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GENETIC VARIABILITY OF WOLVERINES (*GULO GULO*) FROM THE NORTHWEST TERRITORIES, CANADA: CONSERVATION IMPLICATIONS

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Because of anthropogenic factors in the early 1900s that caused populations to decline dramatically, wolverines (*Gulo gulo*) currently are designated as endangered in eastern Canada and classified as vulnerable throughout the Holarctic Region by the International Union for Conservation of Nature and Natural Resources. Although numerous examples exist that illustrate the utility of genetic data for development of conservation plans, no study has investigated the genetic structure of natural populations of wolverines. We assessed allozymic and mitochondrial DNA (mtDNA) variability of wolverines within and among 5 sites from the Northwest Territories, Canada. Five of 46 presumptive allozyme loci were polymorphic. Estimates of heterozygosity (2.6%) and polymorphism (11.6%) were lower than values reported for most mammals but were within the range reported for Carnivora. To evaluate levels of variation in mtDNA, we sequenced the left domain of the control region. Six variable nucleotide sites were observed, resulting in 9 haplotypes of mtDNA. Within-site diversity of haplotypes (h) was high, but within-site diversity of nucleotides (π) was low, indicating little sequence divergence among the 9 haplotypes. Sequence data for mtDNA revealed considerably more genetic partitioning among sites (ϕ_{ST} = 0.536) than did allozyme data (F_{ST} = 0.076). Based on fixation indices, gene flow estimates (Nm) were moderate for nuclear markers but low for mtDNA loci. These findings suggest that, although wolverines maintain large home ranges, they exhibit fidelity to discrete areas, gene flow is predominantly male-mediated, and most sites in the Northwest Territories are genetically independent and thus represent populations. Therefore, any conservation plan for wolverines in the Northwest Territories must consider preservation of populations if genetic diversity of this taxon is to be maintained.

Key words: allozymes, genetic variability, *Gulo gulo,* mitochondrial DNA, mtDNA, wolverine

In northern parts of the Holarctic Region, the wolverine (*Gulo gulo*) occurs at low population densities in wilderness areas of boreal forest and tundra (Pulliainen 1988; Wilson 1982; Wozencraft 1993). By the early 1900s, North American populations of wolverines experienced drastic declines as a result of overharvest for fur, habitat loss, and other anthropogenic factors (Banci 1994; van Zyll de Jong 1975; Wilson 1982). Although populations have increased in density and distribution in some

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areas of North America during past decades, wolverines currently are designated as endangered in eastern Canada (Nowak 1991) and classified as vulnerable throughout the Holarctic Region by the International Union for Conservation of Nature and Natural Resources (Wozencraft 1993).

Studies conducted in Montana (Hornocker and Hash 1981), Alaska (Gardner 1985; Whitman et al. 1986), Yukon Territory, Canada (Banci and Harestad 1988, 1990), and southcentral Norway (Landa et al. 1998) have contributed important ecological, behavioral, and demographic information that have led to a better understanding of the biology of wolverines. However, because wolverines occur in remote areas, exhibit a solitary and secretive lifestyle, and have the potential to be highly vagile (Gardner et al. 1986), our understanding of demographic factors important to their conservation lags behind that of many other vertebrates (Magoun and Copeland 1998; van Zyll de Jong 1975). Several authors have suggested establishment of refugia that contain suitable habitat and den sites interconnected with adequate travel and dispersal corridors to help maintain viable populations of wolverines (Banci 1994; Dias 1996; Magoun and Copeland 1998; McCullough 1996). This proposal focuses on only ecological and demographic aspects, and development of a biologically sound conservation plan also needs to consider genetic aspects to preserve the genetic diversity of the species (Avise 1994; Moritz 1994).

Our purpose was to provide baseline data on genetic variation and genetic structure of wolverines at several sites in the Northwest Territories, Canada. A better understanding of genetic structure of wolverines is important because such data provide insight into past and current evolutionary processes that have generated patterns of biodiversity, the preservation of which should be an important component of conservation plans (Smith et al. 1993). Moreover, ascertaining levels of genetic structure, which is caused primarily by selection, genetic drift, and emigration, can provide additional insight into demographic aspects at large spatial scales that otherwise may be difficult to obtain with direct observations or radiotelemetry.

