

Genetic structure of North American wolverine (*Gulo gulo*) populations

C. J. KYLE and C. STROBECK

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada

Abstract

Wolverines (*Gulo gulo*) are found in low densities throughout their circumpolar distribution. They are also potentially susceptible to human-caused population fragmentation (development, recreation and fur harvesting). The combination of these factors has contributed to this species being listed as having either vulnerable or endangered status across much of its current range. The effects of inherently low densities and anthropogenic pressures on the genetic structure and variation of wolverine populations are, as yet, unknown. In this study, 461 individuals were typed at 12 microsatellite loci to investigate the population genetic structure of wolverines from north-western Alaska to eastern Manitoba. Levels of gene flow and population differentiation among the sampled regions were estimated via a genotype assignment test, pairwise F_{ST} , and two genetic distance measures. Our results suggest that wolverine populations from southernmost regions, in which anthropogenic factors are strongest, revealed more genetic structuring than did northern populations. Furthermore, these results suggest that reductions in this species' range may have led to population fragmentation in the extreme reaches of its southern distribution. The continued reduction of suitable habitat for this species may lead to more populations becoming isolated remnants of a larger distribution of northern wolverines, as documented in other North American carnivore species.

Keywords: forest carnivore, *Gulo gulo*, microsatellite, Mustelidae, population structure, wolverine

Received 14 June 2000; revision received 29 September 2000; accepted 29 September 2000

Introduction

Wolverines (*Gulo gulo*) are the largest terrestrial member of the family Mustelidae (the weasel family). This species has a circumpolar distribution and is found in tundra, taiga and forest zones of North America and Eurasia (Wilson 1982). In North America, prior to human settlement, wolverines were distributed across Canada and Alaska with projections southward into the conterminous US through montane regions as far as New Mexico and Arizona (Hash 1987). Currently, wolverines in Canada west of Hudson's Bay are found in the northern regions of the prairie provinces, in Alberta's western national parks, and throughout most of British Columbia and the Canadian Territories (Banci 1994). East of Hudson's Bay, wolverines are exceedingly rare and are listed as endangered by COSEWIC (1998). In the conterminous US

the range of wolverines has steadily retracted since the 1840s, with animals persisting in isolated regions of Montana, Idaho, Wyoming, Colorado, Oregon and California (Hash 1987). The contracting range of wolverines in North America is the result of habitat loss, overharvest and other anthropogenic factors, which together have led to a decline in the numbers of these animals (Wilson 1982; Banci 1994).

Wolverines are found at low density across their Holarctic distribution with estimates of one animal per 40–800 km² (see review by Banci 1994). This species also establishes large home-ranges (100–900 km²) which seem to be maintained between years (Magoun 1985; Banci 1987) and which vary in size in relation to food abundance (Banci 1994). Males typically maintain larger home-ranges than females and may overlap the home-ranges of several females (Banci 1994).

Wolverines are reproductive from 2 years of age onwards, although the fecundity of females is thought to decrease after the age of 6 (Banci & Harestad 1988). Not all females become pregnant in a given year (53–92% in the Yukon;

Correspondence: C. Kyle. Fax: +1 780 492 9234; E-mail: ckyle@ualberta.ca

Banci & Harestad 1988) depending on food availability. Even when females do give birth, kit mortality is thought to be high with estimates of $\approx 36\%$. These figures are considered to underestimate the true mortality rate (Banci 1994).

Most juvenile females exhibit natal area fidelity and establish home-ranges adjacent to their mothers (Magoun 1985), although some females have been observed to disperse far beyond their natal range. Subadult males typically disperse 30–100 km from their natal home-range (Gardner 1985; Magoun 1985), although several movements further than this have been reported. Gardner *et al.* (1986) reported a 378-km straight-line movement of a 2-year-old from southcentral Alaska to the Yukon Territory in a 7-month period. A yearling female was reported by Magoun (1985) to have dispersed 300 km in a 5-month period. It should be noted, however, that some rare long-distance movements in this species do not seem to be linked to dispersal, but are simply temporary forays from their home-range (Banci 1994). It is believed that half to one-third of all dispersing individuals die, either by starvation, harvest or predation (Krott 1982).

The movement of wolverines does not seem to be influenced by the presence of lakes, rivers, mountain ranges or other topographical features (Hornocker & Hash 1981; Banci 1987). Only human development and major access routes are thought to function as barriers to dispersal for this species. Furthermore, human activity may influence kit survival, thereby limiting the expansion of wolverine populations (Banci 1994).

With increasing concern about the status of this forest carnivore and how it might be managed effectively, it is necessary to determine the genetic distinctiveness of wolverine populations and the levels of gene flow between geographical areas. Population genetic data may also be used to determine whether harvested populations are replaced solely from within or if replacement from other populations plays an important role in maintaining a population's numbers.

Wolverines, where they persist north of the 38th parallel in North America, are currently considered to be a continuous breeding group, however, the extent of movement between the larger Canadian populations and remnant conterminous US populations is unknown (Banci 1994). Recent work by Edelman & Copeland (1999) suggests that movement between Idaho and Oregon populations may be restricted due to a lack of suitable habitat between them. These findings may be reflected in the population genetic structure of wolverine populations in this area. Another recent study by Wilson *et al.* (2000) evaluated the genetic variability of wolverines in the Northwest Territories, Canada using both mitochondrial DNA (mtDNA) and allozyme markers. A high degree of population structure was found using mtDNA ($\phi_{ST} = 0.536$), although much less

structure was observed among the sampled regions using nuclear allozyme markers ($F_{ST} = 0.076$). These results are not surprising given this species' high potential for dispersal, chiefly male-mitigated dispersal, and female fidelity to natal areas. These findings, however, were based on small sample sizes ($n = 3, 3, 3, 12$ and 20 for each of the five studied 'populations', respectively) and limited genetic variation which the authors admit pose considerable constraints on the conclusions which can be drawn from the study.

The first goal of this study was to elucidate the population genetic structure of wolverines across much of their North American distribution, which to date is largely unknown. The second goal was to determine what underlying factors influence the population genetic structure of this species. We hypothesize that the genetic structuring of wolverine populations will be higher in the southern regions of this species' range, where anthropogenic factors potentially act as barriers to dispersal, than those populations found in undisturbed northern habitats.

