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RESEARCH ARTICLE

# Genetic isolation of wolverine (*Gulo gulo*) populations at the eastern periphery of their North American distribution

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**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 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, it remains unclear if similar patterns exist at this range edge. Using neutral microsatellite and mitochondrial DNA markers from a broad geographic extent (>2,500 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.

**Keywords** Microsatellites · Mitochondrial DNA · Genetic structure · Wolverine · Peripheral population · Conservation · *Gulo gulo*

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## Introduction

During a population decline, contraction of 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; Garner et al. 2004; Gaston

1990; Griffith et al. 1989; Lesica and Allendorf 1995). 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., Antunes et al. 2006; Channell and Lomolino 2000) 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 (Brito 2009; Kramer 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 (Dalén et al. 2005; Flagstad and Røed 2003). 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 subalpine zones of North America and Eurasia (Kvam et al. 1988; Ruggiero 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 (Schreiber et al. 1989; Wilson 1982). 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 (COSEWIC

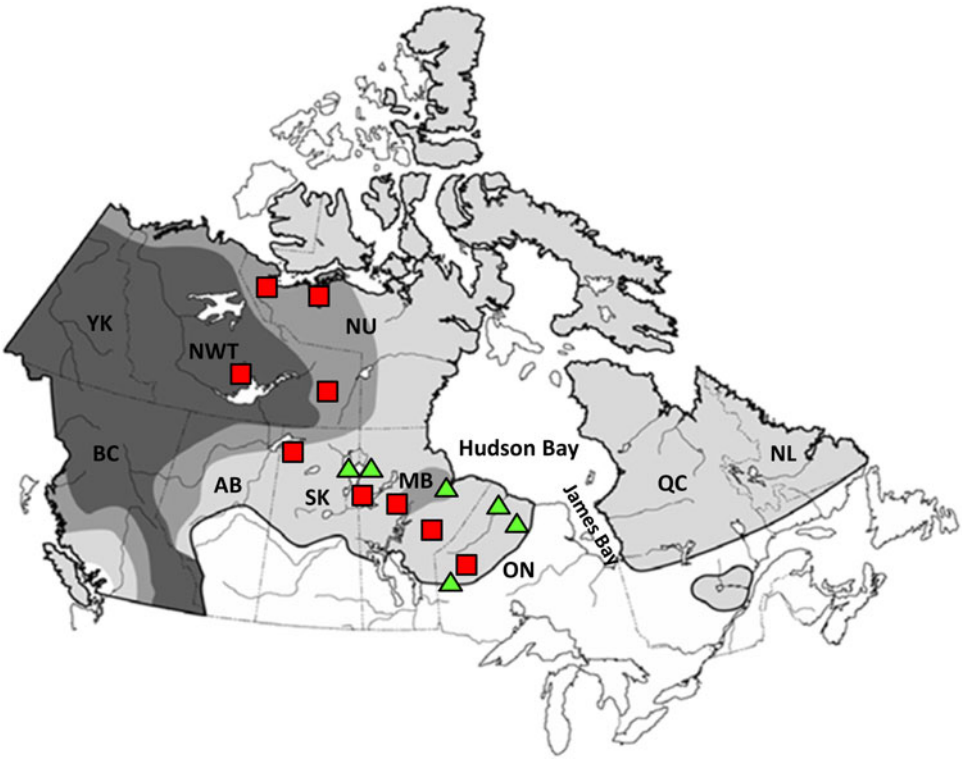


**Fig. 1** Map depicting contemporary distribution and density (low, *light grey*; medium, *grey*; high, *dark grey*, COSEWIC 2003) of *Gulo gulo* in Canada, and sampled localities of wolverines. *Red squares* represent sampled localities from Kyle and Strobeck (2001, 2002) and *green triangles* represent localities sampled in this study. (Color figure online)