Although numerous studies illustrate the utility of genetic data in development and implementation of biologically sound conservation and management plans (Avise and Hamrick 1996; Schonewald-Cox et al. 1983; Smith and Wayne 1996), none has investigated genetic structure of natural populations of wolverines. Genetic data from 2 wolverines were included in a study of hemoglobin variability in carnivores (Seal 1969), and data from 6 captive wolverines (originally from Burwash Landing, Yukon Territory, Canada) were included in a study of lactate dehydrogenase (LDH) in mustelids (LeDoux and Kenyon 1973). Both studies revealed no genetic variability.

Our purpose was to assess genetic variability of wolverines within and among sites in the Northwest Territories using allozymes (biparentally inherited) and sequence data of the mitochondrial DNA (mtDNA, maternally inherited) control region. Combined analyses of slowly evolving nuclear and rapidly evolving mtDNA loci can be a powerful approach for estimating levels of population subdivision, thereby providing insight into historical and contemporary levels of gene flow (Avise 1994; Friesen 1997).

Materials And Methods

Forty-three wolverines from the Northwest Territories were collected by personnel of the Department of Renewable Resources, Government of the Northwest Territories, during November 1988–April 1989. Tissue samples were collected from carcasses and stored at −20° or −70°C. Based on collecting information, all but 2 specimens were assigned to 1 of 5 sites. Fifteen individuals were collected from the Northwest Territories near the border of the Yukon Territory (sites 1 and 2; Fig. 1). Twenty-eight individuals were collected from the central Northwest Territories (26 individuals from sites