To detect genetic differentiation among wolverine populations, high resolution (fast evolving) neutral genetic markers are preferred. Previous studies have found little or no genetic variation in wolverines using other markers (haemoglobin, Seal 1969; lactate dehydrogenase, LeDoux & Kenyon 1973; 337 bp of mtDNA sequence and five allozyme markers, Wilson *et al.* 2000). For this reason, we have chosen to use hypervariable microsatellite loci. These polymorphic, tandem repeats of DNA have proven useful in other studies of mammalian species with high vagility, such as the polar bear (*Ursus maritimus*; Paetkau *et al.* 1995, 1999) and the North American brown bear (*Ursus arctos*; Paetkau *et al.* 1998). Microsatellites identified clear genetic differentiation between populations of these animals despite the long-range movements known to occur in these species, and the fact that little variation was detected using other methods.

Materials and methods

Sample collection

Twelve polymorphic loci were used to examine the population genetic structure of wolverines from 12 geographical regions. Bone samples were acquired from six Alaskan regions (all samples were provided by the University of Alaska, Fairbanks Museum and are sorted according to their quadrat system): Anchorage ($n = 36$); Arctic region, including samples from Arctic, Fort Yukon and Black River ($n = 47$); Nabesna ($n = 38$); Russian Mission region, including samples from Russian Mission and Holy Cross ($n = 34$); Noatak region, including samples from Noatak, Point Hope, Point Lay, Meade River, Survey Pass and Howard Pass ($n = 38$); and the Nome region, including samples from Solomon and Nome ($n = 38$). All samples

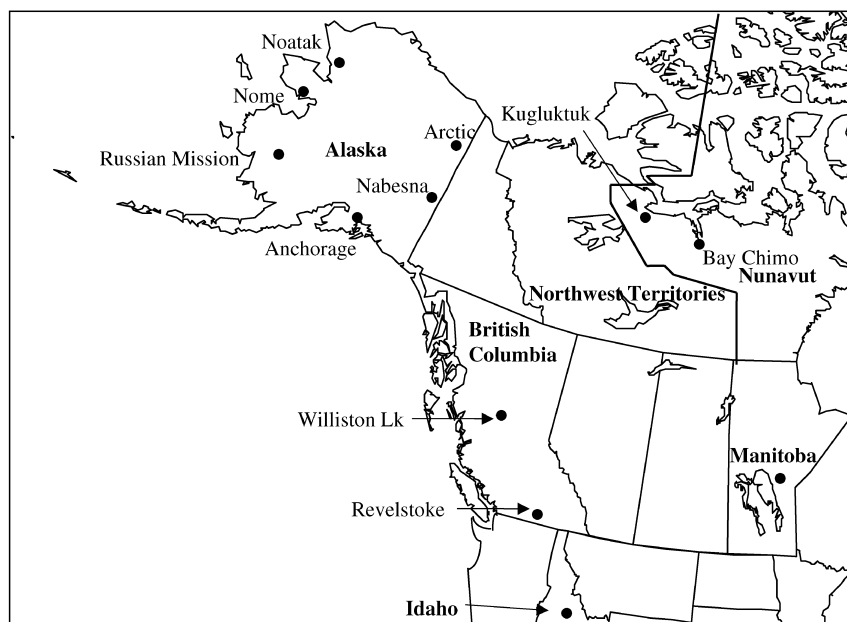


Fig. 1 Map of sampled regions.

from Alaska date from the late 1950s through to the mid-1960s. Tissue samples were obtained from two Nunavut territory regions (then part of the Northwest Territories): Kugluktuk ($n = 67$) and Bay Chimo ($n = 40$). Ear plug and hair samples were obtained from the Revelstoke ($n = 47$) and Williston Lake ($n = 37$) regions in British Columbia, eastern Manitoba ($n = 28$) and central Idaho ($n = 14$) (Fig. 1). All samples, other than those collected from Alaska, are of recent origin (early 1990s).

Laboratory methods

DNA was extracted using a QIAamp® Tissue Extraction Kit (QIAGEN). The primers used to amplify the microsatellites were developed by: Davis & Strobeck (1998) in badgers (BA-1 and BA-4), martens (MA-3) and wolverines (GG-3, GG-4, GG-7, GG-14); by Duffy *et al.* (1998) in wolverines (Ggu 101, Ggu 216 and Ggu 234); by Flemming *et al.* (1999) in mink (Mvis-75); and by Dallas & Pierny (1998) in Eurasian otters (L-604). Amplification of DNA was performed as in Davis & Strobeck (1998). The DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. Analysis of DNA fragments was done using the programs GENESCAN™ ANALYSIS 2.02 and GENOTYPER® 2.0.

Data analysis

A G-test for heterogeneity (Sokal & Rohlf 1995) was performed for each of the sampled areas by making pairwise comparisons of allele distributions. Departures from Hardy–Weinberg equilibrium (HWE) were tested for each of the 12 loci as assessed by GENEPOP version 3.1d

(Raymond & Rousset 1995) which uses a Markov chain method following the algorithm of Guo & Thompson (1992). This software was also used to evaluate genotypic disequilibrium among the loci used.

The relative genetic variation in each population was first assessed using allele frequency data from which the mean number of alleles, unbiased expected heterozygosity, H_E (formula as per Nei & Roychoudhury 1974), and unbiased overall probability of identity, P_{ID} (Paetkau *et al.* 1998) were determined. The genetic distances between the populations were estimated using two measurements: Nei's standard genetic distance, D_S (Nei 1972) which is calculated from genotype frequencies, and the genotype likelihood ratio distance, D_{LR} (Paetkau *et al.* 1997) which is calculated from genotype probabilities. These measures were both identified by Paetkau *et al.* (1997) to perform better than other measures of genetic distance that rely on the stepwise mutation model. Both sets of genetic distance values were calculated by programs from the website, <http://www.biology.ualberta.ca/jbrzusto/Doh/html>, designed by John Brzustowski.

