci with ≤ 2 mismatches. Mismatches consisted of a locus being heterozygous for one sample and homozygous for one of the alleles in the other sample. The identity check was rerun using the combined genotypes of repeatedly sampled individuals. Error rates were calculated by genotyping a subset of the samples twice and having each genotype scored by two independent individuals.

Mitochondrial DNA sequencing

A 360 bp fragment of the control region was amplified with the primers Gulo0F (Schwartz et al. 2007) and H16498 (Ward et al. 1991). Amplifications were conducted in 20 μ l volumes containing 5-10 ng of DNA, 0.7 μ M of each primer, 1X Mg free PCR buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.1 mg/mL BSA, and 0.05 U/ μ l of *Taq* DNA polymerase. Cycling conditions consisted of an initial 5 min denaturation step at 94°C followed by 30 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 60 s. Amplified products were electrophoresed in a 1.5% agarose gel stained with ethidium bromide and run alongside a Low DNA Mass™ Ladder (Invitrogen) at 100 volts for 45 min. Successfully amplified products were purified using ExoSap-IT (New England BioLabs) following the manufacturer's instructions, and sequenced in both directions using a BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were visualized using an ABI™ 3730 DNA Analyzer (Applied Biosystems).

Genetic diversity and population structure

Observed and expected heterozygosity values (H_O and H_E) were calculated for all sampling regions and identified genetic clusters with Cervus 3.0.3 (Kalinowski et al. 2007), and tested for statistical significant differences using a paired *t* test with the Bonferroni correction for multiple comparisons (Zar 1999). GENEPOP 4.0 (Raymond and Rousset 1995) was used to test each locus for deviations from Hardy Weinberg equilibrium (HWE) and to evaluate linkage disequilibrium (LD) among loci. Levels of significance were Bonferroni corrected for multiple comparisons (Zar 1999). We used a rarefaction approach based upon the smallest sample size (ADZE 1.0; Szpiech et al. 2008) to calculate adjusted estimates of allelic richness (A_R) and private alleles (A_P) for each sampling region and identified genetic clusters.

A Bayesian clustering method (STRUCTURE 2.3; Prichard et al. 2000; Falush et al. 2003) was used to infer the number of genetic clusters and assign individuals to them based on allele frequencies and without knowledge of sample origin. The number of genetic clusters was estimated using the most conservative approach by selecting admixture and correlated allele frequency models. Ten independent runs were performed for $K = 1 - 10$ with 200 000 Markov chain Monte Carlo (MCMC) cycles for burn-in and 500 000 MCMC cycles for data collection. At each K , prior and posterior probabilities were assessed to ensure an adequate number of MCMC cycles were used to reach stationarity for all estimations (Excoffier and Heckel 2006). Optimal cluster number was inferred based on the rate of change in the log probability of data between successive K values (Evanno et al. 2005) and verified with the geographic distribution of sampled regions. Individual membership coefficients (q) for each cluster at optimal K were summarized for the 10 runs with CLUMPP (Jakobsson and Rosenberg 2007). Genetic cluster assignment was determined based on three minimum membership thresholds of 0.7, 0.8, and 0.9. Individuals remained unassigned when threshold values were not met for all clusters.

Including each sample's geographic location as prior information may assist clustering processes as individuals are grouped by collection site based on the likelihood that sampling locations may be informative about ancestry (Hubisz et al. 2009). In our analysis of population structure, we used the spatially explicit LOCPRIOR model and the same procedures used for the non-spatial models of STRUCTURE. Locations were considered informative when values of r , which parameterized the amount of information carried by the locations, were ≤ 1 (Hubisz et al. 2009).

We used TESS 2.3 (François et al. 2006; Chen et al. 2007) to account for actual spatial coordinates when estimating probabilities of individual membership, where spatially proximate individuals are assumed to be genetically similar. The non-admixture algorithm was used as we were interested in identifying the maximal number of clusters (Durand et al. 2009). We performed 30 independent simulations of 60 000 sweeps with a burn-in of 40 000 for $K = 2 - 10$. Three interaction parameters, $\psi = 0, 0.6,$ and 1.2 , were used to determine the extent which geographical information influenced individual assignment. The Deviance Information Criterion (DIC) was used to select optimal cluster number. Average membership was calculated in CLUMPP using 10% of the runs having the lowest DIC value for optimal K . Each individual was also tested for its status as a resident or recent immigrant using a Bayesian Monte Carlo re-sampling assignment test of 10 000 simulated individuals (GeneClass2; Piry et al. 2004).

ARLEQUIN 3.5 (Excoffier and Lischer 2010) was used to calculate pairwise F_{ST} estimates (Weir and Cockerham 1984) of sampling regions and a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) of the genetic clusters identified by STRUCTURE. Comparable levels of genetic diversity were also measured using D_{est} (Jost 2008), and were calculated with SMOGD (Crawford 2010). D_{est} was used instead of other diversity measures like G_{ST} (Nei 1973) or G'_{ST} (Hedrick 2005) as it partitions diversity into independent between and within subpopulation components (Jost 2008).

Mitochondrial DNA sequence analysis

The resulting sequences were compiled and edited using MEGA 4.0.2 (Tamura et al. 2007), aligned with Clustal W (Larkin et al. 2007), and verified visually. We used FABOX 1.35 (Villesen 2007) to identify variable nucleotide positions and compile unique sequences for further analysis. Nucleotide (π) and haplotype (h) diversities were

estimated with ARLEQUIN for each sampling region and identified genetic clusters based on nuclear DNA. Departure from the neutral model of evolution (Tajima's D ; Tajima 1989) and population growth (Fu's F_s $P < 0.02$; Fu 1997) were tested with ARLEQUIN using 10 000 permutations. Corrected estimates of haplotype richness (H_R) and the number of private haplotypes (H_P) were calculated based on the smallest sample size with ADZE.

Inferences of geographic structuring among sampling regions were assessed with a spatial analysis of variance (SAMOVA) using the program SAMOVA 1.0 (Dupanloup et al. 2002). We performed SAMOVAs for 10 000 iterations using 100 random initial conditions, with all grouping options for $K = 2 - 4$. We also examined the amount of geographic variability partitioned within and among populations by implementing a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) in ARLEQUIN for 10 100 permutations. Groups were designated based on population clustering as determined from the SAMOVA analysis and microsatellite data using STRUCTURE. Pairwise Φ_{ST} estimates were calculated using ARLEQUIN for each sampling region and genetic clusters identified using microsatellite data.

We constructed a median-joining phylogenetic network using Network 4.5 (Bandelt et al. 1999) to visualize the relationships among the observed haplotypes. Given the lack of diversity observed among our nine haplotypes, phylogenetic analyses were not included as they did not provide any additional information. Intraspecific genetic variation can have low divergence, thus for haplotype data where differences can involve single nucleotide substitutions a network can accurately represent those phylogenetic relationships (Posada and Crandall 2001).

Results

Identification of individuals from hair snags

Thirty-four hair samples collected from ON were recognized as unique wolverine individuals, with another nine samples identified as recaptures. The remaining hair samples from ON either failed to amplify for more than three markers ($n = 26$), were identified based on mtDNA as wolverine but provided partial profiles that could not be used for population analyses ($n = 21$), or were of another species ($n = 1$). Thus, 246 individuals were used for all nuclear DNA analyses in this study (ON [$n = 52$], MB [$n = 30$], SK [$n = 16$], NU [$n = 106$], and NT [$n = 42$]).

Nuclear DNA diversity

Expected heterozygosity of sampling regions ranged from 63.5% in SK to 68.3% in ON (Table 2.1). There were no significant differences between observed and expected heterozygosity for the identified genetic clusters and sampling regions with the exception of MB, which had a significant heterozygosity deficit ($P = 0.03$). All loci were in HWE for each sampling site and for both genetic clusters. In comparison, one of the 11 loci was found not to be in HWE for the data set as a whole. This departure from HWE was not consistent across loci or sampled regions so all loci were retained in further analyses. Linkage disequilibrium was observed between Tt-1 and Gg-7 for the ON sampling region. As these loci did not display genotypic disequilibria in any of the other sampling regions it is unlikely that they are physically linked and were therefore retained for further analyses. Total allelic richness (A_R) adjusted for sample size ranged from 3.93 alleles/locus in SK to 4.14 in ON, and adjusted private allelic (A_P) richness was low for all sampled regions (0.07 - 0.23). Both A_R and A_P values were slightly higher for the

peripheral genetic cluster in comparison to the core (peripheral $A_R = 5.31$, $A_P = 0.60$; core $A_R = 5.17$, $A_P = 0.46$, Table 2.1).

Nuclear genetic structure

The same two genetic clusters were identified by all three of our clustering methods (STRUCTURE, Figure 2.2a; LOCPRIOR, Figure 2.2b; and TESS, Figure 2.2c). Cluster 1 consisted of SK, NU and the NT, whereas MB and ON pooled together to form Cluster 2. We consistently observed these two clusters in STRUCTURE, even when fewer samples successfully assigned to a specific cluster as the minimum membership threshold was increased (Figure 2.2a). Additionally, all three threshold levels in STRUCTURE (Figure 2.2a) identified cross assignments (i.e., individuals assigned to a cluster from which they were not sampled) for both clusters. Analyses involving the LOCPRIOR model found sampling locations to be informative ($r = 0.17$). Higher coefficient values associated with sampling locality for the LOCPRIOR model resulted in three individuals from Cluster 2 to remain unassigned even for the lowest membership threshold and no cross assignments. In comparison, the interaction parameter of $\psi = 0$ in TESS was analogous to STRUCTURE without admixture and with uncorrelated allele frequencies (François et al. 2006; Safner et al. 2011), and corresponded to weak interactions with no identification of distinct genetic clusters for our data. Both moderate and strong interaction parameter values in TESS detected two clusters with no spatial variation in boundary location among runs, but only those of $\psi = 0.6$ are reported. Two individuals from Cluster 1 and three individuals from Cluster 2 remained unassigned for the lowest membership threshold. In addition, TESS identified cross assigned individuals for both Cluster 1 ($n = 1$) and Cluster 2 ($n = 3$) (Figure 2.2c).

Although numerous samples were identified by STRUCTURE and TESS as being cross assigned, only five first-generation dispersers ($P \leq 0.01$) were identified by GeneClass2 (Figure 2.2a, stars). In Cluster 1, a male from SK and a female from NU were identified as originating from Cluster 2 (MB and ON). The remaining three dispersers were all males sampled in MB, but identified as immigrants from Cluster 1 (SK, NU, and NT). All five first-generation dispersers were identified as cross assigned individuals by STRUCTURE without the LOCPRIOR model. In comparison, TESS identified only one disperser for Cluster 1 and all three for Cluster 2 that were first-generation dispersers.

Comparable results were obtained for both pairwise F_{ST} and D_{est} estimates; thus only the D_{est} results are presented (Mantel test $r = 0.98$, $P < 0.001$; Mantel 1967). Relatively low D_{est} estimates were observed when sampling regions within each genetic cluster were compared ($D_{est} < 0.01$). When sampling regions were compared between clusters, D_{est} values were moderately higher (0.04 to 0.08) except for the comparison between SK and MB (0.02, Table 2.2). Pooling the data by cluster produced a somewhat higher estimate, $D_{est} = 0.07$.

Mitochondrial DNA diversity

One hundred and fifty-six individuals were sequenced at the mitochondrial DNA control region, and nine haplotypes were identified with nine variable sites (Table 2.3). All haplotypes were compared to those reported in previous studies (Wilson et al. 2000; Chappell et al. 2004; Tomasik and Cook 2005; Cegelski et al. 2006; Frances 2008). Although similar haplotypes have been identified by multiple studies, the naming of haplotypes has been inconsistent across publications. We catalogued the haplotypes in

the order that each one was first published; thus the labeling of haplotypes in this study is not consecutive but reflective of this listing (Appendix 2.1).

The most common haplotype (Hap1) comprised 29% of all samples, and occurred predominately in ON (Figure 2.3a). The next most frequent haplotypes were Hap7 and Hap25 (Table 2.3). Hap7 was primarily observed in NT, and Hap25 only in MB and ON. Overall, NU, NT, and SK were largely characterized by Hap3, Hap6, Hap7 and Hap8, while the eastern periphery consisted of Hap1, Hap24 and Hap25 (Figure 2.3a). Hap24 was also observed in two individuals from the northwestern core, one from NT and the other from SK. Although only three haplotypes are identified within MB and ON, they occur in different frequencies across the two sampling regions (Table 2.3).

The number of haplotypes in each sampling region ranged from three in MB and ON to six in NU, NT and SK. Haplotype diversity (h) was high for all sampled regions, with the core genetic cluster having a slightly higher value in comparison to the peripheral genetic cluster (Table 2.4). The peripheral cluster had a slightly greater nucleotide diversity value than the core cluster (peripheral $\pi = 0.01$; core $\pi = 0.004$); however, nucleotide diversity was low for all sampled regions, indicating that the haplotypes were closely related. Tests for divergence from neutrality were found to be non-significant for both Tajima's D and Fu's F (Table 2.4). Adjusted total haplotype richness (H_R) was lowest for MB and ON, with SK having the greatest level (Table 2.4). Adjusted private haplotype richness analyses revealed NU having the highest number of private haplotypes and no private haplotypes were observed for NT, MB, and ON (0.00 - 1.55, Table 2.4). Data analysis based on genetic clusters further supported the observed pattern of the core population having greater total haplotype richness and more private

haplotypes in comparison to the peripheral population (core $H_R = 8.00$, $H_P = 6.00$; peripheral $H_R = 3.00$, $H_P = 1.00$).

Mitochondrial population structure

SAMOVA analyses indicated the presence of distinct genetic clusters. Partitions for $K = 2$ identified the eastern periphery and northwestern core genetic clusters. The indicator of differentiation, ϕ_{CT} , decreased only slightly between $K = 3$ and $K = 4$, thus our SAMOVA analyses suggested the presence of possibly three or four groups of maximally differentiated sampling regions (Table 2.5). For $K = 3$, the additional partition subdivided the eastern periphery, while $K = 4$ further separated SK from the northwestern core group. High ϕ_{CT} but low ϕ_{SC} values indicate that the inferred grouping of a northwestern core and a likely subdivided peripheral group is appropriate. Having few populations present in our SAMOVA analyses, however, led to there being a smaller number of populations within each group as K values increased, resulting in fewer differences between populations within groups (a reduction of ϕ_{SC} variance) and an increase in ϕ_{CT} (Dupanloup et al. 2002). Similar results were also obtained for AMOVA analysis.

Pairwise Φ_{ST} estimates (Table 2.2, below diagonal) revealed that wolverines were not genetically differentiated among NT, NU, and SK sampling regions, but that wolverines from these three regions were genetically differentiated from both MB and ON. Pairwise Φ_{ST} estimates also indicated that wolverines were genetically differentiated between the two peripheral sampling regions (Table 2.2), further supporting the genetic structure captured by SAMOVA. Analysis of the data based on nDNA genetic clusters produced a pairwise Φ_{ST} estimate of 0.26, indicating that the two clusters are genetically differentiated.

A median-joining network (Figure 2.3b) of mitochondrial sequences placed the two haplotypes (Hap24 and Hap25) exclusive to the eastern periphery at opposite branch ends, separating them by eight mutational steps and clearly indicating that these two haplotypes are quite divergent. The main body of the median-joining network was composed of haplotypes predominately occurring in the northwestern core.

Discussion

We found evidence that the extant population of wolverines in Canada is composed of two genetically distinct clusters – a range core and an eastern peripheral population. Neutral microsatellite markers revealed higher allelic diversity for the peripheral population than the range core, and phylogenetic analysis of mtDNA haplotypes was suggestive of more longstanding subdivisions between the two genetic clusters. Taken together these results suggest that the eastern peripheral wolverine population in North America should be recognized as a distinct evolutionary unit for conservation.

Nuclear DNA

Bayesian clustering analyses of our data suggest an increase in genetic structure along the eastern edge of the extant population. Although pairwise D_{est} estimates between the eastern periphery and the northwestern core were small (Table 2.2), the conclusion that the extant wolverine population is composed of two genetic clusters was supported by assignment tests from both STRUCTURE (Figure 2.2a) and TESS (Figure 2.2c). Overall, D_{est} estimates from this study were comparable to F_{ST} values observed by Kyle and Strobeck (2002). Comparing wolverine population structure between the eastern periphery and the southwestern edge revealed gene flow was restricted over shorter distances in the south (Cegelski et al. 2006). Factors likely contributing to greater

genetic structure in the south include a higher frequency of anthropogenic activities, and a matrix of lowland valleys and high mountainous regions in comparison to the vast expanses of continuous forest in the north (Banci 1994; Cegelski et al. 2006). Our results also corroborate earlier findings that wolverines from SK, NU and the NT are part of a large panmictic core population (Wilson et al. 2000; Kyle and Strobeck 2001, 2002; Chappell et al. 2004).

Significantly lower heterozygosity was observed for MB ($P = 0.03$, Table 2.1) even though recent population estimates for this region suggest that wolverine abundance is increasing (Slough 2007). This loss of heterozygosity is unlikely a result of the widespread population decline experienced during the mid 1900s (Johnson 1990), as low heterozygosity levels would have also been observed in adjacent regions.

Analysis of heterozygosity, allelic richness, and private alleles between the two genetic clusters revealed a trend towards slightly greater values for the peripheral population (Table 2.1). Although these values for the periphery are only marginally higher than those from the core, these values indicate that genetic variation is comparable across both clusters. A review by Eckert et al. (2008) of genetic variation across species' geographical ranges identified several instances where genetic diversity either remained unchanged or increased for populations at the range edge (e.g., Munwes et al. 2010). Increased genetic variability at the periphery may result from higher fluctuating selection so that peripheral animals are able to persist under frequently extreme climatic and biotic conditions (Brussard 1984; Parsons 1991). Even though empirical results illustrate the difficulty in using neutral genetic diversity as a surrogate indicator for variation in fitness-related loci (Aguilar et al. 2004) and adaptive variation (Zeisset and Beebee 2010), more extreme climatic conditions and unique habitats at range edges are likely to influence

non-neutral loci for peripheral populations. Gene flow, even at low rates, between the two clusters may reduce the genetic differentiation between populations and likely impede local adaptation of peripheral populations. Although the density of wolverines between these two clusters is considered low (COSEWIC 2003), occasional long-range dispersal between regions is possible given observations of long-distance movements (Magoun 1985; Gardner et al. 1986; Flagstad et al. 2004).

A comparison among the three assignment methods revealed an inconsistency in the number of putative dispersers. The LOCPRIOR model did not identify any cross assigned individuals, as membership coefficient values were inflated in favour of sampling locations. Conversely, STRUCTURE without the LOCPRIOR model gave higher estimates of cross assigned individuals than TESS. STRUCTURE cross assigning 14 samples for the lowest membership threshold, which included the five individuals GeneClass2 detected as first-generation dispersers. In comparison, TESS only identified five cross assigned individuals, four of which were recognized by GeneClass2 as first-generation dispersers. This discordance among the different assignment tests for inferring putative dispersers indicates that caution should be exercised when making conclusions based on a single assignment test (Cegelski et al. 2003). This is also a reflection of the continuous nature of these populations that are not clearly bounded by distinct geographic ranges, and the interaction between landscape configuration and the dispersal capacity of the species.

The identification of first-generation dispersers (Figure 2.2a, stars) supports previous reports of inferred long-distance movements by wolverines (Magoun 1985; Gardner et al. 1986; Flagstad et al. 2004). The asymmetrical movement of males from the core to the range edge means nuclear genes are being contributed to the eastern

periphery. This sex-biased dispersal could explain why haplotype richness was lower at the periphery (Table 2.4) but levels of allelic richness remained consistent across sampling regions (Table 2.1). Immigrants into a peripheral population that is genetically divergent from the core will introduce new alleles into this periphery and in turn increase levels of allelic richness. A fourfold reduction in effective population size is expected for mtDNA in comparison to nuclear markers, as a result of mtDNA being haploid and primarily maternally inherited. Higher levels of genetic drift and increased population differentiation for mtDNA due to this difference in effective population size, and our observation of male-biased dispersal from the core to the periphery provide an explanation as to why population pairwise Φ_{ST} estimates were greater for mitochondrial DNA in comparison to D_{est} estimates for nuclear microsatellite data (Table 2.2). While assignment tests can identify dispersers, these results should be interpreted with caution as the number of dispersers may not necessarily equate to the number of 'effective dispersers' (Cegelski et al. 2006).

Despite identifying two genetic clusters, a large percentage of individuals remained unassigned (Figure 2.2a). This was not unexpected given that both clusters were historically considered to comprise a single panmictic population due to the high vagility of the species (Magoun 1985; Gardner et al. 1986; Flagstad et al. 2004). Alternatively, wolverine populations have experienced significant declines as a result of fur harvests during the 20th century (Slough 2007), and the loss of scavenging opportunities (van Dijk et al. 2008) along with incidental poisoning (Lopez 1978) associated with wolf control efforts. This reduction in wolverine abundance, particularly at the eastern periphery where densities have historically been considered low, may have led to smaller isolated populations and increased effects of genetic drift. Anecdotal

evidence of wolverines recolonizing northern ON (Dawson 2000) suggests that populations along the eastern edge may be expanding. An expanding peripheral population may in part explain our observation of slightly elevated levels of heterozygosity, allelic richness and private alleles along this eastern range periphery (Table 2.1). Not enough time has elapsed, about eight generations, since wolf bounties ceased and fur harvests declined (Slough 2007; McKelvey et al. 2011) for wolverine densities to fully recover. This short timescale makes it unclear whether this likely population expansion at the eastern periphery is influencing observed levels of genetic variation or will result in increased gene flow between the two genetic clusters.

Although direct and indirect persecution of wolverines has largely been reduced, additional factors may continue to affect genetic structure for the extant population. The mechanisms influencing connectivity between peripheral and core populations likely include topographic features and environmental conditions (Banci 1994). For instance, a reduction of suitable habitat between these two genetic clusters – due to the vast expanses of prairie/Boreal Plains and Hudson's Bay (Figure 2.3a) – may represent a spatial bottleneck, a narrow corridor limiting the movement of dispersers. An associated reduction in successful colonizers may accentuate founder effects, resulting in genetic drift having a stronger influence at the periphery (McRae and Beier 2007; Rees et al. 2009). Another factor is the persistence of spring snow cover and its strong correlation with wolverine distribution, denning locations and genetic distances (Aubry et al. 2007; Schwartz et al. 2009; Copeland et al. 2010). Loss of spring snow cover due to climate change may limit connectivity among wolverine populations (Copeland et al. 2010). In addition, wolverines also appear to be limited by summer temperatures and human

activities associated with logged landscapes (Aubry et al. 2007; Bowman et al. 2010; Copeland et al. 2010).

Mitochondrial DNA

The genetic structuring of the mitochondrial DNA control region over relatively small geographic scales, and Hap1 as the most widespread haplotype parallel previous findings (Wilson et al. 2000; Chappell et al. 2004; Tomasik and Cook 2005; Cegelski et al. 2006; Schwartz et al. 2007; Frances 2008). Our observation of nearly two thirds of the haplotypes occurring exclusively in the core cluster is analogous with previous observations of increased haplotype diversity at the centre of the species range (Chappell et al. 2004; Tomasik and Cook 2005). Comparisons between the eastern periphery and southwestern range edges (Cegelski et al. 2006) in North America revealed greater haplotype diversity for ON and MB. There was a lower frequency of Hap1 in the eastern periphery, driving the haplotypic frequency differences between the regions.

Additionally, most of the genetic variation observed in the southwestern periphery was also present in the core range. This is in contrast to the genetic diversity at the eastern edge, where Hap24 and Hap25 were restricted primarily to the periphery. This lack of gene flow between the eastern periphery and the northwestern core likely reflects a more longstanding separation from the core. In comparison, the southwestern edge is more reflective of rapid range contraction resulting from anthropogenic activities.

Our SAMOVA results of either three or four genetic clusters (Table 2.5) are in contrast to Frances (2008), who was unable to identify population groups as higher K values resulted in the continuously increasing ϕ_{CT} estimates. The occurrence of three genetic clusters, with MB and ON as separate populations, is likely due to contrasting frequencies of the three haplotypes characterizing the eastern periphery. Haplotype

frequency also appears to explain why populations in SK are separate from the core group of populations for $K = 4$ (Figure 2.3a). Although $K = 3$ had the highest ϕ_{CT} value, the more notable SAMOVA result is the partition observed for $K = 2$ (Table 2.5). This separation of ON and MB from the remaining sampling regions not only supports our microsatellite results of a subdivision into a northwestern core and an eastern periphery, but also suggests that this division is more longstanding and deeply rooted to historical processes.

The two haplotypes largely restricted to the eastern periphery, Hap24 and Hap25, occurred at opposite ends of the median-joining network (Figure 2.3b) and not on the same branch as would be expected if the peripheral genetic cluster originated from a single glacial refugium. We present four hypotheses as explanations for the haplotype distribution observed at the eastern range edge. The first suggests that considerable population declines during the first half of the 20th century, as outlined earlier in the discussion, further decreased already low densities along the eastern periphery, thus increasing the effects of genetic drift where rare haplotypes like Hap24 and Hap25 became more frequent. An alternative explanation focuses on the glacial cycles shaping the genetic structure of biota in North America (Shafer et al. 2010). Fossil (Bryant 1987) and phylogeographic (Tomasik and Cook 2005) data suggest the presence of a single refugium in Beringia during the last glaciation. Over the past decade, however, a growing number of phylogeographic studies in North America have provided evidence for several southeastern refugia (Beatty and Provan 2010), like the coast of Labrador (Godbout et al. 2005), the Grand Banks south of Newfoundland (Holder et al. 1999), and the Appalachian Mountains (Pielou 1991). During the last glaciation wolverines may have experienced range expansions similar to other Holarctic species (Flagstad and Røed

2003; Dalén et al. 2005), and possibly occupied high-latitude refugia within the ice sheets of eastern North America. The coalescence of multiple eastern glacial refugia may explain the nearly exclusive occurrence of Hap24 and Hap25 at the eastern periphery of the extant wolverine range in North America. Alternatively, Hap25 could be a remnant haplotype from the functionally extirpated population of northern Quebec and Labrador, which may have occupied an entirely separate refugium. Finally, it is also likely that there have been several colonization events across the Bering Strait from multiple Pleistocene glaciation periods, as proposed for arctic hares (Waltari and Cook 2005). Phylogeographic analysis of Hap25 with published wolverine haplotypes revealed that Hap25 was more closely related to haplotypes identified in Mongolia and California, USA (Schwartz et al. 2007) than to extant haplotypes occurring in North American (Chapter 3). Even though we did not observe any slightly deviating haplotypes centred around the more widely distributed haplotypes (e.g., Hap1), as would be expected if past glaciation events were the only contributing factor shaping present day patterns of mtDNA diversity, this does not exclude the possibility of previous glacial cycles influencing the genetic diversity of wolverines in North America. A more plausible explanation is that the observed haplotype pattern reflects the combination of former glacial events with contemporary demography.

