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Genetic diversity and population structure of wolverine (*Gulo gulo*) populations at the southern edge of their current distribution in North America with implications for genetic viability

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Abstract

The current range of wolverines (*Gulo gulo*) within the lower 48 states includes small, remnant populations in Idaho, Washington, Wyoming and Montana. The size and trend of each of these populations and connectivity to adjacent populations in the contiguous United States and Canada are poorly understood. In this study, levels of genetic diversity and population genetic structure were examined in three states (Idaho, Wyoming, and Montana) and two Canadian provinces (Alberta and British Columbia) using both mitochondrial (mtDNA) and nuclear microsatellite DNA. Restricted levels of gene flow were detected among these populations with mitochondrial and nuclear DNA and our observations suggest a pattern of male-mediated gene flow. Populations in the United States appear to be receiving migrants from Canada, however, substantial genetic differentiation suggests that gene flow may not be high enough to prevent genetic drift. Our analyses suggest that at least 400 breeding pairs or 1–2 effective migrants per generation would be needed to ensure genetic viability in the long-term for each of the populations in the United States. Significant matrilineal structuring and restricted female gene flow indicates that demographic viability will depend upon the movement of female wolverines into new territories. Results from this study provide guidelines for conservation and management and indicate the need for more ecological data.

Introduction

The wolverine (*Gulo gulo*) is one of the least studied species among large terrestrial carnivores due to its elusive nature, low density and large dispersal ability. The species is currently listed as ''vulnerable'' by the IUCN (Taylor 2000) and is especially sensitive to human disturbance and habitat destruction (Banci 1994). Wolverines have a circumpolar distribution, which historically included the tundra and taiga zones of Scandinavia and Germany to Northeastern Siberia, as well as North America (Hall 1981). In the past 150 years, the worldwide range of the species has been considerably reduced due to anthropogenic factors such as habitat destruction, exploitation for fur, and lethal predator control programs (Schreiber et al. 1989). Wolverines in the Canadian provinces and the state of Alaska may be the most stable and largest contiguous population of wolverines worldwide (Banci 1994). Currently, wolverines can be found in low numbers in the boreal forests of

Norway, Sweden, Finland, China, Russia, and the contiguous United States (Schreiber et al. 1989). In the contiguous United States, wolverine populations were once distributed across the northernmost tier of states and southward to New Mexico and Arizona (Hash 1987), but are currently patchily distributed in Idaho, Washington, Wyoming and Montana (Hash 1987; Banci 1994; Figure 1).

As a consequence of range contraction, a petition was filed in 1995 with the United States Fish and Wildlife Service (USFWS) to list the wolverine as threatened within the contiguous United States. The USFWS did not find sufficient evidence to list the wolverine as threatened (USFWS 1995). A second petition, filed in 2000, was also denied due to insufficient evidence of a change in status (USFWS 2003). Currently, each population in the contiguous United States is managed independently under state jurisdiction. Effective management and conservation for wolverines requires an understanding of the demographics and connectivity of these remnant populations to each other and to the larger core population of wolverines in Canada.

Field data regarding dispersal and connectivity of wolverine populations within the contiguous United States is limited to three published studies (Hornocker and Hash 1981; Copeland 1998; Edelmann and Copeland 1999). Difficulties in assessing ecological and demographic requirements of wolverines can be attributed to life history characteristics of the wolverine. It is a solitary species with a propensity to occupy large, remote, high elevation and high latitude habitats at low densities. Home ranges can extend from 100 to 900 km^2 (Magoun 1985; Banci 1987) and densities vary from one individual per 40–800 km^2 (Banci 1994). Daily movements average 30–40 km (Krott 1960; Haglund 1966; Pulliainen 1968; Banci 1994),

Figure 1. Sampling localities for eight wolverine populations in Southern Canada and Northwestern United States.

and although the propensity for long distance movement exists (Hornacker and Hash 1987), this does not necessarily imply gene flow as wolverines frequently make long exploratory movements prior to dispersal (Vangen et al. 2001).

Genetic analyses can provide an alternative and complimentary method for evaluating population health and demographic connectivity. Genetic diversity may be a useful indicator for identifying populations vulnerable to inbreeding, demographic stochasticity, and reduced viability. A loss in genetic diversity may lead to a decrease in fitness (Coltman et al. 1998; Westemeier et al. 1998; Madsen et al. 1999; Reed and Frankham 2003) although this relationship is frequently debated (Hedrick and Miller 1992; Amos and Balmford 2001; Reed and Frankham 2001). Gene flow between populations is also an important consideration because in small populations, it counters the forces of random genetic drift by increasing effective population size and minimizing possible stochastic (Frankham 1995; Storfer 1999) and inbreeding effects (Westemeier et al. 1998; Madsen et al. 1999). Evidence of reduced gene flow among populations can further identify populations of conservation and management concern.

Population genetic structure of wolverines has previously been evaluated in parts of North America, revealing varying levels of connectivity among adjacent populations. A mitochondrial DNA (mtDNA) study by Wilson et al. (2000) in the Northwest Territories of Canada found significant population differentiation among regions separated by 100 km. Female philopatry was proposed as a potential mechanism, although several females were observed to have moved significant distances. Kyle and Strobeck (2001, 2002) examined genetic diversity and population structure of wolverines from regions across much of their North American distribution using 12 microsatellite loci (nDNA). Genetic distance measures and diversity estimates suggested that the northern populations, from Alaska to Nunavat, Canada are connected through relatively high levels of gene flow and that the Idaho, Wyoming, and Revelstoke populations had become fragmented from northern populations. The differences in genetic structure proposed by Kyle and Strobeck (2001, 2002) and Wilson et al. (2000) prompted a re-evaluation of the genetic structure of wolverines in northern Canada by Chappell et al. (2004). This investigation suggested that there were differences in dispersal patterns between the two sexes (Chappell et al. 2004). In a nDNA evaluation of genetic structure of Montana wolverines, Cegelski et al. (2003) found a high degree of population subdivision and defined three subpopulations; while no such fragmentation had been observed across a similar spatial scale in Northern Canada (Kyle and Strobeck 2001, 2002).