2003; Dauphiné 1989), no sightings have been confirmed over the past 30 years (Fig. 1; Fortin et al. 2005). An extant population occurs west of James Bay (Fig. 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 in very low densities (0.3–6.2 wolverines/1,000 km$^2$, Lofroth and Krebs 2007), have extensive home ranges (100–1,400 km$^2$, Dawson et al. 2010; Magoun 1985), and are highly vagile (straight-line distance $\geq$300 km over a 20 month period, Gardner et al. 1986; Magoun 1985). High levels of gene flow among wolverine populations across northern regions of North America have been noted by several studies using nuclear DNA markers (Chappell et al. 2004; Kyle and Strobeck 2001, 2002; Wilson et al. 2000), with genetic structure progressively increasing towards the southern and eastern peripheries of their distribution (Cegelski et al. 2003, 2006; Kyle and Strobeck 2002). Conversely, investigations based on maternally inherited mitochondrial markers (Cegelski et al. 2006; Frances 2008; Schwartz et al. 2007; Tomasik and Cook 2005; Wilson et al. 2000) have reported genetic structure over relatively small geographic scales despite the long-range dispersal capacity of both males and females (Flagstad et al. 2004; Gardner et al. 1986; Magoun 1985). 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 (Cegelski et al. 2003, 2006; Magoun 1985; Schwartz et al. 2007; Tomasik and Cook 2005; Wilson et al. 2000).

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 (Chappell et al. 2004; Kyle and Strobeck 2001, 2002; Tomasik and Cook 2005; Wilson et al. 2000). 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 (Cegelski et al. 2006; Kyle and Strobeck 2001, 2002) represents only a subset of the genetic diversity found for more northern populations and 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 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 ON (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 (NWT, $n = 42$), Saskatchewan (SK, $n = 15$), Manitoba (MB, $n = 28$) and ON ($n = 12$), with new data from SK ($n = 1$), MB ($n = 9$) and ON ($n = 61$) (Fig. 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, ON, 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, gender identification, and microsatellite genotyping

Genomic DNA was isolated using a Qiagen DNeasy® Tissue Extraction Kit following the manufacturer's instructions. Gender 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, 1× Mg free PCR buffer, 2.5 mM $MgCl_2$, 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 s, 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 *Mustela vison* (Mvis-75, Flemming 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 as null allele(s) were found at this locus for ON animals. However, no evidence of null alleles 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 null alleles 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 gender 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 µl volumes containing 5–10 ng of DNA, 0.7 µM of each primer, 1× Mg free PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 mg/ml BSA, and 0.05 U/µl 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 Exo-Sap-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, Falush et al. 2003; Pritchard et al. 2000) 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 sampling localities 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 $\leq 1$ (Hubisz et al. 2009).

We used TESS 2.3 (Chen et al. 2007; François et al. 2006) 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 resampling 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

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 ($\pi$) 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 1989) and population growth (Fu's F, $P < 0.02$, 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 $\Phi_{ST}$ 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 were 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 NWT ($n = 42$)].

### Nuclear DNA diversity

Expected heterozygosity of sampling regions ranged from 63.5 % in SK to 68.3 % in ON (Table 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 dataset as a whole. This departure from HWE was not consistent across loci or sampled regions so all loci were retained in further analyses. LD was observed between Tt1 and Gg7 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 1).

**Table 1** Estimates of genetic diversity of 11 microsatellite loci for each region sampled and genetic cluster based on STRUCTURE (Pritchard et al. 2000) and TESS (Chen et al. 2007) analyses

| | $n$ | $H_O$ (%) | $H_E$ (%) | $A_R$ | $A_P$ |
|---|---|---|---|---|---|
| Sampling region | | | | | |
| NWT | 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, *NWT* Northwest Territories, *NU* Nunavut, *Core* SK, NWT and NU, *Periphery* ON and MB

### Nuclear genetic structure

The same two genetic clusters were identified by all three of our clustering methods (STRUCTURE, Fig. 2a; LOCPRIOR, Fig. 2b; and TESS, Fig. 2c). Cluster 1 consisted of SK, NU and the NWT, 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 (Fig. 2a). Additionally, all three threshold levels in STRUCTURE (Fig. 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