Conservation implications

The identification of a separate genetic cluster along the eastern edge of the species range in North America has implications for COSEWIC Designatable Units. Designatable Units should be of evolutionary significance, where the unit in question is considered important to the overall evolutionary legacy of the species and, if lost, would not be replaced by natural dispersion (COSEWIC 2012). Pronounced selection pressures

associated with peripheral populations due to larger environmental fluctuations (Cassel-Lundhagen et al. 2009) are likely to result in peripheral populations containing genotypes with a greater capacity to respond to future selection pressures like climate change. In addition, peripheral populations would be more likely to colonize new areas as habitat conditions are generally similar to regions just outside the current species' distribution (Safriel et al. 1994). This is of particular significance for the eastern peripheral genetic cluster, where wolverines in Ontario occupy a lowland boreal forest ecotype different from the larger taiga ecoregion of their distribution. Wolverines along the eastern periphery may be better adapted to inhabit lowland boreal forest habitats, and in turn become a key source population of genetic diversity should northern habitats become replaced by more southern ones due to climate change.

Conclusions

Our results support the view presented in past studies (e.g., Lesica and Allendorf 1995; Vucetich and Waite 2003) that peripheral populations can be a source of genetic diversity not observed elsewhere in the species' range, and that edge populations may comprise a unique evolutionary unit. Although the contemporary subdivision of the extant population may reflect anthropogenic influences during the past century, mitochondrial DNA analyses suggest a more longstanding genetic division. What remains unresolved is whether this peripheral genetic cluster is part of the functionally extirpated population that once extended into the northern Appalachian ecoregion. Additional research is needed in understanding the phylogeography of this boreal carnivore by identifying the underlining processes associated with the more longstanding genetic subdivisions. Furthermore, we suggest that future investigations also examine the genetic diversity of functional markers between the eastern periphery and the core to help

resolve the designation of the eastern periphery as a separate management unit. Disjunct peripheral populations are not only likely to be genetically divergent from core populations but may also harbour distinct genotypes crucial for adaptation to local or new conditions (e.g., García-Ramos and Kirkpatrick 1997). Identifying present-day populations of Holarctic species harbouring genetic diversity associated with unique biogeographical traits that have been preferred for their ecological and evolutionary importance is of high conservation importance, particularly when these populations are located at the periphery of the species' range (Hampe and Petit 2005).

Addendum

Alternative hypothesis of isolation by distance

Wolverines are mainly continuously distributed throughout their northern range from Alaska to Hudson Bay, Canada (Kyle and Strobeck 2001), with no obvious barriers to movement. There is the possibility that the identified eastern peripheral genetic cluster (MB and ON) could be part of a larger continuum. An alternative hypothesis is that population genetic structure of wolverines across Canada would display a pattern of isolation by distance. Subsequent to this study (Chapter 2), clustering analyses were performed across the wolverine's extant distribution in Canada along with a simple Mantel test to assess the relationship between geographical and population genetic distances. Clustering analyses distinguished two genetic clusters, with MB and ON grouping separately from the rest of Canada. The simple Mantel test revealed a strong and significant correlation between genetic and geographical distances ($r = 0.54$, $P = 0.01$; Rico et al. unpublished data), indicating that genetic differentiation of wolverines across their Canadian distribution followed an isolation by distance pattern.

While the structuring pattern supports isolation by distance (Rico et al. unpublished data), the support of these weak clusters at higher membership thresholds (Chapter 2) suggests that the observed clustering pattern reflects spatial changes in genetic variation even though population borders are not defined (Pelletier et al. 2012). Identifying more precisely where this change in genetic variation occurs could be useful for management and conservation decisions. Aside from including additional sampling regions from western Canada, Rico et al. (unpublished data) did not add any new samples to the five regions analyzed in this study (Chapter 2) and based their analyses on the same 11 microsatellites used here. More intensive sampling across a larger geographic area of the wolverine's range and the use of more loci will help to better resolve the two hypotheses (two genetic clusters vs. isolation by distance) and help determine a more precise region where this clinal transition zone occurs.

Relatedness among individuals

Lastly, the estimated levels of genetic structure were unlikely to be influenced by relatedness among individuals from increased sampling in the Red Lake region. Within the Red Lake intensive sampling area, 90.6% of all pairs of individuals ($n = 741$) were unrelated and only 1.21% of putative pairs had a genetic similarity corresponding to either full siblings or parent-offspring.

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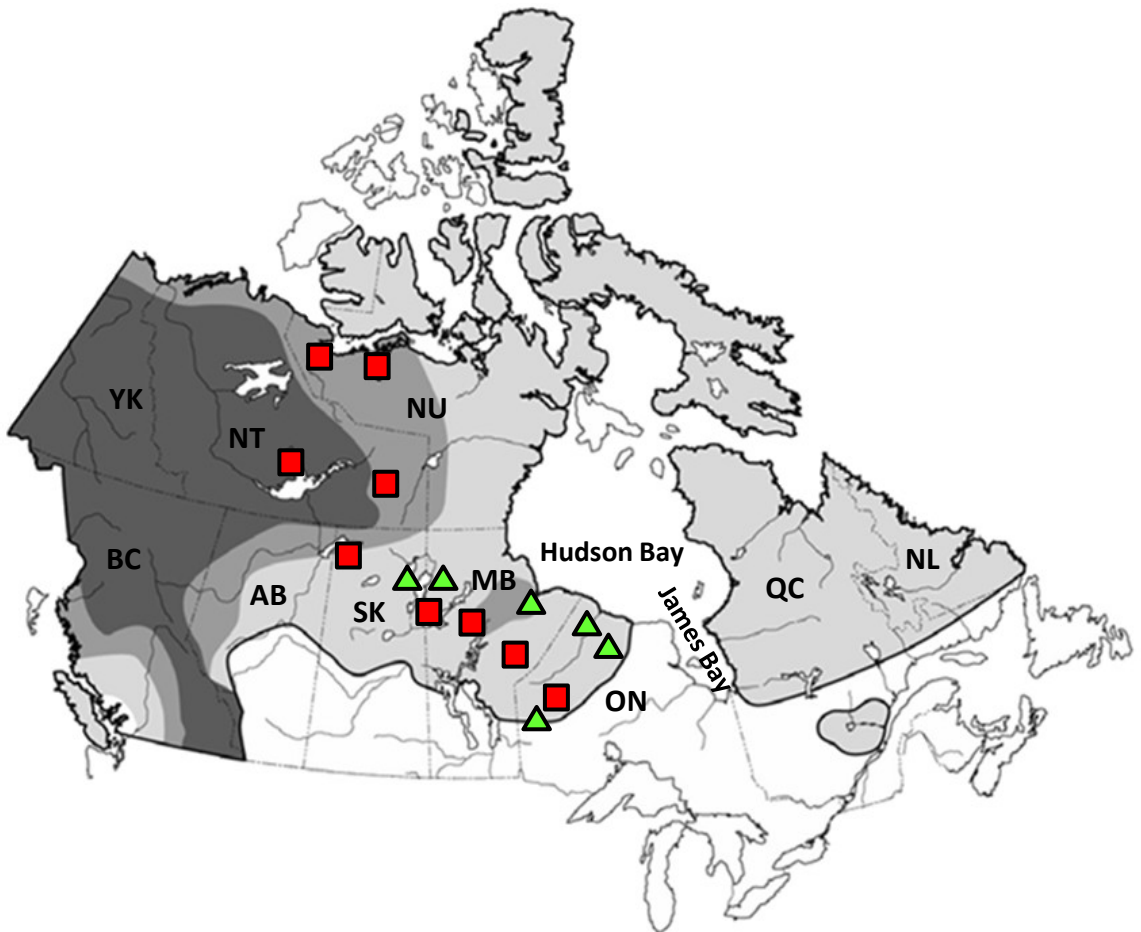


Figure 2.1 Map depicting contemporary distribution and density (low, light grey; medium, grey; high, dark grey, COSEWIC 2003) of *Gulo gulo* in Canada, and sampled localities of wolverines. Red squares represent sampled localities from Kyle and Strobeck (2001, 2002) and green triangles represent localities sampled in this study. Symbols depict the general areas where samples were collected.

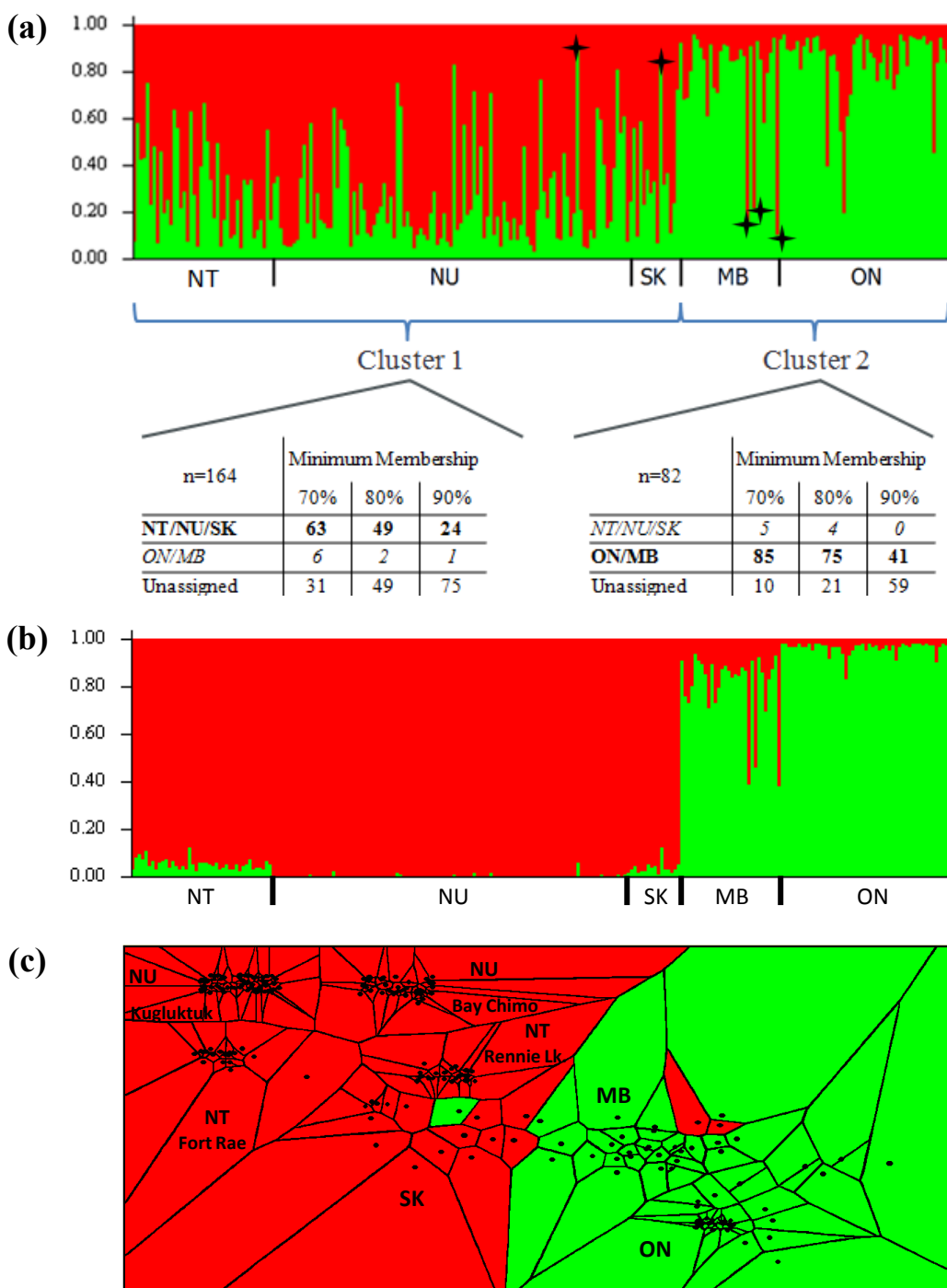


Figure 2.2 Individual based clustering results: **a** STRUCTURE (Pritchard et al. 2000) summary plot of the estimated membership coefficient (x axis) for each sampled region based on 11 microsatellite loci. Each individual is represented by a single column broken

into different colour segments, where segments are proportional to the membership coefficient for each of the inferred K clusters. Individuals are arranged by source populations from which they were sampled, and marked with a star if identified by GeneClass2 (Piry et al. 2004) as first-generation dispersers. Tables represent percentage of number of individuals assigned to each genetic cluster based on varying minimum membership; **b** STRUCTURE (Pritchard et al. 2000) summary plot including geographic sampling locations as prior information; **c** clusters as determined by TESS (Chen et al. 2007), $K = 2$ and interaction parameter $w = 0.6$.

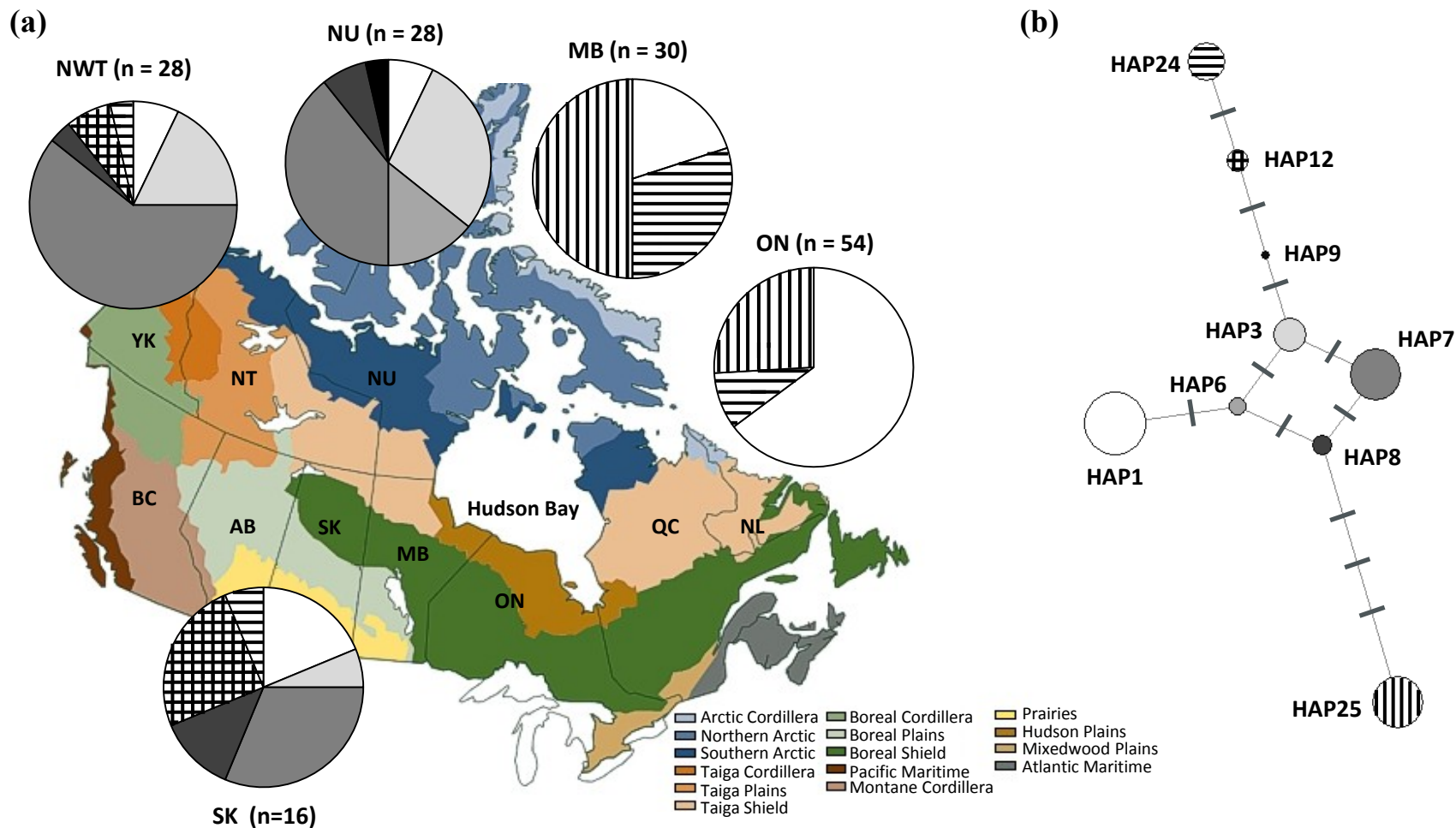


Figure 2.3 a Map of terrestrial eozones in Canada (adapted from Ecological Stratification Working Group 1996, and Canadian Council of Forest Ministries 2006), and geographic distribution of the nine mtDNA control region haplotypes based on frequencies

observed for each sampling region listed in Table 2.3; **b** median-joining network of the haplotypes found in this study, where circle size is proportional to haplotype frequency and black dashes refer to mutational event.

Table 2.1 Estimates of genetic diversity of 11 microsatellite loci for each region sampled and genetic cluster based on STRUCTURE (Prichard et al. 2000) and TESS (Chen et al. 2007) analyses.

	n	H_O (%)	H_E (%)	A_R	A_P
Sampling Region					
NT	42	61.8	64.4	4.08	0.08
NU	106	65.2	65.1	4.02	0.14
SK	16	63.7	63.5	3.93	0.09
MB	30	58.4	67.3	4.09	0.07
ON	52	69.9	68.3	4.14	0.23
Genetic Cluster					
Core	164	64.18	64.78	5.17	0.46
Periphery	82	65.64	68.13	5.31	0.60

n sample size, H_O observed heterozygosity, H_E expected heterozygosity, A_R allelic richness and A_P private alleles standardized to the smallest sample size using ADZE rarefaction (Szpiech et al. 2008)

ON Ontario, *MB* Manitoba, *SK* Saskatchewan, *NT* Northwest Territories, *NU* Nunavut, *Core* SK, NT and NU, *Periphery* ON and MB

Table 2.2 Pairwise estimates of population genetic distance for both nuclear (D_{est} , above diagonal) and mitochondrial (Φ_{ST} , below diagonal) DNA among sampling regions.

		D_{est}				
		NT	NU	SK	MB	ON
Φ_{ST}	NT	-	<0.01	<0.01	0.04	0.07
	NU	0.03**	-	<0.01	0.05	0.08
	SK	0.06**	0.07**	-	0.02	0.05
	MB	0.30**	0.29**	0.18	-	<0.01
	ON	0.36**	0.30**	0.23	0.14	-

ON Ontario, *MB* Manitoba, *SK* Saskatchewan, *NT* Northwest Territories, *NU* Nunavut

**Significance for $\Phi_{ST} P < 0.01$

Table 2.3 Identified mtDNA haplotypes for each region sampled and genetic cluster based on STRUCTURE (Prichard et al. 2000) and TESS (Chen et al. 2007) analyses.

Variable Nucleotide Position									Haplotype	Sampling Region Frequency (%)					Genetic Cluster Frequency (%)	
90	103	165	166	189	220	227	242	248		NT	NU	SK	MB	ON	Core	Periphery
T	C	-	-	C	A	G	T	T	Hap1	7	7	19	20	65	10	49
•	T	•	•	T	•	•	•	•	Hap3	18	29	6	0	0	19	0
•	•	•	•	T	•	•	•	•	Hap6	0	14	0	0	0	6	0
•	T	•	•	T	•	A	•	•	Hap7	60	39	31	0	0	46	0
•	•	•	•	T	•	A	•	•	Hap8	4	7	13	0	0	7	0
•	T	C	•	T	•	•	•	•	Hap9	0	4	0	0	0	1	0
•	T	C	•	T	G	•	•	•	Hap12	7	0	25	0	0	8	0
•	T	C	C	T	G	•	•	•	Hap24	4	0	6	30	9	3	17
C	•	•	•	T	•	A	C	C	Hap25	0	0	0	50	26	0	34
<i>n</i>										28	28	16	30	54	72	84

Variable nucleotide positions are denoted according to their location within the 360bp portion of the D-loop control region.

n sample size

ON Ontario, *MB* Manitoba, *SK* Saskatchewan, *NT* Northwest Territories, *NU* Nunavut, *Core* SK, NT and NU, *Periphery* ON and MB

Table 2.4 Genetic diversity measures of the five sampling regions and two inferred genetic clusters based on STRUCTURE (Prichard et al. 2000) and TESS (Chen et al. 2007) analyses.

	<i>n</i>	π	<i>SD</i> π	<i>h</i>	<i>SD</i> <i>h</i>	H_R	H_P	Tajima's D	<i>P</i>	Fu's F	<i>P</i>
Sampling Region											
NT	28	0.004	0.003	0.61	0.09	4.79	0.00	-0.04	0.50	-1.01	0.27
NU	28	0.003	0.002	0.76	0.05	5.20	1.55	0.95	0.83	-1.33	0.18
SK	16	0.007	0.004	0.83	0.06	6.00	0.25	1.46	0.93	-0.26	0.44
MB	30	0.01	0.006	0.64	0.05	2.99	<0.01	2.51	1.00	8.04	0.99
ON	54	0.008	0.005	0.51	0.06	2.84	<0.01	1.42	0.93	6.83	0.98
Genetic Cluster											
Core	72	0.004	0.003	0.74	0.04	8.00	6.00	1.04	0.85	-0.91	0.37
Periphery	92	0.01	0.006	0.62	0.03	3.00	1.00	2.58	0.99	10.08	0.99

n sample size, π nucleotide diversity, *h* haplotype diversity, *SD* standard deviation, H_R haplotype richness and H_P private haplotypes standardized to the smallest sample size using ADZE rarefaction (Szpiech et al. 2008), Tajima's D (Tajima 1989), Fu's F_s (Fu 1997), *P* *P* value

ON Ontario, *MB* Manitoba, *SK* Saskatchewan, *NT* Northwest Territories, *NU* Nunavut, *Core* SK, NT and NU, *Periphery* ON and MB

Table 2.5 Spatial Analysis of Molecular Variance (SAMOVA, Dupanloup et al. 2002)

results for different population configurations of mtDNA.

Source of Variation	Variance Components	Percentage of Variance	ϕ -statistics	<i>P</i>
<i>K=2 (MB/ON vs. NT/NU/SK)</i>				
Among groups	0.40	26.31	$\phi_{CT} = 0.26$	0.09
Among populations within groups	0.11	7.28	$\phi_{SC} = 0.10$	<0.001
Within populations	1.00	66.41	$\phi_{ST} = 0.34$	<0.001
<i>K=3 (MB vs. ON vs. NT/NU/SK)</i>				
Among groups	0.44	30.20	$\phi_{CT} = 0.30$	0.1
Among populations within groups	<0.01	0.56	$\phi_{SC} < 0.01$	<0.001
Within populations	1.00	69.24	$\phi_{ST} = 0.31$	<0.001
<i>K=4 (MB vs. ON vs. NT/NU vs. SK)</i>				
Among groups	0.40	28.41	$\phi_{CT} = 0.28$	0.09
Among populations within groups	<0.01	0.12	$\phi_{SC} < 0.01$	<0.001
Within populations	1.00	71.74	$\phi_{ST} = 0.29$	<0.001

Appendix 2.1 The control region haplotype labels in this study and their correspondence to those identified in previous studies, where sequential numbering of each haplotype is reflective of when it was first published in the literature.

This Study	Wilson et al. 2000	Chappell et al. 2004	Tomasik and Cook 2005	Cegelski et al. 2006	Frances 2008	GenBank Accession Numbers
Hap1	A		B	A	Hap1	AF210090
Hap3	C		G	C	Hap10	AF210107
Hap6	F		F	F	Hap6	AF210105
Hap7	G		E		Hap9	AF210106
Hap8	H		A	H	Hap2	AF210130
Hap9	I		H		Hap11	AF210112
Hap12			D		Hap3	AF55403
Hap24		K			Hap23	EU812449
Hap25		J			Hap22	EU812445

CHAPTER 3

PHYLOGEOGRAPHY AND POST-GLACIAL RECOLONIZATION IN WOLVERINES (*GULO GULO*) ACROSS THEIR CIRCUMPOLAR DISTRIBUTION

A version of this chapter has been published

Zigouris J, Schaefer JA, Fortin C, Kyle CJ (2013) Phylogeography and post-glacial recolonization in wolverines (*Gulo gulo*) from across their circumpolar distribution. PLoS ONE 8:e83837.

Abstract

Interglacial-glacial cycles of the Quaternary are widely recognized in shaping phylogeographic structure. Patterns from cold-adapted species can be especially informative – in particular, uncovering additional glacial refugia, identifying likely recolonization patterns, and increasing our understanding of species' responses to climate change. We investigated phylogenetic structure of the wolverine, a wide-ranging cold-adapted carnivore, using a 318 bp of the mitochondrial DNA control region for 983 wolverines ($n = 209$ this study, $n = 774$ from GenBank) from across their full Holarctic distribution. Bayesian phylogenetic tree reconstruction and the distribution of observed pairwise haplotype differences (mismatch distribution) provided evidence of a single rapid population expansion across the wolverine's Holarctic range. Even though molecular evidence corroborated a single refugium, significant subdivisions of population genetic structure ($0.01 < \Phi_{ST} < 0.99$, $P < 0.05$) were detected. Pairwise Φ_{ST} estimates separated Scandinavia from Russia and Mongolia, and identified five main divisions within North America – the Central Arctic, a western region, an eastern region consisting of Ontario and Quebec/Labrador, Manitoba, and California. These data are in contrast to the nearly panmictic structure observed in northwestern North America using nuclear microsatellites, but largely support the nuclear DNA separation of contemporary Manitoba and Ontario wolverines from northern populations. Historic samples (c. 1900) from the functionally extirpated eastern population of Quebec/Labrador displayed genetic similarities to contemporary Ontario wolverines. To understand these divergence patterns, four competing recolonization hypotheses were tested using Approximate Bayesian Computation (ABC). The most supported scenario was a single Beringia incursion during the last glacial maximum that established the northwestern population,

followed by a west-to-east colonization during the Holocene. This pattern is suggestive of colonization occurring in accordance with glacial retreat, and supports expansion from a single refugium. These data are significant relative to current discussions on the conservation status of this species across its range.

Introduction

Understanding phylogeographic differentiation and patterns of genetic diversity for many extant species in the Northern Hemisphere hinges on understanding the interglacial-glacial cycles of the Quaternary (Dalén et al. 2005; Shafer et al. 2010). Cold-adapted species can be particularly informative as these species typically experience population increases and range expansions during glacials, and range contractions during interglacials (Flagstad and Røed 2003; Dalén et al. 2005). These species may also undergo range contractions into refugia when continental ice-sheets attain their greatest extent (e.g., Stuart et al. 2004), further shaping present-day patterns of biodiversity in the arctic (Fedorov and Stenseth 2001).