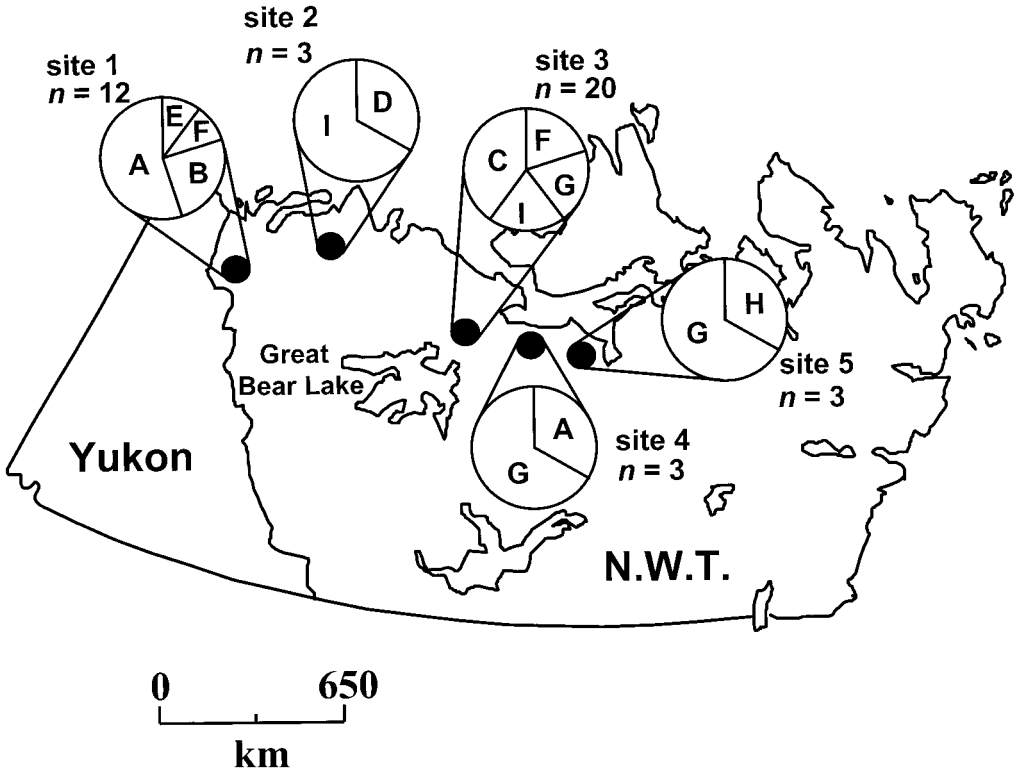

FIG. 1.—Five collection sites (site) of wolverines in the Northwest Territories (NWT), Canada, and geographic distribution of mtDNA haplotypes among sites. Pie diagrams show frequencies of each haplotype. The 5 collecting sites of wolverines examined are: site 1, Aklavik–Fort McPherson; site 2, Rendezvous Lake; site 3, area between Great Bear Lake and Coppermine; site 4, area between Coppermine and Bathurst Inlet; and site 5, Bathurst Inlet. For hierarchical analyses of genetic differentiation, sites 1 and 2 represent the western region and sites 3, 4, and 5 represent the central region. Specific collection data were not available for 2 samples from the central region; therefore, the two samples could not be assigned to specific sites.

3, 4, and 5). Genetic relationships among wolverines were evaluated using allozyme electrophoresis and DNA sequence data from a portion of the mtDNA genome, including the entire left domain of the noncoding control region. Numerous mammalian taxa have been surveyed for genetic variation at allozyme loci, providing a wealth of comparative data for evaluating genetic variability of wolverines. Similarly, we concentrated on the hypervariable left domain (5′ end) of the mtDNA control region because several recent studies of intraspecific relationships (Avise 1994; Brown et al. 1986; González et al. 1998; Taberlet 1996; Waits et al. 1998) have shown it to be especially informative for detecting genetic variation within and among populations of mammals.

Allozymic variation.—Water-soluble proteins from skeletal muscle, liver, and kidney of wolverines were visualized using standard horizontal starch-gel electrophoretic methods (Murphy et al. 1996) with 3 buffer systems: JRP (Ayala et al. 1972); continuous tris-citrate, pH 8.0 (Selander et al. 1971); lithium hydroxide (Ridgway et al. 1970). Protein products examined and locus designations (following Murphy et al. 1996, except where noted) were: acid phosphatase (Enzyme Commission 3.1.3.2; ACP); aconitate hydratase (EC 4.2.1.3; ACOH-1, ACOH-2); adenosine deaminase (EC 3.5.4.4; ADA); adenylate kinase (EC 2.7.4.3; AK); alcohol dehydrogenase (EC 1.1.1.1; ADH); aspartate aminotransferase (EC 2.6.1.1; AAT-1, AAT-2); creatine kinase (EC 2.7.3.2; CK); cytosol aminopep-

tidase (EC 3.4.11.1; CAP); dihydrolipoamide dehydrogenase (EC 1.8.1.4; DDH); esterase (EC 3.1.1.-; EST-1, EST-2, EST-3; alpha-naphthol propionate as substrate); fumarate hydratase (EC 4.2.1.2; FUMH); general proteins (GP-1, GP-2, GP-3); glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PDH); glucose-6-phosphate isomerase (EC 5.3.1.9; GPI); beta-glucuronidase (EC 3.2.1.31; bGLUR); glutamate dehydrogenase (EC 1.4.1.2; GTDH); glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; G3PDH); L-iditol dehydrogenase (EC 1.1.1.14; IDDH); isocitrate dehydrogenase (EC 1.1.1.42; IDH-1, IDH-2); L-lactate dehydrogenase (EC 1.1.1.27; LDH-1, LDH-2); malate dehydrogenase (EC 1.1.1.37; MDH-1, MDH-2); malate dehydrogenase ($NADP^+$) (EC 1.1.1.40; MDHP-1, MDHP-2); mannose-6-phosphate isomerase (EC 5.3.1.8; MPI); menadione reductase (EC 1.6.99.2; MNR—Conkle et al. 1982); peptidase (EC 3.4.-.-; PEP-A, PEP-B, PEP-C, PEP-D; leucyl-alanine, L-leucylglycylglycine, and L-phenylalanyl-L-proline as substrates); phosphoglucomutase (EC 5.4.2.2; PGM-1, PGM-2, PGM-3); phosphogluconate dehydrogenase (EC 1.1.1.44; PGDH); purine-nucleoside phosphorylase (EC 2.4.2.1; PNP); and superoxide dismutase (EC 1.15.1.1; SOD-1, SOD-2, SOD-3). Allelic frequencies, heterozygosity, tests of Hardy–Weinberg equilibrium, Rogers' (1972) genetic similarity coefficients, and Wright's *F*-statistics (Wright 1965) were compiled with BIOSYS-1 (Swofford and Selander 1981). Significance of F_{IS}- and F_{ST}-values was determined by methods of Li and Horvitz (1953) and Workman and Niswander (1970), respectively.

