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Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations

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Abstract

Wolverine (*Gulo gulo*) numbers in Scandinavia were significantly reduced during the early part of the century as a result of predator removal programmes and hunting. Protective legislation in both Sweden and Norway in the 1960s and 1970s has now resulted in increased wolverine densities in Scandinavia. We report here the development of 15 polymorphic microsatellite markers in wolverine and their use to examine the population sub-structure and genetic variability in free-ranging Scandinavian wolverine populations as well as in a sample of individuals collected before 1970. Significant subdivision between extant populations was discovered, in particular for the small and isolated population of southern Norway, which represents a recent recolonization. Overall genetic variability was found to be lower than previously reported for other mustelids, with only two to five alleles per locus and observed heterozygosities (H_O) ranging from 0.269 to 0.376 across the examined populations, being lowest in southern Norway. Analysis of the mitochondrial DNA control region revealed no variation throughout the surveyed populations. As the historical sample did not show higher levels of genetic variability, our results are consistent with a reduction in the genetic variation in Scandinavian wolverines that pre-dates the demographic bottleneck observed during the last century. The observed subdivision between populations calls for management caution when issuing harvest quotas, especially for the geographically isolated south Norwegian population.

Keywords: bottleneck, genetic diversity, microsatellite, Mustelidae, phylogeography, population

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Introduction

The geographical distribution of the wolverine (*Gulo gulo*) originally included most of the taiga and tundra zones of the Holarctic (Nowak 1991; Banci 1994), throughout which it is considered a single species. While smaller than most large carnivores, typically 8–18 kg, it is capable of taking prey that are several times its own body weight (Nowak 1991). As such, the wolverine has been faced with human persecution similar to other large carnivores in Nordic countries due to its habit of preying upon domestic livestock, primarily semi-domestic reindeer (*Rangifer tarandus*, Bjärvall *et al*. 1990) and domestic sheep (*Ovis aries*, Landa & Tømmerås 1996).

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Historically the wolverine has been distributed throughout mountainous and forested habitats in Scandinavia, including the southern-most areas of Norway (Johnsen 1928) and as far south as Värmland in Sweden (Lönnberg 1936). As a result of the predation on livestock, bounties were offered in Sweden from 1827 and in Norway from 1845. The statistics relating to these state-paid bounties reveal a substantial downward trend in the number of wolverines killed (Fig. 1). Bounty figures offer a reasonable indication that the total population was decreasing, at least to the late 1960s or early 1970s, when protective legislation was passed to protect the wolverine (Landa & Skogland 1995; Sandell 1995). The decrease in population size has been associated with contracted distribution, and, for instance, Landa & Skogland (1995) reported that the wolverine in southern Norway was functionally extirpated in 1967. A subsequent re-colonization event occurred

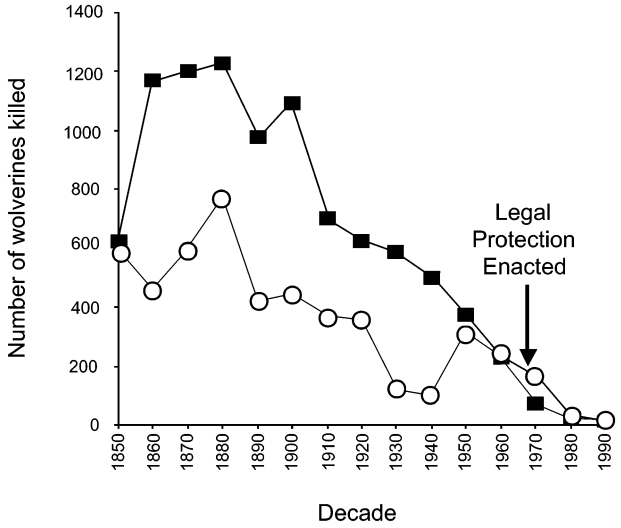

Fig. 1 Hunting statistics for wolverines by decade from 1846 to 1992 in Sweden (■) and Norway (○) (based on Landa & Skogland 1995).

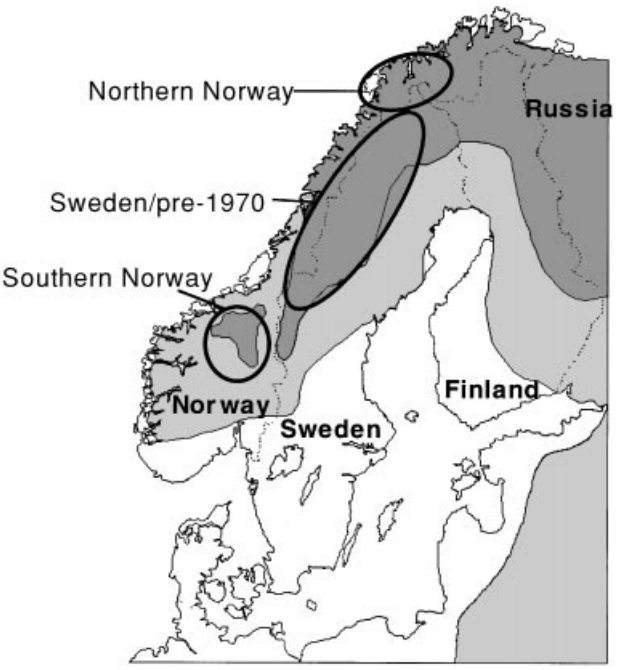

Fig. 2 Wolverine distribution in Europe in the middle of the 19th century (light grey) and current distribution (dark grey) (based on Landa *et al*. 1999).