Refugial history has focused predominately on temperate taxa (Hewitt 2000), revealing major refugia – like Beringia (Pielou 1991). However, progressively more investigations are proposing 'cryptic' glacial refugia beyond the limit of glaciations (see review by Shafer et al. 2010). Such refugia are usually limited and sporadic in geographic extent, and often overlooked in glacial biogeographic reconstructions based on fossil records (Huck et al. 2009; Schmitt and Varga 2012). However, identification of the same regions by multiple studies (see Beatty and Provan 2010) signifies these refugia are not so much cryptic as additional to conventional Pleistocene refugia (Rull 2010). For many taxa, isolation of populations in separate refugia resulted in the formation of distinct genetic lineages (Hewitt 2000; Steele and Storfer 2006). Patterns of genetic differentiation are also reflective of stochastic processes like genetic drift and species-specific dispersal abilities (Davison et al. 2001). Wide-ranging species generally display a lack of population structuring across their range, increasing the likelihood of similar mitochondrial haplotypes being observed across very distant geographical locations (Vila

et al. 1999; Walker et al. 2001). Overall, these data are relevant to understanding how historic processes influence contemporary genetic patterns and how these data should be interpreted in context of management actions for species of conservation concern.

Most phylogeographic studies of cold-adapted taxa have focused on vegetation (Abbott et al. 2000), birds (Holder et al. 1999), and small mammals (Fleming and Cook 2002). Investigations of large mammals remain limited (Loehr et al. 2006; Klütsch et al. 2012), even though large body size (>5.5 kg) is a strong predictor of extinction risk for mammals (Davidson et al. 2009). Furthermore, relic populations may contain biogeographic traits associated with glacial refugia (Bhagwat and Wills 2008), particularly at range peripheries. This has conservation implications where peripheral populations – faced with increased environmental fluctuations (Cassel-Lundhagen et al. 2009), reduced gene flow and low densities (Lesica and Allendorf 1995) – may maintain unique genetic variability necessary in responding to climate change (Hampe and Petit 2005).

Wolverines (*Gulo gulo*) are a cold-adapted carnivore with a circumboreal distribution (Figure 3.1; Landa et al. 2000). Fossil evidence is minimal for this species (Anderson 1977), and from those records that do exist, fossils in Europe extend from the Iberian Peninsula (Döppes 2001) eastwards to the Czech Republic (Diedrich 2009). In North America, late Pleistocene fossil remains have been found in Alaska (Porter 1988) and the Yukon Territory (Bryant 1987). Given the gaps in the fossil record, genetic evidence could provide a clearer picture of how glacial refugia have shaped postglacial recolonization of wolverines and other cold-adapted species.

In the past half-century, wolverines have experienced substantial range reductions along the southern edge of their circumpolar distribution due to habitat alterations,

persecution, and other anthropogenic influences such as indirect poisoning campaigns targeted at wolves (Figure 3.1; Wilson 1982; Schreiber et al. 1989; Landa et al. 2000). In North America, this animal has lost 37% of its historic range (Laliberte and Ripple 2004). Although globally a species of least concern (Abramov et al. 2009), its regional status ranges from stable (Alaska; Banci 1994) to endangered (Norway; Norwegian Biodiversity Information Centre 2010), and in some areas, functionally extirpated (Quebec-Labrador; Fortin et al. 2005). Wolverines occur at low densities (Lofroth and Krebs 2007) and have very large home range sizes (188 - 2563 km² for males; Banci and Harestad 1990; Dawson et al. 2010), increasing their vulnerability to habitat fragmentation and external anthropogenic threats such as logging, fur harvesting and direct persecution (Haskell et al. 2002; Bowman et al. 2010; International Union for Conservation of Nature and Natural Resources, IUCN 2011).

Wolverines are highly vagile (Banci and Harestad 1990; Dawson et al. 2010), largely explaining the high levels of gene flow observed among populations based on nuclear DNA (Wilson et al. 2000; Kyle and Strobeck 2001, 2002; Chappell et al. 2004; Cegelski et al. 2006). Genetic structure, however, tends to increase towards range peripheries in North America (Kyle and Strobeck 2001, 2002; Cegelski et al. 2003, 2006; Zigouris et al. 2012), suggesting irregular distributions of populations (Banci 1994) due to range contractions (IUCN 2011). Additionally, spring snow cover is positively correlated with wolverine distribution (Aubry et al. 2007; Copeland et al. 2010) and genetic differentiation (Schwartz et al. 2009), underlining the sensitivity of wolverines to climate change. Mitochondrial DNA (mtDNA) studies of this species have revealed strong genetic structure over small geographic scales, reflecting female philopatry (e.g., Tomasik and Cook 2005; Cegelski et al. 2006). In North America, the two haplotypes (Hap24 and

Hap25) predominantly found at the eastern periphery were separated by eight mutational steps and three steps between Hap25 and the adjacent Hap8, suggesting a longstanding subdivision reflective of historical processes (Zigouris et al. 2012).

Here, we investigated the phylogeographic patterns of wolverines across the full breadth of their Holarctic range (>5 million km²) using conventional Bayesian approaches. We obtained samples from 209 individuals together with existing data from 774 wolverines to assess the genetic variation of the mtDNA control region. In addition, we apply a coalescent-based approximate Bayesian computation (ABC) method to test among competing hypotheses of postulated recolonization pathways influencing population divergence. We propose four alternative hypotheses that may explain observed patterns of haplotype distribution and resulting groupings that include: (1) present-day populations diverged from a single ancestral population during the last glacial maximum (LGM); (2) a single incursion across Beringia resulted in the divergence between Eastern and Western Hemisphere wolverines, with divergence of North American populations occurring during glacial retreat; (3) a single incursion across Beringia during the LGM, followed by a west-to-east stepping-stone divergence pattern across North America; and (4) two incursions from Beringia during the LGM, with the second incursion being followed by a west-to-east stepping-stone divergence. Understanding the response of cold-adapted species to past climatic fluctuations could help identify evolutionary significant units (Cossíos et al. 2009) and improve predictions of the effects of climate change on arctic wildlife (Provan and Bennett 2008).

Methods

Samples

We processed contemporary samples collected from: Russia (RUS, $n = 49$), Yukon (YK, $n = 26$), British Columbia (BC, $n = 81$), Alberta (AB, $n = 26$) and Manitoba (MB, $n = 1$), and historical samples (Figure 3.1, Appendix 3.1) collected between 1889 - 1944 as identified by the Global Diversity Information Facility (<http://www.gbif.org/>) from the Yukon ($n = 9$, c. 1923 - 1932), Nunavut (NU, $n = 1$, c. 1944), British Columbia ($n = 11$, c. 1910 - 1927; with one sample representing *G. gulo vancouverensis* from Vancouver Island, Canada), Saskatchewan (SK, $n = 1$, c. 1920), Ontario (ON, $n = 1$, c. 1920) and Quebec-Labrador (QC/NL, $n = 15$, c. 1883 - 1900). Contemporary samples for Russia, Yukon, Alberta, and Manitoba were collected from pelts through fur auction houses and pelt dealers. British Columbia samples were obtained from pelt samples through fur auction houses, and tissue samples (ear plug and hair) collected by the Columbia Basin Fish and Wildlife Compensation Program. Permission to acquire tissue samples from archived specimens was obtained from all museums and institutions, and all tissue samples from historic specimens were donated. Our data were combined with mtDNA control region data from previous studies (Figure 3.1; Wilson et al. 2000; Walker et al. 2001; Tomasik and Cook 2005; Cegelski et al. 2006; Arnason et al. 2007; Frances 2008; Zigouris et al. 2012; Rochnov and Meschersky unpublished data) – including samples from: Sweden (SWE, $n = 62$), Norway (NOR, $n = 108$) Mongolia (MNG, $n = 6$), Russia ($n = 5$), Alaska (AK, $n = 148$), Yukon ($n = 23$), Nunavut ($n = 81$), Northwest Territories (NT, $n = 53$), British Columbia ($n = 5$), Saskatchewan ($n = 16$), Manitoba ($n = 30$), Ontario ($n = 54$), Montana (MT, $n = 148$), Wyoming (WY, $n = 13$), Idaho (ID, $n = 15$), and California (CA, $n = 7$). We did not include data reported in Cegelski et al.

(2006) for BC and AB, and for ON reported by Frances (2008) as samples were obtained from C.J. Kyle and likely the same individuals. We also excluded sequence data from Chappell et al. (2004) as the mtDNA control region was 200 bp and did not encompass five known variable sites in the larger fragment.

Preparation of bone samples and DNA extraction

Bone dust (~100 µL) was collected from museum specimens by drilling mandibles or from turbinate bones frozen with liquid nitrogen and crushed into bone powder using a mortar and pestle. Mandibles were initially washed with Decon solution (1:49), rinsed with DNAase-free ddH₂O (Gibco), and the outer surface removed using a Dremel tool. Strict laboratory protocols were followed to minimize risk of cross-contamination from contemporary sources. Specifically, equipment was sterilized with Decon solution (1:9) and rinsed with DNAase-free water between handling each sample, and filter tips and disposable pipettes were used. Extraction blanks were included at the beginning and end of extractions, and after every fifth sample to assess sample cross-contamination. Historic samples were processed in rooms not exposed to contemporary samples.

Extraction procedures followed the manufacturer's protocol for Qiagen's DNeasy tissue extraction kit (Qiagen) for all contemporary samples, with the following modifications for historic samples: (1) historic samples were rotated overnight at room temperature in 1.5 mL of ethylenediaminetetraacetic acid (EDTA; 0.5 M, pH 7.5) to decalcify the bone powder, and after 24 h samples were centrifuged at 9 000 rpm for 60 s, EDTA was poured off and a second treatment applied; (2) used 1.5 mL of Buffer ATL and treated samples with a second dose of 20 µL Proteinase K (600 mAU/mL) (Qiagen, Mississauga), followed by a 2 h incubation at 56°C; (3) incubated for 10 min at 56°C after adding buffer AL; (4) performed two elution steps with heated buffer at 70°C; and

(5) transferred both elutions to an Amicon Ultra-0.5 centrifugal unit that yielded approximately 20 μL of DNA.

Mitochondrial DNA Sequencing

A 360 bp fragment of the control region was independently amplified twice using primers Gulo0F (Schwartz et al. 2007) and H16498 (Ward et al. 1991). Amplification of a shorter sequence length allowed for data from previous studies to be compared with our data set. Sequencing procedures followed Zigouris et al. (2012); however, for the historic samples Taq was increased from 0.05 to 0.1 U/ μL and PCR cycles increased from 30 to 50. Additionally, PCR products that showed extra banding were run on a gel, and the target band was cut out. Excised bands were frozen overnight at -80°C , vortexed and centrifuged. Amplified DNA expelled from excised gel bands and from samples with low DNA concentrations were re-amplified a second time to confirm sequence data.

Data analyses

Sequences were edited with MEGA 4.0.2 (Tamura et al. 2007), aligned with Clustal W (Larkin et al. 2007), and verified visually. We identified variable nucleotide positions and compiled unique sequences using FABOX 1.35 (Villesen 2007). New haplotypes were confirmed only when independent PCR reactions generated the same sequence. Historic and contemporary samples were both treated as separate entries (historic SK omitted due to $n = 1$), as well as combined by sampling region. In this study, historic and contemporary samples are reflective of sampling occurring pre- and post-20th century population declines (Slough 2007). Thus, frequency differences between pre- and post-population declines may provide insight of the underlining processes influencing present-day patterns of haplotype distribution and frequency. Nucleotide (π) and haplotype (h) diversity values were estimated with ARLEQUIN 3.5 (Excoffier and

Lischer 2010). Haplotype richness and number of private haplotypes were calculated manually and with a rarefaction test (ADZE 1.0; Szpiech et al. 2008) based on the smallest sample size. We tested for departures from neutrality (Tajima's D ; Tajima 1989) and population growth (Fu's F_s , $P < 0.02$; Fu 1997) using ARLEQUIN with 10 000 bootstrap replicates.

Differentiation among sampling sites were estimated with pairwise Φ_{ST} values using ARLEQUIN (10 000 permutations, $P < 0.05$). An analysis of molecular variance (AMOVA) was used to quantify genetic variability among groups of populations, as well as within and among populations (Excoffier et al. 1992), in ARLEQUIN for 10 100 permutations. Groupings reflected the sharing of haplotypes among sampled regions irrespective of geographic location. We evaluated optimal grouping of sites by defining groups of samples maximally differentiated, but geographically proximate to each other. We performed a spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 (Dupanloup et al. 2002), $2 \leq K \leq 10$, using 100 random initial conditions and 10 000 iterations. Historic samples from YK, BC and SK were omitted from SAMOVA because they represented some of the main haplotypes observed in contemporary samples. SAMOVA analyses were performed with and without historic samples from CA and QC/NL for all contemporary data and for North American samples only. Historic samples from CA and QC/NL were included, as contemporary data was absent from these two peripheral regions. We also conducted Mantel tests (Mantel 1967) using Isolation by Distance (IBD) Web Service 3.23 (Jensen et al. 2005) to test for correlations between genetic and geographic distances. To minimize error in characterizing IBD (McRae et al. 2005), we excluded sample regions with $n < 10$ individuals. Correlations were tested between the natural logarithm of geographical distances and the regression of paired Φ_{ST} .

$/(1 - \Phi_{ST})$ estimates as proposed by Rousset (1997). Mantel tests were performed on North American regions and the data as a whole. For Eurasia, there were too few distance classes for IBD to be detected (Slatkin and Maddison 1990).

We performed Bayesian analyses in BEAST 1.7.1 (Drummond et al. 2012) to investigate phylogenetic composition of lineages and estimate divergence times (e.g., Rajabi-Maham et al. 2008). The Hasegawa-Kishino-Yano (HKY, Hasegawa et al. 1985) model with a gamma shape distribution and invariant sites best fit our data based on Akaike Information Criterion (AIC) values (jModelTest 0.1.1, Posada 2008). Base frequencies were estimated and rate variation among sites was modeled using four gamma rate categories. Divergence estimates were calculated using two independent calibration points (Rambaut and Drummond 2009a), averaged across two phylogenetic studies (Koepfli et al. 2008; Sato et al. 2009: divergence between the *Gulo/Martes* clade and *Mustela* 11.9 (9.9 - 14.1, 95% CI) million years ago (Mya); and divergence of *Gulo* and *Martes* 6.1 (4.3 - 8.1, 95% CI) Mya. Control region sequences of mtDNA for *Mustela putorius*, *M. frenata* and *M. nivalis* represented the Mustelidae outgroup (GenBank Accession Numbers: AY962032; HM106321; HM106319), and *Martes martes* was used for the *Martes* outgroup (Accession Number: AJ585357). We used an uncorrelated lognormal relaxed clock model with a Coalescent constant size tree and normal distribution priors. Analyses were run for 40 million generations, with a burn-in of the first 4 million, and a sampling frequency of 500 steps. Tracer 1.5 (Rambaut and Drummond 2009b) was used to evaluate estimated values and effective sample size (ESS) for each model parameter. For all parameters, $ESS > 450$ suggested sufficient sampling and acceptable mixing. Bayesian analyses were computed multiple times to check for convergence. The phylogenetic tree was constructed using TreeAnnotator with a 20%

burn-in of total trees generated, and viewed in FigTree 1.3.1 (Rambaut 2009). A median-joining network was created to visualize haplotype relationships using Network 4.5 (Bandelt et al. 1999).

To test for population expansion, we used ARLEQUIN with 10 000 bootstrap replicates to estimate the distribution of observed pairwise haplotype differences (mismatch distribution, Rogers and Harpending 1992) among individuals. Populations that have undergone recent expansion display a unimodal distribution, whereas populations at demographic equilibrium are multimodal (Rogers and Harpending 1992). The validity of the estimated expansion model was evaluated using the sum of squared deviations (SSD) between observed and expected mismatch distribution values (Schneider and Excoffier 1999), and the raggedness index (r , Harpending 1994) that measures the smoothness of the mismatch distribution. Given the low power of r to detect population expansions, we also calculated Fu's F_s statistic on the whole data set.

Based on the results of the preceding analyses, we assumed that all populations had the same colonization origin, and likely located near Beringia. We compared four alternative colonization pathways (Figure 3.2) using the coalescent-based approximate Bayesian computation (ABC, Beaumont et al. 2002) in DIYABC v2.0 (Cornuet et al. 2014). The first two hypotheses take into account the wolverine's vagility and adaptation to cold environments, and postulate that colonization included regions that were not entirely ice-free. Under this assumption, individuals from the ancestral population could have dispersed in multiple directions, colonizing several regions simultaneously (e.g., Tougaard et al. 2008). The first scenario tests if all populations diverged from a single ancestral Holarctic population during the LGM, where the incursion across Beringia and multiple colonization events occurred concurrently. The second scenario includes a time

lag between the single incursion event across Beringia during the LGM, and multiple colonization events. The third and fourth hypotheses assume that colonization was constrained by glaciers, and dispersing individuals followed retreating ice-sheet fronts. The third hypothesis considers a single incursion from Beringia during the LGM giving rise to a northwestern AK-YK-BC-AB-MT-WY-ID population (Pop1), from which colonization occurred in a west-to-east stepping-stone direction during the Holocene. This would result in a 'leading edge' pattern of colonization, with decreasing genetic diversity further away from the putative refugium (Hewitt 1996). Given the likelihood of multiple incursions across Beringia (e.g., red fox, *Vulpes vulpes*, Kutschera et al. 2013), our fourth hypothesis assumes the occurrence of two incursions across Beringia during the LGM, resulting in the divergence between RUS-MNG (Pop5) and AK-YK-BC-AB-MT-WY-ID (Pop1) and between Pop 5 and NU-NT-SK (Pop2). This second incursion was then followed by a sequential, eastward stepping-stone dispersal scenario during the Holocene. We conducted ABC analyses for all regions except for SWE, NOR, and CA_H. These localities were excluded based on either a large geographic gap in information between sampled regions (e.g., SWE-NOR and RUS-MNG) or small sample size (e.g., CA_H, $n < 10$). Although wolverine populations in North America experienced declines during the 20th century due to predator control programs and fur trading (Slough 2007), we assumed that only Pop3 (MB-ON) experienced a genetic bottleneck given the low abundance of wolverines in this region. We applied a 75% reduction in the effective population size (N_e), thus simulating an extreme bottleneck (Hadly et al. 2004). Simulations for all scenarios were performed with and without this bottleneck. For all scenarios, the sex ratio was equal and generation time was four years (Banci 1994). Range limits and associated conditions of parameters used in DIYABC analyses are listed

in Appendix 3.2. We simulated 10^6 data sets for each scenario to build a reference table. The closest 1% of the simulated data sets to the observed data was used to estimate relative posterior probabilities per scenario. Type I and type II errors were calculated from 500 simulated data sets per scenario using the same prior distribution parameter values as the scenarios. Because Bayesian posterior probabilities were used, generated type I and type II errors are not reflective of a classical frequentist hypothesis framework where the null is never accepted but rejected when data are incompatible with it (Bertorelle et al. 2010). When type II errors are small under the ABC approach, there is good confidence in the results, even though type I errors can be large (Bermond et al. 2012).

Results

Haplotype diversity

We sequenced 360 bp of the control region for 183 contemporary samples and 26 out of 38 extracts for museum specimens (Appendix 3.1). Analyses were performed on 318 bp to include data from Walker et al. (2001). Three variable sites were lost when the 360 bp fragment was shortened to 318 bp. However, the inclusion of Walker et al. (2001) data added two different variable sites, resulting in the overall loss of a single variable site. Haplotypes 'C' and 'J' from Tomasik and Cook (2005) were amalgamated with haplotypes 'A' and 'F' from Wilson et al. (2000), respectively (Appendix 3.3). In addition, haplotype 'Mong1' from Schwartz et al. (2007) grouped with haplotype 'L' from Tomasik and Cook (2005). All three amalgamated haplotype pairs differed by one base pair. We compared all haplotypes to reports in the literature (Wilson et al. 2000; Chappell et al. 2004; Tomasik and Cook 2005; Cegelski et al. 2006; Frances 2008; Zigouris et al. 2012). Although multiple studies identified identical haplotypes, naming was inconsistent among

publications. All haplotypes identified for the control region of *G. gulo* were catalogued by publication date (Appendix 3.3).

A total of 39 haplotypes and 28 variable sites were identified from 983 individuals (Appendix 3.4), with 34 haplotypes observed in previous studies. All five new haplotypes identified in this study were sequenced from pelt samples, with the majority of the samples tested having high molecular weight DNA, and confirmed with independent PCRs. Voucher sequences of new haplotypes were submitted to GenBank (Accession Numbers: KC182788 - KC182792). The most common haplotype was Hap1, found within 36% of all individuals. Hap1 was only observed in the Western Hemisphere, comprising 46% of North America samples and even occurring on Vancouver Island. The next most frequent haplotypes were Hap10 and Hap8, comprising 17% and 9% of all samples, respectively (Figure 3.3, Appendix 3.5). Fourteen of the remaining haplotypes occurred at frequencies between 1 - 5% and represented 36% of the data. The remaining 22 haplotypes were considered rare, each < 1% and overall comprising 2% of the data (Figure 3.3, Appendix 3.5).

Population genetic parameters were consistent among groupings of contemporary and historic samples, but we present only results where contemporary and historic samples were treated as separate entries. Nucleotide diversity (π) was low across all regions, but highest in MB and ON (Table 1). Haplotype diversity (h) ranged from 0 (NOR and ID) to 0.83 (NT and SK), and was highest in northwestern North America and Russia (Table 1). Accounting for sample size (standardized to $g_{\text{CONTEMPORARY}} = 6$, and $g_{\text{HISTORIC}} = 5$), private haplotypes were highest in YK and RUS, and absent from NOR, WY, ID, BC_H, and QC/NL_H (0.00 to 1.16, Table 1). All tests, with the exception of two, produced non-significant values for both Tajima's D and Fu's F_s (Table 1). Significant

values were observed for Tajima's D (-1.44 , $P = 0.04$) for Sweden and for Fu's F_s (-5.46 , $P = 0.04$) for Alaska.

Genetic structure

Pairwise Φ_{ST} estimates (Table 2) revealed several differentiated groups. In Eurasia, wolverines were not genetically differentiated between SWE and NOR ($\Phi_{ST} = 0.01$, $P = 0.36$), nor between MNG and RUS ($\Phi_{ST} = -0.06$, $P = 0.79$). However, wolverines from SWE and NOR had high levels of divergence with MNG and RUS ($\Phi_{ST} \geq 0.89$), and all four were differentiated from North America ($\Phi_{ST} \geq 0.21$; Table 2). In North America, five main divisions were observed based on the pairwise Φ_{ST} estimates that largely pooled the Arctic regions (NT, NU, northern SK), western regions (AK, YK, BC, AB, MT, WY, ID), and eastern regions (ON, QC/NL_H). The additional two divisions included MB and CA_H that were differentiated from the other North American regions (Table 2), with divisions being more pronounced for CA_H.

SAMOVA identified negligible differences between ϕ_{CT} values as K increased for all analyses, with the exception of $K = 2$ for North American samples with CA_H and QC/NL_H. This inflection point separated CA_H from the remaining samples. An inflection point of diminished values was also observed for among population within groups variation across all data combinations. The K value for which ϕ_{ST} values greatly decreased varied among data groups; however, a similar hierarchical pattern of genetic divisions was observed for all analyses (Table 3). The overall regional genetic groups delineated by ϕ_{ST} results were SWE-NOR, MNG, RUS, CA_H, QC/NL_H, MB, NT-NU-SK, and the remaining samples. Weaker relationships were found when populations were grouped according to geographic distribution in ANOVA.

Mantel tests revealed a significant correlation between genetic and geographic matrices among sites ($r = 0.190$, $P = 0.001$), indicating that genetic differentiation was partially explained by isolation by distance. Similarly, isolation by distance was also found to be significant among North American localities ($r = 0.254$, $P = 0.033$).

Phylogenetic analysis

The Bayesian phylogenetic tree (not shown) revealed low support (posterior probability <0.75) for all but one node in the derived wolverine phylogeny. The supported node (1.0 posterior probability) was found near the base of the tree, suggesting a single phylogenetic haplogroup across this carnivore's Holarctic range. The predominance of low bootstrap values is likely the result of haplotypes being separated by single point mutations as revealed by the median-joining network (Figure 3.4). The haplotype relationships depicted by the phylogenetic tree were represented by the median-joining network. Although the mismatch distribution did not display the pattern of a typical Poisson distribution, it was unimodal (Appendix 3.6), and consistent with a rapid range expansion. The variance (SSD = 0.0099; $P = 0.636$) and Harpending's raggedness index ($r = 0.026$; $P = 0.799$) suggested that the observed mismatch distribution did not differ from the expected distribution of a population expansion model. Similarly, Fu's F_s test showed a signal of population expansion across all wolverine samples ($F_s = -10.688$, $P = 0.031$). The median-joining network revealed a star shaped topology associated with Hap1. The main body of the network was characterized by several reticulations that included Hap3, Hap6, Hap7, Hap8, which primarily occurred in the northwestern North America and Russia (Figure 3.4). Additionally, the network identified eight mutational steps separating the two haplotypes predominately found in

MB and ON, Hap24 and Hap25 (Figure 3.4). However, both Hap24 and Hap25 were each only one mutational step away from other haplotypes found in North America.

ABC Analysis

The selection of the most optimal model did not change whether or not a bottleneck was modeled within the MB-ON population. Results for both sets of analyses were comparable, thus we only present findings for the reference table with the population bottleneck for MB-ON. The DIYABC analysis revealed the third hypothesis of a single Beringia incursion during the LGM established the northwestern Pop1 and then followed by a west-to-east Holocene colonization was the most supported. The posterior probability of the logistic regression for scenario 3 was 1.0, with a type I error of 0.750 and a type II error of 0.147.

Discussion

Pleistocene influence

Species with widespread contemporary distributions and/or highly mobile species are said to more likely to have occupied multiple glacial refugia during glaciations (Stewart et al. 2010). The star phylogeny and unimodal mismatch distribution, with non-significant sum of square deviations between observed and expected values, indicate a rapid range expansion of wolverines occurred from a single glacial refugium. The mismatch graph (Appendix 3.6) did not display a standard Poisson distribution, but was similar to the mismatch distribution produced by Excoffier and Schneider (1999) when an early population expansion also experienced a historic (400 generations ago) bottleneck. The further back in time bottlenecks take place, the longer the time period for increased genetic drift, resulting in increased variance of the mismatch distribution and higher frequencies of low difference classes (e.g., 0 and 1; Excoffier and Schneider 1999). This

expansion event was also supported by a significantly negative F_u 's F_s value for the whole data set. Although F_u 's F_s statistic was not significant for individual regions, except for AK, the occurrence of negative values implies that there may be some deviation from neutrality for the northwestern region.

This expansion event from a single glacial refugium is in contrast to the phylogeographical structure of other cold-adapted species such as woodland caribou (*Rangifer tarandus caribou*), a widely distributed and highly vagile ungulate. For woodland caribou a multimodal mismatch distribution pattern of mtDNA sequences (Flagstad and Røed 2003) reflected postglacial expansions from three putative regions in North America (Klütsch et al. 2012). Although data presented in this study were collected throughout the Holarctic range of wolverines, certain areas like the region between Scandinavia and the Russian Far East remained unsampled. In this study, Scandinavia represented a geographic outlier. Implementing a more systematic sampling scheme may identify new haplotypes or missing branching lineages, or change the frequency and distribution of known haplotypes. This may help resolve some of the reticulated haplotypes in the network and provide a more comprehensive assessment of the relationship between Hap10 (SWE-NOR) and adjacent haplotypes. Furthermore, severe population declines experienced by Scandinavian populations (Landa et al. 2000), with drift likely undermining our ability to resolve historic processes and links with other populations.