This study provides an examination of genetic diversity and population genetic structure of wolverine populations at the southern extent of their North American range using 217 samples collected from populations in Montana, Idaho, Wyoming, central Alberta and southern and central British Columbia. Unlike previous studies which included these populations, we have combined both nuclear microsatellite and mitochondrial DNA data, enabling a finer-scale investigation of the degree of differentiation among adjacent populations. By combining datasets from previously published studies and adding mtDNA sequence data, we address: (1) the level of gene flow and connectivity among populations in southern Canada and adjacent wolverine populations in the contiguous United States, (2) the potential for sex-biased dispersal among populations and (3) the management implications for long-term persistence of these vulnerable populations at the southern edge of the wolverine's range.

Materials and methods

Sampling

Tissue and/or extracted DNA samples were obtained from the following localities: central Idaho (n = 15), Yellowstone and Grand Tetons National Parks, Wyoming (n = 13), Grande Cache, Alberta (n = 17), Williston Lake, British Columbia (n = 37), Revelstoke, British Columbia (n = 47) and three populations defined in Montana (n = 89, Cegelski et al. 2003): Rocky Mountain Front (n = 44), Gallatin (n = 26), Crazybelts (n = 19; Figure 1, Table 1). Microsatellite data for these populations were previously reported by Kyle and Strobeck (2001, 2002) and Cegelski et al. (2003) and we have sequenced the mtDNA control region of these samples for the current study.

Table 1. Number of mitochondrial DNA control region haplotypes in each wolverine population sampled in Canada and the United States along with total frequency of the haplotype in the dataset

							Haplotype	Frequency in each population								Total
111	*133*	*173*	*197*	*228*	*235*	*251*		RMF	CB	GA	ID	WY	WL	REV	GC	
C	A	–	C	A	G	C	A	40	4	20	15	10	11	4	5	65%
T	A	–	T	A	G	C	C	0	0	0	0	0	0	0	2	1%
C	A	–	T	A	G	C	F	0	0	0	0	0	0	0	2	1%
C	A	–	T	A	A	C	H	0	0	0	0	1	0	5	0	4%
T	A	C	T	A	G	C	I	4	13	5	0	2	0	0	0	14%
C	A	–	C	A	G	T	L*	0	2	0	0	0	6	4	8	12%
C	A	–	C	G	G	C	M*	0	0	0	0	0	1	0	0	< 1%
C	G	–	C	A	G	C	N**	0	0	0	0	0	1	0	0	< 1%
C	A	–	C	A	A	C	O*	0	0	0	0	0	0	3	0	2%
							Total	44	19	25	15	13	19	16	17	168
							h	0.17	0.50	0.33	N/a	0.41	0.59	0.79	0.71	
							π	0.002	0.006	0.004	0.0	0.004	0.002	0.006	0.004	

Abbreviations refer to the following populations: RMF = Rocky Mountain Front; CB = Crazybelts; GA = Gallatin, ID = Idaho; WY = Wyoming; WL = Williston Lake; GC = Grande Cache. *indicates haplotypes that were not previously identified; – denotes a deletion; **N = Tomasik and Cook 2005 (Haplotype B); *h* = haplotype diversity; π = nucleotide diversity.

DNA extraction and microsatellite analysis

DNA was extracted from a 1-mm piece of tissue using a modified Qiagen tissue protocol (Qiagen Co., USA). Buffer ATL was substituted with 1× Nucleic Acid Purified Lysis Buffer (Applied Biosystems, USA), which aids in the lysis of older tissue samples (Guglich et al. 1996). DNA was re-suspended in 400 μl of Buffer AE (Qiagen Co., USA).

Ten microsatellite loci were amplified: Lut604 (Dallas and Piertney 1998), Ggu101B, Ggu216, Ggu234 (Duffy et al. 1998) Gg3, Gg4, Gg7, Gg14 (Davis and Strobeck 1998), Tt-4 and Ma-3 (Davis and Strobeck 1998). Polymerase chain reaction (PCR) conditions are presented in Kyle and Strobeck (2001) and Cegelski et al. (2003).

Mitochondrial DNA sequencing

A short segment (300 bp) of the mitochondrial DNA (mtDNA) control region was amplified for a subset of samples in all of the populations, using the primers, L15926 and H16498 as described in Wilson et al. (2000). PCR products were purified using ExoSap (USB Co., USA) and Sephadex (Sigma Co., USA). Both heavy and light strands were cycle-sequenced using the PRISM DyeDeoxy Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Inc., USA) and loaded on an ABI 377. Sequences were analyzed using Sequencher 3.1 and manually aligned with PAUP 3.0 (Swafford 1998). Haplotypes observed only once were re-amplified and sequenced to verify accuracy.

Statistical analysis

Hardy–Weinberg and linkage disequilibrium

Each population was tested for Hardy–Weinberg equilibrium and linkage equilibrium using GENEPOP (available at http://www.wbiomed.curtin.edu.au/genepop/; Raymond and Rousset 1995). A sequential Bonferroni correction ($\alpha = 0.05$) was used to correct for multiple comparisons (Rice 1989).

Genetic diversity

Mitochondrial DNA diversity was evaluated using nucleotide diversity (π), and haplotype diversity values (*h*) for each population using ARLEQUIN 2.0 (Schneider et al. 2000). Nuclear diversity was measured by both mean number of alleles per locus (*A*) and Nei's unbiased expected heterozygosity (H_e; Nei 1978). A rarefaction option performed by FSTAT 2.9.3 (available at http://www.unil.ch/izea/softwares/fstat.html) was used to correct A

for unequal sample sizes. Corrected estimates of allelic diversity (R_t) were obtained based upon the smallest sample size of this study (n = 12). Statistical differences in H_e were evaluated using an arcsine log transformation and paired *t*-test (Archie 1985; Nei 1987).