in this area in the late 1970s (Kvam 1980; Landa 1997), which resulted in a small but still extant population there (Kvam *et al*. 1988).

While protection has resulted in an apparent increase in the overall numbers of wolverines in Scandinavia (Heggberget & Myrberget 1980; Landa 1997), densities are still lower than at the turn of the century and the species has not re-occupied all of its former range (Landa & Skogland 1995; Landa *et al*. 1999) (Fig. 2). Recent estimates place the total number of wolverines in Scandinavia at approximately 400. Of that number, approximately 250 are in Sweden and 150 in Norway (Landa *et al*. 1998). The wolverines in the Snøhetta region of southern Norway, with about 30 animals (Landa *et al*. 1998, 1999), are geographically separated from the northern populations.

While many wild carnivore populations are faced with possible extinction due to loss or fragmentation of habitat, the wolverines in Scandinavia have experienced population reduction and fragmentation primarily due to predator removal programmes. A major factor associated with this fragmentation into small populations is changes in genetic variation, an essential component for both the short- and long-term persistence of populations (Lande 1988). The implications are twofold. First, small populations have an increased probability of losing genetic variation due to stochastic processes, whether demographic (due to differential survival and reproduction), environmental (due to variation in the biotic or physical environment) or genetic (due to inbreeding, genetic drift, founder effects, etc.) (Frankel & Soulé 1981; Shaffer 1981, 1987; Brussard & Gilpin 1989). Second, populations with more profound spatial fragmentation should show greater amounts of between-population heterogeneity than populations with a more continuous distribution (Nei *et al*. 1975; Hartl & Clark 1989).

Of the four large carnivores present in Scandinavia, including the wolf (*Canis lupus*), brown bear (*Ursus arctos*) and lynx (*Lynx lynx*), the wolverine is probably the least known in terms of its biology and population genetic structure. This project was initiated to characterize the degree of genetic variability in the extant population as well as to compare that variability with wolverines sampled earlier in the century. If wolverines had gone through a bottleneck in Scandinavia in recent decades, we would expect overall lower genetic diversity compared to samples from earlier in the century. In addition to quantifying the genetic diversity of the entire population, comparisons of variation seen in local populations were performed to test the hypothesis that wolverines in Scandinavia are genetically subdivided, as might be suggested by geographical discontinuity. Finally, the population in southern Norway was compared to the larger northern populations in order to characterize the reduction of variation resulting from a recent founder event. If this population was founded by a low number of immigrants from the northern population, then we would expect both reduced genetic variation and potentially significant differentiation. However, if gene flow is ongoing, then the expected genetic differentiation should be small.

Materials and methods

Sample collection

As wolverines are known to have a high mobility and their range in Scandinavia has expanded in the last 30 years, we decided to consider just three geographical regions. The boundaries between regions corresponded to a gap in the distribution of the species (for southern Norway) and to a limit separating northern (northern Norway) and southern samples (Sweden), that also corresponded to the mountain ranges between the two Scandinavian countries (Fig. 2). The Swedish population consisted of 51 wolverine blood or tissue samples from the counties of Norrbotten, Västerbotten and Jämtland collected between 1978 and 1997. The northern Norway population consisted of 87 wolverine blood or tissue samples collected between 1983 and 1998 in the counties of Finnmark, Troms, Nordland and Nord-Trøndelag. The southern Norwegian population consisted of 21 wolverine tissue samples collected between 1983 and 1996 from the counties of Møre, Oppland, Hedmark, Romsdal and Sør-Trøndelag. This southern Norwegian population is from an area including the Snøhetta reindeer area and was presumably founded in the late 1970s. All samples were collected either from dead animals (tissue biopsies) or from animals captured as part of ongoing radio-telemetry projects. Consequently, sampling bias due to inclusion of multiple samples from the same individual has been effectively eliminated. A pre-1970 Swedish population was also included and consisted of an additional 10 tissue samples collected from the same counties as the extant Swedish population in 1922, 1928, 1929, 1932, 1934, 1934, 1938, 1965, 1968(2). The 1970 division between historical and current samples was chosen as this is the approximate date when wolverines came under legal protection and this date seems to correspond to the minimum population size. The pre-1970 samples were all from tanned or dried skins, a small piece of which had been subsequently stored in ethanol.