Interestingly, a very similar phylogeographic study by McKelvey et al. (2014) proposed a southern ice-free refugium in addition to Beringia. While historic samples were also included in McKelvey et al. (2014), they largely represented the southwestern range periphery. These historic samples revealed a localized distribution of the extirpated

California haplotypes (Hap21, Hap22), and the basis for a likely southern refugium during the LGM. Historic sampling from Quebec-Labrador in this study identified the presence of Hap21 in northeastern North America, indicating this haplotype was more widely distributed than indicated by McKelvey et al. (2014), bringing to question the occurrence of a southern refugium. These contradicting results could reflect the low nucleotide diversity among wolverine sequences, which were unable to provide the resolution needed for making phylogenetic inferences.

Our observation of low nucleotide diversity among sequences may be attributed to the amplification of a 318 bp fragment of the mtDNA control region. This sequence length is likely not sufficient to provide the necessary resolution needed as observed with the unsupported phylogenetic tree. This small fragment size was used to allow us to expand our study and compare samples from existing databanks and get the largest distribution possible, and is clearly a compromise that does put some limits on our interpretations. The presence of low nucleotide diversity has also been observed in other mustelid species like pine marten (*Martes martes*; Davison et al. 2001) and fisher (*Martes pennanti*; Drew et al. 2003). In the case of pine marten, similarly truncated mtDNA control region sequences were also used (320 bp), with results suggesting European colonization from a single refugium following a recent glaciation (Davison et al. 2001). Although a slightly longer sequence of the mtDNA control region was used for woodland caribou (429 bp; Klütsch et al. 2012), the finding of three highly supported phylogroups may be reflective of woodland caribou's classification into different ecotypes. This shows that such a small mtDNA fragment can provide insight into phylogeographic processes.

If multiple refugial lineages persisted in present-day wolverine populations, we would expect to find clearly delineated subclades for each glacial refugium. Our network

would also have numerous substitutions along branches connecting each of the subclades, as observed in the Nearctic clade d-loop network of the red fox (Aubry et al. 2009). The most differentiated haplotype was Hap10 found in SWE and NOR, but this haplotype was separated by only three mutations from Hap15 found in RU and AB. This step-wise mutation pattern does not provide evidence of longstanding genetic differences of animals isolated by multiple glacial refugia, but is suggestive of long-range dispersal movements characteristic of wolverines. Single mutational step differences among control region haplotypes were also observed for the arctic fox (*Alopex lagopus*), with several haplotypes having a Holarctic distribution indicative of the long-distance dispersal capabilities of this carnivore (Dalén et al. 2005). Although our data suggest wolverines likely underwent a postglacial expansion from a single glacial refugium, the colonization pattern of this expansion and its effect on contemporary genetic structure of mtDNA remains unknown. A hypothesis testing approach could provide insights into different recolonization scenarios, and distinguish among these alternative hypotheses the most likely one.

Hypothesis testing of population divergence scenarios

The most supported ABC model was a single incursion from Beringia during the LGM giving rise to a northwestern AK-YK-BC-AB-MT-WY-ID population, followed by a west-to-east stepping-stone divergence scenario during the Holocene. This pattern is suggestive of recolonization occurring in accordance with glacial retreat, where initial recolonization along the western coast of North America was followed by an inland recolonization pattern (Latch et al. 2009). The inclusion of a recent bottleneck for the MB-ON populations did not change which scenario was best supported. Although scenario 3 had the highest posterior probability and a low type II error, summary statistics

for all simulated data sets did not surround the observed data set, reflecting a poor fit by all four scenarios. This does not necessarily mean that scenario 3 was erroneously selected, but that additional events may need to be incorporated in the proposed colonization patterns. One likely explanation for the poor fit may be the omission of a historic bottleneck across multiple populations as indicated by the mismatch distribution. While the mismatch analysis identified a historic bottleneck, additional information such as duration, time period, and geographic extent were not provided, but required for DIYABC simulations when modeling a bottleneck event. In addition, the DIYABC limitation that dispersal is absent among populations once they have diverged (Cornuet et al. 2008) may contribute to our poor fit between simulated and observed data sets. Both male and female wolverines have demonstrated extensive dispersal distances (Gardner et al. 1986; Zigouris et al. 2012), resulting in gene flow among diverged populations. Finally, our use of a short mtDNA fragment may not provide enough resolution to establish a good fit between simulated and observed data sets despite ABC suggesting some models are highly probable. We suggest that a cautionary approach be taken regarding ABC model choice, and this method be viewed as an exploratory tool (Robert et al. 2011).

Historic and contemporary influences

The Bering Strait represents a barrier to wolverine movement across Eastern and Western Hemispheres. However, excluding the Eurasian samples from Mantel tests still produced a low r value ($r = 0.254$) for North America. Tomasik and Cook (2005) and Cegelski et al. (2006) found no support for IBD and suggested barriers may be influencing differentiation patterns. Extrinsic factors that appear to influence wolverine genetic structure include summer temperatures, spring snow cover and ecological changes

associated with anthropogenic land-use activities (Banci 1994; Aubry et al. 2007; Schwartz et al. 2009; Bowman et al. 2010; Copeland et al. 2010). Additionally, our use of a maternal marker for a carnivore with male-biased dispersal (Cegelski et al. 2003) would result in a stronger isolation by distance value particularly for the philopatric sex (Handley and Parrin 2007), but also be more apparent across all individuals. This absence of a stronger IBD further supports the influence of external factors on population differentiation.

Negligible differences between ϕ_{CT} statistics of SAMOVA for K values before and after inflection points were also observed by Schwartz et al. (2007), while Frances (2008) found an overall lack of geographic structure. Our SAMOVA analyses revealed lower K values being representative of SWE-NOR, CA_H, MNG, QC/NL_H, and MB. Schwartz et al. (2007) also observed CA_H as a separate group for $K > 2$, and that SWE-NOR separated from all other locations at $K = 2$. These lower K groupings represent peripheral regions of the wolverine's contemporary distribution. Range peripheries are generally characterized by decreased density, ecologically marginal habitats and isolation (Gaston 1990; Lesica and Allendorf 1995) that may bring about more frequent extinction-recolonization events, and likely result in a distinct diversity gradient as the species range edge is approached (see Peterman et al. 2013). Alternatively, more pronounced selection pressures in conjunction with reduced gene flow at range peripheries may lead to increased genetic distinctiveness of edge populations (Lesica and Allendorf 1995; Cassel-Lundhagen et al. 2009).

Even though we observed spatial genetic structuring of mtDNA over small geographic scales, reflecting female philopatry (Tomasik and Cook 2005; Cegelski et al. 2006), we also found haplotypes displaying disjointed distributions. Hap15 was found in

SWE and RUS, while Hap21 occurred in CA_H and QC/NL_H - the two most peripheral populations in North America. The presence of Hap21 and Hap15 in QC/NL_H and RUS samples in this study, and their independent observation in CA_H (Schwartz et al. 2007) and SWE (Arnason et al. 2007) supports that our observation of these haplotypes are not the result of sequencing errors. The patchy distribution of Hap15 and Hap21 may reflect leptokurtic dispersal (Ibrahim et al. 1996) following post-glacial colonization.

Alternatively, both haplotypes may have been more widely distributed in the past but have since decreased in frequency or become lost from adjacent regions due to direct human persecution in both hemispheres (Landa et al. 2000; Slough 2007), extensive range contractions in North America (Aubry et al. 2007), and random effects of genetic drift. To distinguish between long-distance dispersal and fragmentation, additional sampling is needed between Scandinavia and the Russian Far East, along with the inclusion of supplementary history samples from adjacent regions of CA_H and QC/NL_H.

Manitoba and Ontario were characterized by Hap1, Hap24, and Hap25, with the latter two haplotypes occurring almost exclusively in these two regions (Figure 3.3). The separation of MB and ON by both SAMOVA and pairwise Φ_{ST} estimates was unexpected based on the very different composition of MB and ON in comparison to the other regions, and that microsatellite data group MB and ON as a separate genetic cluster from the panmictic northwest population (Zigouris et al. 2012). Based on this, MB and ON should be pooled together as a separate genetic group. Contrasting haplotype frequencies among the three regions likely explains why MB and ON were separated into different genetic clusters (Zigouris et al. 2012). In particular, the higher frequency of Hap1 in ON (65%) compared to MB (19%) is presumed to have influenced the grouping of ON with other regions also having high Hap1 frequencies. This high Hap1 frequency in ON may

have occurred as a result of samples consisting of more closely related individuals than expected by chance. The majority of samples from ON were collected using baited hair snares encompassing 2000 km² (Koen et al. 2008), increasing the likelihood of close relatives. Alternatively, substantial population declines during the early 1900s (Slough 2007), where densities have historically been considered low, likely increased the effects of genetic drift and strong female philopatry continuing to maintain altered haplotype frequencies (Wilson et al. 2000; Tomasik and Cook 2005; Schwartz et al. 2007).

Conservation implications

Pairwise Φ_{ST} estimates pooled QC/NL_H with contemporary ON. This grouping is likely due to Hap21 (QC/NL_H) and Hap25 (ON) being derived from the same missing intermediate haplotype (Figure 3.4), and the high frequency of Hap1 in both regions. This genetic clustering of QC/NL_H with the extant peripheral cluster of MB-ON brings into question the classification of wolverines from Quebec-Labrador as the 'eastern' population. This potential grouping will also influence the selection of a source population for the proposed translocation of wild animals as outlined in the national recovery plan for the eastern wolverine population in Quebec-Labrador (Fortin et al. 2005). However, this grouping does not preclude the possibility of QC/NL_H with MB-ON being considered a designatable unit. For a population to be identified as a designatable unit (DU), the population needs to be recognized as being both 'discrete' and 'evolutionary significant' (Committee on the Status of Endangered Wildlife in Canada, COSEWIC 2004). Using both mtDNA and microsatellites, Zigouris et al. (2012) found MB-ON formed a separate genetic cluster from core regions (NT-NU-SK). In addition, genetic analysis of multilocus genes found in the major histocompatibility complex (MHC) for wolverines across their Canadian range revealed a duplicated DRB exon 2

with the peptide binding region having a significant excess of non-synonymous substitutions – indicative of positive selection acting on MHC of wolverines (Oomen et al. 2013). Further research is needed on wolverines along their eastern range edge in North America, including combining ecological data with genetic markers under natural selection (Crandall et al. 2000; Allendorf et al. 2010), in order to appropriately define a population as a DU (COSEWIC 2004). Furthermore, the historic sample from Vancouver Island was representative of the widely distributed Hap1. Wolverines from Vancouver Island are classified as a separate subspecies, *G. g. vancouverensis* (Goldman 1935); however, morphological comparisons between island and mainland specimens provided minimal evidence for designating Vancouver Island animals as a separate subspecies (Banci 1982). The presence of Hap1 for the Vancouver Island sample highlights the need to further investigate the genetic structure and diversity of Vancouver Island wolverines.

Conclusions

In contrast to previous glacial refugia studies of arctic species (e.g., Fleming and Cook 2002; Loehr et al. 2006; Klütsch et al. 2012), we found no molecular evidence of wolverines inhabiting multiple glacial refugia during the last glacial maximum. In addition, approximate Bayesian computations supported a wolverine colonization of North America where individuals followed retreating glaciers. Even with molecular evidence of a single expansion event, significant subdivisions of population genetic structure over small spatial scales were observed. This genetic structure reflected historic population declines throughout the wolverine's Holarctic range, as indicated by the mismatch distribution, along with the influence of subsequent genetic drift and strong female philopatry (e.g., Wilson et al. 2000; Tomasik and Cook 2005; Schwartz et al. 2007). We acknowledge that the mtDNA analyses in this study were based on a short

sequence fragment of the control region, restricting the resolution needed for us to make conclusive inferences. However, these data provide some insights into the post-glacial colonization and phylogeographic relationships among contemporary wolverine populations. In particular, our observation of low genetic differentiation between QC/NL_H and ON, which puts into question the designation of Quebec-Labrador as the 'eastern' population (COSEWIC 2003). These underlining genetic associations among regions highlight key areas and questions where future research should focus, including using longer mtDNA fragment or alternative markers like single nucleotide polymorphisms (SNPs) to obtain deeper resolution of phylogeographic history. We also suggest further research be undertaken with functional markers to investigate the possibility of local adaptation among the different genetic clusters. This information will have strong implications for the identification of designatable units (COSEWIC 2004), and future conservation and recovery activities of wolverines, particularly at the eastern periphery of their North American range.

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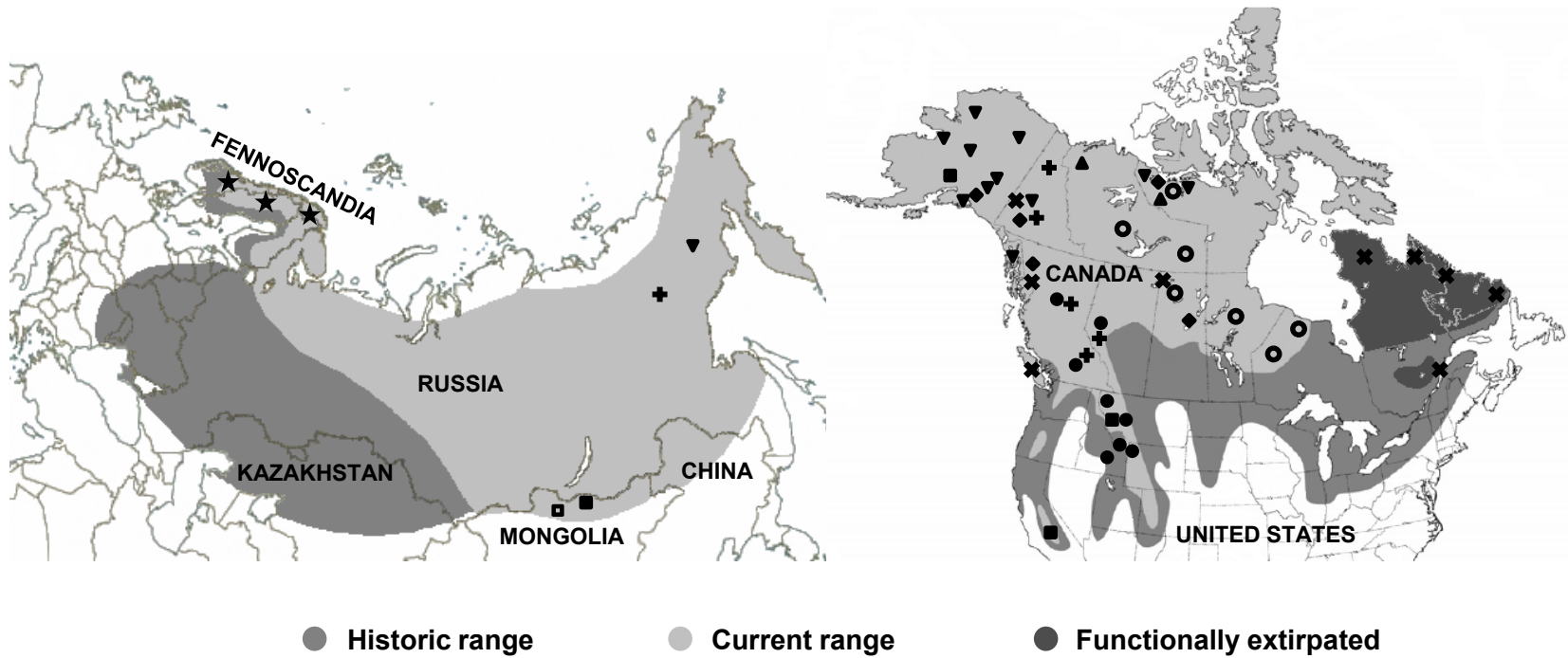


Figure 3.1 Historic and current distribution of wolverines in Eurasia (adapted from National Geographic Society 2002) and North America (adapted from COSEWIC 2003), and sampled localities from ▲ Wilson et al. (2000); ★ Walker et al. (2001); ▼ Tomasik and Cook (2005); ● Cegelski et al. (2006); ■ Schwartz et al. (2007); ◆ Frances (2008); ● Zigouris et al. (2012); ■ Rochnov and Meschersky unpub.; + New contemporary samples; and ✱ New historic samples. Symbols depict the general areas where samples were collected.

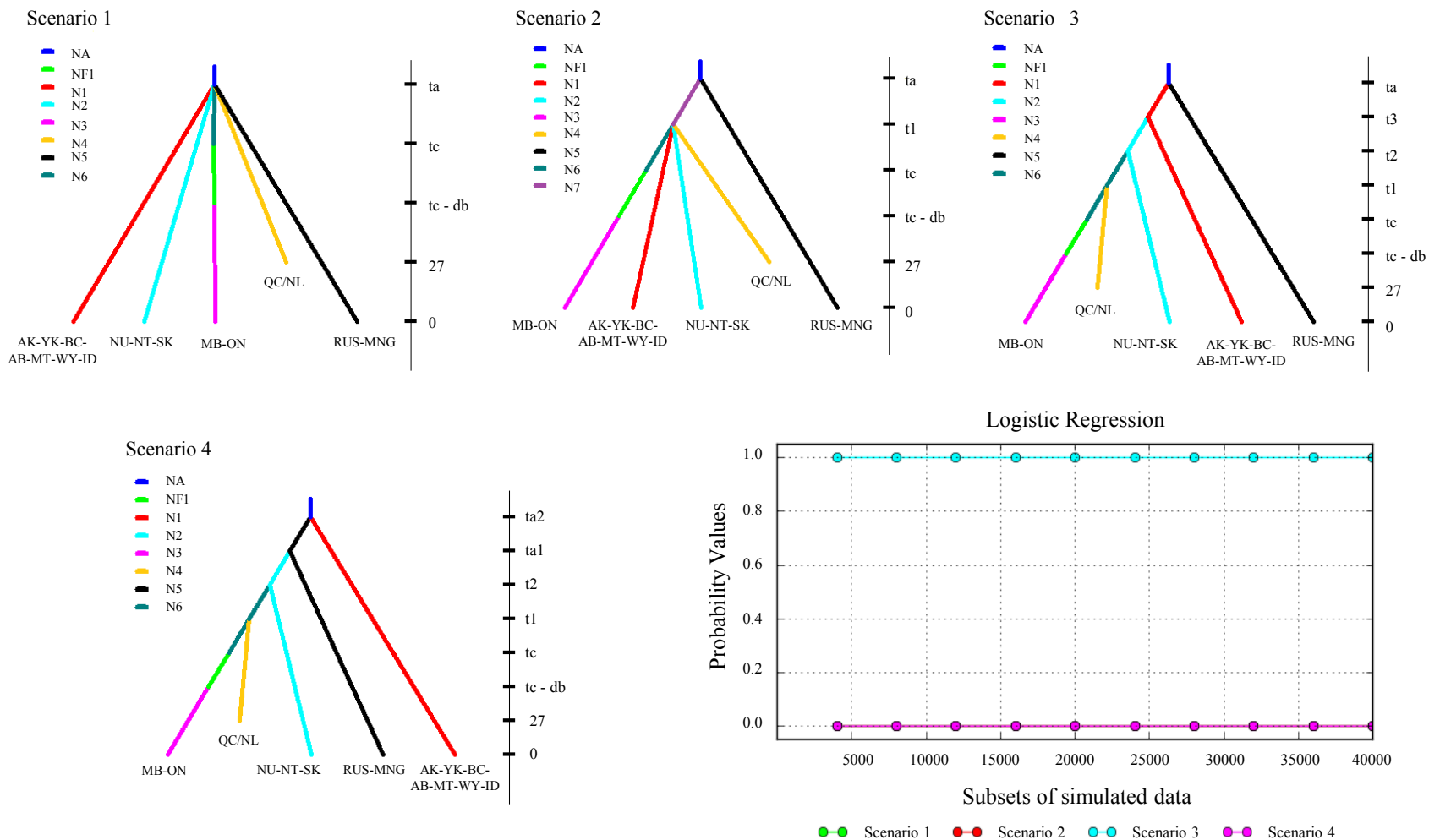


Figure 3.2 Graphic representation of the four scenarios used in DIYABC and logistic regression of posterior probabilities with a recent bottleneck (20th century) for MB-ON. Please note that time is not to scale.

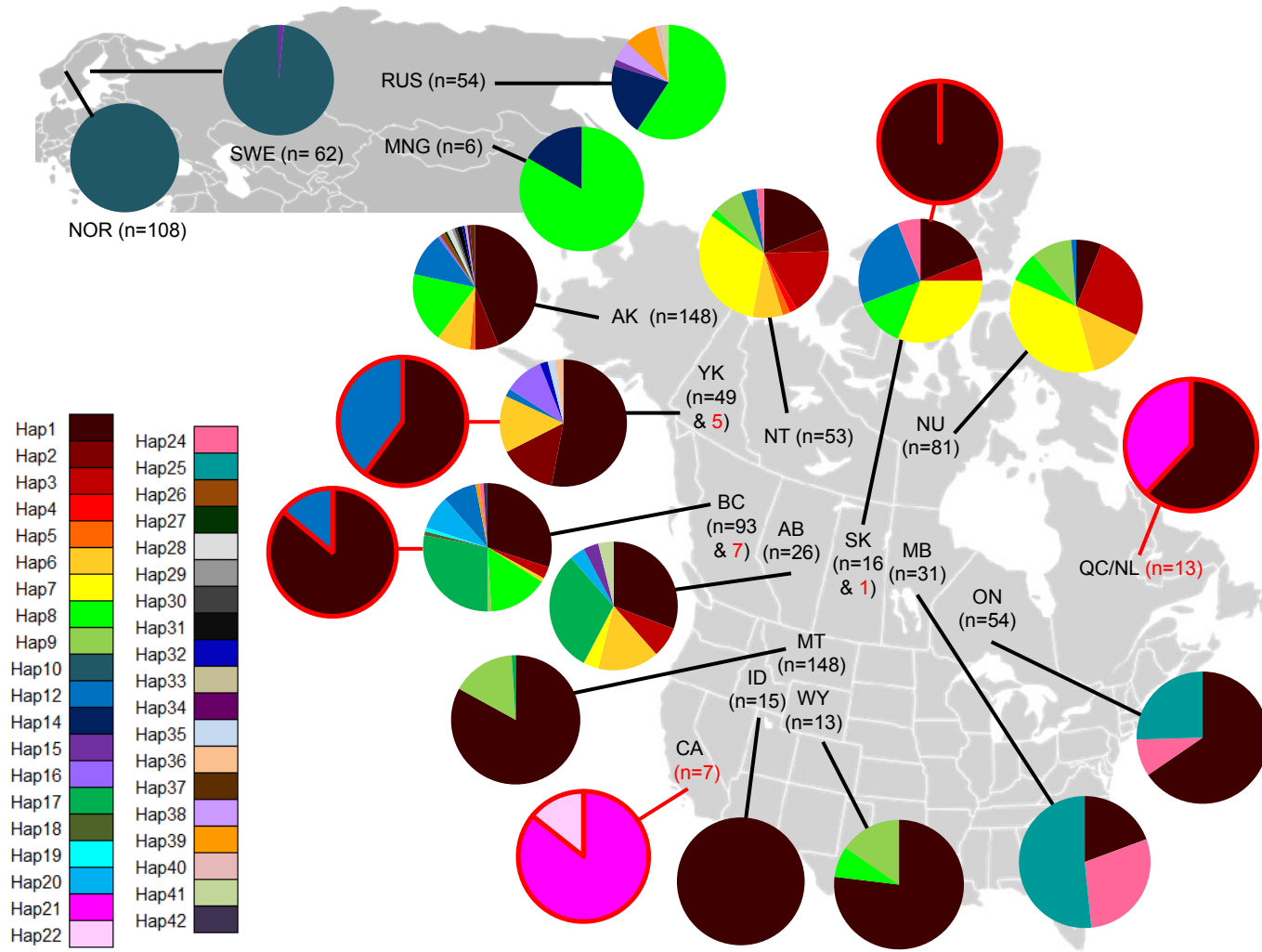


Figure 3.3 The geographic distribution of mtDNA control region haplotypes based on frequencies observed for each region.

Historic samples are presented separately and identified with a red border and red sample size number.

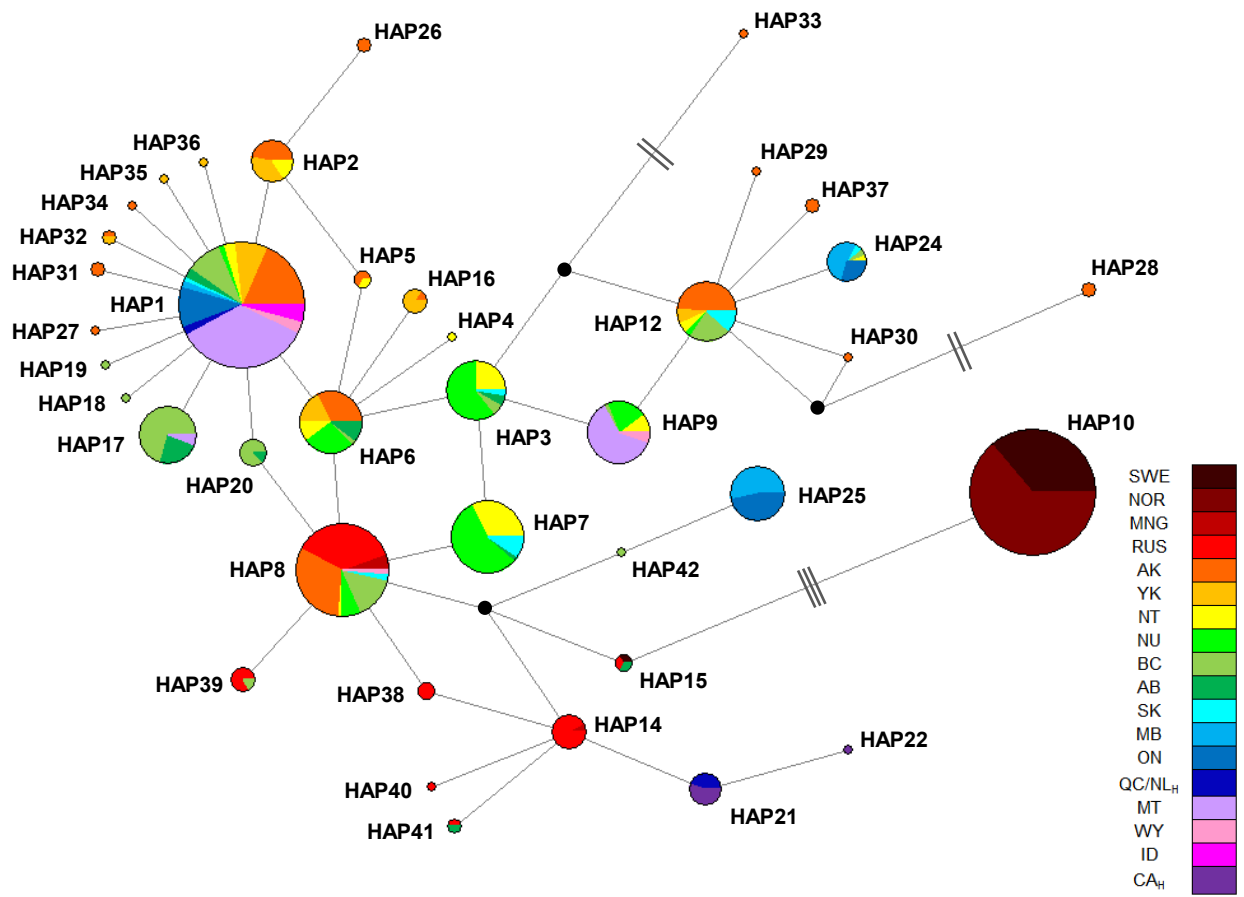


Figure 3.4 Median-joining network of the mtDNA control region haplotypes for all samples combined. Haplotype size reflects relative frequency. Each branch represents one mutational step, unless otherwise noted. Black circles represent missing intermediate haplotypes.