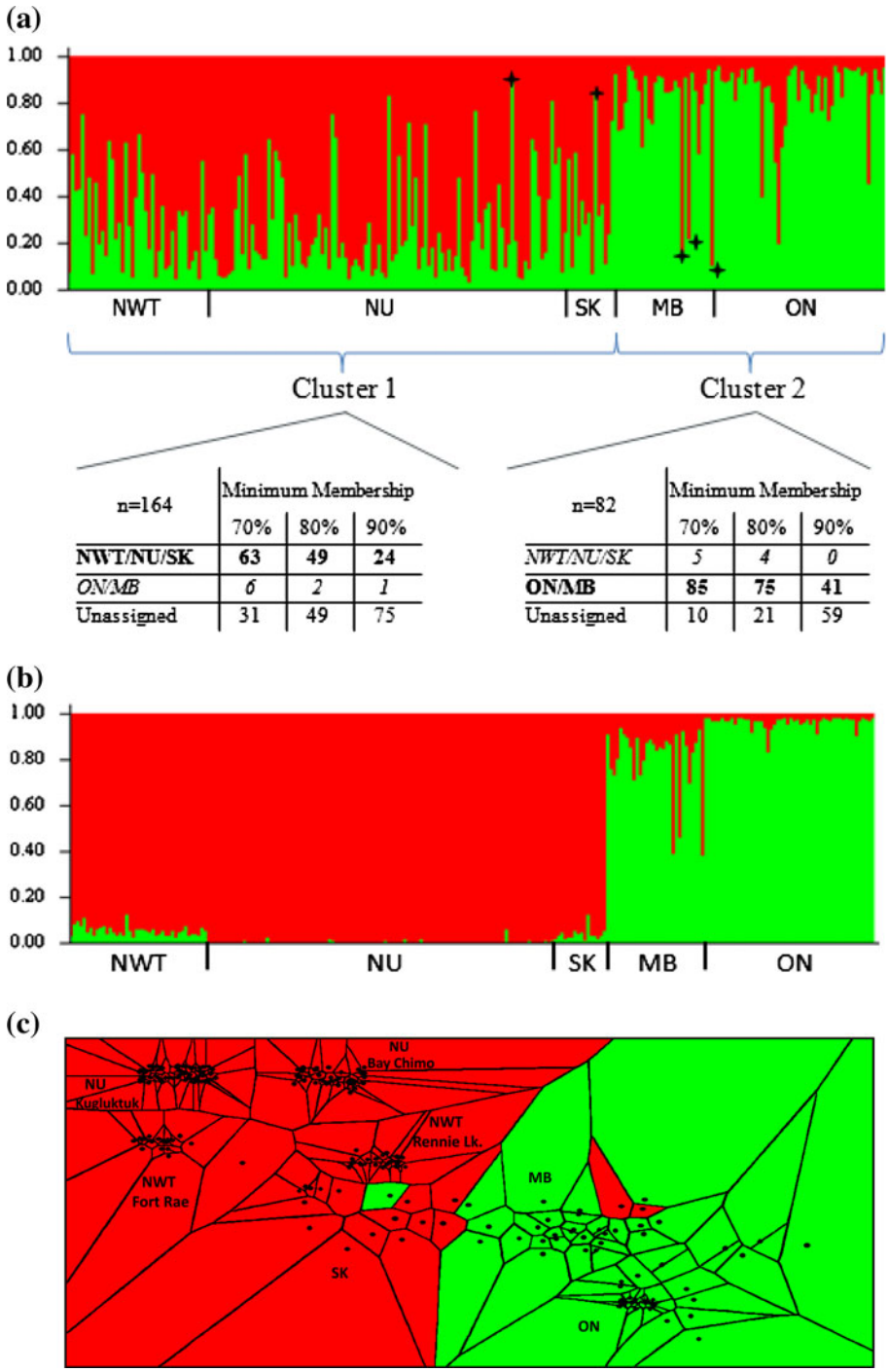


**Fig. 2** Individual based clustering results: **a** STRUCTURE (Pritchard et al. 2000) summary plot of the estimated membership coefficient (*x* axis) for each sampled region based on 11 microsatellite loci. Each individual is represented by a *single column broken* into *different colour segments*, 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 migrants. *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 $\psi = 0.6$. (Color figure online)

individuals for both Cluster 1 ($n = 1$) and Cluster 2 ($n = 3$) (Fig. 2c).