mtDNA variation.—Whole genomic DNA was extracted from liver or kidney tissue (Longmire et al. 1997). The polymerase chain reaction (PCR) was used to amplify a portion of mtDNA extending from $tRNA^{thr}$ to $tRNA^{phe}$ using primers L15926 and H00651 (Kocher et al. 1989). Amplifications were performed in 10-μl volumes (100–500 ng of DNA) using Epicentre MasterAmp 2× PCR PreMix FN solution (100mM Tris-HCL [pH 9.0, 25°C], 40 mM $(NH_4)_2SO_4$, 400 μM each dNTP, 7.0 mM $MgCl_2$, and 4× MasterAmp Enhancer), and AmpliTherm or *Taq* DNA polymerase (Epicentre Technologies, Madison, Wisconsin). Cycling parameters were: 94°C denaturation for 3 min, followed by 35 cycles of 94°C denaturation for 30 s, 54°C annealing for 60 s, and 72°C extension for 90 s, followed by a single 30-min final extension at 72°C. These amplicons (1.4 kilobases [kb]) were used as template DNA in a subsequent PCR (50 μl) with primers L15926 and H16498 (Shields and Kocher 1991; Taberlet 1996) to amplify a segment extending from $tRNA^{thr}$ to the central domain of the control region. This PCR followed the profile described with the exception of increasing the annealing temperature to 60°C for 30 s and performing the 72°C extension for 60 s for 35 cycles. Resulting amplicons were electrophoresed through a 1% Tris-boric acid–EDTA (TBE) agarose gel stained with ethidium bromide and visualized with exposure to ultraviolet light. The PCR products were purified using Wizard PCR Preps (Promega Corporation, Madison, Wisconsin) and sequenced using an ABI (Applied Biosystems, Inc., Foster City, California) 373 automated sequencer. Sequences reported herein have been deposited in GenBank under accession numbers AF210090-AF210132.

Sequences were compiled and aligned using CLUSTAL W (Thompson et al. 1994), followed by visual verification. Percentage sequence divergence within and between sites was calculated using the Kimura 2-parameter method (Kimura 1980) of PAUP, version 4.0b1 (Swofford 1998). Differences in frequency of haplotypes among sites were examined using a heterogeneity chi-square test with Monte Carlo simulations (1,000 randomizations; Roff and Bentzen 1989). Estimates of diversity of haplotypes (h) and nucleotides (π—Nei 1987) within sites were calculated using the computer program Arlequin (Schneider et al. 1997). The extent to which variation in mtDNA sequence was partitioned among sites and among regions (sites 1 and 2 represented the western region, whereas sites 3, 4, 5 and the 2 individuals that could not be specifically assigned to a site comprised the central region) was analyzed in a hierarchical fashion by the ϕ-statistic using the nested analysis of molecular variance (AMOVA—Excoffier et al. 1992) option of Arlequin. Significance of variance estimates for ϕ-statistics was obtained using a randomized procedure with 1,000 permutations (Excoffier et al. 1992).

Although accuracy of gene flow estimates is influenced by satisfaction of many assumptions, some of which may not be met in this study (populations in migration–selection equilibrium, island model of population structure), estimates

TABLE 1.—All frequencies for 5 polymorphic loci, percentage of polymorphic loci, and percentage of heterozygosity per locus based on 43 samples of *Gulo gulo* from 5 sites and 2 regions (Fig. 1) in the Northwest Territories, Canada.