Table 3.1 MtDNA nucleotide (π) and haplotype (h) diversity, their standard deviations ($SD \pi$, $SD h$), haplotype richness (H_R), private haplotype (H_P) counts, standardized haplotype richness (H_{Rstd}) and private haplotype (H_{Pstd}) counts to the smallest sample size for both contemporary ($g = 6$) and historic ($g = 5$) samples using ADZE rarefaction, Tajima's D , and Fu's F_s .

Region	n	π	$SD \pi$	h	$SD h$	H_R	H_{Rstd}	H_P	H_{Pstd}	Tajima's D	P	Fu's F_s	P
Contemporary													
SWE	62	< 0.001	0.001	0.03	0.03	2	1.10	0	0.07	-1.44	0.04	-0.57	0.14
NOR	108	0	0	0	0	1	1	0	0	0	1	-	-
MNG	6	0.002	0.002	0.33	0.22	2	2	0	0.24	-1.13	0.15	0.95	0.61
RUS	54	0.003	0.003	0.61	0.06	7	2.86	2	1.00	-0.08	0.52	-1.56	0.21
AK	148	0.006	0.004	0.75	0.03	17	3.64	9	0.77	-0.59	0.31	-5.46	0.04
YK	49	0.003	0.003	0.68	0.06	8	3.23	2	1.16	-0.74	0.27	-2.72	0.07
NT	53	0.006	0.004	0.83	0.03	11	4.10	1	0.42	0.53	0.74	-2.97	0.09
NU	81	0.004	0.003	0.78	0.03	7	3.63	0	0.20	0.60	0.75	-0.69	0.40
BC	86	0.006	0.004	0.80	0.02	13	3.84	3	0.65	-0.08	0.53	-3.39	0.09
AB	26	0.005	0.004	0.81	0.05	8	3.85	0	0.68	0.14	0.60	-2.10	0.10
SK	16	0.007	0.005	0.83	0.06	6	3.99	0	0.19	1.46	0.93	-0.26	0.45
MB	31	0.013	0.007	0.63	0.05	3	2.65	0	0.40	2.49	0.99	8.07	0.99
ON	54	0.009	0.005	0.51	0.06	3	2.31	0	0.03	1.42	0.92	6.83	0.98
MT	148	0.003	0.002	0.30	0.04	3	1.74	0	0.20	0.11	0.62	2.47	0.87
WY	13	0.003	0.003	0.41	0.15	3	1	0	0	-0.48	0.30	0.98	0.71
ID	15	0	0	0	0	1	1	0	0	0	1	-	-
Historic													
YK	5	0.008	0.006	0.60	0.18	2	2.00	0	0.29	1.57	0.96	3.02	0.91
BC	7	0.004	0.003	0.29	0.20	2	1.71	0	0	-1.36	0.08	2.05	0.83
QC/NL	13	0.008	0.005	0.51	0.08	2	1.96	0	0	2.11	0.99	5.45	0.99
CA	7	0.001	0.001	0.29	0.20	2	1.71	1	0.76	-1.01	0.23	-0.09	0.23

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; AK = Alaska; YT = Yukon; NT = Northwest Territories; NU = Nunavut;
BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC/NL = Quebec-Labrador; MT = Montana;
WY = Wyoming; ID = Idaho; CA = California

Table 3.2 Pairwise estimates of population genetic distance for mtDNA among sampling localities (Φ_{ST} , below diagonal), and associated P values (above diagonal).

	SWE	NOR	MNG	RUS	AK	YT _C	YT _H	NT	NU	BC _C	BC _H	AB	SK	MB	ON	QC/ NL _H	MT	WY	ID	CA _H	
SWE	•	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NOR	0.01	•	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MNG	0.97	0.99	•	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.01	0.03	0.00	0.00	0.00	0.00	0.00
RUS	0.89	0.93	-0.06	•	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AK	0.81	0.84	0.33	0.40	•	0.00	0.33	0.00	0.00	0.00	0.40	0.01	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00
YT _C	0.92	0.95	0.60	0.61	0.06	•	0.02	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.03	0.00	0.00
YT _H	0.67	0.99	0.53	0.61	0.01	0.23	•	0.06	0.00	0.09	0.53	0.05	0.25	0.06	0.14	0.05	0.04	0.29	0.06	0.00	0.00
NT	0.86	0.91	0.31	0.41	0.14	0.33	0.13	•	0.04	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NU	0.89	0.92	0.40	0.46	0.27	0.49	0.34	0.03	•	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BC _C	0.84	0.88	0.33	0.40	0.05	0.10	0.09	0.21	0.35	•	0.28	0.52	0.00	0.00	0.00	0.01	0.00	0.16	0.01	0.00	0.00
BC _H	0.97	0.99	0.65	0.53	-0.01	<0.01	-0.01	0.25	0.47	0.02	•	0.18	0.01	0.01	0.16	0.11	0.62	0.80	0.32	0.00	0.00
AB	0.91	0.95	0.40	0.47	0.07	0.11	0.15	0.23	0.40	-0.01	0.05	•	0.00	0.00	0.01	0.05	0.00	0.12	0.00	0.00	0.00
SK	0.92	0.95	0.30	0.45	0.19	0.44	0.06	0.02	0.07	0.25	0.28	0.30	•	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MB	0.79	0.86	0.21	0.36	0.33	0.42	0.19	0.28	0.37	0.34	0.27	0.31	0.19	•	0.00	0.01	0.00	0.00	0.00	0.00	0.00
ON	0.81	0.86	0.27	0.39	0.09	0.13	0.09	0.21	0.35	0.10	0.05	0.10	0.23	0.15	•	0.09	0.00	0.08	0.01	0.00	0.00
QC/NL _H	0.91	0.95	0.27	0.39	0.19	0.29	0.22	0.31	0.47	0.12	0.19	0.09	0.32	0.18	0.06	•	0.00	0.04	0.01	0.01	0.01
MT	0.91	0.93	0.67	0.67	0.09	0.07	0.21	0.38	0.53	0.12	-0.05	0.15	0.50	0.54	0.19	0.40	•	0.71	0.12	0.00	0.00
WY	0.96	0.98	0.59	0.59	0.01	0.02	0.08	0.22	0.43	0.03	-0.09	0.05	0.30	0.29	0.06	0.20	-0.03	•	0.09	0.00	0.00
ID	0.99	1.00	0.92	0.71	0.12	0.07	0.52	0.41	0.60	0.11	0.12	0.17	0.54	0.41	0.14	0.36	0.06	0.12	•	0.00	0.00
CA _H	0.98	1.00	0.84	0.66	0.67	0.82	0.80	0.69	0.76	0.65	0.87	0.70	0.69	0.37	0.55	0.49	0.85	0.84	0.98	•	0.00

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; AK = Alaska; YT = Yukon; NT = Northwest Territories; NU = Nunavut;

BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC/NL = Quebec-Labrador; MT = Montana;

WY = Wyoming; ID = Idaho; CA = California; _C = Contemporary; _H = Historic

Table 3.3 Results of delineated genetic groupings identified by SAMOVA for different population configurations.

Data Assemblage	Delineated Genetic Grouping	Variance Components	Percentage of Variance	ϕ -statistics	<i>P</i>
Eurasia, North America, CA _H , & QC/NL _H	<i>K</i> =8 (SWE-NOR vs. MNG vs. RUS vs. CA _H vs. QC/NL _H vs. MB vs. NT-NU-SK vs. Rest)				
	Among groups	1.38	68.55	$\phi_{CT} = 0.68549$	< 0.001
	Among populations within groups	0.06	2.90	$\phi_{SC} = 0.09226$	< 0.001
	Within populations	0.57	28.55	$\phi_{ST} = 0.71451$	< 0.001
Eurasia & North America	<i>K</i> =7 (SWE-NOR vs. MNG vs. RUS vs. MB vs. NT-NU vs. SK vs. Rest)				
	Among groups	1.39	68.88	$\phi_{CT} = 0.68876$	< 0.001
	Among populations within groups	0.06	2.95	$\phi_{SC} = 0.09490$	< 0.001
	Within populations	0.57	28.17	$\phi_{ST} = 0.71829$	< 0.001
North America, CA _H & QC/NL _H	<i>K</i> =5 (CA _H vs. QC/NL _H vs. MB vs. NT-NU-SK vs. Rest)				
	Among groups	0.43	35.54	$\phi_{CT} = 0.35540$	< 0.001
	Among populations within groups	0.07	5.49	$\phi_{SC} = 0.08510$	< 0.001
	Within populations	0.71	58.97	$\phi_{ST} = 0.41026$	< 0.001
North America	<i>K</i> =3 (MB vs. NT-NU-SK vs. Rest)				
	Among groups	0.37	32.62	$\phi_{CT} = 0.32624$	< 0.001
	Among populations within groups	0.07	5.78	$\phi_{SC} = 0.08575$	< 0.001
	Within populations	0.70	61.60	$\phi_{ST} = 0.38401$	0.001

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; NT = Northwest Territories; NU = Nunavut; SK = Saskatchewan;
MB = Manitoba; QC/NL_H = Historic Quebec-Labrador; CA_H = Historic California

Appendix 3.1 Catalogue information of all wolverine samples obtained from collections and if samples were sequenced.

Provider	Catalogue Number	Collection Date	Locality	Province	Sequenced
University of Colorado Museum of Natural History	10678	01 January 1910	Vancouver Island	British Columbia	Yes
Museum of Comparative Zoology, Harvard University	18935	01 August 1920	Athabasca Lake	Saskatchewan	Yes
Museum of Comparative Zoology, Harvard University	BANGS-7429	Summer 1897	Okak	Labrador	Yes
Museum of Comparative Zoology, Harvard University	BANGS-7430	Summer 1897	Okak	Labrador	Yes
Museum of Comparative Zoology, Harvard University	BOM-7483	1898	Big Bay	Labrador	Yes
Museum of Comparative Zoology, Harvard University	BOM-7484	30 January 1899	Kippokak Bay	Labrador	Yes
Museum of Comparative Zoology, Harvard University	BANGS-8881	24 October 1898	Black Bay	Labrador	Yes
Museum of Comparative Zoology, Harvard University	BANGS-8882	9 January 1899	Black Bay	Labrador	Yes
Museum of Comparative Zoology, Harvard University	BANGS-8962	10 November 1899	Lance au Loup	Labrador	Yes
National Museum of Natural History	USNM A23142	Pre 1900	Ungava	Quebec	Yes
National Museum of Natural History	USNM 188245	24 April 1888	Godbout	Quebec	No
National Museum of Natural History	USNM 189000	1883	Near Godbout	Quebec	Yes
National Museum of Natural History	USNM A23256	Pre 1900	Ungava	Quebec	Yes
National Museum of Natural History	USNM 14781	Pre 1900	Ungava, Rapids	Quebec	Yes

National Museum of Natural History	USNM A23254	21 February 1889	Ungava	Quebec	No
National Museum of Natural History	USNM 188244	1889	Godbout	Quebec	Yes
National Museum of Natural History	USNM 141911	Pre 1900	Ungava	Quebec	Yes
The Museum of Vertebrate Zoology, Berkeley	33270	1916	Lower Iskut River near junction with Stikine River	British Columbia	No
The Museum of Vertebrate Zoology, Berkeley	33271	1916	Lower Iskut River near junction with Stikine River	British Columbia	Yes
The Museum of Vertebrate Zoology, Berkeley	43625	11 April 1929	Indianpoint Lake, Cariboo District	British Columbia	No
The Museum of Vertebrate Zoology, Berkeley	33269	1916	Lower Iskut River near junction with Stikine River	British Columbia	Yes
The Museum of Vertebrate Zoology, Berkeley	43631	Winter 1926-1927	McClary Lake region	British Columbia	No
The Museum of Vertebrate Zoology, Berkeley	43634	Winter 1927-1928	McClary Lake region	British Columbia	Yes
The Museum of Vertebrate Zoology, Berkeley	34396	Winter 1923-1924	97 km W Carcross	Yukon	No
The Museum of Vertebrate Zoology, Berkeley	159320*	Winter 1974-1975	vicinity of Paint Lake, 30 km S Thompson	Manitoba	Yes
The Museum of Vertebrate Zoology, Berkeley	43632	Spring 1926-1927	McClary Lake region	British Columbia	No
The Museum of Vertebrate Zoology, Berkeley	43633	Winter 1926-1927	McClary Lake region	British Columbia	Yes
The Museum of Vertebrate Zoology, Berkeley	33267	Winter 1916-1917	Lower Iskut River near junction with Stikine River	British Columbia	Yes
The Museum of Vertebrate Zoology, Berkeley	33268	Winter 1916-1917	Lower Iskut River near junction with Stikine River	British Columbia	Yes
Royal Ontario Museum	35.4.3.1	1920	Thunder Bay	Ontario	No

Royal Ontario Museum	16261	1944	Qamani'tuaq (Baker Lake), Keewatin	Nunavut	No
Royal Ontario Museum	31.2.16.9	January 1930	White River	Yukon	Yes
Royal Ontario Museum	32.10.16.21	26 December 1931	Yukon River	Yukon	No
Royal Ontario Museum	32.10.16.22	5 March 1932	White River	Yukon	Yes
Royal Ontario Museum	32.10.16.23	10 February 1931	White River	Yukon	Yes
Royal Ontario Museum	32.10.16.24	1 March 1932	White River	Yukon	Yes
Royal Ontario Museum	32.10.16.25	1 December 1931	White River	Yukon	Yes
Royal Ontario Museum	32.10.16.27	March 1931	White River	Yukon	No
Royal Ontario Museum	32.10.16.28	March 1931	White River	Yukon	No

*Specimen was grouped with contemporary samples as it was collected post-1945.

Appendix 3.2 Minimum and maximum limits and associated conditions of parameters used in DIYABC analyses.

Parameter	Parameter Symbol	Minimum	Maximum	Conditions	References
Ancestral population (Beringia)	NA	1000	10000		
<i>Ne</i> sample 1 (Pop1) - AK, YK, BC, AB, MT, WY, ID	N1	1000	10000		
<i>Ne</i> sample 2 (Pop2) - NU, NT, SK	N2	1000	10000		Slough 2007
<i>Ne</i> sample 3 (Pop3) - MB, ON	N3	50	1000		Slough 2007
<i>Ne</i> sample 4 (Pop4) - QC/NL	N4	50	75		Slough 2007
<i>Ne</i> sample 5 (Pop 5) - RUS, MNG	N5	1000	10000		Slough 2007
<i>Ne</i> sample 3 prior to bottleneck - not sampled	N6	50	1000	$N6 > N3$	Slough 2007
<i>Ne</i> of ancestral North American Population	N7	1000	10000	$N7 > N1$; $N7 > N2$; $N7 > N4$; $N7 > N6$	
<i>Ne</i> sample 3 during the bottleneck	NF3	6	125	$NF1 < N3$	
Start of bottleneck (generations)	Tc	22.3	22.3		Slough 2007
Duration of bottleneck (generations)	Db	12.5	12.5		Slough 2007
Divergence of Pop4 for Hypothesis 3 (generations)	Td	28	28		
Divergence of populations since the start of the Holocene and European colonization (generations)	t1, t2, t3	125	2900	$t1 < t2$; $t2 < t3$	Dykoski et al. 2005; Cole 2010
Divergence from ancestral population during LGM - crossing of Beringia (generations)	ta, ta1, ta2	3125	15000	$ta1 < ta2$	Elias et al. 1996; Pitulko et al. 2004

Appendix 3.3 Control region haplotype labels discussed in this study and their corresponding designation(s) as identified in previous studies. Sequential numbering of each haplotype is reflective of when it was first published in the literature.

This Study	Wilson et al. 2000	Walker et al. 2001	Chappell et al. 2004	Tomasik and Cook 2005	Cegelski et al. 2006	Schwartz et al. 2007	Frances 2008	Accession Numbers
Hap1	A		A	B	A		Hap1	AF210090
Hap2	B		B	I			Hap12	AF210094
Hap3	C		C	G	C		Hap10	AF210107
Hap4	D		D	P			Hap27	AF210097
Hap5	E		E	K			Hap26	AF210098
Hap6	F		F	F	F		Hap6	AF210105
Hap7	G		G	E			Hap9	AF210106
Hap8	H		H	A	H		Hap2	AF210130
Hap9	I		I	H			Hap11	AF210112
Hap10		Scandinavia					Hap35	AF245496
Hap11*				C				AF56914
Hap12				D			Hap3	AF55403

Hap13*				J				AF56912
Hap14				L			Hap31	AF52358
Hap15				M			Hap32	AF52388
Hap16				N			Hap13	AF15901
Hap17					L		Hap8	see Cegelski et al. 2006
Hap18					M		Hap28	"
Hap19					N		Hap29	"
Hap20					O		Hap30	"
Hap21						Cali1	Hap33	AY880313
Hap22						Cali2	Hap34	AY880314
Hap23*						Mng1		AY880315
Hap24			K				Hap23	AY185168
Hap25			J				Hap22	AY185167
Hap26							Hap4	EU812347
Hap27							Hap5	EU812353
Hap28							Hap7	EU812365
Hap29							Hap14	EU812407

Hap30							Hap15	EU812416
Hap31							Hap16	EU812422
Hap32							Hap17	EU812424
Hap33							Hap18	EU812430
Hap34							Hap19	EU812431
Hap35							Hap21	EU812442
Hap36							Hap24	EU812451
Hap37								EU812432
Hap38								KC182788
Hap39								KC182789
Hap40								KC182790
Hap41								KC182791
Hap42								KC182792

Amalgamated haplotypes (Hap11 → Hap1; Hap13 → Hap6; Hap23 → Hap14) when 360 bp control region fragment was reduced to 318 bp to include sequence data from Walker et al. (2001).

Appendix 3.4 Variable nucleotide positions of identified haplotypes are denoted according to their location within the 318 bp portion of the D-loop mtDNA control region.

Haplotype	Variable Nucleotide Position																											
	8	10	18	26	29	47	56	57	59	60	65	68	81	83	84	85	86	87	88	88	90	91	96	99				
Hap1	G	A	C	C	A	T	A	T	C	C	—	A	T	—	—	T	C	C	A	G	T	T	C	T	G	G	A	T
Hap2	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Hap3	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
Hap4	•	•	A	•	•	•	•	test•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
Hap5	•	•	•	•	•	•	•	C	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
Hap6	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
Hap7	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•	•	T	•	•	A	•	•	•	•	•	•	•	•
Hap8	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	A	•	•	•	•	•	•	•	•
Hap9	•	•	•	•	•	•	•	•	•	T	•	•	•	C	•	•	T	•	•	•	•	•	•	•	•	•	•	•
Hap10	A	T	•	•	•	•	—	•	•	•	•	•	•	•	•	•	T	•	•	A	•	C	•	•	•	•	G	•
Hap12	•	•	•	•	•	•	•	•	•	T	•	•	•	C	•	•	T	•	G	•	•	•	•	•	•	•	•	•
Hap14	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	A	•	C	T	•	•	•	•	•
Hap15	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	A	•	C	•	•	•	•	G	•
Hap16	•	•	•	•	•	•	•	•	•	•	•	•	C	•	•	•	T	•	•	•	•	•	•	•	•	•	•	•
Hap17	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	•
Hap18	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•
Hap19	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Hap20	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•
Hap21	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	T	•	•	A	•	C	T	•	•	•	•	•
Hap22	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	T	T	•	A	•	C	T	•	•	•	•	•
Hap24	•	•	•	•	•	•	•	•	•	T	•	•	•	C	C	•	T	•	G	•	•	•	•	•	•	•	•	•
Hap25	•	•	•	•	•	C	•	•	•	•	•	•	•	•	•	•	T	•	•	A	•	C	•	C	•	•	•	•

Hap26	C	C	
Hap27	.	.	G	
Hap28	.	.	G	T	C	.	.	T	.	G	
Hap29	T	.	.	C	.	.	T	.	G	G	
Hap30	.	.	-	T	.	.	C	.	.	T	.	G	
Hap31	A	.	.	
Hap32	A	
Hap33	T	T	.	G	A	A	.	.
Hap34	T	
Hap35	.	.	.	T	
Hap36	-	
Hap37	T	.	.	C	.	-	T	.	G	
Hap38	T	.	.	A	.	.	T	
Hap39	T	.	.	A	C	
Hap40	A	T	.	.	A	.	C	T	
Hap41	C	.	.	T	.	.	A	.	C	T	
Hap42	T	.	.	A	.	C	.	C	

Appendix 3.5 Percent frequencies of wolverine mtDNA control region haplotypes within and across regions.

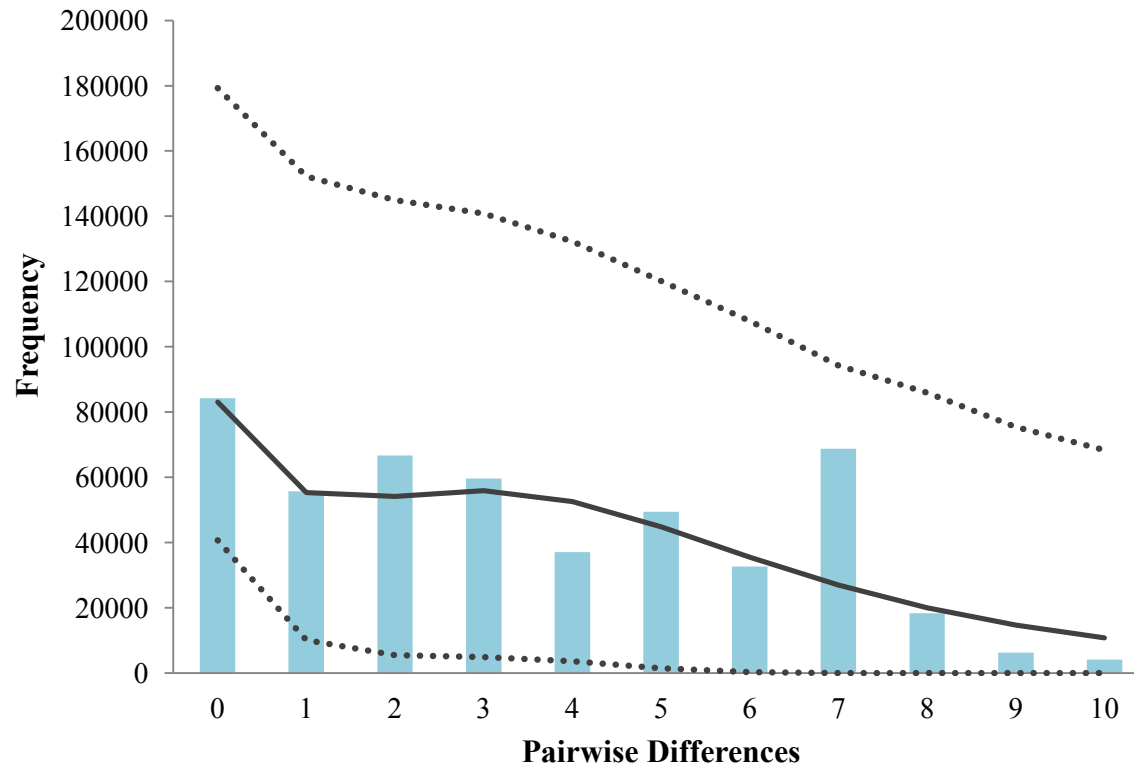
Haplotype	Within Region Frequency (%)																				Total Frequency (%) Across Regions
	SWE	NOR	MNG	RUS	AK	YT _C	YT _H	NT	NU	BC _C	BC _H	AB	SK _C	MB	ON	QC/ NL _H	MT	WY	ID	CA _H	
Hap1					43	53	60	19	6	31	86	31	19	19	65	62	83	77	100		36
Hap2					6	14		6													2
Hap3								17	26	3		7	6								4
Hap4								2													<1
Hap5					1			2													<1
Hap6					9	14		7	14	1		15									4
Hap7								32	36			4	31								5
Hap8			83	59	18			2	7	15			13					8			9
Hap9								7	10	1								16	15		4
Hap10	98	100																			17
Hap12					12	2	40	4	1	8	14		25								4
Hap14			17	20																	1
Hap15	2			2								4									<1
Hap16					1	11															1
Hap17										28		31					1				3
Hap18										1											<1
Hap19										1											<1
Hap20										8		4									1
Hap21																38					86
Hap22																					14
Hap24								2		1			6	29	9						2
Hap25														52	26						3
Hap26					1																<1
Hap27					1																<1
Hap28					1																<1
Hap29					1																<1
Hap30					1																<1
Hap31					1																<1
Hap32					1	2															<1
Hap33					1																<1

Hap34					1																	<1	
Hap35																							<1
Hap36																							<1
Hap37						1																	<1
Hap38				6																			<1
Hap39				9						1													1
Hap40				2																			<1
Hap41				2																		4	<1
Hap42										1													<1
<i>n</i>	62	108	6	54	148	49	5	53	81	86	7	26	16	31	54	13	148	13	15	7			

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; AK = Alaska; YT = Yukon; NT = Northwest Territories; NU = Nunavut;

BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC/NL = Quebec-Labrador; MT = Montana;

WY = Wyoming; ID = Idaho; CA = California; _c = Contemporary; _H = Historic; *n* = sample size



Appendix 3.6 Pairwise mismatch distribution performed on mtDNA haplotypes among individuals. Bars represent observed values, a solid line represents the expected distribution according to the sudden expansion model, and dotted lines show \pm 95% confidence intervals.