An unrooted neighbour-joining tree of the D_S values was created using PHYLIP 3.572 (Felsenstein 1995). The geographical and genetic distance values were also entered into a two-way Mantel test (Mantel 1967) within the set of programs called the 'R' Package for multivariate analysis designed by Alain Vaudor (software package can be found on Pierre Legendre's website: <http://www.fas.umontreal.ca/BIOL/legendre/>) to determine the correlation between genetic distance and geographical proximity. This software package was also used to run a three-way Mantel test (as per Smouse *et al.* 1986). We used this method to determine

whether the curves on the scatter plot of genetic distance against geographical distance had significantly different intercepts. Populations which had significantly different curves from the expectation of isolation by distance were taken to have some barrier to gene flow other than geographical distance present. Each population was tested by creating a matrix of ones and zeros for the presence or absence of a barrier, while controlling for the geographical distance. All geographical distances were calculated using the DISTGEO program, also found within the 'R' package, from approximate latitudes and longitudes for each region.

Pairwise F_{ST} estimates were obtained from the software package GENEPOP 3.1d (Raymond & Rousset 1995; as per Weir & Cockerham 1984).

The assignment test (Paetkau *et al.* 1995), also found on the aforementioned web site, was run for all populations. This program determines both the probability of a genotype occurring in the region from which it was sampled and the probability of it occurring in the regions with which it is being compared. It then assigns each individual to the population in which that individual's genotype has the highest probability of occurring (see Waser & Strobeck 1998). Unlike other pairwise population statistics, the assignment test uses the information from each individual's genotype to determine how similar are the gene pools of the two populations. The test was always run with the option of replacing allele frequencies of 0 with 0.01.

The significance of the assignment test results was obtained using two types of randomization tests. In the first randomization test, for each replicate, the same number of individual genotypes as found in a population is drawn using the allele frequencies at each locus in the combined gene pool (all populations) assuming HWE. This randomization notes the proportion of replicates in which the number of individuals in population A assigned to population B is less than or equal to the observed number of individuals assigned from population A to population B. When the proportion is < 0.05 , there is evidence that significant heterogeneity exists between the genotype frequencies in populations A and B.

In the second randomization test, for each replicate, the same number of individual genotypes as found in a population is drawn using the allele frequencies at each locus found in that population, assuming HWE. This randomization notes the proportion of replicates in which the number of individuals in population A assigned to population B is less than or equal to the observed number of individuals in population A assigned to population B. When this proportion is > 0.95 then there is a significant number of cross-assignments from A to B. This implies that some of the individuals in A must be in genotypic disequilibrium with population A and are therefore presumably immigrants into A from a population with allele frequencies different

for those found in A. Ten thousand replicates were used to obtain the significance values for both randomization tests.

Results

Tests of disequilibrium

All 12 loci used in this study were tested for departures from HWE. After accounting for sample-wise error (as per the Dunn-Sidak method; Sokal & Rohlf 1995), four departures from HWE were found: locus GG-3 in the Williston Lake British Columbia (BC) region, locus BA-4 in the Manitoba region, and locus Ggu 234 in the Anchorage and Nabesna regions. All departures from HWE were found to be heterozygote deficits which may imply the presence of null alleles in these populations. Low copy number may also be responsible for the heterozygote deficit in the two Alaskan populations as the DNA was extracted from bone samples. As deviations from HWE were not found in other populations for these loci, they were retained for analyses. Genotypic disequilibrium was suggested for the following pairs of loci: GG-7 with GG-14 in the Manitoba population, GG-3 with BA-4 in the Bay Chimo region, and GG-4 with GG-14 in the Revelstoke region. As these genotypic disequilibria were not found in more than one population it is unlikely that any loci are physically linked. Hence, all loci were retained for analyses. It is possible that these disequilibria are the result of linkage disequilibrium in the founding population that has not yet dissipated or that a recent admixture of populations with differing gametic frequencies has occurred (Hartl & Clark 1989).

Heterogeneity of sampled regions

Twelve geographical areas were sampled in this study (Fig. 1). All sampled regions were found to have significantly ($P < 0.05$) different allele frequencies by a G-test for heterogeneity. The comparison of the allele frequencies in the Russian Mission and Arctic regions and the comparison of the Russian Mission and Nabesna regions were both significant at the 5% level. These differences, however, were found not to be significant when the experiment-wise error rate was applied (as per the Dunn-Sidak method; Sokal & Rohlf 1995). Despite these findings we treated these three regions as populations because of their geographical separation from one another (≈ 1000 km).

Genetic variation

The population with the highest mean number of alleles was in Arctic, Alaska (AK) with a value of 5.83, whereas Idaho had the lowest value of 2.83 (Table 1). H_E values ranged from 68% in Nabesna, AK to 42% in Idaho (Table 1).

	Abbreviation	<i>n</i>	A	<i>H_E</i> (%)	<i>P_{ID}</i>
Anchorage, AK	Anch	36	5.17	65.45	6 450 000 000
Arctic, AK	Arct	47	5.83	66.90	10 190 000 000
Nabesna, AK	Nabe	38	5.25	68.48	36 100 000 000
Russian Mission, AK	Russ	34	5.00	63.53	1 647 000 000
Noatak, AK	Noat	38	5.00	65.31	5 090 000 000
Nome, AK	Nome	35	4.67	62.31	1 004 000 000
Bay Chimo, NU	BayC	40	4.83	63.61	1 278 000 000
Kugluktuk, NU	Kugl	67	5.00	64.68	2 540 000 000
Revelstoke, BC	Reve	47	5.17	60.98	377 000 000
Williston Lake, BC	Will	37	4.92	61.18	1 231 000 000
Idaho	Idah	14	2.83	42.09	452 000
Manitoba	Mani	28	4.50	66.99	1 865 000 000

Table 1 Genetic variation estimates from sampled regions including the mean number of alleles (A), the mean unbiased expected heterozygosity (*H_E*), and the unbiased probability of identity (*P_{ID}*)

Table 2 Population assignments from the genotype assignment test

	<i>N</i>	Anch	Arcti	Nabe	Russ	Noat	Nom	Bay	Kugl	Reve	Willi	Idah	Mani
Anch	36	8	7**	1	7	2	2	0	5*	1	1	0	1
Arct	47	4	10	4	4	9*	3	6*	1	1	4	0	1
Nabe	38	5	4	11	2	9**	2	1	3	0	1	0	0
Russ	34	3	7**	3	6	4	6**	2	2	1	0	0	0
Noat	38	2	5	3	7*	13	2	1	2	1	2	0	0
Nome	35	2	3	2	2	1	21	2	2	0	0	0	0
BayC	40	0	2	1	7**	2	0	19	7	0	0	0	2
Kugl	67	2	1	2	3	3	3	20**	23	0	5	0	5*
Reve	47	1	2*	0	1	0	0	2	1	35	4	0	1
Will	37	3	0	2	3	3	3	1	1	5	15	1*	0
Idah	14	0	0	0	0	0	0	0	0	0	0	14	0
Mani	28	0	0	0	0	1	1	0	3*	0	0	0	23

*Significant at the 5% level for randomizations within each gene pool. **Significant at the 1% level for randomizations within each gene pool.