Simulations were carried out using the program GENELOSS (England and Osler 2001) to determine the minimum number of breeders needed to maintain genetic variation (heterozygosity) in the United States populations. Genetic drift was simulated over 100 generations for each population using observed allele frequencies, no migration, and no mutation. The number of migrants (Nm) needed to maintain genetic diversity was also calculated using the following equation (Miller and Waits 2003):

$$H_{e(source)} = H_{e(sampled)} \times [4Nm/(4Nm+1)]$$

Population genetic structure

Female population structure was evaluated using mtDNA sequence data. Analysis of Molecular Variance (AMOVA) and pairwise F_{st} estimates were calculated using ARLEQUIN 2.0 (Schneider et al. 2000). A hierarchical AMOVA analysis (Excoffier et al. 1992) was performed to reflect how mtDNA is partitioned at different levels of a hierarchy. Two groups were *a priori* designated following geographic location (Canada, United States) to determine how genetic variation was partitioned between these two groups, among populations within these groups, and within populations.

A nested clade analysis (NCA) was used to gain a more complete depiction of the patterns and processes responsible for the observed mtDNA population structure (Templeton et al. 1992, 1995; Templeton and Sing 1993). NCA uses statistical tests based upon a minimum spanning network to explain the genetic structure of a population. Hypotheses such as fragmentation, isolation by distance effects, and range expansion can be simultaneously evaluated (Templeton et al. 1998). TCS 1.13 (Clement et al. 2000) was used to estimate the minimum spanning network using the algorithm of Templeton et al. (1992). The network and geographic coordinates were then entered into the program GeoDIS (Posada et al. 2000) to perform the cladistic analysis described in Templeton et al. (1992). Templeton's updated key (2004) was used to infer population processes (available at http://www.darwin.uvigo.es/software/geodis.html).

Nuclear genetic differentiation was measured by pairwise F_{st} estimates (Weir and Cockerham 1984) and an AMOVA analysis. The AMOVA analysis was conducted for the same hierarchical relationships as the mtDNA. The relationship between geographic distance and genetic distance (F_{st}) was further investigated with a Mantel test (Mantel 1967). Geographic distance was measured in kilometers for each pair of sampling locations. A regression of $F_{st}/(1-F_{st})$ on the logarithm of geographic distance for all population pairs was conducted with Genepop on the Web (Raymond and Rousset 1995). Genetic structure was also visualized using principle component analysis (PCA) of GENETIX (available at http://www.univ-montp2.fr/~genetix/genetix.htm) and a dendrogram generated with Cavalli-Sforza and Edward's (1967) chord distance using the program POPULATIONS (Langella et al. 2001).

Two Bayesian assignment tests (STRUCTURE; Pritchard et al. 2000 and GENECLASS; Paetkau et al. 2004) were also utilized to detect migration and the degree of reproductive discreetness among sampling locations. The Bayesian assignment test of STRUCTURE was used to assign individuals to clusters based upon multi-locus genotypes. Five independent runs for each starting number of populations ($K = 1$–10) were performed at 200,000 MCMC repetitions following 200,000 burn-in period using no prior information and assuming correlated allele frequencies and admixture. The posterior probability was then calculated for each value of K using the estimated log likelihood of K to choose the optimal K. Individuals were assigned to respective populations based upon percentage of membership (q). The Bayesian assignment test of GENECLASS was used to test each individual for its status as resident or recent immigrant using a Monte Carlo re-sampling method (Paetkau et al. 2004) of 10,000 simulated individuals. The likelihood ratio of drawing a genotype from the population of sampling origin (L_h) over the likelihood of observing the genotype in any of the sampled populations (L_{max}) was computed using the program GENECLASS and an alpha level of 0.01 was used for significance.

Results

Hardy–Weinberg and linkage equilibrium

Eighty exact tests for H–W equilibrium were performed, of which, nineteen were significant at the 0.05 level prior to a Bonferroni correction. None of the significant tests clustered around a specific population or locus and only one of the tests was rejected at the 0.05 level following a sequential Bonferroni correction. None of the tests for linkage equilibrium were significant at the 0.05 level with a sequential Bonferroni correction.

Mitochondrial DNA diversity

A 300 bp fragment of the mtDNA control region was sequenced for 168 individuals representing geographic areas in Montana, Idaho, Wyoming, British Columbia and Alberta. Seven variable sites including six transitions and one insertion–deletion identified nine haplotypes (A, C, F, H, I, L, M, N, O); Figure 2). All haplotypes were compared against those previously reported (Wilson et al. 2000; Chappell et al. 2004; Tomasik and Cook 2005) and haplotypes L, M, and O were not previously identified. Our haplotype N matched haplotype B reported by Tomasik and Cook (2005). Haplotype A was the most widespread haplotype, occurring in 53% of the samples. The next most frequent haplotypes, I and L, were found in 14% and 13% of the samples, respectively. Haplotypes C, F, H, M, N, and O accounted for 20% of the samples (Table 1). Haplotype diversity values (h) ranged from 0.00 in Idaho where only one haplotype was recorded to 0.79 in Revelstoke. Five haplotypes (C, F, M, N, and O) were identified in the Canadian populations only, and one haplotype (I) was seen only in the United States populations. Nucleotide diversity values (π) ranged from 0.001 to 0.006, indicating a close relationship among haplotypes (Table 1, Figure 2).

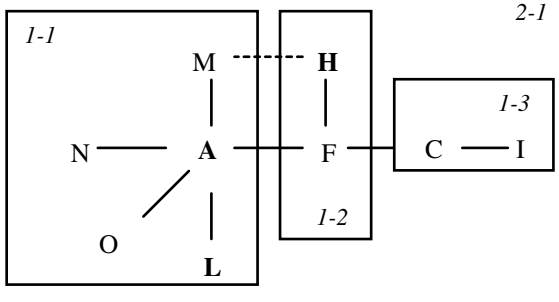

Figure 2. Haplotype network of the wolverine mitochondrial DNA haplotypes with TCS 1.13 (Clement et al. 2000). Each line represents a mutational change. Boxes are drawn around clades.