CHAPTER 4

THE INFLUENCE OF LANDSCAPE FEATURES ON GENETIC CONNECTIVITY OF WOLVERINES IN MANITOBA AND ONTARIO

Abstract

Habitat loss and fragmentation can disrupt population connectivity, resulting in small, geographically isolated populations and reduced genetic variability. Identifying landscape features influencing connectivity within a population can inform preventative management activities, limiting potential loss of genetic variation. Wolverines have a low resiliency to disturbances, limiting their ability to persist in modified or fragmented landscapes. Based on previously identified habitat associations, we investigated the influence of five landscape features (forest age, road density, land cover, spring snow cover mean and variance) on the genetic connectivity of wolverines at the periphery of their extant range in North America. We genotyped 67 wolverines at 18 microsatellite loci, and applied an individual-based genetic distance measure in Manitoba and Ontario. We evaluated landscape genetic relationships using multiple regressions on distance matrices and model averaging. Road density and Euclidean distance were negatively associated with wolverine genetic distance, while spring snow cover was positively associated with genetic distance. Sex-specific analyses revealed road density was associated with female genetic distance, while spring snow cover variance was associated with male genetic distance. Our results suggest that the northward expansion of anthropogenic disturbances have the potential to affect genetic connectivity. The high temporal resolution of individuals helped identify landscape features potentially influencing genetic connectivity within the population. Landscape genetics can provide valuable insight regarding the influence of habitat variability on genetic connectivity of wide-ranging low density species where traditional monitoring approaches are not practical.

Introduction

Habitat loss and fragmentation are recognized as two of the most serious threats to biodiversity (Fahrig 2003). A fragmented landscape can heighten resistance to movement, where habitat suitability reflects changes in limiting factors, such as predation, intraspecific competition, and disease (Wiens 1992; Robertson and Hamilton 2012; Pilfold et al. 2014). In addition to habitat fragmentation, wildlife movements can also be influenced by naturally occurring elements like elevation, topology and climate (Cushman et al. 2006; Pilot et al. 2006). Altered movement patterns and restricted dispersal can change the distribution of wildlife and indirectly affect population genetic structure (Banks et al. 2005; Coster and Kovach 2012). Disrupted connectivity can ultimately lead to small, geographically isolated populations that may over time become genetically distinct and have an overall reduction of genetic variability within each population (Keyghobadi et al. 2005). Inbreeding in isolated populations together with low genetic variability could lead to the possible loss of adaptive potential (Spielman et al. 2004; Keyghobadi 2007). Smaller, isolated populations are also more susceptible to stochastic events (Vandergast et al. 2009). It is important for declining populations to be detected early, so that management actions can be quickly applied and prevent irreversible loss of genetic variation (Antao et al. 2011).

Until recently, studies assessing landscape effects on animal movement for wide-ranging species commonly relied on radio telemetry; these studies, however, are restricted by sample size (Miller et al. 2010; Kelly et al. 2012). Genetic investigations on rare and elusive species generally capitalize on multiple sampling methods in order to increase sample size and spatial coverage of sampling. Genetic monitoring is also useful for wide-ranging species, where movements are difficult to monitor directly with other field-based

approaches (Vandergast et al. 2009). Sampling approaches typically used in tandem are snare surveys, road kills and trapper harvests (Comer et al. 2011; Schregel et al. 2012). While genetic sampling can provide information into population connectivity, landscape genetics focuses on the interaction between genetic connectivity and landscape features (Manel et al. 2003).

Landscape genetics represents an approach to quantify the effects of landscape and environmental features on spatial genetic variation and gene flow (Manel et al. 2003; Storfer et al. 2007). Important baseline measures can be quantified, such as detecting the presence of a barrier and providing insight on a species' dispersal patterns (Mu and Radke 2009; Keller and Holderegger 2013). Identifying habitat variables that influence dispersal, especially for species where direct monitoring is not possible, can inform conservation and management strategies how to best facilitate functional connectivity among populations (Epps et al. 2007; Roever et al. 2013). Additionally, landscape genetics can be used to evaluate the effectiveness of connectivity measures already in place (Holderegger and Wagner 2008). However, combining different sampling schemes within a study may result in uneven sampling intensity across the study area, which could affect the inferred relationship between landscape features and genetic connectivity (Oyler-McCance et al. 2013).

Neutral genetic markers and their associated distance measures remain the most widely used form of genetic data in landscape genetic studies (Bolliger et al. 2014). Landscape genetics continues to face challenges regarding the appropriateness of various statistical analyses for evaluating the influence of landscape features on genetic variation, especially for neutral markers using pairwise distance matrices (Bolliger et al. 2014). Mantel tests have been regularly used to assess how genetic distances relate to

geographical distances (Cushman and Landguth 2010; Storfer et al. 2010). However, the validity of Mantel tests has been questioned due to high type I error rates (Balkenhol et al. 2009; Guillot and Rousset 2013), suggesting this approach may be inadequate (Graves et al. 2013). Inflated type I errors have been associated with the occurrence of spatially autocorrelated data (Guillot and Rousset 2013). One suggested solution is to apply a lower significance level (e.g., $\alpha = 0.001$, Oden and Sokal 1992; Diniz-Filho et al. 2013). Although linear correlation, regression and canonical analyses do not address the problem of spatial autocorrelation, these approaches have demonstrated greater power than Mantel tests in detecting a relationship between landscape and genetic data when autocorrelation was present (Legendre and Fortin 2010). Akaike's Information Criterion (AIC) for regression model selection is increasingly being applied in landscape genetics (e.g., Goldberg and Waits 2010; Garroway et al. 2011; Richardson 2012).

Landscape genetics studies are largely centred on identifying landscape features that influence genetic connectivity (Spear et al. 2010). Of these studies, many use population-based measures (e.g., F_{ST}) to quantify genetic variation (Blair et al. 2013; Reding et al. 2013). However, individual-based methods provide a more powerful approach for identifying landscape genetic relationships (Bolliger et al. 2014). A higher temporal resolution is achieved by individual-based methods for detecting the influence of landscape features on genetic distances (Landguth et al. 2010), making this approach particularly valuable for analyses where landscape gradients or populations are continuously distributed (Bolliger et al. 2014). An individual-based approach also allows for the identification of landscape features that may have sex-specific effects (Talbot et al. 2012). Genetic structure can also reflect inherent life-history strategies (McDonald et al. 1999; Clark et al. 2008), like sex-biased dispersal (Lawson Hadley and Perrin 2007).

Male-biased dispersal is common in mammalian species (Greenwood 1980; Lawson Hadley and Perrin 2007), with dispersal occurring over large distances for highly vagile species (Bowman et al. 2002). Sex-biased dispersal will result in variable genetic structure between males and females, and in turn may influence the relationship between genetic structure and landscape heterogeneity. To date, many of the published landscape genetics studies examine the potential effect of different landscape features on genetically differentiated populations. Few studies have investigated the influence of landscape variables on a single population (i.e., no genetic structure, $K = 1$) to identify landscape features influencing movement, which could over time lead to increased genetic structuring.

The wolverine (*Gulo gulo*) is the largest of the terrestrial Mustelidae and has a Holarctic distribution (Hash 1987; Landa et al. 2000). Range contraction along the southern edge of the species distribution as occurred in both Europe and North America (Hash 1987; Pasitschniak-Arts and Larivière 1995) due to habitat loss, persecution and other anthropogenic activities (Wilson 1982; Schreiber et al. 1989; Landa et al. 2000). Recently, May 2014, the eastern population of Quebec and Labrador and the western population west of James Bay were deemed a single unit across their Canadian range, with an overall designation of special concern (Committee on the Status of Endangered Wildlife in Canada, COSEWIC 2014a). However, wolverines in Ontario are designated provincially as threatened (Species at Risk in Ontario, SARO 2012), and those in Quebec and Labrador have an endangered SARA (Species at Risk Act) status (SARA 2014). Wolverines in Quebec and Labrador are considered functionally extirpated as no sightings have been confirmed over the past three decades (Fortin et al. 2005), resulting in

Ontario being the extant eastern range edge of the wolverine's North American distribution.

Phylogenetic analysis across the wolverine's Holarctic distribution (Chapter 3) supported a single refugium. However, increased sampling along the extant eastern range edge (Manitoba and Ontario) revealed that the contemporary distribution of wolverines in Canada is composed of two genetic clusters – a panmictic core and an extant eastern peripheral group (Chapter 2). Increasing genetic differentiation of wide-ranging wolverines toward the northeastern edge of their extant distribution may reflect extrinsic factors like landscape features and environmental conditions influencing dispersal patterns.

Wolverines share similar life history characteristics observed in large-sized predators, like large home range size (e.g., males 209 - 2563 km², Banci and Harestad 1990; Dawson et al. 2010), low population density (1 wolverine/40 km² to 800 km², Banci 1994) and low fecundity (Banci and Harestad 1988; Copeland 1996; Persson et al. 2006). The lifetime productivity of wolverines appears to be lower than grizzly bears (*Ursus arctos horribilis*), suggesting low resilience to population disturbance and a low potential for population growth (Weaver et al. 1996; Persson et al. 2006). That is, wolverines appear to be intolerant of habitat change and may not be able to persist in areas with added mortality due to human presence.

In North America, the majority of wolverine studies on habitat associations have occurred in mountainous regions and are based primarily on radio telemetry data (e.g., Banci and Harestad 1990; Krebs and Lewis 2000; Schwartz et al. 2009). Wolverines are positively associated with late successional stands (Lofroth 2001; Wright and Ernst 2004a,b), and are rarely observed in recent burns and clear-cuts of any size (Hornocker

and Hash 1981). In Ontario, wolverines appear to avoid deciduous forests, as these higher ungulate biomass environments are likely associated with an abundant and diverse predator community, which could limit wolverines (Bowman et al. 2010). Although avoidance distances for different types of roads vary (Austin 1998; Krebs et al. 2007, May 2007; Dawson et al. 2010), the overall negative correlation between wolverines and roads likely reflects increased anthropogenic mortality associated with human access (e.g., Dawson et al. 2010). Additionally, spring snow cover (representing the reproductive denning period) is correlated with wolverine gene flow in the northern US Rockies (Schwartz et al. 2009), global wolverine distribution, and denning locations (Magoun and Copeland 1998; Krebs and Lewis 2000; Lofroth 2001; Copeland et al. 2010). Spring snow cover is important for successful natal denning in wolverines, as it provides thermoregulatory advantages and protection from predators (Magoun and Copeland 1998; Persson et al. 2006).

Higher rates of temperature increase at northern latitudes (Intergovernmental Panel on Climate Change, IPCC 2007) have resulted in more pronounced effects of climate change for arctic and subarctic regions (Scholze et al. 2006). In addition, human presence and resource development are expanding northward and encroaching onto wolverine habitat at the extant eastern edge of the wolverines' North American range, permanently altering the landscape and reducing the likelihood of wolverine persistence (Koen et al. 2008; Ontario Wolverine Recovery Team 2013).

Based on habitat associations identified by previous studies, we investigated the influence of five landscape features (forest age, road density, land cover, spring snow cover mean and variance) on the genetic connectivity of wolverines comprising the extant eastern peripheral population in North America. We expected that unsuitable landscape

features for wolverines (i.e., young forests, low mean snow cover, high snow cover variability, high road density, and water-mixed/deciduous forests/anthropogenic land cover types) to have high movement costs (Table 1). We predicted that forest age, land cover, and road density would explain, at least in part, the genetic distance patterns among individuals due to increased mortality associated with associated landscape changes. Given the fairly contiguous uniform landscape of our study area, we predicted spring snow cover would not influence genetic variability in contrast to its demonstrated importance in montane environments (Schwartz et al. 2009).

Methods

Sampling and microsatellite genotyping

Our data set consisted of 73 wolverine samples from Ontario (ON; $n = 12$, Kyle and Strobeck 2002; $n = 61$, Zigouris et al. 2012) and 36 samples from Manitoba (MB; $n = 27$, Kyle and Strobeck 2001; $n = 9$, Zigouris et al. 2012). The majority of samples (93%) were collected between 1999 and 2009; however, five samples collected prior to 1999 ($n = 1$, 1962; $n = 1$, 1975; $n = 2$, 1980s; $n = 1$, 1996) were included to increase sample size along the extant eastern periphery. All samples were genotyped at 25 microsatellite loci, developed in wolverines (Gg-3, Gg-4, Gg-7, Gg-10, Gg-14, Gg-25, Gg-37, Gg-42, Gg-443, Gg-452, Gg-454, Gg-465, Gg-471, Ggu-192, Walker et al. 2001; Ggu-101, Ggu-216, Ggu-234, Duffy et al. 1998), marten *Martes americana* (Ma-19, Davis and Strobeck 1998), mink *Neovison vison* (Mvis-20, Mvis-72, Mvis-75, Fleming et al. 1999), ermine *Mustela erminea* (Mer-41, Fleming et al. 1999), Eurasian otter *Lutra lutra* (Lut-604, Dallas and Piertney 1998), and badger *Taxidea taxus* (Tt-1, Tt-4, Davis and Strobeck 1998), to increase the resolution of finer spatial scale analyses and to identify close relatives (i.e., parent-offspring and full siblings). DNA extraction, genotyping, and

identity analyses followed Davis and Strobeck (1998) and Chapter 2, with the following changes. Seven primers were omitted from subsequent analyses due to heterozygote deficiencies (Mvis-72, Mvis-20) or failure to amplify (Gg-25, Gg-452, Gg-465, Gg-471, Mer-41). The remaining 18 loci (Gg-3, Gg-4, Gg-7, Gg-10, Gg-14, Gg-37, Gg-42, Gg-443, Gg-454, Ggu-192, Ggu-101, Ggu-216, Ggu-234, Ma-19, Mvis-75, Lut-604, Tt-1, Tt-4) were used for all genetic analyses described herein. Sex was determined using primer pairs P1-5EZ/P2-3EZ (Aasen and Medrano 1990) and Y53-3C/Y53-3D (Fain and LeMay 1995).

Descriptive genetic analyses

An intensive hair snare survey in the Red Lake area resulted in a high abundance of individuals sampled in a 2000 km² sampling area (Magoun et al. 2004). To avoid biasing allele frequencies from sampling kin (Goldberg and Waits 2010), we randomly filtered the samples from the Red Lake area to include only one individual from each full-sibling or parent-offspring pair. Inbreeding coefficient and relatedness values were estimated with Coancestry 1.0.1.2 (Wang 2011). The inbreeding coefficient was moderate ($f = 0.09$, Tuckwell and Everet 2009; Ruiz-Lopez et al. 2010). Using allele frequencies from the data set, we simulated 100 pairs of parent-offspring, full-sibs, half-sibs and unrelated individuals to determine expected values of relatedness from this population. We used the triadic likelihood estimator (TrioML, Wang 2007) to calculate pairwise relatedness, as this method allows for inbreeding and estimates relatedness using a triad of individuals. This reduces the chance of mistakenly inferring a gene identical in state as being identical by descent (Wang 2007).

To determine if intensive sampling of the Red Lake area influenced the results, we analyzed the data with and without the Red Lake sampling cluster. To mimic the

sampling density of the study area, we omitted all but four randomly selected samples from Red Lake for the data set without the cluster. Differences in dispersal distances between sexes was quantified by comparing genetic diversity (D_{est} ; Jost 2008) of males and females between Manitoba and Ontario using SMOGD (Crawford 2010). The greater dispersing sex is expected to have lower between-subpopulation differentiation (Prugnolle and de Meeus 2002). In order to determine the presence of sex-specific effects on the relationship between genetic structure and landscape heterogeneity, we also conducted analyses by sex for the full data set.

The four data sets (both sexes, both sexes no Red Lake, females, males) were tested for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using Genepop 3.4 (Raymond and Rousset 1995) with a sequential Bonferroni correction. Genetic distances, ($D_{PS} = 1 - \text{proportion of shared alleles}$), were calculated among individuals for all data sets as this genetic distance measure is free of equilibrium assumptions and is able to reveal fine-scale contemporary genetic structure (Bowcock et al. 1994; Murphy et al. 2010). We used Microsatellite Analyzer (MSA) 4.05 (Dieringer and Schlötterer 2003) to calculate D_{PS} matrices to test the influence of landscape and environmental variables on genetic distance. D_{PS} values can range from zero (genetic identity) to one (no alleles shared). Summary statistics (mean and value ranges) of D_{PS} were calculated for all four data sets. We performed an initial isolation by distance test to ensure that the observed patterns of genetic distances were not exclusively reflective of geographic distances. Mantel tests (Mantel 1967) were performed using *ecodist* package (ver. 1.2.9, Goslee and Urban 2013) in R (ver. 3.0.2, R Development Core Team 2013) to determine the correlation between genetic variability (pairwise D_{PS} values) and geographic distance for the four data sets.

Landscape variables and resistance matrices

We selected five predictor variables (forest age, land cover type, spring snow cover mean and variability, and road density; Table 1) hypothesized to have relevance to wolverine dispersal for our study area based on snow-tracking and radio telemetry (e.g., Hornocker and Hash 1981; Austin 1998; Magoun and Copeland 1998; Bowman et al. 2010; Dawson et al. 2010), climate correlation analyses (Copeland et al. 2010) and a landscape genetic study at the southwestern range periphery (Schwartz et al. 2009). Forest age was obtained from North American Carbon Program Forest Age Maps, compiled in 2004 at 1-km² resolution (Pan et al. 2012). Landcover type was based on Advanced Very High Resolution Radiometer satellite imagery at 1-km² resolution, 1981 - 1994 (Hansen et al. 2000). We obtained ten 8-day (15-23 April, corresponding to the end of the denning season; Magoun and Copeland 1998) composite snow cover maps, 2000 - 2009, at a resolution of 0.1 degrees (10 x 10 km) from NASA's Earth Observatory (<http://earthobservatory.nasa.gov/GlobalMaps/index.php>). To ensure map resolution remained consistent across all variables, spring snow cover maps were rescaled to 1-km² grid cells using the nearest resampling method in ArcGIS 10.0 (Environmental Systems Research Institute, ERSI 2011). Spring snow cover mean and variance were recalculated using the rescaled maps. A road map that included winter road networks was obtained from GeoGratis (www.geogratis.gc.ca), and road density (km/km²) was calculated for a 5-km radius for each 1 km x 1 km cell. The radius represented the observed distance of a wolverine den in Ontario to the nearest access trail (Dawson et al. 2010).

Based on the expected effect of each variable on wolverine movement, resistance for forest age, spring snow cover mean and variance, and road density was modeled as a linear function ranging between 1 and 101, representing low and high cost respectively

(i.e., map cells coded as a resistance surface, Koen et al. 2012b; Table 4.1). For land cover, we grouped similar habitats together, reducing the number of habitat types from twelve to six (Appendix 4.1). To address the uncertainty regarding resistance of water, categorical land cover classes were ranked in two different orders. To assess the sensitivity of categorical data to resistance variation, both orders were assigned four resistance ratios (Table 4.1). We also calculated isolation by Euclidean distance (IBD) by creating a resistance surface where all grid cells were assigned a cost value of one (Trumbo et al. 2013).

Pairwise resistance distances were calculated between individuals for each variable using circuit theory (McRae and Beier 2007; McRae et al. 2008) with Circuitscape 3.5 (McRae and Shah 2009). The algorithm applies a multiple-path process to evaluate landscape resistance between each pairwise comparison (McRae 2006). We chose this multi-path approach over a least-cost path method as a single pathway would be unable to elucidate the movement over multiple generations. Model organisms within Circuitscape behave like random walkers, but with the restriction that map edges function as artificial barriers. These artificial edges constrain the movement of model organisms, and may result in the overestimation of effective resistance (Koen et al. 2010). This bias can be reduced by including a buffer around the edge of the map or study area. We used a 100-km buffer (e.g., Koen et al. 2012a) around the study area of our terrestrial resistance surfaces (i.e., excluding Hudson Bay and Lake Superior, as these were treated as infinite barriers; Figure 4.1). All maps were larger than the study area, resulting in the buffer having resistance values reflective of map data. For all analyses in Circuitscape, we used a pairwise mode and an eight-neighbouring cell connection scheme (McRae and Shah 2009).

Data analysis

We evaluated all eight resistance ratios for the categorical land cover variable using a causal modeling framework (Cushman et al. 2006) to identify the ones most supported by the four data sets (Table 4.1). To help remedy inflated type I errors, we used a slightly more conservative P value of 0.001 (Oden and Sokal 1992; Diniz-Filho et al. 2013). Mantel correlation coefficients were calculated between pairwise D_{PS} values and each alternative land cover resistance ratio (Table 4.1) using *ecodist* package in R with 10 000 permutations. Based on simple Mantel tests between the eight land cover resistance matrices and pairwise D_{PS} values, we did not identify any models as significant for any of the data sets. Although not significant, land cover was negatively correlated with male genetic distance, but positively correlated for the remaining data sets (Table 4.2). The majority (83%) of our study area was composed of the three lowest resistance categories (35% evergreen forest; 32% woodland; 16% grassland/shrubland). The three remaining categories of higher resistance occurred mainly along the edge of our study area, and likely not included in the estimation of pairwise resistances. Gene flow (or movement) will likely not be restricted for a highly connected variable or when resistance contrast between categories is low (Cushman et al. 2013). The land cover variable was omitted from further analyses given its low geographic variation for our study area.

Models containing different predictor variables that are correlated may have similar fits to the data, making it difficult to determine proper relationships (Freckleton 2011). Multicollinearity can inflate the standard errors of estimated coefficients and affect the magnitude of regression weights, adversely influencing coefficients of statistical significance (Kraha et al. 2012). Removing one of the correlated predictor variables reduces biological and statistical redundancy (Quinn and Keough 2002).

Correlations were performed among pairwise resistance values of explanatory variables to test for multicollinearity. Highly correlated variables ($|r_s| > 0.8$, Katz 2006) were evaluated to determine if one or more variables should be removed. Spearman rank correlations (r_s) with a Bonferroni adjustment and Variance Inflation Factors (VIFs, Kutner et al. 2004) were calculated for predictor variables using R packages *Hmisc* (ver. 3.14-3, Harrell 2014) and *usdm* (ver. 1.1-12, Naimi 2013), respectively. Similar to previous studies, $VIF > 10$ was used as evidence for significant multicollinearity (Dyer et al. 2010). We found $|r_s| < 0.80$ for pairwise comparisons among the predictor variables, with the exception of forest age and Euclidean distance ($r_s \geq 0.88$) across all data sets. The presence of multicollinearity between forest age and Euclidean distance was also detected by the variance inflation factor ($VIF \geq 10$), with all other comparisons having $VIF \leq 5.38$ (Table 4.3). The two correlated variables, forest age and Euclidean distance, were considered in competing models, but not within the same model (Dextrase et al. 2014).

We evaluated 23 a-priori resistance models to pairwise D_{PS} estimates using an information-theoretic approach (Burnham and Anderson 2002). This multiple regression approach not only tests for significant relationships between distance matrices but also quantifies each explanatory variables contribution to the overall fit of the model (Legendre et al. 1994). An uncertainty associated with the use of information theoretic values (e.g., AIC) based on multiple regression equations is the potential bias in model-selection due to the non-independence of pairwise distances (Goldberg and Waits 2010). When pairwise observations are falsely assumed to be independent, AIC differences between the top model and other models can be inflated, resulting in increased apparent support of the top model (Burnham and Andersen 2002; van Strien et al. 2012). Model

averaging may decrease the bias associated with non-independent pairwise observations, as the predictor model is derived from a number of candidate models and not simply based on the model with the highest AIC weight. We assessed the differences in Akaike's Information Criterion for small sample size (AIC_C) between the top-ranked model and each subsequent model (Δ_i), and selected those with $\Delta_i < 4$ for model-averaging. Choosing a higher Δ_i threshold for model-averaging ensures that the top Kullback-Leibler model will be retained (Burnham and Anderson 2002). We model-averaged parameter estimates (β) and unconditional 95% confidence intervals (CI) for all variables within the selected set. For each variable, we examined the unconditional 95% CI to assess the biological importance of the model-averaged parameter estimate. Biological importance was considered to be negligible if the CI contained zero. Multivariate resistance surfaces were then constructed using biologically important model-averaged parameter estimates. All model averaging analyses were done using *MuMIn* package (ver. 1.9.13, Bartón 2013) in R. Fit of modeled estimates of effective resistance to genetic distances was evaluated with a simple Mantel test with the *ecodist* package.

Results

Descriptive genetic analyses

Based on simulations using allele frequencies from the data set, we identified a relatedness value of $r = 0.6$ for parent-offspring and full-sibling pairs. Seven kin groupings were identified, resulting in the removal of eight individuals from the Red Lake area to avoid biasing allele frequencies from sampling close relatives (i.e., parent-offspring or full-sibling). Of the remaining samples, 16 failed to amplify for more than four loci and were removed. A total of 67 individuals was used in this study (MB [$n = 26$], ON [$n = 41$], Figure 4.1, Appendix 4.2). All 18 loci were consistent with HWE for

the whole data set, Red Lake cluster removed, and for each sex. A single pair of loci (Gg7 and Gg442) exhibited linkage disequilibrium for the data set as a whole. Since linkage disequilibrium for this pairing was not consistent across the different data subsets, these loci were retained for further analyses. Genetic differentiation was higher among MB and ON females ($D_{\text{est}} = 0.010$) in comparison to males ($D_{\text{est}} = 0.001$). Summary statistics of individual pairwise genetic distance estimates remained unchanged with or without the Red Lake cluster. D_{PS} values for the whole data set ranged from 0.14 to 0.84, with the range being narrower for females and males (Appendix 4.3). The results of the Mantel test showed the correlation between genetic and geographic distances was significant but that genetic distance patterns were only partly explained by isolation by distance for the whole data set ($r = 0.18$, $P < 0.001$), females ($r = 0.16$, $P = 0.018$) and males ($r = 0.18$, $P = 0.005$). Similar results were obtained with the removal of the Red Lake cluster ($r = 0.20$, $P < 0.001$). Isolation by distance plots (Appendix 4.4) also revealed a weak pattern for all data sets.

Data analysis - both sexes

Comparable results (selected models and model-averaged coefficient estimates) were obtained with and without the Red Lake cluster; thus only the results using the whole data set are presented. Model ranking for the whole data set identified a single model that noticeably outperformed the other models. The selected set ($\Delta i < 4$) comprised two models. The first-ranked model for the whole data set had a best approximating model ranking probability of 63%, and contained the variables road density, mean spring snow cover and Euclidean distance (Table 4.4). All three predictor variables had positive coefficient estimates (Table 4.5). The other model had a ranking probability of 27%, and included spring snow cover variance (positive coefficient

estimate; Table 4.5) in addition to the three variables from the top ranked model (Table 4.4). Only three of the independent variables (road density, mean spring snow cover and Euclidean distance) included in the selected set had 95% CI that did not overlap zero, suggesting that these variables appeared to have some predictive power. The significance of road density, mean spring snow cover and Euclidean distance was further supported with variable importance weights of 1.00, and model-averaged coefficient estimates indicated all three predictor variables were associated with genetic distance (Table 4.5). There was no overlap among confidence intervals of parameter estimates for the three variables, indicating that all three possibly influenced genetic variability. Coefficient estimates revealed Euclidean distance may have the largest effect, followed by road density and spring snow cover mean (Table 4.5).