Although numerous samples were identified by STRUCTURE and TESS as being cross assigned, only five first generation migrants ($P \leq 0.01$) were identified by GeneClass2 (Fig. 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 migrants were all males sampled in MB, but identified as immigrants from Cluster 1 (SK, NU, and NWT). All five first generation migrants were identified as cross assigned individuals by STRUCTURE without the LOCPRIOR model. In comparison, TESS identified only one migrant for Cluster 1 and all three for Cluster 2 that were first generation migrants.

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

**Table 2** Pairwise estimates of population genetic distance for both nuclear ($D_{est}$, above diagonal) and mitochondrial ($\Phi_{ST}$, below diagonal) DNA among sampling regions

| | $D_{est}$ | | | | |
|---|---|---|---|---|---|
| | NWT | NU | SK | MB | ON |
| $\Phi_{ST}$ | | | | | |
| NWT | – | <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, *NWT* Northwest Territories, *NU* Nunavut

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

within each genetic cluster were compared ($D_{est} < 0.01$). When sampling regions were compared between clusters, $D_{est}$ values were moderately higher (0.04–0.08) except for the comparison between SK and MB (0.02, Table 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 3). All haplotypes were compared to those reported in previous studies (Cegelski et al. 2006; Chappell et al. 2004; Frances 2008; Tomasik and Cook 2005; Wilson et al. 2000). 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).

The most common haplotype (Hap1) comprised 29 % of all samples, and occurred predominately in ON (Fig. 3a). The next most frequent haplotypes were Hap7 and Hap25 (Table 3). Hap7 was primarily observed in NWT, and Hap25 only in MB and ON. Overall, NU, NWT, and SK were largely characterized by Hap3, Hap6, Hap7 and Hap8, while the eastern periphery consisted of Hap1, Hap24 and Hap25 (Fig. 3a). Hap24 was also observed in two individuals from the northwestern core, one from NWT 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 3).

The number of haplotypes in each sampling region ranged from three in MB and ON to six in NU, NWT 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 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 4). Adjusted total haplotype richness ($H_R$) was lowest for MB and ON, with SK having the greatest level (Table 4). Adjusted private haplotype richness analyses revealed NU having the highest number of private haplotypes and no private haplotypes were

**Table 3** Identified mtDNA haplotypes for each region sampled and genetic cluster based on STRUCTURE (Pritchard 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 | | NWT | 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 | • | • | • | Hap11 | 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 |
| | | | | | | | | | n | 28 | 28 | 16 | 30 | 54 | 72 | 84 |

Variable nucleotide positions are denoted according to their location within the 360 bp portion of the Dloop control region