Isolation and analysis of microsatellites

Total genomic DNA was extracted from the samples via proteinase K digestion and phenol/chloroform extraction using the methods of Hillis *et al*. (1996). A total of 10 μg DNA pooled from five individuals was digested to completion with the restriction enzyme *Mbo*I following the manufacturer's protocols (Pharmacia). The resulting fragments were size-fractionated by electrophoresis in a 1% agarose gel and then purified from the gel slice containing the 400–1100 bp fragments (QIAex*II*, Qiagen). The size-fractionated wolverine DNA fragments were ligated overnight at 14 °C in equimolar amounts with *Bam*HI-digested pUC19 vector (Pharmacia). The ligands were then transformed into *Escherichia coli* DH5-α cells by electroporation.

Blue and white screening was used to estimate that about 7500 recombinant clones were grown, and these clones were subsequently transferred to Hybond N+ (Amersham) nylon membranes following the manufacturer's protocols. Filters were pre-hybridized at 65 °C for 1 h in a buffer containing 0.26 M Na_2HPO_4, 1% BSA, 1 mM EDTA-Na_2 and 7% SDS, before adding a mixture of radiolabelled simple repeat motif oligonucleotide probes. The probe mixture contained 50 pmol each of $(CA)_{15}$ and $(GA)_{15}$ oligonucleotides previously end-labelled with [γ^{32}P]dATP in a reaction containing T4 polynucleotide kinase following the method of Sambrook *et al*. (1989). Hybridization was allowed to continue for 12–16 h. After hybridization, filters were washed for 5 min in 2 × SSC, 0.1% SDS at room temperature, followed by successive washes for 15 min at 55 °C with a final stringency of 0.3 × SSC, 0.1% SDS. Positive colonies were identified by autoradiography. Stripped filters (boiled in 0.5% SDS) were then re-hybridized using a probe mixture containing 10 pmol of each of the following radiolabelled simple repeat motif oligonucleotides: $(GATA)_8$, $(GGAA)_8$, $(GAAT)_8$, $(GGAT)_8$, $(AAAG)_8$ and $(AAAT)_8$. Filters were washed and subjected to autoradiography as above. Clones identified as positive were grown in 5 mL overnight cultures and plasmids were purified using a JET STAR (Genomed) plasmid purification kit. The resulting purified plasmids were then sequenced by dye primer cycle sequencing chemistry (Perkin Elmer) using M13 forward and/or reverse primers, and recorded using an ABI 377 sequencer (Perkin Elmer).

Primers for polymerase chain reaction (PCR) amplification of microsatellites were designed with the aid of the computer package OLIGO (National Biosciences Inc., version 4.0) for clones that contained a minimum of 13 uninterrupted tandem repeats (exceptions, see Gg466 and Gg470, Table 1). One primer from each pair was end-labelled with [γ^{32}P]dATP (as above), using 0.5 μCi isotope per pmol of primer. PCR amplifications were carried out on a Techne PHC3 thermal cycler in 10 μL reactions containing 2 pmol of each primer, 0.25 units AmpliTaq (Perkin Elmer), 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% w/v gelatin, 30 mM dNTP and 25 ng genomic DNA. PCR profiles comprised 3 min denaturation at 94 °C followed by 32 cycles of 30 s at 94 °C, 45 s at the corresponding annealing temperature (Table 1), and 75 s at 72 °C. A final extension at 72 °C for 5 min followed all reactions. Following PCR, the resulting products were diluted with 4 μL formamide loading dye before loading 3 μL on a standard 6% denaturing polyacrylamide sequencing gel and visualization by autoradiography. Several individuals previously characterized were run on all gels

Table 1 Microsatellite loci characterized in wolverine (*Gulo gulo*). Motifs and length refer to the cloned alleles