P_{ID} values ranged from 1/36 000 000 000 in Nabesna, AK to 1/452 000 in Idaho (Table 1). The Revelstoke population also had a low *P_{ID}* value (1/377 000 000) relative to the other populations. The low estimates of the mean number of alleles in Idaho may be explained, in part, by the low sample size (*n* = 14) relative to the other populations (*n* > 30), although measures of heterozygosity are less affected by sample size.

Assignment test

The results from the genotype assignment test revealed little genotypic distinctiveness between the Alaskan regions sampled, as evidenced by the relatively low number of correctly assigned individuals (1/4 to 1/6). The one exception was Nome, in which 21/35 individuals were assigned to the region from which they were sampled (Table 2). Most cross-assignments in Alaskan regions went to other Alaskan regions. A relatively high number of cross-assignments

also went to the Nunavut regions in which 5/36 Anchorage individuals were assigned to Kugluktuk and 6/47 Arctic individuals were assigned to Bay Chimo.

Slightly more structure was observed in the Nunavut populations, with 19/40 and 23/67 individuals assigned correctly to Bay Chimo and Kugluktuk, respectively. The majority of the cross-assignments in Kugluktuk went to Bay Chimo (20/67). In Bay Chimo, 7/40 individuals were cross-assigned to both Kugluktuk and Russian Mission, AK.

Of the two British Columbian populations, Revelstoke demonstrated more population genetic structure than did Williston Lake. In Revelstoke, 35/47 individuals were assigned correctly, whereas the Williston Lake population had only 15/37 individuals assigned correctly to itself. In both British Columbian populations the cross-assignments were most likely to go to the other British Columbian region with 5/37 Williston Lake individuals assigned to Revelstoke and 4/47 individuals from Revelstoke assigned to Williston Lake.

Table 3 Upper diagonal represents pairwise F_{ST} values (Weir & Cockerham 1984); lower diagonal as calculated by the GENEPOP 3.1d software package. Lower diagonal is the approximate geographical distances (km) between populations as calculated by the program DISTGEO using approximate latitudes and longitudes of from the centre of each sampled region

	Anch	Arct	Nabe	Rus	Noat	Nom	Bay	Kug	Reve	Will	Idah	Mani
Anch		.0088	.0102	.0017	.0092	.0178	.0346	.0201	.0720	.0344	.2087	.0897
Arcti	730		.0117	.0066	.0044	.0251	.0255	.0182	.0611	.0301	.1961	.0820
Nabe	390	560		.0134	.0093	.0232	.0432	.0290	.0680	.0301	.1787	.0816
RusM	590	980	940		.0088	.0165	.0267	.0135	.0626	.0278	.2157	.0915
Noat	950	865	1140	575		.0247	.0293	.0166	.0651	.0213	.1948	.0943
Nome	875	970	1135	390	230		.0408	.0242	.0692	.0412	.2079	.1016
BayC	2235	1665	1870	2640	2470	2615		.0070	.0597	.0469	.2067	.0915
Kugl	1760	1205	1395	2170	2035	2170	480		.0534	.0272	.1916	.0963
Revel	2245	2285	1940	2840	3070	3075	1920	1780		.0359	.1686	.1205
Will	1605	1670	1295	2200	2430	2430	1700	1415	645		.1670	.1109
Idaho	2885	2980	2610	3475	3745	3735	2515	2445	700	1320		.1838
Manit	3210	2860	2820	3735	3720	3820	1435	1730	1595	1900	1800	

Both Idaho and Manitoba were found to be highly structured populations with 14/14 individuals correctly assigned to Idaho and 23/28 individuals correctly assigned to Manitoba.

Pairwise F_{ST} , and genetic distance measures

Pairwise F_{ST} values ranged from 0.0017 to 0.2157 (Table 3). The smallest F_{ST} values were found within Alaska (0.0017–0.0251) and Nunavut (0.0070). There was also little structure between the Alaskan and Nunavut regions (pairwise F_{ST} ranged from 0.0135 to 0.0432). The highest pairwise F_{ST} estimates were found for the Revelstoke (0.0359–0.0720), Manitoba (0.0816–0.1205) and Idaho (0.1670–0.2157) populations.

Estimates of the likelihood ratio (D_{LR}) and Nei's standard (D_S) genetic distances paralleled the results from both the assignment test and pairwise F_{ST} estimates. D_{LR} values ranged from 0.24 to 8.87 and D_S values ranged from 0.03 to 0.36. The Alaskan populations were all found to be genetically similar using these measures with values of D_{LR} ranging from 0.24 to 0.68 and D_S values ranging from 0.03 to 0.05 with the exception of Nome. Nome was more genetically distinct from other Alaskan populations, with larger genetic distances relative to between the other regions. The two Nunavut populations were also genetically similar ($D_{LR} = 0.25$; $D_S = 0.03$). Pairwise comparisons of the Alaskan populations to the Nunavut populations yielded D_{LR} values ranging from 0.65 to 1.39 and D_S values ranging from 0.05 to 0.10, again suggesting little distinction between these regions. Similar to the findings of the assignment test and pairwise F_{ST} values, these genetic distance measures identified Idaho, Revelstoke and Manitoba as the most genetically distinct regions relative to all other regions sampled.