*n* sample size

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

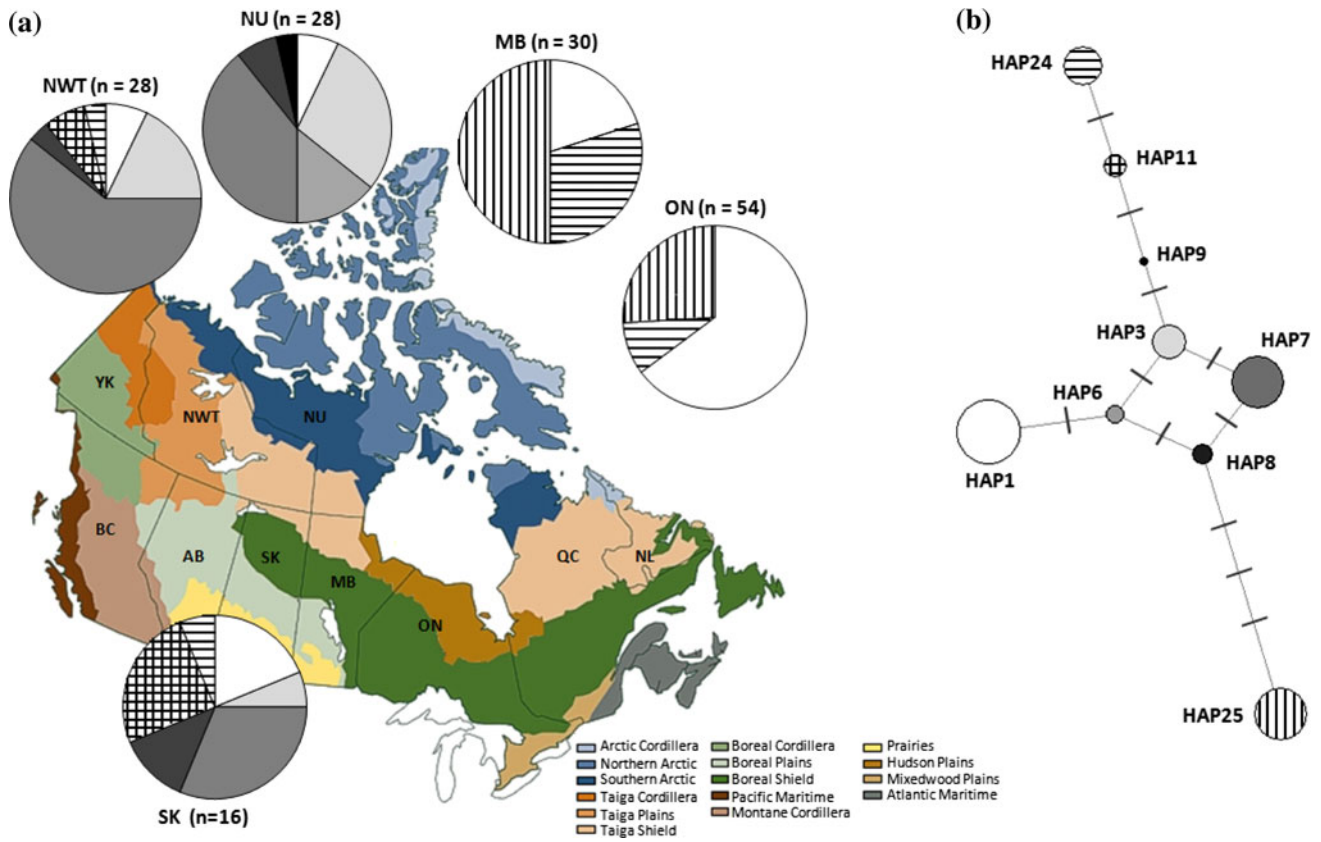


**Fig. 3** **a** Map of terrestrial ecozones in Canada (adapted from Ecological Stratification Working Group 1996, and Canadian Council of Forest Ministries 2006), and geographic distribution of the nine mtDNA control region haplotypes based on frequencies observed for each sampling region listed in Table 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. (Color figure online)

**Table 4** Genetic diversity measures of the five sampling region and two inferred genetic clusters based on STRUCTURE (Pritchard et al. 2000) and TESS (Chen et al. 2007) analyses

| | *n* | $\pi$ | SD $\pi$ | *h* | SD *h* | $H_R$ | $H_P$ | Tajima's D | *P* | Fu's F | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling region | | | | | | | | | | | |
| NWT | 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, $\pi$ 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 Fs (Fu 1997), *P P* value

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

observed for NWT, MB, and ON (0.00–1.55, Table 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, $\phi_{CT}$, 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 5). For $K = 3$, the additional partition subdivided the eastern periphery, while $K = 4$ further separated SK from the northwestern core group. High $\phi_{CT}$ but low $\phi_{SC}$ 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 $\phi_{SC}$ variance) and an increase in $\phi_{CT}$ (Dupanloup et al. 2002). Similar results were also obtained for AMOVA analysis.