Locus	GenBank accession no.	Primer sequence (5′ → 3′)	Repeat motif	Length (bp)	T_a (°C)	N	Number of alleles	H_E	H_O
Gg10*	AF247747	F: TTGCAAAACTACAGGACATT R: CCTGGTACAGGTTTTATATCAG	$(CA)_{22}$	176	55	173	5	0.522	0.566
Gg14*	AF247748	F: GCCAGTTTTTACCCATCT R: GCCATTAAGAAAGTATCAAG	$(CT)_{12}(CA)_{15}$	199	53	172	5	0.570	0.547
Gg25	AF247750	F: GGCTGAGTAATATTCCATCA R: GCACCTTCTTAAAGAGCTAT	$(CA)_{16}$	165	60	172	4	0.573	0.581
Gg37	AF247754	F: GATCCTCACAGTGCCACACA R: GAGCCAGTACAAGTGAAGACC	$(CA)_{16}$	200	60	172	3	0.192	0.186
Gg42	AF247753	F: CCGTGCTACTTTTCATTCAG R: CCCAATTCCCTCTTAACCA	$(CA)_{15}$	203	52	172	3	0.388	0.384
Gg50	AF247746	F: AGCCACTTCACTGTTCCAAG R: GCGCCCCTATAAACTTAT	$(CA)_{16}$	225	59	170	2	0.023	0.024
Gg192	AF247749	F: GAGCCAATTCCTTATATCTC R: CCCTTTTTCATAACCAGAGT	$(CA)_{16}$	186	55	171	3	0.023	0.012
Gg443	AF247752	F: GATCATGTTTGCAATTAAATGT R: GATCCTCCGGTAACTGTTGT	$(CA)_{14}$	95	58	166	4	0.433	0.422
Gg452	AF247751	F: GATCTGTCACTAAGCACACA R: TCCGGTCAATAGCCAGT	$(CA)_{14}$	117	58	162	4	0.681	0.630
Gg454	AF247757	F: CTTCTTACATAGTCAATGTTTTG R: TGCCATTTTCTCCAGAA	$(CA)_{20}$	137	56	161	5	0.685	0.634
Gg465	AF247758	F: GATCTTCACAAACAAGCTTC R: GATCTCCTTTCCTCTCTTTG	$(CA)_{15}(CT)_{14}$	183	58	171	4	0.585	0.544
Gg466	AF247759	F: GCCCCTCACCTAGAAAGAAC R: CTGCGTCTAAGGATTGAGTG	(CA) repetitive element 210 bp	286	58	165	2	0.407	0.364
Gg470	AF247756	F: GGCATTGCACCTTTCTAG R: CCAATTACAATGTGACCATGAAG	$(CA)_7TA(CA)_7$	116	58	171	2	0.426	0.298
Gg471	AF247755	F: CCAGAATTTAAAATCACATA R: AATTTCTCTGTCTTATATGC	$(CA)_{14}$	115	56	163	3	0.365	0.380
Gg473	AF247760	F: GGAAAACCTAAATTGTTTAG R: CTTCCACAAGTCATTTAGTA	$(CA)_{14}$	138	56	157	2	0.305	0.312
Lut604†	Y16300	F: TTTCAACAATTCATGCTGGAAC R: TATGATCCTGGTAGATTAACTTTGTG	$(CA)_{15}$	78	55	171	2	0.428	0.398
Lut615†	Y16301	F: ATTCTCTTTTGCCCTTTGCTTC R: TGCAAAATTAGGCATTTCATTCC	$(CA)_{14}$	182	55	172	2	0.130	0.128

*Loci found to be linked; †from Dallas & Piertney (1998). F, forward primer; R, reverse primer; T_a (°C), annealing temperature; N, number of individuals scored; H_E, expected heterozygosity; H_O, observed heterozygosity.

to ensure standardization during scoring. For Lut604 and Lut615, as the originally cloned alleles were not available in our laboratory, two alleles were sequenced from each locus in order to determine the structure of the repeat.

Statistical analysis of microsatellite data

Microsatellites were tested for linkage disequilibrium using an exact test based on a Markov chain algorithm as implemented in the program GENEPOP version 3.1 (Raymond & Rousset 1995). The test was performed for each population (excluding pre-1970 because of its small sample size), and the statistical significance was assessed using Bonferroni's correction (Rice 1988).

Genetic variation in terms of observed heterozygosity (H_O) and Nei's unbiased expected heterozygosity (H_E) under Hardy–Weinberg equilibrium (HWE), as well as their standard errors (SE), were calculated using the program BIOSYS-1 version 1.7 (Swofford & Selander 1989). To test whether the differences in H_O and the average number of alleles were due to sample size bias, 100 random draws of 10 individuals (pre-1970 was the smallest sample with only 10 individuals included) from each of the extant populations were made. The average number of alleles, H_E and H_O were calculated for each of these groups of 10, and tests for significant differences between populations were done using the Kruskal–Wallis and Mann–Whitney tests. Ninety-five per cent confidence intervals were calculated and compared with the value

observed for the pre-1970 sample. An exact test based on a Markov chain algorithm was conducted to test deviations from HWE (Guo & Thompson 1992) across loci in each of the four populations, using the software package GENEPOP.

We used an exact test of genic differentiation to study the significance of the differentiation across populations, as implemented in the program GENEPOP. As populations have passed through a demographic bottleneck that may have induced strong genetic drift and therefore the random survival of different alleles, independent of ancestry, we used an infinite allele model approach when estimating genetic differentiation (Slatkin 1993; Jarne & Lagoda 1996) rather than the stepwise mutation model (Slatkin 1995). Genetic differentiation between populations under the infinite alleles model was calculated using the θ estimator (Weir & Cockerham 1984) of Wright's F_{ST} (Wright, 1965), using the program FSTAT version 1.2 (Goudet 1995). The pairwise differentiation between populations was characterized using pairwise estimates of θ. The significance of these values was tested comparing the observed value with the distribution of values obtained from 1000 permutations of the individuals in the two populations. The level of gene flow between populations was estimated as the number of migrants per generation, Nm, where N is the effective population size and m the migration rate per generation. Nm was estimated from θ using the expression $\theta = 1/(1 + 4Nm)$ (Wright 1978; Hedrick 2000). These tests and estimations were performed using the program GENETIX (Belkhir *et al*. 2000). The pairwise θ values were used as distances to construct an unrooted phenogram in PAUP* 4.0 (Swofford 1998) using the neighbour-joining algorithm of Saitou & Nei (1987). To analyse the support of the groupings presented by this tree, we built a consensus tree from 150 bootstrap replicates of the distance matrix generated by the program MICROSAT (http://human.stanford.edu/microsat).