		Sites					Regions	
Locus	Allele	1	2	3	4	5	Western	Central
GTDH	Medium	0.458	0.667	0.650	0.333	0.333	0.500	0.544
	Slow	0.542	0.333	0.350	0.667	0.667	0.500	0.446
MDH-1	Medium	1.000	1.000	0.975	1.000	1.000	1.000	0.982
	Slow	0.000	0.000	0.025	0.000	0.000	0.000	0.182
MDPH-2	Medium	0.833	1.000	0.825	1.000	0.750	0.867	0.846
	Slow	0.167	0.000	0.175	0.000	0.250	0.133	0.154
MPI	Medium	0.875	1.000	0.975	0.833	1.000	0.900	0.964
	Slow	0.125	0.000	0.025	0.167	0.000	0.100	0.036
PGM-1	Medium	0.625	0.333	0.675	0.500	0.500	0.567	0.661
	Slow	0.375	0.667	0.325	0.500	0.500	0.433	0.339
% polymorphic loci		9.3	4.6	11.6	6.9	6.9	9.3	11.6
% heterozygosity		2.5	1.6	3.0	1.5	3.5	2.3	2.8

provide a basis for comparing gene flow among sites. Based on allozymic data, the effective number of migrants per generation (N_em) was estimated from the mean F_{ST}-value using Wright's (1969) island model where $N_em = ((1/F_{ST}) - 1)/4$. Using mtDNA data, the effective number of dispersing females (Takahata and Palumbi 1985) was estimated from the approximation $N_fm = ((1/\phi_{ST}) - 1)/2$.

RESULTS

Allozymic variation.—Of the 46 allozyme loci examined, 3 (ADA, MNR, and PNP) could not be scored consistently and, therefore, were excluded from analyses. Thirty-eight loci were monomorphic, and 5 were polymorphic (frequency of the common allele in at least 1 population <0.99; Table 1). Significant ($P < 0.05$ with Bonferroni adjustment—Rice 1989) deviation from Hardy–Weinberg expectations were observed for 1 locus (MPI). That significant deficiency of heterozygotes occurred within site 1 and was present in the western region and in the total sample. That deficiency also was indicated by positive F_{IS}-values for sites and regions (Table 2). Deviation from Hardy–Weinberg equilibrium also was indicated for GTDH ($P = 0.051$ for central region and 0.021 for populations 2 and 4; not significant at $P < 0.05$ with Bonferroni adjustment). Positive F_{IS}-values (significantly different from 0) were observed for that locus (Table 2) for sites and regions. Percentage of polymorphic loci ranged from 4.6% (site 2) to 11.6% (site 5) among sites, was 9.3% and 11.6% for the western and central regions, respectively, and was 11.6% for the total sample. Average heterozygosity per locus ranged from 1.5% (site 2) to 3.5% (site 5) among sites, was 2.3% and 2.8% for the 2 regions (western and central, respectively), and was 2.6% for the total sample.

Although no significant differentiation

TABLE 2.—Summary of *F*-statistics for 5 polymorphic loci for samples of *Gulo gulo* from 5 sites and 2 regions (Fig. 1) in the Northwest Territories, Canada.

	Sites		Regions	
Locus	F_{IS}	F_{ST}	F_{IS}	F_{ST}
GTDH	0.395	0.086	0.341*	0.003
MDH-1	−0.026	0.020	−0.018	0.009
MDHP-2	−0.080	0.098	0.103	0.001
MPI	0.144	0.081	0.445*	0.016
PGM-1	0.022	0.057	0.009	0.009
Mean	0.155	0.076*	0.188	0.006

* Significantly different from 0 at $P < 0.05$.

TABLE 3.—Matrix of Rogers' genetic similarity coefficients for pairwise comparisons of 5 sites (Fig. 1) of *Gulo gulo* from the Northwest Territories, Canada.