**Table 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 | $\phi$ Statistics | $P$ |
|---|---|---|---|---|
| $K = 2$ (MB/ON vs. NWT/NU/SK) | | | | |
| 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 |
| $K = 3$ (MB vs. ON vs. NWT/NU/SK) | | | | |
| 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 |
| $K = 4$ (MB vs. ON vs. NWT/NU vs. SK) | | | | |
| 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 |

Pairwise $\Phi_{ST}$ estimates (Table 2, below diagonal) revealed that wolverines were not genetically differentiated among NWT, NU, and SK sampling regions, but that wolverines from these three regions were genetically differentiated from both MB and ON. Pairwise $\Phi_{ST}$ estimates also indicated that wolverines were genetically differentiated between the two peripheral sampling regions (Table 2), further supporting the genetic structure captured by SAMOVA. Analysis of the data based on nDNA genetic clusters produced a pairwise $\Phi_{ST}$ estimate of 0.26, indicating that the two clusters are genetically differentiated.

A median-joining network (Fig. 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), the conclusion that the extant wolverine population is composed of two genetic clusters was supported by assignment tests from both STRUCTURE (Fig. 2a) and TESS (Fig. 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 NWT are part of a large panmictic core population (Chappell et al. 2004; Kyle and Strobeck 2001, 2002; Wilson et al. 2000).

Significantly lower heterozygosity was observed for MB ($P = 0.03$, Table 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 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 (Flagstad et al. 2004; Gardner et al. 1986; Magoun 1985).

A comparison among the three assignment methods revealed an inconsistency in the number of putative migrants. 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 assigned 14 samples for the lowest membership threshold, which included the five individuals GeneClass2 detected as first generation migrants. In comparison, TESS only identified five cross assigned individuals, four of which were recognized by GeneClass2 as first generation migrants. This discordance among the different assignment tests for inferring putative migrants 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 migrants (Fig. 2a, stars) supports previous reports of inferred long distance movements by wolverines (Flagstad et al. 2004; Gardner et al. 1986; Magoun 1985). 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 4) but levels of allelic richness remained consistent across sampling regions (Table 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 $\Phi_{ST}$ estimates were greater for mitochondrial DNA in comparison to $D_{est}$ estimates for nuclear microsatellite data (Table 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 migrants' (Cegelski et al. 2006).

Despite identifying two genetic clusters, a large percentage of individuals remained unassigned (Fig. 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 (Flagstad et al. 2004; Gardner et al. 1986; Magoun 1985). Alternatively, wolverine populations have experienced significant declines as a result of fur harvests during the 20th century (Slough 2007), and the loss scavenging opportunities (van Dijk 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 1). Not enough time has elapsed, about eight generations, since wolf bounties ceased and fur harvests declined (McKelvey et al. 2010; Slough 2007) 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 (Fig. 3a)—may represent a spatial bottleneck, a narrow corridor limiting the movement of migrants. 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; Copeland et al. 2010; Schwartz et al. 2009). 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 (Cegelski et al. 2006; Chappell et al. 2004; Frances 2008; Schwartz et al. 2007; Tomasik and Cook 2005; Wilson et al. 2000). 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 center 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 at 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, which was restricted primarily to the periphery. This lack of gene flow between the eastern periphery and the northwestern core likely reflects a more longstanding separtaion 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 5) are in contrast to Frances (2008), who was unable to identify population groups as higher $K$ values resulted in the continuously increasing $\phi_{CT}$ 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 $K = 4$ (Fig. 3a). Although $K = 3$ had the highest $\phi_{CT}$ value, the more notable SAMOVA result is the partition observed for $K = 2$ (Table 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 (Fig. 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 (Dalén et al. 2005; Flagstad and Røed 2003), and possibly occupied high altitude 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 (Zigouris unpublished). Even though we did not observe any slightly deviating haplotypes centered 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 2010). 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 ON 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).

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## Appendix

See Table 6.

**Table 6** 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 |
| Hap11 | | | D | | Hap3 | AF55403 |
| Hap24 | | K | | | Hap23 | EU812449 |
| Hap25 | | J | | | Hap22 | EU812445 |

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