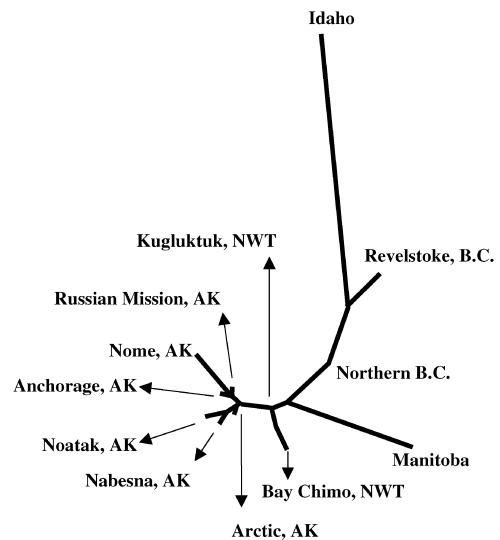


Fig. 2 Unrooted neighbour-joining trees of the D_S genetic distance values (Nei's standard). The length of the tree branches is relative to the genetic distances.

An unrooted neighbour-joining tree of the D_S values is illustrated in Fig. 2 which reveals the relationships of genetic distances to all populations. The length of the tree branches is relative to the genetic distances. The tree shows that the Alaskan populations cluster, the Nunavut populations cluster, the British Columbia populations do not cluster, but were closely associated, and the Manitoba and Idaho populations are each on their own branches.

The results from both genetic distance measures were entered into a two-way Mantel test with geographical distance. The results from these two tests suggest that both genetic distance measures are correlated to geographical

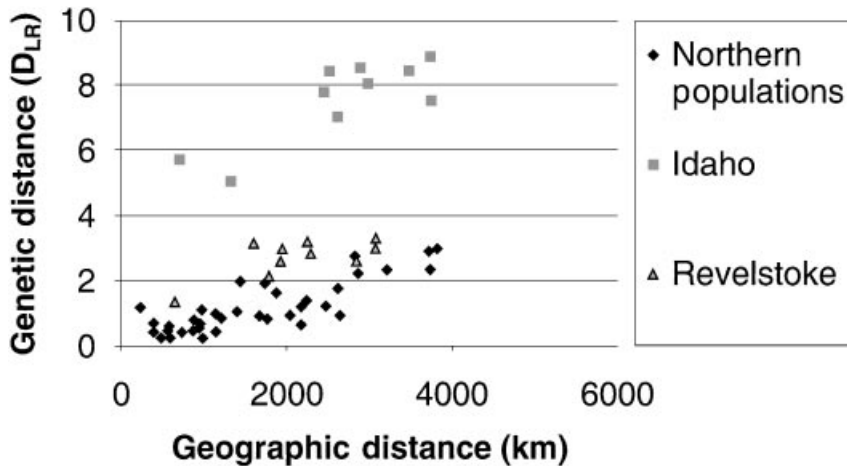


Fig. 3 Relationship of likelihood ratio genetic distance, D_{LR} , to geographical distance.

distance (D_{LR} vs. geographical distance $r = 0.63$ $P = 0.00004$; D_S vs. geographical distance $r = 0.63$ $P = 0.00003$). D_{LR} and D_S were also found to be highly correlated to one another ($r = 0.99$ $P = 0.00009$). The D_{LR} values were also entered into a three-way Mantel test to test for the presence or absence of a barrier to gene flow while controlling for geographical distance. The results revealed that the Idaho population is highly correlated to a barrier to gene flow other than distance ($r = 0.90$ $P = 0.0002$). The Revelstoke population was not as highly associated with a barrier to gene flow other than distance, yet the result was still significant ($r = 0.46$ $P = 0.01$) (Fig. 3).

Discussion

This study has elucidated the population genetic structure of wolverine populations across a large part of their North American distribution. Our results suggest that there is little genetic structuring of populations in the northern regions of this species' range, as reflected by an assignment test, pairwise F_{ST} estimates, and two genetic distance measures, D_S and D_{LR} . Furthermore, we found the more southerly populations, in which anthropogenic factors are most pronounced, to be more genetically structured than those found in the north. These results reflect similar findings to another recent study of wolverines using allozymes (Wilson *et al.* 2000) which revealed little genetic structure between regions in the Northwest Territories using nuclear markers ($F_{ST} = 0.076$). A study by Paetkau *et al.* (1998) of North American brown bears (*Ursus arctos*) also reflects findings similar to those presented here. This is significant as both brown bears and wolverines have a similar distribution, density and potential for dispersal. In both these species, populations in the southern reaches of their range are more genetically distinct, and have less within-population variation, than those found in less

disturbed northern habitats. Our results are also consistent with those found for another mustelid species, the North American pine marten (*Martes americana*). Marten populations sampled from the Yukon and Northwest Territories revealed little population genetic structure and much gene flow was evident across the entire northern sampling range (Kyle *et al.*, 2000). We suggest that the level of genetic structuring in the southern reaches of this species' distribution is the result of low effective population sizes, restricted gene flow and potentially population fragmentation, whereas the low level of structure in the northern regions is consistent with high levels of gene flow and few barriers to dispersal.

Sampling in time and space

It is important to mention, as noted in Materials and methods, that all Alaskan samples collected in the late 1950s to mid-1960s were treated as a contemporary to samples collected in the 1990s. We made the assumption that 30–40 years difference (≈ 6 –8 generations) between the collection times of the samples has had little effect on the genotype frequencies in these regions. There is little evidence to suggest that large demographic changes have taken place during the time separating the collection of these samples. We are unaware of any drastic population bottlenecks or large range expansions that may have greatly affected the genotype frequencies in these Alaskan regions during this time. We have also presented results suggesting that extensive gene flow exists among northern regions. With an extensive mixing of populations genotype frequencies would be less likely to change significantly over a period of six to eight generations. Despite our assumptions, we have no direct evidence that genotype frequencies have not changed in this time, therefore our findings may not reflect current levels of genetic variation and gene flow in these regions.