MtDNA population structure

A hierarchical AMOVA analysis was performed to detect population genetic structure in females. For the two defined groups (Canada, USA): 8% of the variation was detected among groups, 22.21% of the variation was partitioned among populations within groups, and 69.8% of the variation was partitioned within populations. Significant Φ_{st} values were found for United States populations paired with Alberta and for all populations paired with the Crazybelts population (Table 2). Furthermore, the Rocky Mountain Front population was significantly differentiated from the Williston Lake, BC population and the Wyoming population was differentiated from the Idaho population. The Idaho population was fixed for one haplotype, and was not significantly differentiated from the Rocky Mountain Front or Gallatin populations due to the prevalence of that haplotype in those populations.

The NCA revealed a network with three distinct clades. A significant geographical association of haplotypes was found for clade 1-1 ($P < 0.01$), clade 1-2 ($P = 0.035$) clade 1-3 ($P < 0.01$) and clade 2-1 ($P < 0.01$). The ancestral and most widespread haplotype found was haplotype A (Figure 2). Clade 1-1 included haplotype A which was found in high frequency in all of the populations; haplotype L which was found in high frequency in the Canadian populations and one population in Montana, and haplotypes N and O which were found only in Canada. Clade 1-1 revealed restricted gene flow with isolation by distance. Clade 1-2 provided an inconclusive outcome due to sampling two haplotypes in only eight individuals but the limited distribution of each haplotype may also indicate limited gene flow among the sampled populations within and between regions. Clade 1-3 provided evidence for restricted gene flow and dispersal with some dispersal among the populations in the contiguous United States.

Table 2. Levels of genetic differentiation measured by Φ_{st} for mitochondrial DNA data (below) and F_{st} for microsatellite data (above) calculated using ARLEQUIN (Schneider et al. 2000)

	RMF	WY	CB	GA	ID	GC	WL	RE
RMF		0.008	0.105*	0.084*	0.124*	0.090*	0.064*	0.055*
WY	0.002		0.036*	0.067*	0.136*	0.075*	0.053*	0.034*
CB	0.558*	0.334*		0.095*	0.213*	0.126*	0.120*	0.078*
GA	0.020	−0.050	0.345*		0.192*	0.156*	0.173*	0.120*
ID	0.019	0.121	0.607*	0.120		0.200*	0.112*	0.142*
GC	0.248*	0.136*	0.434*	0.171*	0.295*		0.044*	0.053*
WL	0.164*	0.151*	0.555*	0.177*	0.181*	0.052		0.037*
RE	0.220*	0.072	0.415*	0.152*	0.219*	0.078	0.114*	

Population abbreviations follow Table 1.
*Indicates significance at the 0.05 level.

Haplotype I was common to the populations in the contiguous U.S. (in unequal frequencies) but not Canada and haploytpe C was only found in Canada. Clade 2-1 revealed restricted gene flow/dispersal with occasional long distance dispersal.

Nuclear DNA diversity

Average heterozygosity levels ranged from 41.1% in Idaho to 62.8% in the Williston Lake population (Table 3). Allelic richness (R_t) measured 2.9 alleles/locus in Crazybelts to 4.3 alleles/locus in Grande Cache, Alberta (Table 3). A Wilcoxon-sign rank test revealed that both the Gallatin and Crazybelt populations in Montana had significantly lower allelic richness than all other populations sampled in the United States and Canada ($P < 0.05$). Heterozygosity levels for the Rocky Mountain Front population were not significantly different than the Wyoming population, Revelstoke population, or Canadian populations (Revelstoke, Williston Lake, Grande Cache; $P > 0.16$–0.84). Heterozygosity levels of the Gallatin, Crazybelts, and Idaho populations also were not statistically different from each other ($P > 0.80$). However, the Rocky Mountain Front population, Wyoming population, and populations in Canada exhibited significantly higher heterozygosities than the other populations in Montana and Idaho ($P < 0.05$).

Table 3. Estimates of genetic diversity for wolverine populations at 10 microsatellite loci based upon mean number of alleles per locus (A), allelic richness (R_t), mean expected heterozygosity (H_e; Nei 1978) and mean observed heterozygosity (H_o) per population

	n	A	R_t	H_e (%)	H_o (%)
United States					
Rocky Mountain Front	44	4.6	3.6	56.7	50.2
Crazybelts	19	3.4	2.9	48.8	41.5
Gallatin	26	3.7	3.0	42.2	40.0
Wyoming	12	3.6	3.6	56.3	55.4
Idaho	15	3.2	3.2	41.1	43.6
Canada					
Revelstoke, BC	47	5.1	4.0	54.7	60.2
Williston Lake, BC	37	4.9	4.3	62.8	56.6
Grande Cache, Alberta	17	4.7	4.1	59.8	52.9

Nuclear DNA population structure

F_{st} values were significantly greater than zero for all pairwise comparisons except those involving Wyoming (Table 2), which had the lowest F_{st} values for each comparison (Table 2). The Idaho population had the highest F_{st} values for all pairwise comparisons. The Mantel test indicated that there was no relationship between genetic distance and geographic distance ($r^2 = 0.06$; $P = 0.11$; Figure 3). A distinct break was identified between the U.S. populations and Canadian populations by both the dendrogram (Figure 4) and PCA analyses (Figure 5). Population scores for the PCA were plotted on two principle axes (PC1, PC2), which cumulatively explained 52% of the genetic variation. The PCA plot and dendrogram both showed that all of the Canadian populations clustered together; the Rocky Mountain Front population clustered with the Wyoming population, the Crazybelts population clustered with the Gallatin population, and the Idaho population was highly differentiated. The AMOVA analysis partitioned