To test for evidence consistent with recent bottlenecks in all population samples, the program BOTTLENECK (Cornuet & Luikart 1997; Luikart & Cornuet 1998) was used to perform a Wilcoxon sign-rank test on both measures of heterozygosity. This program calculates the expected heterozygosity for each locus and population based on the number of alleles and population size assuming mutation-drift equilibrium, and then tests for significant heterozygosity excess or deficiency for each population.

Mitochondrial DNA single-strand conformation polymorphism (SSCP) and sequence analysis

A 338 bp fragment of mitochondrial DNA (mtDNA) control region I (left domain) was amplified from genomic DNA preparations from all individuals, using primers L15997 (5′-GCCATCAACTCCCAAAGCT-3′) and H16498 (5′-CCTGAAGTAGGAACCAGATG-3′; Ward *et al*. 1991). PCR amplifications were conducted in 10 μL reaction mixtures similar to those for microsatellites. Resulting products were diluted with formamide loading dye and separated on an 8% non-denaturing polyacrylamide gel for 12–17 h at constant power (1 W) and temperature (6 °C). Individual bands were visualized by silver-staining (Bassam *et al*. 1991) and selected electromorphs were sequenced as above.

Results

Microsatellite development

Eighty-five positive clones from the wolverine DNA library of approximately 7500 clones were sequenced, from which 22 pairs of primers were constructed. Seventeen of these successfully amplified microsatellite loci. Two of the 17 primer pairs detected monomorphic loci and the remaining 15 (Table 1) were used to analyse the genetic variability in the Scandinavian wolverine population. All polymorphic microsatellites contained CA as the repetitive element. Two additional microsatellite loci, Lut604 and Lut615, developed for *Lutra lutra* and shown to be polymorphic in wolverines (Dallas & Piertney 1998), were also scored (Table 1) and included in the analysis. Linkage disequilibrium analysis (data not shown) suggested that two of these loci were linked (Gg10 and Gg14) and one of these (Gg14) was removed from subsequent analyses, which thus included the remaining 16 loci.

Population variability and structure: microsatellites

Microsatellites showed low levels of polymorphism, with the total number of alleles per locus ranging only from two to five (Table 1). The average number of alleles per locus for all populations combined was 3.0 ± 0.29 ($\pm$ SE), with the highest and lowest in Sweden and in the pre-1970 sample, respectively (2.88 ± 0.26 and 2.19 ± 0.16; Table 2). The historic sample also had the lowest H_O (0.269 ± 0.05), the mean for all populations being 0.368 ± 0.050. As the variation in at least the number of alleles between populations could be due in part to differences in sample size, 100 random draws of 10 individuals were analysed for each extant population (Table 2). The observed heterozygosity in the pre-1970 sample was lower than the three 95% confidence intervals calculated for the extant populations after 100 random draws. Moreover, the average number of alleles per locus for the pre-1970 sample was lower than the corresponding confidence intervals for two of the extant populations (northern Norway and Sweden).

	H_E	H_O	Average number of alleles
Northern Norway	0.393 (0.053) **0.393; 0.350–0.436**	0.376 (0.051) **0.376; 0.307–0.445**	2.63 (0.30) **2.43; 2.27–2.58**
Southern Norway	0.347 (0.057) **0.345; 0.304–0.386**	0.347 (0.064) **0.346; 0.277–0.415**	2.50 (0.26) **2.21; 2.00–2.41**
Sweden	0.369 (0.049) **0.371; 0.324–0.418**	0.374 (0.055) **0.374; 0.319–0.429**	2.88 (0.26) **2.51; 2.30–2.71**
Pre-1970	0.348 (0.572)	0.269 (0.050)	2.19 (0.16)

Table 2 Expected H_E and observed H_O heterozygosities, and average number of alleles per locus (standard errors in parentheses). Figures in bold are mean values from 100 random samplings of 10 individuals from each extant population and their 95% confidence intervals

Population sample	Southern Norway	Northern Norway	Sweden	Pre-1970
Southern Norway	—	5.42	2.59	1.51
Northern Norway	0.044*	—	10.46	4.03
Sweden	0.088*	0.023*	—	2.70
Pre-1970	0.142*	0.058 (NS)	0.085*	—

*Significantly different from 0 at $P < 0.01$; NS, not significantly different from 0.