Table 4 Genetic distances: likelihood ratio genetic distance (D_{LR} , Paetkau *et al.* 1997) in the lower diagonal and Nei's standard genetic distance (D_S , Nei 1972) in the upper diagonal

	Anch	Arcti	Nabe	Russ	Noat	Nom	Bay	Kugl	Reve	Willi	Idah	Mani
Anch		0.04	0.05	0.03	0.04	0.06	0.09	0.06	0.16	0.08	0.36	0.15
Arct	0.42		0.04	0.04	0.03	0.07	0.07	0.05	0.14	0.08	0.32	0.12
Nabe	0.43	0.47		0.05	0.04	0.06	0.10	0.07	0.14	0.06	0.27	0.14
Russ	0.25	0.24	0.55		0.04	0.06	0.07	0.05	0.14	0.07	0.35	0.13
Noat	0.68	0.47	0.44	0.61		0.07	0.08	0.05	0.14	0.06	0.30	0.15
Nome	0.8	1.11	0.99	0.7	1.18		0.10	0.07	0.15	0.09	0.33	0.16
BayC	1.39	0.92	1.63	0.93	1.22	1.76		0.03	0.13	0.11	0.33	0.12
Kugl	0.83	0.85	1.05	0.65	0.94	1.2	0.25		0.12	0.07	0.31	0.13
Reve	3.2	2.83	2.98	2.6	2.99	3.3	2.59	2.14		0.08	0.21	0.18
Will	1.12	1.22	1.08	1.09	1.14	1.71	1.59	1.02	1.35		0.20	0.15
Idah	8.53	8.04	7.01	8.44	7.51	8.87	8.42	7.78	5.71	5.05		0.35
Mani	2.33	2.22	2.75	2.34	2.9	2.98	1.98	1.92	3.15	2.59	8.14	

Lack of genetic structure in northern regions

Little structure was observed among the northern regions sampled in Alaska and Nunavut. Measures of F_{ST} , D_S and D_{LR} among the Alaskan regions revealed the smallest pairwise differences, supporting the conclusions from the assignment test that little structure exists between these regions despite large geographical distances separating them. To put these values into some perspective, although they are not directly comparable as different microsatellite markers were used, the smallest D_S value reported in brown bears by Paetkau *et al.* (1998) was between two adjacent Northwest Territory populations with a value of 0.053. The range of D_S genetic distances among wolverine populations in Alaska and Nunavut vary between 0.03 and 0.07 (Table 4).

The one Alaskan exception, which did reveal a slightly higher level of genetic structure relative to the other Alaskan regions, was Nome. This population had almost 2/3 of the animals sampled assigned to itself compared to only 1/6 to 1/4 of the individuals being correctly assigned in the other Alaskan regions (Table 2). It is unclear why this region would be more structured than other Alaskan regions, although the fact that Nome is found on a peninsular projection of Alaska may have somehow isolated this region.

The two Nunavut regions, Bay Chimo and Kugluktuk, are separated from each other by 400–500 km and reveal a similar pattern to that found in Alaska. There seems to be little genetic structuring between these northern regions, as reflected by the relatively high number of cross-assignments shared between these two regions and the estimates of F_{ST} , D_{LR} and D_S (Tables 3 and 4).

There also seems to be little genetic structure between the Alaska and Nunavut populations. Of the two populations sampled in Nunavut many of the cross-assigned individuals went to Alaskan populations (Table 2). The

genetic distance measures (Table 3) between Nunavut and Alaska are also relatively low, with Kugluktuk having slightly smaller pairwise estimates of genetic distance to the Alaskan populations than Bay Chimo, as follows from their geographical proximity to these regions (Fig. 1).

The lack of genetic structuring in the northern reaches of this species' range is most likely the result of extensive gene flow among these regions. This conclusion is supported by several wolverine life history characteristics: topographical features such as rivers, lakes and mountain ranges do not limit dispersal (Hornocker & Hash 1981; Banci 1987), subadult males are known to disperse large distances from their natal range to establish their own home ranges (Magoun 1985; Copeland, personal communication), and wolverines are capable of moving long distances with relative ease as documented by Gardner (1985), Gardner *et al.* (1986), and Magoun (1985). The combination of these life history characteristics has most likely had the effect of genetically homogenizing wolverine populations in undisturbed habitats in which few barriers to dispersal exist. Only a few migrants per generation are capable of genetically homogenizing populations over time (Slatkin 1985).

An alternative to the aforementioned hypothesis is that the lack of structure observed in the northern reaches of this species' range may not represent extensive gene flow, but rather a relatively recent postglacial colonization of the north. However, wolverines were thought to exist in several refugia during the Wisconsin glaciation, both south of the ice sheets and in Beringia as revealed by fossil evidence (Bryant 1987). The current pattern of genetic structure therefore has most likely not been influenced by a rapid radiation of wolverines northward from a southern glacial refugium.

Without samples from the Yukon or Northwest Territories it is unclear whether the lack of genetic differentiation observed between northern regions in this study is true of

all northern regions. Given that no large barriers to dispersal are present in these areas, and the life history traits of wolverines, we would expect a similar pattern of genetic structure to persist across all of these northern regions east of Hudson's Bay.

Despite the lack of genetic differentiation in the northern reaches of this species distribution we cannot say that all the animals are similar in adaptive traits. Adaptive traits can occur between populations showing little genetic structure at neutral genetic loci (Karhu *et al.* 1996).

Genetic structuring of western Canadian populations

Only two regions were sampled from western Canada, both from British Columbia. These regions, however, revealed very different levels of genetic structure. The Williston Lake population had a relatively high level of cross-assignments to all regions (only 15/37 individuals correctly assigned) and pairwise measures of F_{ST} , D_S and D_{LR} were comparable with those found for the Bay Chimo region in Nunavut (Tables 3 and 4). This central British Columbia region also seemed to be more closely associated with populations sampled in Nunavut and Alaska than the southern British Columbia population of Revelstoke with respect to these estimates (Tables 3 and 4).

The Revelstoke population was found to be relatively distinct from all other regions sampled, with 35/47 individuals correctly assigned to itself (Table 2). A plot of geographical vs. genetic distance (Fig. 3) also revealed that the D_{LR} measures in Revelstoke were higher than expected from the values in the northern regions. This difference was found to be significantly correlated to some barrier to gene flow by a three-way Mantel test ($r = 0.46$ $P = 0.01$).