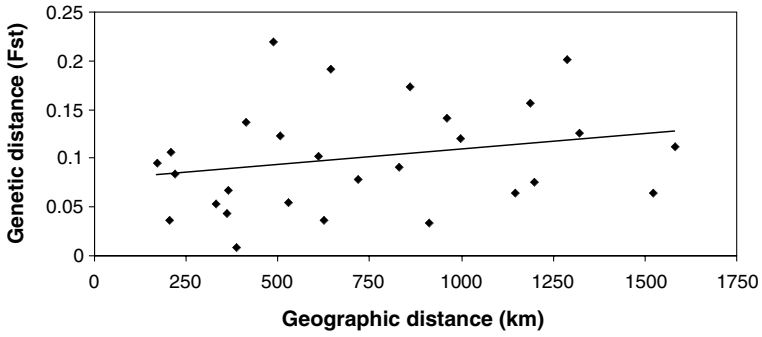

Figure 3. Relationship between genetic distance (F_{st}) and geographic distance (km) in southern wolverine populations in North America.

3% of the variance among groups, 7% among populations within groups and 90% within populations.

The Bayesian assignment test of STRUCTURE was used to identify the number of population clusters and simultaneously assign individuals to each of the identified clusters. Seven population clusters were identified without any prior population information (Table 4). The highest proportion of membership for the Revelstoke population was found in cluster 1 (Table 5). Cluster 2 mainly consisted of individuals from the Alberta population. Cluster 3 mainly consisted of Idaho samples. In cluster 4, the highest proportion of membership was from Williston Lake. Clusters 5, 6, and 7 represent the Crazybelts, Gallatin, and

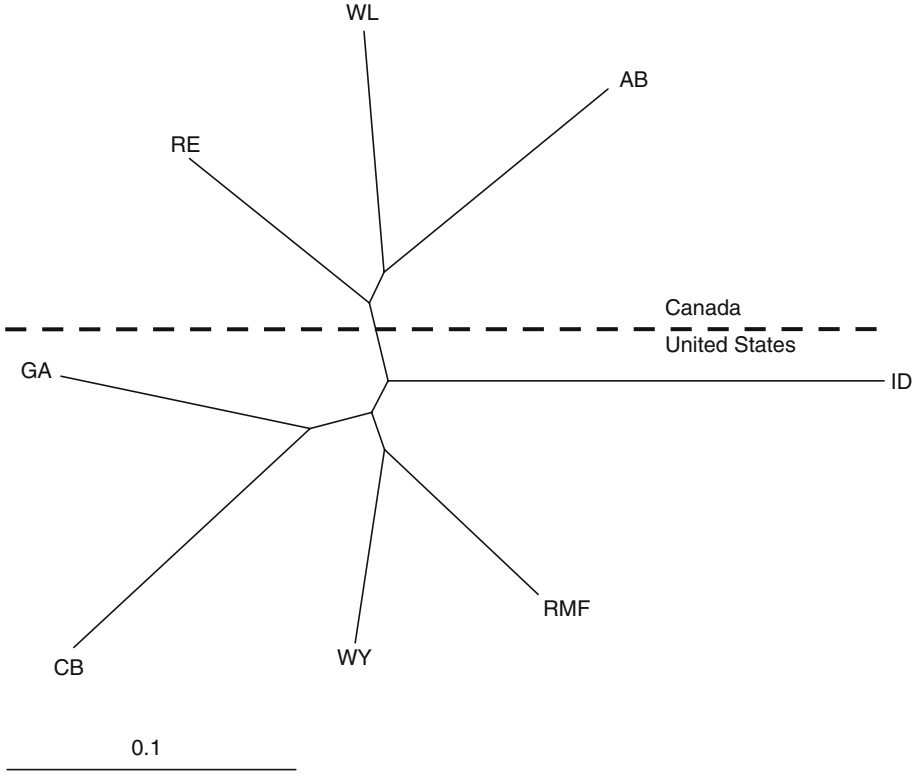

Figure 4. Neighbor-joining dendrogram of the genetic relationships among eight wolverine populations inferred from a matrix of Cavalli-Sforza and Edwards chord distance using 10 microsatellite loci.