Table 3 F_{ST} estimated by θ (Weir & Cockerham 1984), below the diagonal, and number of migrants per generation (Nm) calculated from θ, above the diagonal

The average values for H_E and H_O in extant populations remained almost the same after random draws and proved to be significantly different between the populations (Kruskal–Wallis, $P < 0.001$). In pairwise comparisons, both northern Norway and Sweden showed significantly higher heterozygosities than southern Norway (Mann–Whitney, $P < 0.001$ in both cases). The same pattern was seen for the average number of alleles, i.e. a significantly larger number of alleles in both northern Norway and Sweden than in southern Norway (Mann–Whitney, $P < 0.001$).

Allelic frequencies across loci were not significantly different from expectations under Hardy–Weinberg equilibrium for any population (exact test, $P > 0.05$ in all cases). The results of both an exact test ($P < 0.001$) and F-statistics revealed significant substructure among the population samples. The mean global θ estimate of F_{ST} was 0.045 (95% confidence interval 0.024–0.066). F_{ST} showed statistically significant population differentiation for all possible pairwise comparisons (Table 3), except for the comparison between northern Norway and the pre-1970 sample. Despite the significant structuring, the number of migrants per generation between populations indicates strong gene flow between Sweden and northern Norway, i.e. about 10 migrants per generation (Table 3). An unrooted neighbour-joining tree, based on F_{ST} distances, placed southern and northern Norwegian populations on the same branch with a bootstrap support of 91% (Fig. 3). Both southern

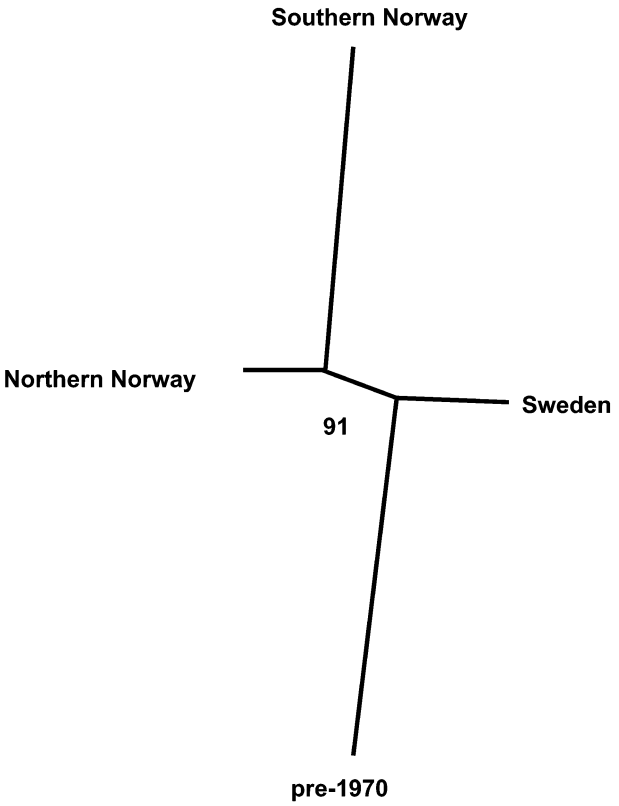

Fig. 3 Neighbour-joining phenogram using θ estimates of F_{ST} as distances. Numbers at nodes represent the support derived from 150 bootstrap replicates.

Norway and the pre-1970 sample are characterized by very long branches, suggestive of population differentiation.

The Wilcoxon sign-rank tests for heterozygosity excess (consistent with a recent bottleneck) within each of the population samples revealed a significant deviation from mutation-drift equilibrium in all population samples except the pre-1970 sample (southern Norway, $P = 0.029$; northern Norway, $P < 0.001$; Sweden, $P = 0.022$; pre-1970, $P = 0.360$). As the sample size for the pre-1970 sample is very limited, the test for this population is very conservative. Five alleles private to either of the three extant or to the historic population were found, one in southern Norway, one in the pre-1970 sample and three in Sweden (Appendix I). Additionally, one allele was only found in Sweden and in the pre-1970 sample. On the other hand, three alleles were missing in southern Norway and nine in the pre-1970 sample. In seven cases, one population was monomorphic for a microsatellite locus.

Population variability and structure: mitochondrial DNA

Only a single SSCP electromorph was identified for a 338 bp mtDNA control region fragment analysed among all 169 wolverines. Subsequent sequencing of two individuals from each of the populations confirmed that they were identical in sequence (GenBank accession number AF245496).

Discussion

Population structure in Scandinavian wolverines

The analysis of population structure among Scandinavian wolverines revealed significant differentiation between extant populations. Despite this, levels of gene flow appeared high and it is likely that the detection of significant differentiation was a consequence of the use of a relatively large number of microsatellite markers. The estimated effective number of migrants per generation was highest (above 10) between Sweden and northern Norway, and these animals may thus partly be seen as belonging to the same interbreeding population (Fig. 2). However, restricted mobility due to limited dispersal capabilities or habitat fragmentation can prevent complete panmixia, inducing differentiation with distance even in a continuous distribution.