The difference in genetic structuring between these two regions may potentially be attributed to differences in anthropogenic pressures. Both regions are intensely forested, yet the Revelstoke region also has major transportation corridors present (Canadian Pacific Railway and the Trans Canada Highway) which are known to account for a high percentage of the mortality of wolverines in this area (J. Krebs, personal communication). The Revelstoke region also has more human inhabitation around it than the Williston Lake region. These and other anthropogenic factors may contribute to decreased gene flow to and from the more southerly population. Our results may also reflect that the Revelstoke population simply has a smaller effective population size than more northern populations.

Potential isolation of the Idaho population

The decreased level of H_E and the increased levels of genetic structure based on the assignment test, F_{ST} , D_S and D_{LR} in Idaho are consistent with other studies which have identified insular populations. In a study of black bears

(*Ursus americanus*) Paetkau & Strobeck (1994) found H_E to be $\approx 80\%$ in all regions with one exception. Bears found in Terra Nova National Park, which are isolated on the island of Newfoundland, were found to have an H_E of 49%. A study of brown bears by Paetkau *et al.* (1998) found H_E to range from ≈ 60 to 80% with the exception of two insular populations, Kodiak Island, AK and Yellowstone National Park which had H_E values of 26.5 and 55.4%, respectively. The decreased genetic variability of the Yellowstone brown bears was attributed to population fragmentation in the early 1900s from the larger continuous population in the north. Here, the lack of genetic variation in the Idaho population, relative to the homogenous levels of variation in most other regions sampled suggests that this population may have become fragmented from the larger distribution of wolverines in the north (Table 1). An alternative to this suggestion would be that a historical bottleneck is responsible for the lack of genetic variation found in this population. Although wolverine numbers are thought to have been declining in the southern reaches of their distribution since the 1840s (Hash 1987), there is no evidence that a historical population bottleneck has caused this decrease in genetic variation.

The results from the assignment test (Table 2) also support our suggestion that the Idaho population has restricted gene flow and may be fragmented from the other sampled regions. The Idaho population is only 700 km from the Revelstoke population, yet it reveals no cross-assignments to Revelstoke, or any other regions. This would suggest that there is a potential barrier to gene flow between these two areas other than isolation by distance.

The genetic measures of F_{ST} , D_S and D_{LR} (Tables 3 and 4) all revealed that the Idaho population was the most genetically distinct region sampled. The genetic distance estimates from Idaho to all other regions are not proportional to the geographical proximity of this population to others (Fig. 3). When values of D_{LR} were entered into a three-way Mantel test, controlling for distance, it was found that these values were strongly and significantly associated with a barrier to gene flow ($r = 0.932$ $P = 0.0012$).

In the conterminous US wolverines are only thought to persist in isolated regions of Montana, Idaho, Wyoming, Colorado, Oregon and California from a once continuous population ranging as far south as Arizona and New Mexico (Hash 1987), supporting the suggestion that this population may be fragmented. The Idaho population also presents a similar example to that of insular Yellowstone brown bears (Paetkau *et al.* 1998). A recent study by Edelman & Copeland (1999) also suggests that the central Idaho population may be isolated from other north-west US wolverine populations. As wolverine dispersal is not thought to be limited by topographical features (Banci 1994), the fragmentation of southern populations may be attributed to habitat loss, overharvest and other anthropogenic factors as suggested by Banci (1994) and Wilson (1982).

In the American north-west, more populations will be needed to determine if these regions all represent genetically insular groups or if gene flow does occur among them and the more northern populations. It is not clear whether other remaining north-western US populations are presently self-sustaining populations or if they are dependent on emigration from Canadian populations (Montana, Newby & McDougal 1964; Idaho, Groves 1988; Oregon, Johnson 1977). It would appear that the surveyed Idaho population is not maintained by Canadian immigrants, as they are genetically distinct from all other sampled populations. It should be noted, however, that the most likely dispersal corridors for wolverines in the American north-west run north/south in the mountain ranges, as most valleys are developed with transportation corridors and human habitation, potentially restricting wolverine movements. The north/south corridor from Idaho to Canada would run into Glacier National Park in southern Alberta. Samples were not available from this region and so we were unable to determine whether animals from central Idaho are genetically connected to them.

The conclusions from the analyses of the Idaho populations must be tempered, however, due to the fact that only 14 individuals were sampled. A small sample size may potentially skew results by providing genotype frequencies that do not reflect those actually present in the population and making the population appear more distinct than it is. A further bias may also have been introduced into our results by including two known relationships in this sample. A potential father was also identified for these relationships. Despite the inclusion of these relationships we still believe that the Idaho population is genetically depauperate as all other potential fathers for the identified mother/offspring relationships matched at 13–15 of 16 loci (data not shown), reflecting the level of genetic relatedness in this region. Furthermore, running the data with and without the known relationships had little effect on our presented results (data not shown).

Eastern populations

In the east only one population from Manitoba was sampled. This population was found to be genetically distinct from all other regions in this study. The two-way Mantel tests suggests that the genetic distances observed for this population are consistent with isolation by distance. However, with no immediately adjacent populations sampled in the east we are unable to say that Manitoba is not genetically isolated from other wolverine populations.

Conclusions

Although this study elucidated the levels of genetic structure between wolverine populations, we are unable to

fully describe the likely complicated local social structure of wolverines. Local wolverine groups, which may exist with juvenile females establishing home ranges adjacent to their mothers, will not be revealed by sampling over a broad scale, as in this study.

This study has shown that populations of wolverines that are exposed to fewer anthropogenic factors potentially have much gene flow among them. In direct contrast, the southern populations of Idaho and to a certain degree Revelstoke, which are exposed to greater pressure from humans (settlement, road systems, recreation in remote areas, and so on), seem to be more genetically isolated. The viability of these populations seems to be dependent, to a great extent, on large areas of undisturbed habitat and corridors among them.

Acknowledgements

We would like to thank the following people for providing samples for this study: John Krebs and Davis Lewis from the Columbia Basin Fish & Wildlife Compensation Program, Don Reid and Eric Lofroth (British Columbia Ministry of the Environment) from the Northern Wolverine Project, Rob Mulders and Albert Bourque from the Department of Resources, Wildlife and Economic Development, Northwest Territories, the University of Alaska Museum (Dr J. Cook and Dr G. Jarrell), Jeff Copeland, Idaho Fish & Game, Neil Dawson (Ontario Ministry of Natural Resources), and Barry Verbiwski (Manitoba Wildlife Branch). We would also like to thank Dr D.W. Coltman, two anonymous reviewers and Dr R.K. Wayne for their comments which have greatly improved this manuscript. This research was funded by a NSERC and Parks Canada grants to C. Strobeck.