	Sites				
Sites	1	2	3	4	5
1					
2	0.792				
3	0.808	0.983			
4	0.875	0.667	0.683		
5	0.875	0.667	0.683	1.000	

among sites was indicated by F_{ST}-values for individual loci, the value for loci combined (F_{ST} of 0.076 significantly different from 0 at $P < 0.05$) indicated a low (Hartl 1980) level of differentiation among the 5 sites (Table 2). The pattern of relationships among sites, as revealed by Rogers' genetic similarity values (Table 3), generally shows some distinction of sites 2 and 3 from sites 4 and 5 (S = 0.983 and 1.000 within each pair, respectively; S ranging from 0.667 to 0.683 between the 2 pairs). Site 1 generally had the same level of similarity with all other sites (S ranging from 0.792 to 0.875). Based on the mean F_{ST}-value, the effective number of dispersing individuals among sites (N_em) was 3.04 individuals per generation. Wright's F_{ST} also detected no significant differences between regions for any of the 5 polymorphic loci or for these loci combined (Table 2).

mtDNA variation.—For each individual, 366–367 base pairs (bp) were obtained, including 28 bp of the gene coding for t-RNA^{thr}, 66 bp of the gene coding for t-RNA^{pro}, and 271–272 bp of the left domain of the control region. Six nucleotide positions were variable (4 transitions, 1 transversion, and 1 insertion–deletion). Based on those 6 variable positions, all 43 individuals were assigned to 1 of 9 mtDNA haplotypes (Fig. 2; Table 4). Mean percent sequence divergence among individuals was 0.41%, with a range of 0.00%–1.11%. Frequencies of mtDNA haplotypes revealed significant ($P < 0.001$) geographic variation among

	Variable nucleotide position					
Haplotype	69	108	111	173	196	234
B	C	C	C	-	C	G
E	.	.	.	-	T	.
D	A	T	.	-	T	.
F	.	T	.	-	T	.
A	.	T	.	-	.	.
I	.	T	T	C	T	.
C	.	T	T	-	T	.
G	.	T	T	-	T	A
H	.	T	.	-	T	A

FIG. 2.—Polymorphic sites within mtDNA tRNA^{pro} and control region (left domain, reported 5′ to 3′) sequences and types of haplotypes (A–I) of 43 wolverines from the Northwest Territories, Canada. Numbers (at top of figure) represent nucleotide position of 367 aligned positions for tRNA^{thr}, tRNA^{pro}, and the complete left domain of the mitochondrial control region. Identical nucleotide characters, with the reference sequence (haplotype B), are indicated with dots, and insertion–deletion events are indicated with dashes. Geographic distribution of each haplotype is shown in Fig. 1.

sites of wolverines in the Northwest Territories based on Monte Carlo simulations (Table 5). When the 2 regions were considered independently, mean sequence divergence for individuals within the central region (0.84%) was higher than that for individuals within the western region (0.35%).

Proportion of genetic diversity attributable to variation among sites within regions and within sites were 32.7% and 46.4%, respectively, but proportion of genetic diversity between regions was 21.0%. Variation among sites relative to variation present within regions and total variation differed from 0 ($P < 0.001$). About one-half (53.6%) of the differences in genetic diversity was attributable to differences among sites (ϕ_{ST}; haplotypic correlation measure analogous to F_{ST}—Excoffier et al. 1992). Those data suggested significant ($P < 0.05$)

TABLE 4.—Number of individuals of each mtDNA haplotype, diversity of haplotypes (h), and diversity of nucleotides (π) of *Gulo gulo* within 5 sites from the Northwest Territories, Canada. Sites 1 and 2 represent the western region and sites 3, 4, and 5 represent the central region (Fig. 1). Two samples from the central region, which could not be assigned to a specific site, had haplotypes G and I.

	mtDNA haplotypes													
Sites	B	E	D	F	A	I	C	G	H	*n*	h	SE	π	SE
1	3	1		1	7					12	0.636	0.128	0.0022	0.0019
2			1			2				3	0.667	0.314	0.0055	0.0051
3				4		4	8	4		20	0.758	0.054	0.0028	0.0022
4					1			2		3	0.667	0.314	0.0055	0.0052
5								2	1	3	0.667	0.314	0.0018	0.0023

geographical structuring of sites. However, grouping sites into central and western regions revealed no significant geographical structuring due to greater variation existing among sites than between regions. Based on mean ϕ_{ST}, number of dispersing females ($N_f m$) was 0.42 individuals/generation.