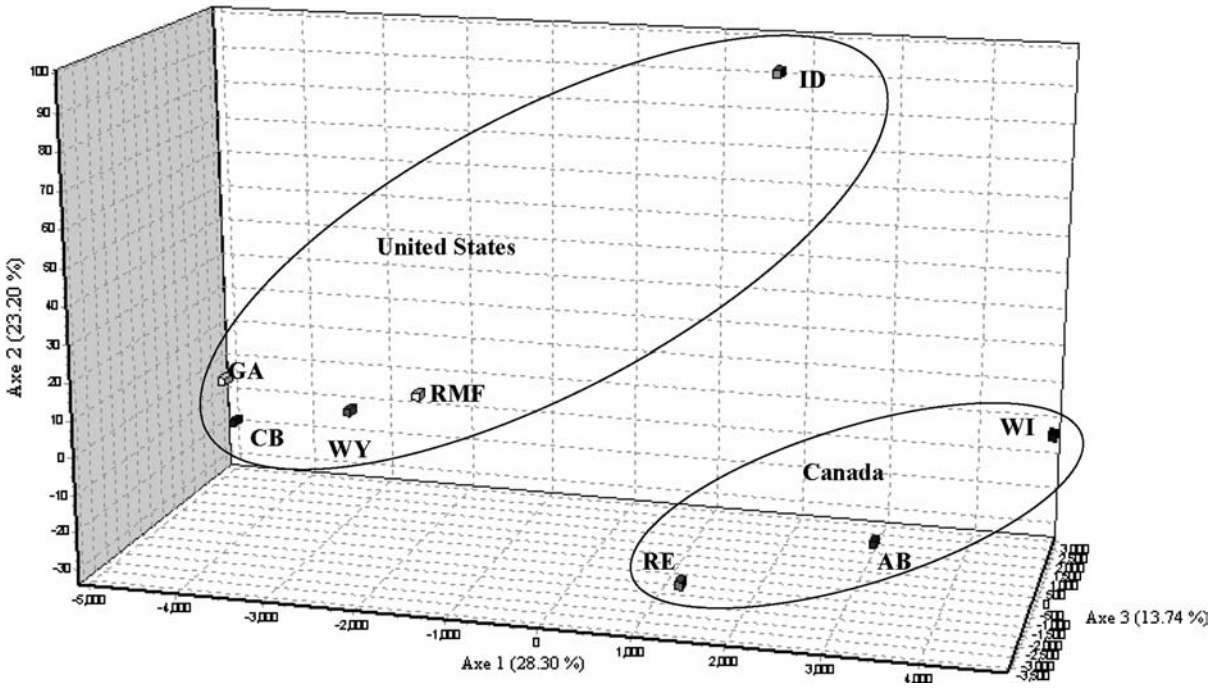

Figure 5. Principle components analysis (PCA) scores of wolverine microsatellite genotypes plotted on the two first axes (PC1, PC2) of a PCA using GENETIX.

Rocky Mountain Front populations, respectively. There was no cluster assigned to the population in Wyoming. The proportion of individuals that were self-assigned to a cluster appeared to be correlated with levels of genetic variability. A regression analysis indicated that self-assignment increased by decreasing levels of expected heterozygosity ($R^2 = 0.73$; $P = 0.014$).

The Bayesian analysis of STRUCTURE was not used to detect migrants since the posterior probabilities for all of the individuals in the sampled populations was low ($q < 0.90$). Therefore the Bayesian assignment test of GENECLASS was used to identify recent migrants. The Bayesian analysis of Geneclass identified nine migrants in total with high stringency ($P = 0.01$; Table 5b). For the United States populations, three males immigrated to the Rocky Mountain Front population; two females immigrated to the Crazybelts population; one male and one female immigrated to the Gallatin population; one male immigrated to the Wyoming population; and one male immigrated to the Idaho population. Two individuals (1 male, 1 female) were migrants from Canada to the US, but no migration was detected from US to Canada or between the Canadian populations.

Discussion

Population genetic structure

Wolverines are believed to have expanded southward following the last glaciation event from a single refugia in Beringia (Bryant 1987; Tomasik and Cook 2005). Our mitochondrial data are consistent with this hypothesis. Haplotype A was the center of the star phylogeny and most widespread haplotype while the limited distribution of recently derived haplotypes suggest that these haploytypes arose more recently and have not become widespread due to restricted gene flow. Haplotype A was also the most widely distributed haplotype in previous studies of Alaska and northern Canada (Chappell et al. 2004; Tomasik and Cook 2005). Five of the haplotypes were only present in the Canadian populations while there was some overlap in the geographic distribution of haplotypes H and L among populations in both the United States and Canada. Substantial frequency differences and lack of geographic overlap among the majority of the haplotypes within populations in each region and between regions support limited contemporary gene flow. The

NCA provided additional support that the association of haplotype distribution with geography was due to restricted gene flow with some long distance dispersal.

Nested clade analysis has been criticized in recent literature for its inability to distinguish the correct history of a species using simulation data (Knowles and Maddison 2002). While a new key has been developed to resolve most of the previous criticism (Templeton 2004), the main criticism of the approach was errors with range expansion inferences. Since the NCA was able to accurately infer fragmentation events with simulation data (Templeton 2004), there is increased support that NCA is accurately depicting population processes for our dataset. Our F_{st} estimates using mtDNA data also substantiated the NCA results, with high degrees of population differentiation observed among the sampled populations.

The amount of genetic differentiation was larger for mitochondrial DNA compared to the nuclear DNA analyses. In the AMOVA analyses, the nDNA Φ_{st} value was 0.09 compared to the mtDNA Φ_{st} value of 0.22. Population pairwise F_{st} estimates were also higher for mtDNA compared to nDNA. This could be a reflection of historical founding events and sex-biased dispersal. While the nDNA is representative of both female and male-mediated gene flow, the mtDNA only reveals female-mediated gene flow. Female wolverines are generally philopatric while males are the predominant dispersers (Banci 1994; Vangen et al. 2001), but occasional long-distance dispersal has been documented for female wolverines in ecological studies (Vangen et al. 2001). The NCA revealed restricted gene flow and dispersal with occasional long-distance dispersal. These data supports the findings of Chappell et al. (2004), which detected a similar pattern of mtDNA and nDNA structuring in Canada and concluded that male-mediated gene flow was predominant. The microsatellite data also indicated that while female migration was occurring, males are the predominant dispersers. Sixty-six percent of the migrants detected by the Bayesian assignment test were males and a previous analysis of Montana wolverines documented differences in male and female gene flow using F_{st} and q values (Cegelski et al. 2003). Therefore, our data indicate that there is limited contemporary female-mediated gene flow among the sampled populations and significant matrilineal structuring.

Table 4. Bayesian clustering results of STRUCTURE, without using any prior population information

K	Log P (k/x)	Variance Log P (k/x)
1	−5326	27.8
2	−5135	173.3
3	−4998	228.3
4	−4891	286.7
5	−4843	366.5
6	−4750	408.8
7	**−4714**	**493.8**
8	−4821	754.8
9	−4879	871.2
10	−4859	978.2

K represents the number of subpopulations. The value in bold indicates the most likely value for K.

Although the level of genetic differentiation is much lower for microsatellite DNA, there is still strong evidence for restricted gene flow among the sampled populations. Earlier nDNA studies of gene flow among populations of wolverines in northern North America have documented high gene flow and connectivity of populations across thousands of kilometers (Kyle and Strobeck 2001, 2002; Chappell et al. 2004). In an evaluation of wolverine population structure in northern Canada across over 4000 km from the Northwest Territories to Manatoba, Chappell et al. (2004) documented a global F_{st} of 0.0004 and only a single population unit using STRUCTURE. In contrast, we observed an optimal K value of 7 and a global F_{st} of 0.08 across a much smaller spatial scale. Earlier research has documented this increase in population structure at the southern extent of the range (Kyle and Strobeck 2001, 2002; Cegelski et al. 2003); however, these studies were not able to address the connectivity of wolverines in Idaho or Montana to each other or to adjacent populations in Canada.