Data analysis - by sex

Model ranking for the single-sex data sets did not identify a single model that noticeably outperformed the other models. Low Akaike weights among competing models and small differences of ΔAIC_C among ranked models were observed for both female and male data sets (Table 4.4). This resulted in 12 and 11 models comprising the candidate set for females and males, respectively. The only independent variable found to have some predictive power for the female data set was road density, having a variable importance weight of 1.00. Road density was the only predictor identified by model-averaged coefficient estimates to be associated with genetic distance among females (Table 4.5). In comparison, spring snow cover variance was the only independent variable that appeared to have some predictive power for males. Spring snow cover variance had a variable importance weight of 1.00, and is likely to have an effect on male genetic distance as suggested by model-averaged coefficient estimates.

Overall modeled resistance rasters to wolverine genetic connectivity were created using model-averaged parameter estimates (Table 4.5) for each of the data sets (Both sexes: $4.77E-01 + 2.51E-03 \cdot \text{road density} + 6.30E-04 \cdot \text{snow cover mean} + 3.45E-02 \cdot \text{euclidean distance}$; Females: $5.04E-01 + 6.34E-03 \cdot \text{road density}$; Males: $4.69E-01 + 1.14E-03 \cdot \text{snow cover variance}$). Simple Mantel tests between genetic distance (D_{PS}) and predicted resistance map revealed that the fit of the model was low across all data sets (Table 4.6).

Discussion

This study highlights the applicability of individual-based landscape genetic approaches in assessing the influence of landscape and environmental features on the genetic structure of continuous populations. Additionally, we demonstrate the importance of examining landscape features that may have sex-specific effects for species demonstrating sex-biased dispersal. We found that road density, mean spring snow cover, and Euclidean distance were associated with genetic variability of wolverines at the eastern periphery of their North American range (Table 4.5). Gender-specific effects were also found; the structure of genetic relatedness of females appeared to be influenced by road density, while males appeared to be influenced by spring snow cover variance (Table 4.5). These findings show the applicability of landscape genetics in identifying landscape features likely influencing genetic connectivity within a population.

Study design and analysis

Although non-invasive hair snare surveys generate large sample sizes and can be deployed over large areas (e.g., Pelletier et al. 2012), studies on elusive, low-density species may include opportunistic samples (trapper and road kills) to increase spatial coverage of sampling and sample size (Dixon et al. 2006; Schregel et al. 2012).

Opportunistically collected samples were unsystematically distributed across the study area, while hair-snare surveys reflected an intensive sampling area near Red Lake. Oyler-McCance et al. (2013) found random sampling regimes identified landscape features likely influencing genetic structure, while cluster or single study site layouts were unable to do so. Intensively sampled areas, such as our Red Lake area, likely consist of closely related individuals (Oyler-McCance et al. 2013), which could influence the relationship between genetic and resistance distances. In our study, the removal or inclusion of the Red Lake cluster did not alter which predictor variables were identified to influence genetic distance (See Results, Data analysis - both sexes). One possible explanation for this could be our removal of closely related animals prior to analysis.

Influence of predictor variables on genetic distance

Our results showed forest age did not appear to influence wolverine genetic distance (Table 4.5). The majority of our study area was represented by an intact boreal forest landscape, with timber harvesting occurring at the southern edge of the study area. This northern landscape is disturbance-driven, with fire the primary agent (Weber and Flannigan 1997). Young forests originated from fire may not limit wolverine movements as timber harvested areas might in the south. In northwestern Ontario, wolverine occurrence was negatively related to deciduous forest, suggesting that an important limiting factor at this carnivore's southern range boundary is habitat alteration (Bowman et al. 2010). Logged areas are characterized by young deciduous forests, an increased abundance of moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), and wolves (*Canis lupus*; Vors et al. 2007; Bowman et al. 2010). Increased predator and scavenger populations due to higher productivity of deciduous forests could limit wolverines through interspecific competition (Bowman et al. 2010). However,

recolonization of wolverines in southern Norway may have been facilitated by increased ungulate availability during winter as a result of wolf recovery in southern boreal forests (van Dijk et al. 2008b). Although wolverines may occupy the same regions as wolves, this mustelid appears to select higher elevation habitat, suggesting the importance of topography in limiting intraguild predation (May et al. 2008; van Dijk et al. 2008b, Khalil et al. 2014). Wolverines may benefit from low-to-moderate wolf densities due to carrion provisioning (Bowman et al. 2010). The average size of packs declines with wolf density (Thurber and Peterson 1993), thus low-to-moderate wolf densities are presumed to result in wolf packs of small-to-intermediate size. The availability of biomass from carcasses to scavengers is maximized by wolf packs of intermediate size, where the kill rate is relatively high and only part of the carcass is consumed (Wilmers et al. 2003). Smaller packs may also incur greater energetic costs associated with guarding the carcass against scavengers, increasing the likelihood of carcass abandonment (Mech 1970, Wilmers et al. 2003). In addition, forest management in Ontario was found not to have an effect on American marten gene flow, suggesting that the boreal forest landscape was well connected for marten. One possible explanation could be that habitat alteration influences wolverine movements at small scales and that this factor may not have a strong enough effect to disrupt genetic connectivity at broad scales (Koen et al. 2012a).

A latitudinal gradient of increasing resistance from north to south was observed for road density (Appendix 4.5a,b). Roads often decrease functional connectivity, resulting in increased genetic distance among individuals or genetic differentiation of populations (Holderegger and Di Giulio 2010). In this study, pairwise genetic distances of wolverines from the eastern periphery of their North American range were associated with road density (Table 4.5). Two direct threats related to roads are increased mortality

from trapping and collisions from vehicles (Banci 1994; Krebs et al. 2004). Although there has been a zero harvest quota for wolverines in Ontario since 2001 (Bowman et al. 2010), harvests may still be occurring (COSEWIC 2014b) in addition to incidental captures (OMNR unpub. data as cited in Ontario Wolverine Recovery Team 2013). In Manitoba, wolverines remain listed as a furbearer and continue to be actively harvested, with furbearer harvest totals of 69 and 47 animals in 2011-12 and 2012-13, respectively (Manitoba Wildlife Branch 2013). However, pelts used domestically may be omitted from official statistics, leading to the under-reporting of harvest levels (COSEWIC 2014b). The degree of unreported harvests varies across the wolverine's range (COSEWIC 2014b), and can be underestimated by 50-90% (Lee 1994). All-season or winter road development can make remote areas more accessible, increasing wolverine susceptibility to overharvest (Mulders 2000). A small number of hunters or trappers targeting a species can result in population declines (e.g., fisher population in California; Lewis and Zielinski 1996). In the Northwest Territories, two hunters were responsible for 27% of the 1992-93 wolverine harvest (Lee 1994). Jensen et al. (1986) found that wolves in Ontario were able to persist at low road densities, but persecution significantly increased where road densities exceeded 6 km/km². Vehicle collisions also contribute to wolverine road-associated mortality, with 11 deaths in Ontario occurring between 1990 and 2013 (Ontario Wolverine Recovery Team 2013). Mortality associated with roads is likely to be additive to natural mortality, with low fecundity of wolverines (Copeland 1996; Inman et al. 2007) limiting their capacity to compensate for anthropogenic causes of death. Roads also affected landscape resistance for fishers (*Martes pennanti*), where road density likely reflected human activities that may impede gene flow (Garroway et al. 2011).

Roads appear to have immediate effects on genetic connectivity, as negative effects were seen within a few generations following road construction (Holderegger and Di Giulio 2010). Landguth et al. (2010) found time of barrier detection was short (1 - 3 generations) for individual-based landscape genetic approaches involving species with dispersal abilities >10 km. This short response time suggests that landscape features such as roads and deforestation will produce a detectable genetic response for individual-based landscape genetics methods (Landguth et al. 2010). However, an underlining factor that needs to be considered is generation time. Short generation intervals will produce a detectable response in a few years compared to species with longer generation intervals. Based on the findings of Landguth et al. (2010), a genetic response by wolverines to road development should be measurable between 4 to 12 years. While some disturbances such as logging and road building cause sudden and abrupt landscape changes, others like increasing temperature generally produce more gradual changes to the landscape that over time can influence genetic connectivity.

Increasing global temperatures are expected to reduce snow cover and depth, in turn likely affecting diversity and distribution of species in northern latitudes. Ye and Cohen (2013) observed a reduction in the snowfall season length for northern Eurasia by 6.2 days per degree of increase, with 3.4 days being lost in the spring. At broad scales, elevation has been identified as an important variable to wolverine habitat, as elevation affects snow depth and persistence (May et al. 2006; Copeland et al. 2007). As such, gene flow among wolverines inhabiting the US Rockies positively correlated with persistent spring snow cover (Schwartz et al. 2009). Given the low topographic relief for Manitoba and Ontario (500 m, Natural Resources Canada 2004) in comparison to mountain ranges with a relief of 2500 m (e.g., Schwartz et al. 2009), we did not expect

spring snow cover to be associated with genetic distance in our study. However, we found lower mean spring snow cover was correlated with increased genetic distance (Table 4.5). The latitudinal gradient of decreasing mean spring snow cover from north to south appears sufficient to be identified as a possible influence on wolverine genetic distance. The influence of snow on genetic distances has also been suggested for Canadian lynx (*Lynx canadensis*, Stenseth et al. 1999; Rueness et al. 2003) and fisher populations in Ontario (Garroway et al. 2011). While one possible explanation for restricted dispersal by lynx may be habitat imprinting on snow conditions (Row et al. 2014), inefficient traveling through deep snow due to high foot loading (ratio of body mass to total foot area) appears to be the underlining mechanism restricting dispersal for fisher (Krohn et al. 2003). Low foot loading for wolverines (Burdukov and Kozlov 1979) suggests that spring snow cover does not influence wolverine movement *per se*. However, this mustelid is an inefficient hunter of large ungulates; wolverines require snow conditions where the snow can carry wolverines but not their larger prey (Pasitschniak-Arts and Larivière 1995; van Dijk et al. 2008a; Mattisson et al. 2011). Reproductive females during winter have smaller prey species in their diet relative to males (Lofroth et al. 2007; van Dijk et al. 2008a; Koskela et al. 2013). Thus the importance of snow conditions influencing ungulate availability may be of greater significance for males (see next section). Spring snow cover is also an important component to the reproductive denning habitat of wolverines, as it provides kits a thermoregulatory advantage and refuge from predators until post-weaning den abandonment in late April to early May (Pulliainen 1968; Magoun and Copeland 1998). These results provide additional support for the idea that wolverines at their southern distribution are limited by spring snow cover (Aubry et al. 2007; Copeland et al. 2010).

Sex-specific effects

We found that the genetic distance among male wolverines was associated with spring snow cover variance (Table 4.5), where increased variance was associated with increased *Dps* values. Spring snow cover variance can be considered a proxy to air temperature. Air temperatures over mid-latitude areas explain ~50% of spring snow cover variability (Brown and Robinson 2011). Although our study area was characterized by a north-south gradient of increasing resistance for mean snow cover, high resistance values for spring snow cover variability were observed for mid-range snow cover resistance values (Appendix 4.5c). The spring snow cover variability measure could indicate where loss of snow cover is likely to occur. Trends in snow cover extent are driven primarily by warmer air temperatures. In the Northern Hemisphere, a 7% and 11% decrease in snow cover extent has been observed for March and April, respectively, from pre-1970 values (Brown and Robinson 2011). Dispersal of juveniles from natal areas is hypothesized to occur anytime from January to May (Magoun 1985). While long distance dispersal of subadults and young-of-the-year, particularly males, make it difficult to determine cause of mortality (Banci 1994), 33-50% of subadult wolverines are considered to perish during dispersal (Krott 1982). Higher mortality rates are expected for transients as they do not have familiar home ranges in which to hunt as do residents (Banci 1994). Carrion is an important winter and spring food resource for wolverines (van Dijk et al. 2008a). Increased availability of ungulate carcasses during snowy winters (e.g., Okarma et al. 1995) is expected to improve food availability for wolverines, especially dispersing males, by providing large localized food sources (Gese et al. 1996). During mild winters, when carrion availability is low, wolverines appear susceptible to trapping with baits (Weaver et al. 1996). Resident coyotes (*Canis latrans*) in

Yellowstone National Park, Wyoming, spent more time feeding and resting instead of traveling and hunting during winters with high ungulate carcass biomass due to deeper snow (Gese et al. 1996).

Road density had an effect on genetic distance for female individuals but not males (Table 4.5). Previous observations based on radio telemetry found females were positively associated with roadless areas during summer months (Krebs et al. 2007) and abandoned their den when disturbed (Copeland 1996), suggesting that females respond negatively to human presence. Female wolverines appear to be affected both directly (trapping and road kill mortality) and indirectly (habitat associations and quality) to roads, which can have significant population level effects.

Predictive model

Although the information theoretic approach may have identified the underlying variables influencing genetic variability and given insight on the importance of these variables to the model, low model fit (Table 4.6) of the predicted model to the genetic data decreases our confidence in the estimated coefficient values. However, for all data sets, most of the multivariate models incorporating landscape variables had higher AIC_C weights than the model of simple isolation by distance. This poor model fit may be due to our assumption of a linear relationship between predictor variables and genetic distance. Graves (2012) suggested incorporating the underlying process of individual movement into the model may reduce this estimate bias. Few studies have utilized non-genetic data to inform resistance assignment, with some reasons being that for many species, especially rare and elusive ones, non-genetic field data are difficult to collect, are likely of low sample size or of limited extent, or temporally inconsistent with genetic data (Spear et al. 2010). Another reason for having poor model fit may be that our resistance

values reflected landscape composition, and did not take into account landscape configuration - the spatial arrangement of landscape elements. Jaquiere et al. (2011) have shown that detectability of factors affecting genetic variation was influenced by both the composition and configuration of landscapes.

Conservation implications

Multiple aerial surveys of wolverine over the past decade in Ontario continue to provide evidence supporting an eastward range expansion and the possibility of an increasing population abundance in Ontario (Magoun et al. 2004; Ontario Wolverine Recovery Team 2013). Populations found at the leading edge of a step-wise range expansion generally exhibit lower genetic diversity, showing a genetic signal of isolation by distance (Le Corre and Kremer 1998; Pruett and Winker 2005). This may explain why Euclidean distance was associated with genetic distance (Table 4.5). Although this apparent expansion is occurring predominately in northern regions that are primarily roadless, human presence and resource development are expanding northward and encroaching onto this area (Koen et al. 2008; Ontario Wolverine Recovery Team 2013). This northward expansion of industrial development has the potential to affect genetic connectivity. Careful road planning will be needed to ensure that connectivity is maintained. Additionally, the negative association of road density with genetic distance further supports the wolverine's low tolerance of industrial development, particularly roads. This carnivore is one of the first species to disappear following the onset of anthropogenic disturbance (Ontario Wolverine Recovery Team 2013). The wolverine can be considered a good ecological indicator species for landscape connectivity due to their dependence on large, intact tracts of suitable habitat (COSEWIC 2003; Ontario Wolverine Recovery Team 2013).

Conclusions

The increased temporal resolution associated with an individual-based genetic distance measure (Landguth et al. 2010) allowed us to identify landscape features potentially influencing pairwise genetic distances within a single population for a wide-ranging, low density carnivore. This approach not only associated spring snow cover and road density with genetic distance, but also allowed the identification of landscape variables that may have sex-specific effects (Talbot et al. 2012). The indication of a highly connected boreal forest landscape (e.g., American marten; Koen et al. 2012a), suggests the negative effect of road density on wolverine genetic distance is not due to the fragmentation of habitat, but likely due to direct mortality. Additionally, persistence of spring snow cover is not only important for wolverines inhabiting the northern U.S. Rockies (Schwartz et al. 2009) but snow cover also influences wolverine genetic connectivity for areas having lower topographic relief. Spring snow cover is important for successful denning (Magoun and Copeland 1998, Persson et al. 2006) and may also affect survival of dispersing subadults, particularly males, by increasing ungulate biomass (Gese et al. 1996) during late winter and early spring. To further develop the findings of this study, we suggest that future research on wolverine landscape genetics expand our study area to encompass the genetically differentiated northwestern region to determine if the landscape features affecting within population genetic connectivity are also associated with between population genetic connectivity.

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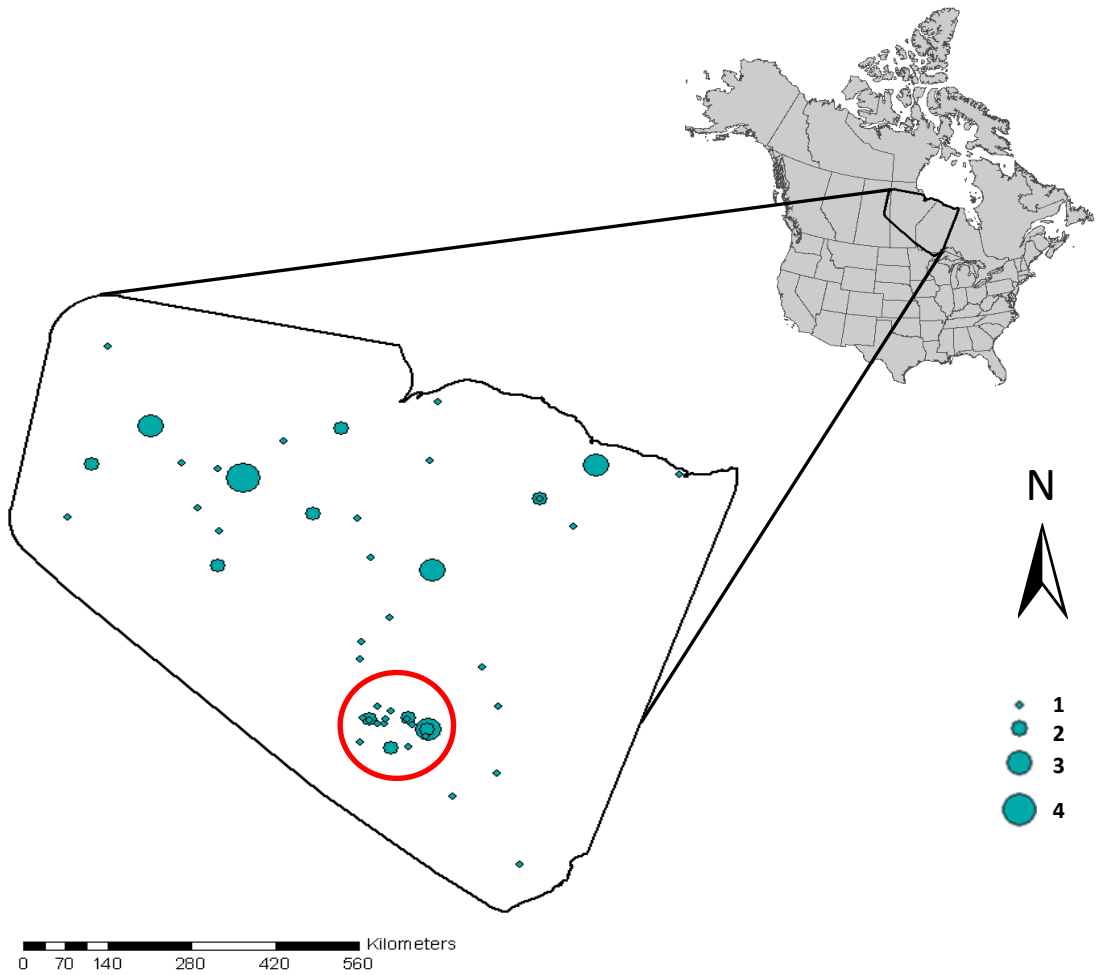


Figure 4.1 Study area of the extant eastern peripheral wolverine population in North America. Size of each circle depicts the number of individuals sampled at that site. Red circle identifies intensively sampled area of Red Lake.

Table 4.1 Assignment of resistance values for each of the landscape features and the six categories of land cover for two sets of rankings of land cover types.

Predictor Variable (expected direction of relationship)	Variable Measure	Resistance Values			
Forest Age (+)	Young Forest	101			
	Old Forest	1			
Snow Cover Mean (+)	Low Cover	101			
	High Cover	1			
Snow Cover Variance (-)	Low Variance	1			
	High Variance	101			
Road Density (-)	Low Density	1			
	High Density	101			
Land Cover (+)					
High Water Resistance	Evergreen Forest	1	1	1	1
	Woodland	20	5	10	30
	Grassland/Shrubland	40	40	20	45
	Mixed/Deciduous Forests	60	60	80	55
	Anthropogenic	80	97	90	70
	Water	101	101	101	101
Land Cover (+)					
Moderate Water Resistance	Evergreen Forest	1	1	1	1
	Woodland	20	5	10	30
	Grassland/Shrubland	40	40	20	45
	Water	60	60	80	55
	Mixed/Deciduous Forests	80	97	90	70
	Anthropogenic	101	101	101	101

Table 4.2 Statistical results of the top resistance model of land cover selected by the Mantel r test for each of the data sets.

Data Set	Mantel r	95% Confidence Interval		P
		Lower	Upper	
Both Sexes	0.065	0.020	0.114	0.319
Both Sexes No Red Lake	-0.074	-0.132	-0.023	0.352
Females	0.092	-0.012	0.194	0.428
Males	0.144	0.061	0.217	0.158

Table 4.3 Variance Inflation Factor values for each independent variable across data sets.

Predictor Variables	Variance Inflation Factor		
	Both Sexes	Females	Males
Forest age	13.63	17.35	21.90
Land cover	1.52	3.72	1.75
Road density	1.24	1.34	1.39
Snow cover mean	2.07	4.05	2.61
Snow cover variance	3.01	5.38	3.14
Euclidean distance	11.03	18.30	18.76

Table 4.4 Model ranking based on the difference between Akaike's Information Criterion (AIC_C) of the top-ranked model and each subsequent model (ΔAIC_C). Akaike weights (w_i) are the probability that the given model is the best model. Models with $\Delta AIC_C > 4$ were not included.

Data Set	Model	AIC_c	ΔAIC_c	w_i
Both Sexes	Road density + snow mean + euclidean distance	-4788.31	-	0.63
	Road density + snow mean + snow variance + euclidean distance	-4786.61	1.7	0.27
Females	Road density + euclidean distance	-893.59	-	0.14
	Road density + snow mean + snow variance	-893.52	0.07	0.14
	Forest age + road density	-893.23	0.36	0.12
	Road density	-892.99	0.60	0.11
	Road density + snow mean + euclidean distance	-892.75	0.84	0.10
	Road density + snow variance	-892.15	1.44	0.07
	Forest age + road density + snow mean	-892.14	1.45	0.07
	Road density + snow mean + snow variance + euclidean distance	-891.87	1.72	0.06
	Road density + snow variance + euclidean distance	-891.54	2.05	0.05
	Forest age + road density + snow mean + snow variance	-891.52	2.07	0.05
	Road density + snow mean	-891.20	2.39	0.04
Forest age + road density + snow variance	-891.15	2.41	0.04	
Males	Snow variance	-1046.64	-	0.20
	Snow mean + snow variance	-1045.88	0.76	0.14
	Road density + snow variance	-1045.25	1.40	0.10
	Forest age + snow variance	-1045.03	1.61	0.09
	Snow variance + euclidean distance	-1044.68	1.96	0.08
	Road density + snow mean + snow variance	-1044.47	2.17	0.07
	Forest age + snow mean + +snow variance	-1044.36	2.29	0.07
	Snow mean + +snow variance + euclidean distance	-1044.09	2.55	0.06
	Forest age + road density + snow variance	-1043.38	3.26	0.04
	Road density + snow variance + euclidean distance	-1043.22	3.42	0.04
	Forest age + road density + snow mean + snow variance	-1042.66	3.98	0.03

Table 4.5 Model averaged coefficients and 95% confidence interval based on the selected model sets for each data set. Predictor variables inferred to have some predictive power are in bold.

Data Set	Predictor Variable	Coefficient Estimate	95% Confidence Interval	
			Lower	Upper
Both Sexes	Intercept	4.77E-01	4.60E-01	4.95E-01
	Road density	2.51E-03	1.63E-03	3.39E-03
	Snow cover mean	6.30E-04	3.01E-04	9.60E-04
	Euclidean distance	3.45E-02	1.78E-02	5.12E-02
	Snow cover variance	3.35E-05	-2.80E-04	5.08E-04
Females	Intercept	5.04E-01	4.64E-01	5.44E-01
	Road density	6.34E-03	3.49E-03	9.19E-03
	Euclidean distance	9.70E-03	-1.34E-02	6.84E-02
	Snow cover variance	2.20E-04	-5.90E-04	1.64E-03
	Snow cover mean	-3.05E-04	-1.75E-03	4.29E-04
	Forest age	8.40E-05	-2.50E-04	8.39E-04
Males	Intercept	4.69E-01	4.31E-01	5.06E-01
	Snow cover variance	1.14E-03	4.30E-04	1.84E-03
	Snow cover mean	1.82E-04	-3.30E-04	1.25E-03
	Road density	1.85E-04	-1.01E-03	2.23E-03
	Forest age	3.57E-05	-3.30E-04	6.20E-04
	Euclidean distance	1.07E-03	-3.08E-02	4.22E-02

Table 4.6 Mantel test between predicted resistance maps and genetic distance for the data sets.

Data Set	Mantel r	95% Confidence Interval		P
		Lower	Upper	
Both Sexes	0.18	0.13	0.22	< 0.001
Females	0.19	0.09	0.27	0.01
Males	0.17	0.07	0.24	0.01

Appendix 4.1 Grouping of the land cover variable into six categories.

Grouping	Cover type	Description*
Evergreen Forest	Evergreen Needleleaf Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 5 m. Almost all trees remain green all year. Canopy is never without green.
Woodland	Woodland	Lands with herbaceous or wood understories and tree canopy cover of >40% and <60%. Trees exceed 5m in height and can be either evergreen or deciduous.
Grassland-Shrubland	Wooded Grassland	Lands with herbaceous or woody understories and tree canopy cover of >10% and <40%. Trees exceed 5 m in height and can also be either evergreen or deciduous.
	Closed Shrubland	Lands dominated by bushes or shrubs. Bush and shrub percent canopy cover is >40%. Bushes do not exceed 5 m in height. Shrubs or bushes can be either evergreen or deciduous. Tree canopy cover is <10%. The remaining cover is either barren or herbaceous.
	Open Shrubland	Lands dominated by shrubs. Shrub canopy is >10% and <40%. Shrubs do not exceed 2 m in height and can be either evergreen or deciduous. The remaining cover is either barren or of annual herbaceous type.
	Grassland	Lands with continuous herbaceous cover and <10% tree or shrub canopy cover.
Mixed-Deciduous Forest	Mixed Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 5 m. Consists of tree communities with interspersed mixtures or mosaics of needleleaf and broadleaf forest types. Neither type has <25% or >75% landscape coverage.
	Deciduous Broadleaf Forest	Lands dominated by trees with a percent canopy cover >60% and height exceeding 5 m. Trees shed their leaves simultaneously in response to dry or cold seasons.
Anthropogenic	Cropland	Lands with >80% of the landscape covered in crop-producing fields. Note that the perennial woody crops will be classified as the appropriate forest or shrub land cover type.
	Bare Ground - Barren	Lands of exposed soil, sand, rocks, snow or ice which never have more than 10% vegetated cover during any time of the year.
	Urban and Built	Land covered by man-made structures.
Water	Water	Water

* Description based on University of Maryland vegetation class definitions as cited in Hansen et al. (2000).