Gene flow was estimated to be lower between southern Norway and the other two other extant populations, consistent with its geographical isolation. Probably because of founding effects (Hedrick 2000), this population had a significantly lower average number of alleles and lower heterozygosity than the other populations. In other words, only part of the genetic variability available in the source population may have been transmitted to the south Norwegian population when it was founded in the late 1970s. In line with this, no private alleles were found in the latter population, and three alleles that were found in the other populations were absent here (Appendix I).

Demographic fluctuations

The comparison of samples collected before the demographic minimum with extant samples fails to provide evidence of a decrease of genetic variability over time. In fact, the average number of alleles was smaller for the historic sample and the observed heterozygosity was lower than in the extant populations, suggestive of a recent increase in genetic diversity. This has to be treated with caution since the observed and expected heterozygosities were quite different in the historic sample (0.269 versus 0.348, Table 2). Low quality of the historical samples might have resulted in frequent allelic drop-outs (Taberlet *et al*. 1996), i.e. one allele not amplifying in heterozygous individuals. As a consequence, some alleles may be missing from the sample, and, importantly, the frequency of homozygotes may have been over-estimated (i.e. homozygote excess or low H_O). Because of this, it is premature to speculate about a true genetic change. In fact, the observation of one private allele at high frequency in the historical sample (allele 2 at locus Lut604, Appendix I) might rather suggest that some alleles have been lost during the recent demographic decline.

In testing for recent bottlenecks within each of the populations, the Wilcoxon sign-rank test indicated that the three extant populations showed significant levels of heterozygosity excess. The results of this test suggest that the extant populations have still not reached the balance between mutation and drift (Hedrick 2000) after the demographic decline during this century (Fig. 1). Consequently, some genetic variability is likely to be lost in the near future if the wolverine population does not rapidly expand.

Overall genetic variability

This study revealed relatively low levels of overall genetic variation in Scandinavian wolverines, both in extant and in historical populations. The average H_E at microsatellite loci across populations was 0.390, which is indeed low when compared with that observed in other mustelids, e.g. American mink *Mustela vison* (captive populations) $H_E = 0.61$ for 12 loci (O'Connell *et al*. 1996; Brusgaard *et al*. 1998a,b), Eurasian otter *Lutra lutra* $H_E = 0.54$ for 13 loci (Dallas & Piertney 1998), American marten *Martes americana* $H_E = 0.67$ for 14 loci, and American badger *Taxidea taxus* $H_E = 0.83$ for four loci (Davis & Strobeck 1998). Clearly, the variability is also

lower than generally seen in other mammals. Duffy *et al*. (1998) isolated four microsatellites from Scandinavian wolverines and reported an average H_E of 0.55, with 4.25 alleles per locus. Although higher than the values observed in our study, the difference is not significant and is still at the low end of the genetic variability seen for other mustelids.

As a consequence of its high overall rate of nucleotide substitution, the mtDNA control region has revealed patterns of population structure and geographical variation in many carnivores (e.g. Girman *et al*. 1993; Taberlet & Bouvet 1994; Vilà *et al*. 1999). In combination with the microsatellite data, the failure to identify variation in the mtDNA control region of Scandinavian wolverines, both in modern and old samples, lends support to the conclusion that (i) Scandinavian wolverines are low in genetic variability, and (ii) a significant proportion of the likely loss must have occurred prior to recent declines in population size. Our data are therefore suggestive of the low genetic variability of wolverines being an older phenomenon, e.g. due to post-glacial founder events. This has recently been suggested to explain the low levels of genetic variability in another European mustelid, the otter *Lutra lutra* (Effenberger & Suchentrunk 1999; Cassen *et al*. 2000).

Davis & Strobeck (1998) isolated five microsatellites from North American wolverines and found an average expected heterozygosity of 0.63 in a sample of 16 North American animals, which is significantly higher than observed here (Mann–Whitney, $P = 0.01$). Moreover, these authors tested a number of microsatellite primers isolated from marten and American badger on several mustelid species. The number of alleles amplified in North American wolverines with this suite of primers was among the highest for the different mustelids tested. These lines of evidence suggest that the low genetic diversity seen in Scandinavian wolverines is not a species-specific phenomenon, for instance resulting from a recent species origin, a peculiar mating system, or odd life history traits.