DISCUSSION

Intrapopulation variation.—Although levels of heterozygosity (2.6%) and polymorphism (11.6%) for allozymes were lower in wolverines than generally reported for other mammals (Nevo 1978; Selander and Johnson 1973; Smith et al. 1978; Wooten and Smith 1985), these levels were within the range reported for other mustelids (Kilpatrick et al. 1986; Lidicker and McCollum 1997; Mitton and Raphael 1990; Serfass et al. 1998; Simonsen 1982). Diversity of mtDNA haplotypes (h), which is based on within-site frequency of each haplotype, was moderate to high (maximum 0.758 ± 0.054; Table 4). Thus, diversity of haplotypes is considerable even with small samples for some sites. In contrast, diversity of nucleotides (π), which is based on frequencies of haplotypes and sequence divergence among haplotypes within sites, was low (maximum 0.0055 ± 0.0052; Table 4). Hence, the contrasting values for h and π describe sites that contain a moderately large number of closely related haplotypes (9 haplotypes detected among 43 individuals).

TABLE 5.—Mitochondrial (mtDNA) genetic differentiation (ϕ_{ST}), upper triangular matrix, and estimates of gene flow ($N_f m$), lower triangular matrix, for all pairwise comparisons of 5 sites (Fig. 1) of wolverines, *Gulo gulo*, from the Northwest Territories, Canada.

Site	1	2	3	4	5
1		0.622*	0.612*	0.518	0.731*
2	0.304		0.137*	0.182	0.455
3	0.317	3.158		0.137*	0.343*
4	0.465	2.250	3.158		0.000
5	0.184	0.600	0.958	∞	

* Significantly different from 0 at $P < 0.05$.

Population structure and gene flow.—Population descriptors based on allozyme and mtDNA sequence data reveal contrasting patterns with regard to magnitude of genetic structuring among sites of wolverines. Of the 5 polymorphic allozyme loci, only MPI revealed significant among-site heterogeneity. Moreover, a low degree of genetic differentiation (F_{ST} = 0.076) was detected based on these data. In contrast, distribution and frequency of mtDNA haplotypes revealed significant geographic structuring (ϕ_{ST} = 0.536) among sites. Although no significant genetic differentiation was detected between regions, haplotypes C, G, and H occurred only in the central region, and haplotypes B, D, and E occurred only in the western region. Failure to detect significant genetic differentiation between regions, although 6 of 9 haplotypes clearly are differentiated, most likely resulted from

small samples for some sites. Interestingly, sites with the largest samples, which were separated by a distance of about 900 km, shared 1 haplotype (Fig. 1; Table 4).

F_{ST}-values (and appropriate analogs such as ϕ_{ST}) measure the extent to which species are organized into subpopulations (Wright 1965). Because many factors affect these indices, it is difficult to know if the magnitude of genetic differentiation detected is biologically significant. However, if populations are in selection–mutation equilibrium, differentiation among subpopulations is related to genetic drift and magnitude and direction of gene flow. Therefore, biological significance of the degree of genetic differentiation can be inferred by evaluating levels of gene flow. For example, only a single migrant per generation is necessary to prevent differentiation among subpopulations by genetic drift when gene flow among subpopulations is random (island model—Wright 1965). Similarly, 2 to 4 migrants per generation are necessary assuming a stepping-stone model of gene flow among subpopulations (Crow and Aoki 1982). Assuming the island model with F_{ST} = 0.076 results in an estimate of 3.04 individuals being exchanged among sites per generation. In contrast, assuming the same model and ϕ_{ST} = 0.536 (for mtDNA data), only 0.42 migrants are exchanged among sites per generation.