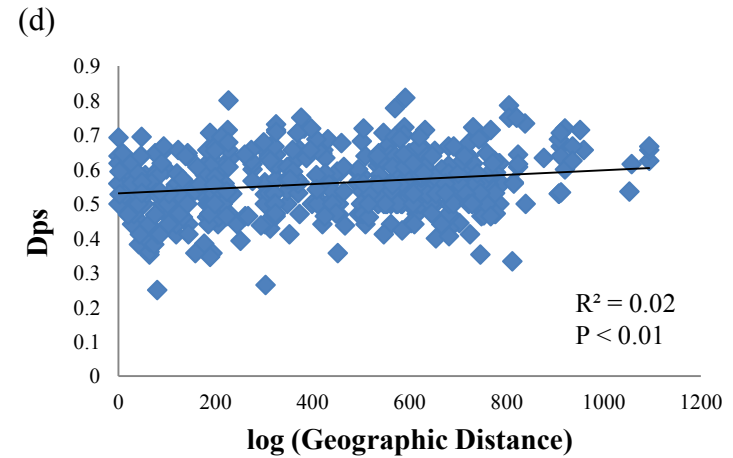
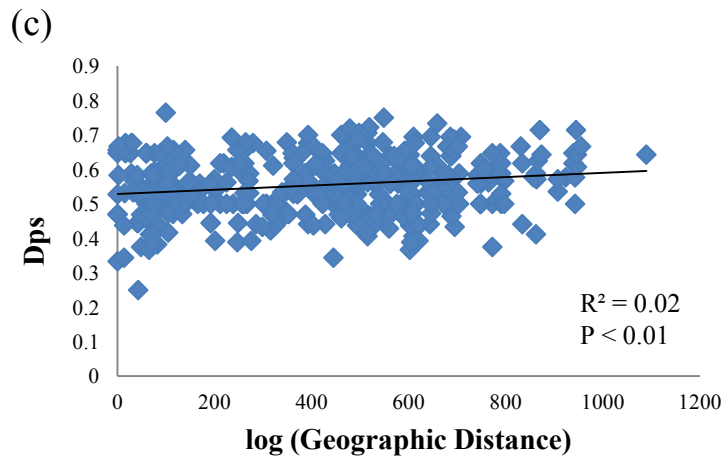
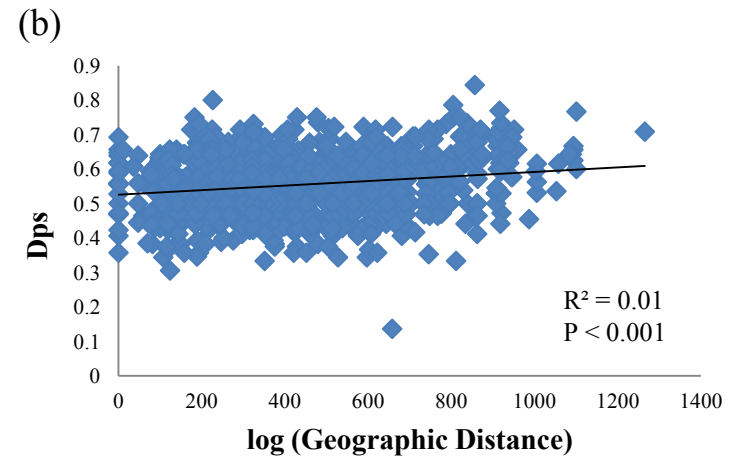
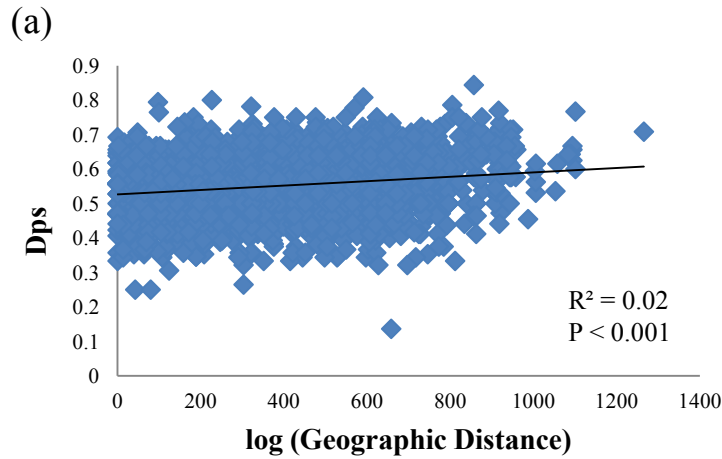
Appendix 4.2 Geographical location of samples analyzed in this study. Coordinates for Manitoba (MB) samples reflect trap line areas or fur house locations, while those for Ontario (ON) samples represent known GPS locations.

Sample Name	Latitude (decimal degrees)	Longitude (decimal degrees)
MB-WL4	54.626	-97.780
MB-WL5	55.588	-97.156
MB-WL6	55.588	-97.156
MB-WL8	56.467	-99.750
MB-WL9	54.948	-95.266
MB-WL11	55.743	-97.855
MB-WOLV4	54.133	-93.750
MB-WOLV7	56.467	-99.750
MB-WOLV9	55.742	-101.316
MB-WOLV12	54.948	-95.266
MB-WOLV14	54.768	-101.864
MB-WOLV15	56.467	-99.750
MB-WOLV21	54.842	-94.090
MB-WOLV23	56.245	-96.094
MB-WOLV30	55.853	-92.086
MB-WOLV34	55.742	-101.316
MB-WOLV37	55.827	-98.852
MB-WOLV39	55.588	-97.156
MB-WOLV45	55.588	-97.156
MB-WOLV48	53.990	-97.816
MB-WOLV54	53.990	-97.816
MB-08-02	56.891	-91.769
MB-08-04	57.854	-101.107
MB-08-06	55.029	-98.391
MB-08-07	56.466	-94.466
MB-08-08	56.466	-94.466
ON-960421	48.419	-90.432
ON-35	53.050	-93.333
ON-55	55.050	-89.167
ON-56	52.617	-94.083
ON-58	53.867	-92.167
ON-59	53.867	-92.167
ON-60	55.581	-87.556
ON-Ignace61	50.117	-90.833
ON-2800	51.117	-93.733
ON-802	53.867	-92.167
ON-803	51.068	-92.845
ON-804	51.196	-92.960

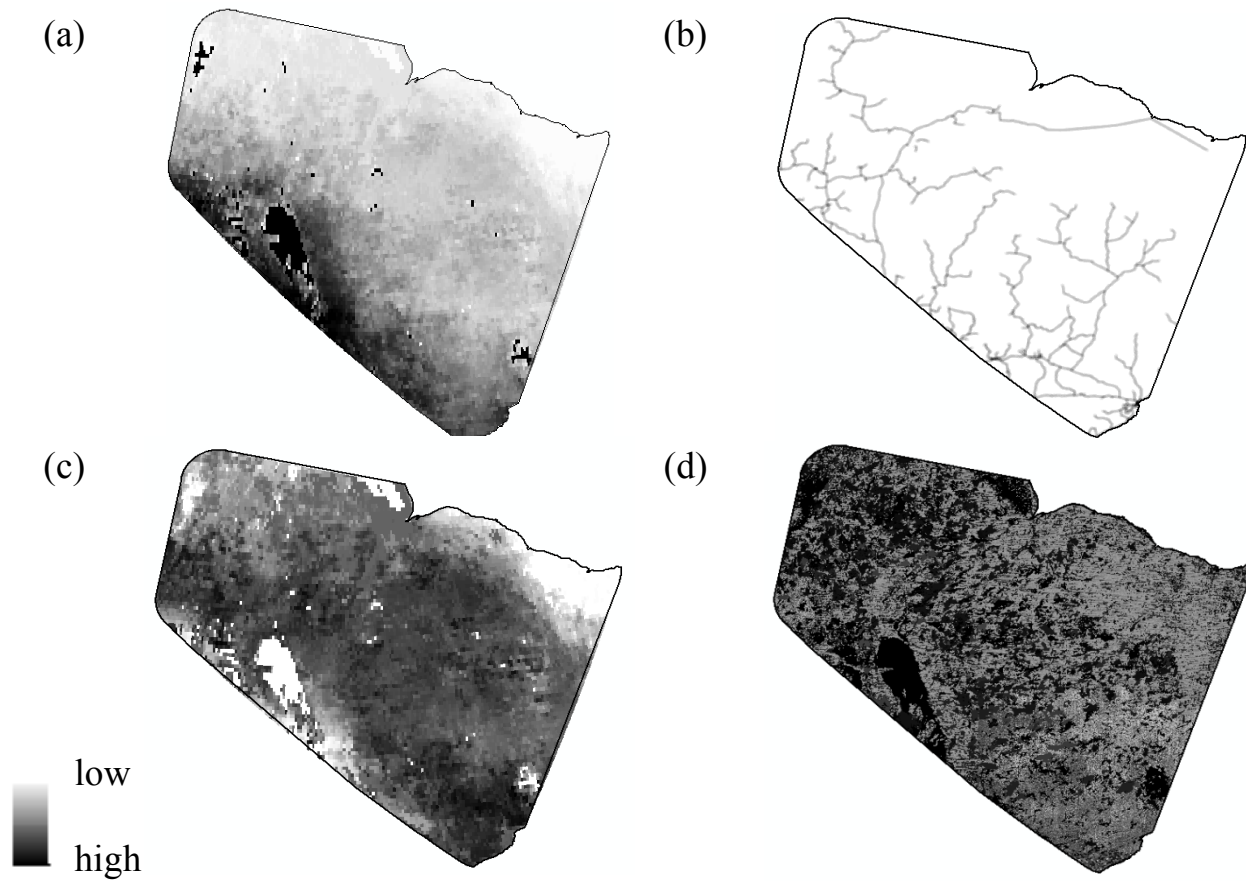
ON-806	51.432	-93.721
ON-808	51.105	-93.550
ON-810	51.213	-94.074
ON-812	51.190	-92.992
ON-814	51.182	-93.524
ON-816	51.196	-92.960
ON-833	51.334	-93.385
ON-839	50.669	-92.960
ON-870	50.984	-92.488
ON-873	50.984	-92.488
ON-874	55.050	-89.167
ON-dump	55.267	-85.342
ON-NWSI-007	52.296	-94.135
ON-NWSI-008	54.527	-88.353
ON-NWSI-009	55.581	-87.556
ON-NWSI-010	55.581	-87.556
ON-NWSI-013	49.740	-91.930
ON-NWSI-014	51.171	-93.908
ON-NWSI-015	50.660	-93.400
ON-NWSI-016	50.970	-92.470
ON-NWSI-017	50.970	-92.470
ON-NWSI-018	50.790	-94.160
ON-NWSI-019	50.660	-93.400
ON-NWSI-020	51.190	-93.920
ON-NWSI-021	51.190	-93.920
ON-NWSI-022	50.970	-92.470
ON-NWSI-023	50.855	-92.531
ON-NWSI-036	51.336	-90.670
ON-NWSI-038	52.071	-91.021

Appendix 4.3 Summary statistics of genetic distance measure (D_{PS}) for all four data sets.

Data Set	D_{PS}		
	Mean	Minimum	Maximum
Both Sexes	0.55	0.14	0.84
Both Sexes No Red Lake	0.55	0.14	0.84
Females	0.55	0.25	0.76
Males	0.56	0.25	0.81



Appendix 4.4 Isolation by distance (IBD) plots of geographic distance (log geographic distance in kilometers) and genetic distance (D_{ps}) for the four data sets: **a** both sexes; **b** both sexes no Red Lake; **c** females; **d** males.



Appendix 4.5 Map of landscape resistance for each of the four landscape variables: **a** spring snow cover mean; **b** road density; **c** spring snow cover variance; **d** forest age.

CHAPTER 5

SYNTHESIS

This thesis contributes insights into the spatial and temporal genetic connectivity patterns of wolverines (*Gulo gulo*). Neutral microsatellite markers and individual assignment tests revealed wolverines are genetically structured at the extant eastern periphery of their North American distribution, forming a separate genetic cluster that is characterized by high allelic diversity. The population genetic analyses were complemented by broad scale phylogeographic analyses of mtDNA control region that identified a single refugium near Beringia during the last glaciation, and the occurrence of historic population bottlenecks (400 generations ago) influencing wolverine genetic structure across their North American distribution. The application of an individual-based genetic distance measure in assessing the influence of landscape features on wolverine genetic structure allowed patterns of genetic connectivity to be investigated at a small spatial scale. Collectively, these studies link present-day patterns of genetic connectivity to natural and anthropogenic disturbances operating across multiple spatial and temporal scales. Understanding connectivity patterns in space and time is critical in conservation and management planning.

Contemporary and historical influences on genetic structure of wolverines

The majority of wolverine research to date in North America has occurred along the western region that encompasses the Rocky Mountain corridor. Wolverine densities are comparatively high for these northern, mountainous areas (e.g., Yukon: 5.64 - 10.75 wolverines/1000 km², Banci and Harestad 1990). Range contractions have also occurred along the eastern periphery of the wolverine's North American distribution, and although

information from this region is limited by sample size (Ontario $n = 8$, Frances 2008; Manitoba $n = 12$, Kyle and Strobeck 2002), it is suggestive of reduced gene flow with the panmictic northwestern continuum (Kyle and Strobeck 2002). In this study (Chapter 2), I relied on non-invasive hair snare surveys and opportunistic sampling from road kills and trapper harvests to increase sample size along the extant eastern periphery (Ontario $n = 44$; Manitoba $n = 18$) of the wolverine's North American distribution. Samples were added to existing data (Kyle and Strobeck 2002) and compared to adjacent regions (Saskatchewan, Northwest Territories and Nunavut) from the larger continuum of populations. This allowed me to examine connectivity between regions and to determine if the peripheral region harbours genetic variants not observed in the population core. Neutral microsatellite markers indicated that the extant Canadian population of wolverines is composed of two genetic clusters – a range core and an extant eastern peripheral population (Chapter 2), supporting previous findings of increasing genetic structure towards the eastern range edge (Kyle and Strobeck. 2002). However, due to the continuous distribution of wolverines across their northern range in North America (Kyle and Strobeck 2001), an alternative hypothesis is that the population genetic structure could reflect isolation by distance. Comparable values of genetic diversity were observed between the eastern periphery and core population (Chapter 2). This was unexpected given that peripheral populations occur at low densities and occupy ecologically marginal habitats (Brown 1984; Lesica and Allendorf 1995). Pronounced selection pressures at range peripheries due to frequent environmental fluctuations (Cassel-Lundhagen et al. 2009) may increase genetic variability of range edge populations, allowing population persistence under extreme climatic and biotic conditions (Brussard 1984; Parsons 1991). Hence, range edge populations can provide a source of genetic diversity that is distinct

from other populations in the species range (e.g., Lesica and Allendorf 1995; Vucetich and Waite 2003). These findings contribute to the growing body of evidence that persistence along historical range edges is more common than expected (e.g., Channell and Lomolino 2000; Antunes et al. 2006). Although increased genetic diversity was observed along the extant eastern range edge, this pattern was not reflected at the southwestern periphery - where gene flow was restricted over shorter distances and genetic diversity represented a fraction of the genetic diversity found in more northern populations (Cegelski et al. 2006; Kyle and Strobeck 2001, 2002). This pattern of genetic diversity at the southwestern periphery demonstrated what would be expected when animals disperse from the range centre into a new area, and what would have been predicted for a west to east colonization of North America by wolverines. This difference in genetic structure between peripheries may reflect different levels of disturbance (Banci 1994; Cegelski et al. 2006).

Phylogeographic analysis of the same samples (Chapter 2) supported the presence of an eastern peripheral cluster, suggesting this division may be rooted in longstanding historical processes. Furthermore, the two haplotypes occurring exclusively at the eastern periphery were highly divergent with each other, suggesting previous glacial cycles may have had a role in shaping present-day patterns of genetic diversity for wolverines in North America. In order to examine the influence of interglacial-glacial cycles of the Pleistocene, I expanded sampling across the wolverine's Holarctic distribution, and included historic samples from the extirpated population of Quebec-Labrador. All new samples ($n = 209$) were combined with existing data ($n = 774$) from previous studies (Wilson et al. 2000; Walker et al. 2001; Tomasik and Cook 2005; Cegelski et al. 2006; Arnason et al. 2007; Frances 2008; Zigouris et al. 2012). This comprehensive data set

(Chapter 3) allowed me to investigate the phylogeographic structure across this carnivore's Holarctic distribution (>5 million km²). Spatial analysis of molecular variance identified five main divisions in North America: the Central Arctic, a western region, Manitoba, California, and an eastern region of Ontario and Quebec/Labrador. This genetic structure of mtDNA control region reflects strong female philopatry and subsequent genetic drift (e.g., Wilson et al. 2000; Tomasik and Cook 2005; Schwartz et al. 2007).

Bayesian phylogenetic tree reconstruction supported a single expansion event originating near Beringia during the last glacial maximum in North America. This single expansion event likely reflected a single refugium rather than the initial entry point of wolverines into the Americas. Although wolverine fossils are not abundant in North America, two fossil records - one from Cumberland Cave, Maryland (Gidley and Gazin 1938) and the other from Port Kennedy, Pennsylvania (Cope 1899) date back to the Irvingtonian (1.8 million to 240,000 years before present). Using an Approximate Bayesian Computation (ABC) approach, I selected the hypothesis of a post-glacial west-to-east recolonization route from a single refugium (Chapter 3). This is in contrast to what I predicted based on findings of multiple refugia for other cold-adapted species (e.g., ermine *Mustela erminea*, Fleming and Cook 2002; woodland caribou *Rangifer tarandus caribou*, Klütsch et al. 2012). A similar phylogeographic study on wolverines (McKelvey et al. 2014) proposed a southern ice-free refugium in addition to Beringia. Historic samples in McKelvey et al. (2014) were largely from the southwestern range periphery, resulting in the extirpated California haplotype (Cali1) being observed in a localized region. Historic sampling from Quebec-Labrador (Chapter 3) identified the presence of Cali1 in northeastern North America, indicating this haplotype was more widely

distributed and a southern refugium may not have occurred. These contradicting results could reflect the low nucleotide diversity among wolverine sequences, due to the amplification of a 318 bp fragment of the mtDNA control region, which were unable to provide the resolution needed for making phylogenetic inferences.

Although phylogeographic analyses revealed a single glacial refugium for wolverines, contemporary spatial distribution of genetic variability reflects a more complex history of fluctuating population abundance since the last glaciation. The mismatch distribution (Chapter 3) suggested the occurrence of a historic bottleneck event approximately 400 generations ago (Excoffier and Schneider 1999). More recently, significant population declines during the 20th century as a result of fur harvesting (Slough 2007) and incidental poisoning from wolf control efforts (Lopez 1978) may have led to smaller, isolated populations and increased effects of genetic drift at the eastern periphery where densities have historically been considered low. Since the cessation of wolf bounties and overall decline in fur harvests (Slough 2007; McKelvey et al. 2011) about eight generations have elapsed, not enough time for wolverine densities to fully recover. Furthermore, harvest levels may be under-reported as most pelts used domestically are generally omitted from official counts (Committee on the Status of Endangered Wildlife in Canada, COSEWIC 2014). Assignment tests (Chapter 2) revealed a large proportion of individuals remained unassigned despite the indication of two genetic clusters. Anecdotal evidence suggests wolverine populations along the eastern periphery may be expanding (Dawson 2000). This short timescale for recovery, however, makes it difficult to determine if this expansion will result in increased gene flow between the two genetic clusters. Pleistocene refugia and population declines with associated effects of genetic drift are not the only mechanisms that may be contributing to

contemporary patterns of genetic structure and connectivity among and within populations of widely distributed species. Rueness et al. (2014) found Eurasian lynx (*Lynx lynx*) populations consisted of three phylogenetic clades with structuring occurring along an east-to-west gradient. While long-distance dispersal was evident (widespread haplotypes), gene flow of Eurasian lynx was restricted as there was only slight overlap among the three clades. Restricted gene flow of Eurasian lynx in the absence of physical barriers may reflect the influence of ecological features (Rueness et al. 2014).

Studies examining the potential effect of different landscape features on genetic distances have worked primarily with populations that are genetically differentiated. However, identifying landscape features influencing connectivity within a population prior to detecting genetic structure may limit potential loss of genetic variation. Using an individual-based genetic distance measure with 18 microsatellites, I investigated the influence of five landscape features (forest age, road density, land cover, spring snow cover mean and variance) on the genetic connectivity of wolverines at the extant periphery of their extant range in North America (Chapter 4). Road density and spring snow cover were associated with wolverine genetic distances (Chapter 4). One of the most direct affects of roads influencing not only wolverines but other carnivores is human persecution. Wolverines are actively harvested in Manitoba (Manitoba Wildlife Branch 2013), with kills likely being additive to natural mortality (Copeland 1996; Inman et al. 2007). Road density effects on the genetic variance of females may have a double effect on wolverine populations, affecting both female survival and reproductive success. Den establishment occurred in an area where human access points were avoided (Dawson et al. 2010), with abandonment occurring as a result of human disturbance (Copeland 1996). Recent aerial surveys suggest an eastward range expansion in Ontario (Ontario Wolverine

Recovery Team 2013). The association of road density to genetic distance suggests that the northward expansion of anthropogenic land-use activities have the potential to affect genetic connectivity.

I did not expect spring snow cover to be associated with genetic distance in this study (Chapter 4) given the low topographic relief for Manitoba and Ontario (500 m, Natural Resources Canada 2004). However, increased genetic distance was correlated to lower mean spring snow cover. The importance of spring snow cover in explaining patterns of genetic variability is consistent with similar observations at the southwestern range edge in the US Rockies (Schwartz et al. 2009). Decreasing snow cover will likely result in the reduction of snow depth, which could reduce den quality and in turn affect offspring mortality rates. Den establishment in deep snow provides a thermoregulatory advantage, increases the probability of den persistence till early spring, and discourages predation attempts, ensuring higher survival rates of offspring (Pulliainen 1968; Magoun and Copeland 1998). Additionally, dispersal of subadults and young-of-the-year occurs between January and May (Magoun 1985). Transient wolverines are expected to have higher mortality rates than residents as these dispersers have no familiar territory in which to hunt (Banci 1994). Snowy winters increase the availability of ungulate carcasses (e.g., Okarma et al. 1995), likely improving food availability for dispersers (Gese et al. 1996), particularly males who disperse farther and more frequent than females. Behavioural effects have also been suggested for snow cover conditions influencing genetic distances, where habitat imprinting on snow conditions has been suggested as a likely possible explanation for restricted dispersal by lynx (Row et al. 2014). This phenomenon may be applicable at a larger scale of connectivity, as an alternative explanation for low dispersal

events between the extant eastern periphery and the larger northwest continuum (Chapter 2).

This study (Chapter 4) revealed wolverine connectivity in Manitoba and Ontario is potentially threatened by the northward expansion of anthropogenic disturbance and ongoing climate change. Landscape genetics can provide important information regarding landscape features impeding species movement, which can also be used as a baseline for future monitoring and assessment. Landscape genetics can be especially useful for elusive, low-density species where traditional monitoring approaches such as radio-telemetry have not been successful in assessing species connectivity.

Conservation implications for wolverines in Ontario

Identifying designatable units is fundamental to conservation – especially for a species such as the wolverine, listed as endangered, threatened, or of special concern in parts of its range. Here, genetic analysis can be informative. Results from Chapter 3 – wolverines in North America originated from a single glacial refugium – support the recent grouping of wolverines into a single unit in Canada. The grouping of historic samples from Quebec-Labrador with the extant peripheral cluster of Manitoba and Ontario raised several questions regarding the classification of wolverines as COSEWIC Designatable Units (DUs). Based on COSEWIC guidelines, a population can become recognized as a DU if it contains attributes that make it both 'discrete' and 'evolutionarily significant' (COSEWIC 2012). Under the 'Discreteness' category, there is evidence of genetic discreteness for the extant eastern peripheral genetic cluster (Manitoba and Ontario) based on neutral genetic markers (DNA microsatellites, Chapter 2). Under the 'Significance' category, our finding of a single glacial refugium expansion during the last glacial maximum (Chapter 3) is not reflective of a deep intraspecific phylogenetic

divergence event (criteria 1), which precludes the identification of the extant eastern peripheral genetic cluster as a DU. However, criteria 2 – persistence in a unique ecological setting such that likely or known local adaptations may arise (COSEWIC 2012) – seems probable for this extant eastern peripheral population and possibly Quebec-Labrador.

In addition to identifying a distinct genetic cluster, Chapter 2 revealed the diversity displayed by extant eastern peripheral genetic cluster to be high (especially for Ontario), something that was not expected given that these animals represent an edge population and occur at low densities. This high genetic diversity may be reflective of different climatic and environmental conditions being experienced at the eastern periphery, where peripheral individuals may contain genotypes that can better respond to climate change or support dispersal to new areas outside the current species' distribution (Safriel et al. 1994). Genetic structure of wolverines from Manitoba and Ontario appears to be influenced by spring snow cover and road density (Chapter 4). Expanding landscape genetic analyses to encompass the genetically differentiated northwestern region will examine the potential influence of these two variables in explaining the presence to two genetic clusters across the wolverine's extant distribution in Canada.

Conclusions

In this thesis, I have shown that present-day patterns of wolverine genetic structure are shaped by multiple processes – Pleistocene glaciations, recent and historic bottleneck events, and present-day anthropogenic activities and climatic features. I also used a coalescent-based approximate Bayesian computation method to identify likely post-glacial recolonization pathways and applied an individual-based approach to identify landscape genetic relationships for a continuously distributed population. This

information provides added genetic data from the extant eastern periphery of the wolverines North American range, which was absent from previous studies. Similarly, phylogeographic analyses resulted in increased sampling across the wolverine's Holarctic distribution, including historic samples from the functionally extirpated region of Quebec-Labrador. These additional historic samples were important in helping to understand historic connectivity of wolverines following glacial retreat.

Additionally, the application of individual-based methods to examine within population connectivity helped to identifying potential landscape features that may over time influence genetic connectivity. Finally, this thesis illustrates the utility of non-invasive genetic approaches in sampling elusive, low-density species and providing information on population structure that can serve as a baseline for future monitoring and assessment.

To further develop the findings presented in this thesis, I recommend the following directions for future wolverine research: (1) investigate the genetic diversity of functional markers, particularly candidate genes potential involved in climate change adaptation; (2) phylogenetic analysis of the complete mitogenome, as it provides higher resolution in comparison to the control region; (3) utilize non-genetic data to inform resistance assignment, such as telemetry data or habitat use *vs.* resource availability information; and (4) expand landscape genetic study to encompass adjacent core region to identify landscapes features influencing genetic connectivity between the extant eastern periphery and core populations.

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