This study places all of the sampled populations into context by analyzing them with adjacent populations in the region to determine the extent of gene flow. Our findings suggest that the Idaho population is isolated from neighboring populations in Montana as well as all other populations. The Idaho population had the lowest diversity levels and largest proportion of membership in a single cluster (92%), indicating significant isolation. Only one individual was detected as a disperser from the Rocky Mountain Front

population into Idaho and this individual was killed while crossing a highway (Copeland, personal communication). In Montana, the greatest concentration of wolverines resides within the Rocky Mountain Front population. This population is also the most geographically proximate population to Canada. The Rocky Mountain Front population had diversity levels comparable to Canada and the assignment test of GENECLASS suggested that this population had received one recent migrant from the Canadian populations. However, STRUCTURE did not detect a signal of admixture among any of the Canadian populations and the Rocky Mountain Front population that would result from ongoing migration. Other immigrants, from the Gallatin population, were detected in this population. Therefore, this population appears to be receiving migrants from the other sampled populations. The Gallatin and Crazybelt populations also appear to be exchanging some migrants, although they are more genetically differentiated and isolated from the other sampled populations compared to the Rocky Mountain Front population (Cegelski et al. 2003).

The population in Wyoming is also geographically proximate to those in Idaho and Montana. The Wyoming samples were collected in Yellowstone and Grand Teton National Parks which comprise over 900,000 ha of protected land. Minimal differentiation detected between the Rocky Mountain Front and Wyoming populations compared to the Gallatin population suggests that wolverines may be moving through the Gallatin range without establishing residency or finding alternate routes into these protected areas. The lack of differentiation and assignment of all individuals from Wyoming to other population clusters

Table 5. Assignment test results using 10 microsatellite loci. (a) Bayesian assignment test for $K=7$ without using any prior population information (Pritchard et al. 2000). Columns indicate the clusters and each row indicates the proportion of individuals from each population assigned to a cluster. The highest proportion of membership is indicated in bold. Population abbreviations follow Table 1. (b) Bayesian assignment test (Paetkau et al. 2004). Number of migrants identified in each population ($P=0.01$) and population of origin followed by the sex of each identified migrant

	N	Clusters						
		1	2	3	4	5	6	7
(a)								
RMF	44	0.09	0.09	0.09	0.05	0.06	0.12	**0.47**
CB	19	0.02	0.02	0.02	0.05	**0.68**	0.17	0.03
GA	26	0.02	0.05	0.05	0.02	0.10	**0.70**	0.05
ID	15	0.02	0.01	**0.85**	0.02	0.01	0.03	0.06
WY	12	0.04	0.04	0.09	0.03	0.25	0.19	0.34
RE	47	**0.42**	0.14	0.04	0.22	0.03	0.06	0.06
WL	37	0.12	0.23	0.08	**0.47**	0.02	0.03	0.04
GC	17	0.08	**0.56**	0.03	0.25	0.02	0.03	0.03

(b)		
Populations	# Immigrants (0.01)	Sex of immigrants
Rocky Mountain Front		
From Grande Cache	1	Male
From Gallatin	2	Male, male
Crazybelts		
From Gallatin	2	Female, female
Gallatin		
From Wyoming	1	Male
From Grande Cache	1	Female
Idaho		
From Rocky Mountain Front	1	Male
Wyoming		
From Crazybelts	1	Male

may be indicative of a recent colonization of Wyoming from the Rocky Mountain Front, while the Crazybelts and Gallatin populations are not as recently established and more differentiated. This result is puzzling and additional field research is underway to understand this pattern (Inman et al. 2003). Although pairwise F_{st} estimates among the populations in Canada and Wyoming were equivalent or lower than comparisons between U.S. populations and Wyoming, the assignment test did not detect any migrants or signatures of admixture between Canada and Wyoming. The lack of a genetic signature could also be an artifact of a prior introduction of wolverines or accidental release of wolverines into Wyoming as hypothesized by Murray (1987) and Kyle and Strobeck (2001), although there is no documentation to support this idea. Additional samples from Wyoming are needed to address this anomaly.

The United States populations do not appear to be sources for dispersing individuals into Canada and the Canadian populations sampled in this study were also highly differentiated from one another. Our data indicates that significant differentiation has resulted between most of the populations in Canada and the United States despite evidence of some migration. The F_{st} estimates, dendrogram, and PCA results indicated a distinct break between populations in Canada and the United States and significant differentiation between the Crazybelts, Gallatin and Idaho population compared to the Wyoming and Rocky Mountain Front populations. The assignment test can detect dispersers but cannot detect "effective migrants", therefore, some caution should be used when using assignment tests to detect migrants. Furthermore, the detection of dispersers is dependent upon sample size and may not be easily converted into an effective migration rate in our case, since wolverines are difficult to capture and our samples from Montana were collected across 20 years. With these cautions in mind, our data indicated that some migration is occurring between populations in Canada and the Rocky Mountain Front and among populations in the United States (excluding Idaho). However, substantial allele frequency differences suggest that the number of migrants may not be large enough to counter genetic drift and indicates that migration may be rare and/or not result in successful reproduction.

While the mechanisms that promote and impede gene flow are not well understood, both topographical features and anthropogenic factors likely play a role in the current genetic structure of wolverines at the southern extent of its North American range. The matrix of high mountainous areas surrounded by lowland valleys contrasts the continuously forested areas in the northern parts of the range and may support fewer numbers of wolverines with historically less movement. Anthropogenic effects on wolverine habitat, such as development, recreational use, agriculture and mining activities, are also greater at the southern end of their North American distribution (Banci 1994) and likely limit migration. Major highways including the Trans Canada Highway, Interstate 90, and Interstate 15 bisect all of the sampled populations and approximately 5% of wolverines studying these study areas were found dead alongside highways (Kyle, unpublished data). Wolverines have low tolerance towards disturbance and may also be influenced by habitat changes and fragmentation in this region (Carroll et al. 2001).