Contrasting degrees of genetic differentiation between nuclear and mitochondrial markers may be due to heightened genetic drift due to the reduced effective size for mtDNA compared with nuclear DNA, differences in age of reproductive maturity between sexes, or male-biased dispersal (Birky et al. 1983; Palumbi and Baker 1994, 1996). In the absence of sex-biased gene flow, we expect a 2-fold difference between estimates of gene flow from allozymes (N_em) and mtDNA (N_fm), because N_fm corrects for haploid, but not maternal, inheritance. The observed difference between N_em and N_fm for wolverines from the Northwest Territories is about 7-fold. Interpreting estimates of gene flow in light of generation time is difficult for wolverines due to delayed implantation, small number of individuals examined, and differences in how age classes were grouped in previous studies addressing aspects of reproduction in wolverines (Banci 1994). Banci (1994) summarized available data on reproduction in wolverines and reported that 0%–85% of subadult females (1–2 years) breed, whereas most males are immature until ≥2 years of age. Therefore, assuming a generation time of about 2 years for male and female wolverines, estimates of gene flow per generation suggest that dispersal among sites is male-biased—a situation common among mammals (Greenwood 1980). Based on direct observations, wolverines have been reported to make periodic long-distance movements. Males typically are more mobile than females, exemplified by an adult male dispersing 378 km from southcentral Alaska to the Yukon Territory over an 8-month period (Gardner et al. 1986). Hence, both male and female wolverines could move between any 2 adjacent sample sites. Despite this potential vagility, lack of genetic differentiation and high estimate of nuclear gene flow suggest that most successful dispersal and subsequent reproduction occurs by males, whereas minimal female-mediated gene flow occurs between sites. Melnick and Hoelzer (1992) and Palumbi and Baker (1994, 1996) also reported lower magnitudes of differentiation for nuclear relative to mtDNA loci and attributed those mirror-image patterns to extensive intergroup movement by males and natal-group fidelity by females.

Conservation implications.—Analysis of nuclear allozymes and frequencies of mtDNA haplotypes confirm that sites of wolverines from the Northwest Territories are structured genetically, even at distances of 100 km. Identification of wolverines from sites that show significant genetic divergence is important, because these sites typically are independent demographically and, thus, should be treated as different

populations (Avise 1995; Moritz 1994). Based on ϕ_{ST}-values, significant genetic differentiation was detected between many pairwise comparisons of sites regardless of region (Table 5). Moreover, degree of gene flow between those sites with significant genetic differentiation are insufficient to overcome effects of genetic drift (Crow and Aoki 1982; Wright 1965), further documenting the independence of many of these populations. Therefore, genetic data indicate that wolverines at sites in the Northwest Territories are demographically independent. Therefore, the 5 sites examined in our study represent populations that could be grouped into at least 2 management units (Moritz 1994). This is supported by the presence of only 3 (C, G, H) of the 9 haplotypes in populations from the central region and 3 other haplotypes (B, D, E) restricted to the western region.

Our data corroborate demographic data that show wolverines are highly mobile and possess large home ranges, but most individuals exhibit fidelity to their natal site (Hornocker and Hash 1981; Jackson 1961; Lee and Niptanatiak 1996; Magoun and Copeland 1998). Therefore, based on our data, wolverines need to be conserved at the population level and populations of wolverines in the Northwest Territories need to be conserved to maintain genetic diversity. Because populations of wolverines <350 km apart are genetically different, an inordinate number of refugia over a broad spatial scale would be required to maintain genetic diversity across their geographic range. Hence, conservation efforts must include provisions for maintaining wolverines in the matrix, with appropriate corridors for travel and dispersal, and not rely solely on a system of reserves. At the same time, we recognize that although this is the most comprehensive genetic study of wolverines, samples for many populations were small and additional individuals are needed to test our conclusions. Moreover, before any permanent conservation plan for wolverines is developed, other populations from throughout the Northwest Territories should be sampled. By increasing samples (in terms of numbers of individuals and collection sites), a better understanding of genetic variability within and genetic differentiation among populations of wolverines will be obtained, thereby assisting in development of a solid conservation plan.

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