Management implications

It is likely that the most important management implication from this study is that of finding significant genetic differentiation between populations, in particular between the southern Norway population and the more northern populations in Norway and Sweden. Although this differentiation was due to differences in the frequency of a relatively static set of alleles at each locus, rather than to many unique alleles in the various populations, any local extinction will increase the risk of additional genetic variation being lost from the already fairly genetically uniform Scandinavian wolverine population. Moreover, as morphological divergence and even speciation can occur with significant gene flow (Smith *et al*. 1997), this might suggest that some of the populations should be managed separately and that caution is required when issuing harvest quotas in different areas. Wildlife management should aim at the preservation of natural processes rather than species (Moritz 1994). The colonization of southern Norway in the late 1970s can be seen as an expression of such processes. To preserve the evolutionary potential of this population and to increase its chances of long-term survival, it will be important to allow natural communication with northern populations.

The levels of genetic variation seen in Scandinavian wolverines are low and the long-term consequences of this reduced variation may remain unrecognized. While documentation of a contributory link between low genetic variation and population viability continues to be difficult to verify, a number of studies have linked low genetic variation with a reduction in fitness characteristics (for example, Wayne *et al*. 1991; Bijlsma *et al*. 1997; Saccheri *et al*. 1998). For Scandinavian wolverines, we cannot determine whether such a risk lies on the horizon, as it is unclear for how long the population has been able to cope with low genetic diversity. It will therefore be important to ascertain whether the loss of genetic variability in the Scandinavian wolverine is a recent or historical phenomenon.

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This work is part of a larger Uppsala University project aimed at addressing conservation genetics issues in Scandinavian carnivores. C. Walker and C. Vilà are postdoctoral researchers in Hans Ellegren's laboratory, concentrating on the use of molecular markers to characterize levels of variation and the potential substructure of that variation in Scandinavian carnivores. A. Landa and M. Lindén are ecologists specializing in wolverine biology.

Appendix I

The table shows the allelic frequencies for each locus and population, with sample size in parentheses. Private alleles (only present in one population) are indicated in bold while alleles missing in only one population are in italic

	Southern Norway	Northern Norway	Sweden	Pre-1970		Southern Norway	Northern Norway	Sweden	Pre-1970
Gg10	(21)	(88)	(51)	(6)	Gg454	(20)	(81)	(49)	(5)
1	0.167	0.114	0.226	*0.000*	1	0.000	0.161	0.102	0.100
2	*0.000*	0.011	0.029	0.167	2	0.325	0.482	0.459	0.700
3	0.024	0.028	0.069	*0.000*	3	0.025	0.049	0.112	*0.000*
4	0.095	0.0102	0.147	0.167	4	0.475	0.204	0.296	0.200
5	0.714	0.744	0.529	0.667	5	0.175	0.105	0.031	*0.000*
Gg25	(21)	(87)	(51)	(9)	Gg465	(21)	(86)	(51)	(7)
1	0.024	0.121	0.088	*0.000*	1	0.643	0.517	0.333	0.571
2	0.405	0.391	0.373	0.167	2	0.024	0.081	0.078	0.357
3	0.571	0.489	0.539	0.833	3	0.333	0.401	0.588	0.071
Gg37	(21)	(88)	(51)	(9)	Gg466	(21)	(87)	(48)	(3)
1	0.905	0.869	0.922	0.944	1	0.548	0.701	0.760	1.000
2	0.024	0.028	0.069	0.056	2	0.452	0.299	0.240	*0.000*
3	0.071	0.108	0.010	*0.000*	Gg470	(21)	(87)	(51)	(6)
Gg42	(21)	(87)	(51)	(9)	1	0.619	0.356	0.147	0.167
1	0.762	0.764	0.745	0.611	2	0.381	0.644	0.853	0.833
2	0.048	0.109	0.137	0.389	Gg471	(21)	(79)	(50)	(6)
3	0.191	0.126	0.118	0.000	1	**0.024**	0.000	0.000	0.000
Gg50	(21)	(87)	(50)	(5)	2	0.595	0.766	0.810	0.917
1	1.000	1.000	0.960	1.000	3	0.381	0.234	0.190	0.083
2	0.000	0.000	**0.040**	*0.000*	Gg473	(20)	(78)	(48)	(5)
Gg192	(21)	(87)	(50)	(6)	1	0.050	0.231	0.188	0.100
1	0.000	0.000	0.020	0.083	2	0.950	0.769	0.813	0.900
2	1.000	1.000	0.970	0.917	Lut604	(21)	(87)	(51)	(9)
3	0.000	0.000	**0.010**	0.000	1	0.881	0.609	0.726	0.278
Gg443	(21)	(82)	(50)	(7)	2	0.000	0.000	0.000	**0.389**
1	0.714	0.683	0.690	0.643	3	0.119	0.391	0.275	0.333
2	0.000	0.000	**0.020**	0.000	Lut615	(21)	(87)	(51)	(8)
3	0.286	0.317	0.290	0.357	1	*0.000*	0.086	0.078	0.188
Gg452	(21)	(83)	(45)	(7)	2	1.000	0.914	0.922	0.813
1	0.143	0.229	0.089	0.214					
2	0.524	0.241	0.144	0.500					
3	0.310	0.416	0.656	0.286					
4	0.024	0.115	0.111	*0.000*					