References

- Banci VA (1987) *Ecology and Behaviour of Wolverine in the Yukon*. MSc Thesis. Simon Fraser University, Burnaby, B.C.
- Banci VA (1994) Wolverine. In: *The Scientific Basis for Conserving Forest Carnivores, American Marten, Fisher, Lynx, and Wolverine in the Western United States* (eds Ruggiero LF, Aubry KB, Buskirk SW, Lyon LJ, Zielinski WJ), pp. 99–127. United States Department of Agriculture, Forest Service, Rocky Mountain Range Experiment Station, General Technical Report, RM-254. Fort Collins, CO.
- Banci VA, Harestad AS (1988) Reproduction and natality of wolverine (*Gulo gulo*) in Yukon. *Annals Zoologica Fennici*, **25**, 265–270.
- Bryant HN (1987) Wolverine from the Pleistocene of the Yukon: evolutionary trends and taxonomy of *Gulo* (Carnivora: Mustelidae). *Canadian Journal of Earth Sciences*, **24**, 654–663.
- COSEWIC (1998) Canadian species at risk. Committee on the Status of Endangered Wildlife in Canada, Ottawa, ON. 18 pp.
- Dallas JF, Pieltney SB (1998) Microsatellite primers for the Eurasian otter. *Molecular Ecology*, **7**, 1248–1251.
- Davis CS, Strobeck C (1998) Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. *Molecular Ecology*, **7**, 1776–1778.
- Duffy AJ, Landa A, O'Connell M, Stratton C, Wright JM (1998) Four polymorphic microsatellites in wolverine, *Gulo gulo*. *Animal Genetics*, **29**, 63–72.

- Edelman F, Copeland J (1999) Wolverine distribution in the northwestern United States and a survey in the Seven Devils Mountains of Idaho. *Northwest Science*, **73**, 295–300.
- Felsenstein J (1995) *PHYLIP (Phylogeny Inference Package)*, Version 3.572. Department of Genetics, University of Washington, Seattle.
- Flemming MA, Ostrander EA, Cook JA (1999) Microsatellite markers for American mink (*Mustela vison*) and ermine (*Mustela erminea*). *Molecular Ecology*, **8**, 1352–1354.
- Gardner CL (1985) *The Ecology of Wolverines in Southcentral Alaska*. MSc Thesis, University of Alaska, Fairbanks.
- Gardner CL, Ballard WB, Jessup RH (1986) Long distance movement by an adult wolverine. *Journal of Mammalogy*, **67**, 603.
- Groves CR (1988) Distribution of the wolverine in Idaho as determined by mail questionnaire. *Northwest Science*, **62**, 181–185.
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.
- Hartl DL, Clark AG (1989) *Principles of Population Genetics*. Sinauer, Sunderland, MA.
- Hash HS (1987) Wolverine. In: *Wild Furbearer Management and Conservation in North America* (eds Novak M, Baker JA, Obbard ME, Malloch B), pp. 575–585 Ontario Ministry of Natural Resources, Ontario.
- Hornocker MG, Hash HS (1981) Ecology of the wolverine in northwestern Montana. *Canadian Journal of Zoology*, **59**, 1286–1301.
- Johnson RE (1977) An historical analysis of wolverine abundance and distribution in Washington, USA. *Murrelet*, **58**, 13–16.
- Karhu A, Hurme P, Karjalainen M *et al.* (1996) Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? *Theoretical and Applied Genetics*, **93**, 215–221.
- Krott P (1982) The wolverine (*Gulo gulo* Linnaeus 1758) in the ecosystem. *Saugetierkundliche Mitteilungen*, **30**, 136–150.
- Kyle CJ, Davis CS, Strobeck C (2000) Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories. *Canadian Journal of Zoology*, **78**, 1150–1157.
- LeDoux RG, Kenyon AJ (1973) Protides of the Mustelidae: comparative study of plasma lactate dehydrogenases. *Comparative Biochemistry and Physiology, Part B.*, **45**, 225–231.
- Magoun AJ (1985) *Population characteristics. Ecology and Management of Wolverines in Northwestern Alaska*. PhD Thesis, University of Alaska, Fairbanks.
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- Nei M (1972) Genetic distances between populations. *American Naturalists*, **106**, 283–292.
- Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics*, **76**, 379–390.
- Newby FE, McDougal JJ (1964) Range extension of the wolverine in Montana. *Journal of Mammalogy*, **45**, 485–486.
- Paetkau D, Amstrup C, Born EW *et al.* (1999) Genetic structure of the world's polar bear populations. *Molecular Ecology*, **8**, 1571–1584.
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.
- Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489–495.
- Paetkau D, Waits LP, Clarkson LP *et al.* (1998) Variation in genetic diversity across the range of North American brown bears. *Conservation Biology*, **12**, 418–429.
- Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.
- Raymond M, Rousset F (1995) GENEPOP (Version 3.1d): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Seal US (1969) Carnivore systematics: a study of hemoglobins. *Comparative Biochemistry and Biochemistry*, **31**, 799–811.
- Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.
- Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edn. WH Freeman, New York.
- Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology and Evolution*, **13**, 43–44.
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Wilson DE (1982) Wolverine. In: *Wild Mammals of North America. Biology, Management and Economics* (eds Chapman JA, Feldhamer GA), pp. 744–652 John Hopkins University Press, Baltimore.
- Wilson GM, Van Den Bussche RA, Kennedy PK, Gunn A, Poole K (2000) Genetic variability of wolverines (*Gulo gulo*) from the Northwest Territories, Canada: conservation implications. *Journal of Mammalogy*, **81**, 186–196.

This project is one of several ongoing population genetic studies of mustelid species by Christopher Kyle as part of his PhD thesis. Various other projects on worldwide mammalian species are currently taking place in Dr Curtis Strobeck's laboratory.