Conservation and management implications

Our data suggest that while some migration is occurring among populations, it may not be large enough to counter the effects of isolation and genetic drift. The long-term persistence of wolverines in the contiguous United States will require maintaining an adequate effective population size to buffer against genetic risks associated with inbreeding and genetic drift. Given generally low population densities and elusive nature of wolverines, estimates of population abundance are not currently available for our sampled populations. Using density estimates of one individual per 105 sq. km (Krebs and Lewis 1999) and the minimum convex polygons created in Cegelski et al. (2003), the estimated habitat available in Montana would support a population size of ~300 individuals in the Crazybelts populations, ~500 individuals in the Gallatin population (independent of Wyoming) and ~1000–2000 individuals in the Rocky Mountain Front population. The software GENELOSS was used to simulate the number of effective breeders necessary to maintain genetic variation in the sampled populations in the lower 48 states in the absence of gene flow. Results indicated that 300 and 200 breeding pairs are needed in the

Rocky Mountain Front and Wyoming populations, respectively, to maintain at least 95% of the variation in the next 100 generations and 200 breeding pairs are needed in the Gallatin, Crazybelts, and Idaho populations to maintain variation (Figure 6). Given a N/Ne ratio of 0.33 used for wolverines in Scanadinavia, (Flagstad et al. 2004), a minimum census size of 1200 adults would be needed for each of the populations.

Gene flow is also an important component to the maintenance of genetic diversity because small amounts of gene flow can prevent genetic drift in the long-term, especially when the effective population size cannot be attained due to habitat constraints or low population densities. Two effective migrants from Canada or Wyoming into the Rocky Mountain Front population will maintain current levels of genetic diversity while one effective migrant from the Canadian, RMF or Wyoming populations is needed to maintain current levels of diversity in the Gallatin, Crazybelts or Idaho populations. Low population densities indicate that the required effective population size may never be realized, therefore, migration is critical for maintaining diversity in these populations. This information provides management guidelines regarding population status and persistence, when ecological information becomes available.

Demographic effects such as vulnerability to stochastic disturbances can also be detrimental in isolated populations and result in localized extinctions (Holsinger 2000). Re-colonization of extirpated areas requires that both sexes migrate (Hanski and Gilpin 1997). While male-mediated gene flow may maintain genetic variation in the long-term, female-mediated gene flow is needed for demographic security. Restricted female-mediated gene flow among these populations suggests that re-colonization of female wolverines into extirpated territories may be rare. The populations sampled in this study were more isolated than those in northern Canada and Alaska. Efforts should be targeted at these populations to ensure that they maintain adequate available habitat to avoid demographic risks as well as genetic risks.

Genetics can provide a basis for understanding population dynamics and for prioritizing populations of management concern. However, additional demographic and ecological studies are greatly needed to better understand the appropriate needs and actions for elusive species such as the wolverine. In particular, population size and density studies are greatly needed to provide better evaluations of population status. Our understanding of wolverine habitat use is also limited, thus additional research is needed. The results of this genetic analysis should complement field

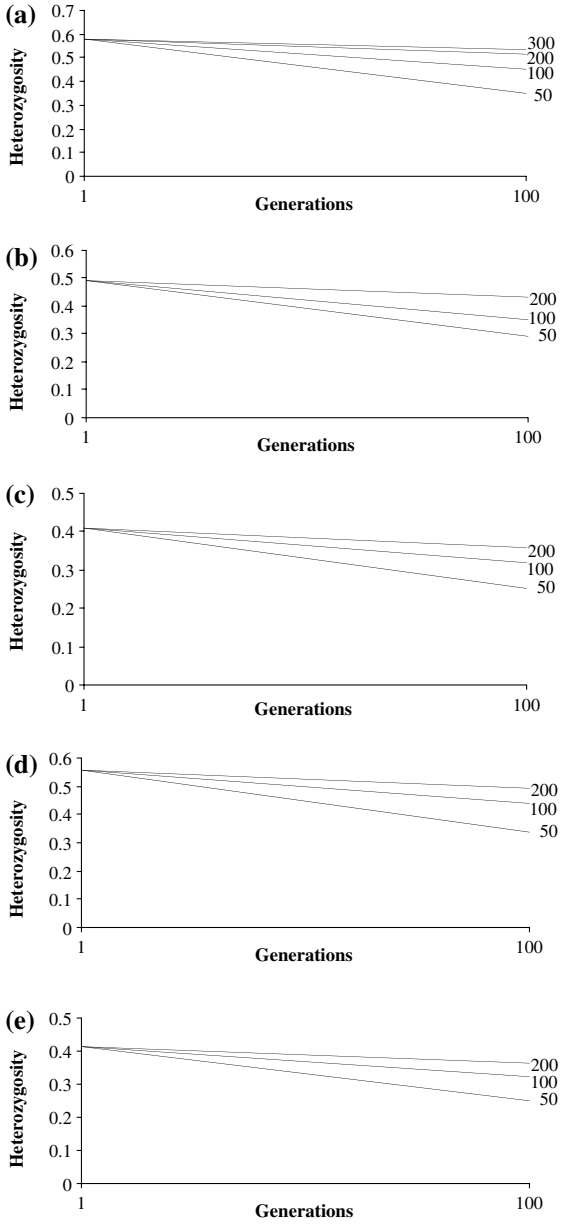

Figure 6. Simulations of the number of breeders needed to maintain heterozygosity in each of the wolverine populations in the United States over 100 generations assuming no migration. (a) Rocky Mountain Front (b) Crazybelts (c) Gallatin (d) Wyoming and (e) Idaho.

studies to determine the type of management actions most appropriate for each of the isolated populations in the contiguous United States.

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