Colonization History and Noninvasive Monitoring of a Reestablished Wolverine Population

ØYSTEIN FLAGSTAD,*§ EVA HEDMARK,* ARILD LANDA,† HENRIK BRØSETH,† JENS PERSSON,‡ ROY ANDERSEN,† PETER SEGERSTRÖM,‡ AND HANS ELLEGREN*

*Department of Evolutionary Biology, Uppsala University, Norbyvägen 18D, S-752 36 Uppsala, Sweden
†Norwegian Institute for Nature Research, Tungasletta 2, N-7485 Trondheim, Norway
‡Swedish University of Agricultural Sciences, Department of Animal Ecology, S-90183 Umeå, Sweden

Abstract: *The southern Norwegian wolverine (*Gulo gulo*) population was considered functionally extinct in the 1960s but has partly recovered in recent years. Proper management of this population is highly dependent on reliable estimates of critical population parameters such as population size, sex ratio, immigration rate, and reproductive contribution from immigrants. We report on a large-scale population monitoring project assessing these parameters through genetic tagging of individuals, with feces as the source of DNA. Sixty-eight different individuals were detected among 147 successfully genotyped samples collected in 2000 and 2001. Sixty of these individuals were represented in the 2001 sample, which may be considered a minimum estimate of the population size. Almost 50% of these animals were sampled only once, however, indicating that the true population size may be markedly higher. Accordingly, a capture-recapture estimate based on the observed resampling rates suggested a population size of 89 wolverines (95% confidence interval [CI] = 74–104), which is approximately 35% higher than an estimate of 64 obtained from the number of active natal dens (95% CI = 46–95;* $p = 0.08$*). Indirect estimates of dispersal distances inferred from mother-offspring relationships suggested that wolverine males have the ability to disperse up to 500 km, a distance exceeding anything previously reported in the literature. Dispersal distances of more than 100 km were detected for females. Bayesian clustering analysis and subsequent assessment of individual relationships suggest that immigrants from northern Scandinavia have contributed and still contribute to the southern Norwegian gene pool, counteracting genetic erosion and reducing the risk of inbreeding depression. Additional sampling efforts will be undertaken during the coming years to allow for observations of population trends, immigration rate, and reproductive variance among individuals. Such data will provide an important basis for the design of an appropriate conservation plan for this small and vulnerable population.*

Key Words: capture-recapture, dispersal, feces, DNA analysis, genetic monitoring, immigration, population size, recolonization, relationship analysis

Historia de Colonización y Monitoreo No Invasivo de una Población Reestablecida de *Gulo gulo*

Resumen: *La población sureña de* Gulo gulo *se consideraba funcionalmente extinta en la década de 1960 pero se ha recuperado parcialmente en años recientes. El manejo adecuado de esta población depende, en gran medida, de estimaciones confiables de parámetros poblacionales críticos como por ejemplo el tamaño poblacional, la proporción de sexos, la tasa de inmigración y la contribución reproductiva de inmigrantes. Reportamos un proyecto de monitoreo poblacional a gran escala que evaluó estos parámetros por medio del marcaje genético de individuos, con heces como la fuente de ADN. Se detectaron 68 individuos diferentes entre 147 muestras de genotipos identificados exitosamente y colectadas en 2000 y 2001. Sesenta de estos individuos estuvieron representados en la muestra de 2001, lo que puede considerarse como una estimación*

§*Current address: Centre for Ecological and Evolutionary Synthesis, Department of Biology, P.O. Box 1066, Blindern, N-0316 Oslo, Norway, email oystein.flagstad@bio.uio.no*
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mínima del tamaño poblacional. Sin embargo, casi 50% de estos animales sólo fueron muestreados una vez, lo que indica que el verdadero tamaño poblacional puede ser marcadamente mayor. Como resultado, una estimación mediante captura-recaptura basada en las tasas de re-muestreo observadas sugirió un tamaño poblacional de 89 individuos (95% IC = 74-104), que es aproximadamente 35% mayor que una estimación de 64 obtenida del número de madrigueras natales activas (95% IC = 46-95; p = *0.08). Estimaciones indirectas de distancias de dispersión inferidas de relaciones madre-cría sugirieron que los* Gulo gulo *machos tienen la habilidad de dispersarse hasta 500 km, una distancia que excede cualquiera reportada previamente en la bibliografía. Se detectaron distancias de dispersión de más 100 km para hembras. Un análisis de cluster Bayesiano y la posterior evaluación de las relaciones individuales sugieren que los inmigrantes del norte de Escandinavia han contribuido y contribuyen al pool génico del sur de Noruega, contrarrestando la erosión genética y reduciendo el riesgo de depresión por endogamia. Se harán esfuerzos de muestreo adicionales en los próximos años para tener observaciones de las tendencias poblacionales, la tasa de inmigración y la varianza reproductiva entre individuos. Tales datos proporcionarán una base importante para el diseño de un plan apropiado de conservación para esta población pequeña y vulnerable.*

Palabras Clave: análisis de ADN, análisis de relaciones, captura-recaptura, dispersión, heces, inmigración, monitoreo genético, tamaño poblacional, recolonización

Introduction

Proper management of small and vulnerable populations is highly dependent on reliable estimates of parameters such as population size, dispersal distance, immigration rate, and reproductive contribution from immigrants. Recently, noninvasive molecular techniques—the use of material such as feces, urine, hair, or feathers as the source of DNA—have emerged as particularly promising tools with which to assess these important parameters (Höss et al. 1992; Morin et al. 1993; Morin & Woodruff 1996; Taberlet et al. 1999). A noninvasive approach allows collection of samples without disturbing or even handling the target animal. Especially in studies of species living in areas difficult to access, where time-consuming and expensive capturing and tracking of animals may give poor results, the technique should be particularly useful.

Despite initially promising results (reviewed in Kohn & Wayne 1997), the number of large-scale projects based on noninvasive samples is still fairly limited (Morin et al. 1994; Taberlet et al. 1997; Kohn et al. 1999; Woods et al. 1999; Constable et al. 2001; Lucchini et al. 2002; Eggert et al. 2003). Increased awareness of the many problems associated with the genetic analysis of dilute DNA samples has apparently reduced the optimism and enthusiasm initially associated with the technique. Rather, cautionary tales against noncritical use of these techniques have emerged (Taberlet et al. 1999; Waits & Leberg 2000), emphasizing the many pitfalls but also providing guidelines for minimizing the problems.

The main problem in genetic analysis of dilute DNA samples from noninvasive sources is the risk of genotyping errors such as allelic dropout (where one allele at a heterozygous locus fails to amplify, producing a false homozygote; cf. Taberlet et al. 1996) and false alleles (spurious amplification of an allele that does not exist). The former type of error is the most prominent, with reported frequencies of >30% of the replicates that should have produced a heterozygous pattern (e.g., Gagneaux et al. 1997). Obviously, without an appropriate number of replicates, such high error rates would lead to large biases if the resultant genotypes were incorporated in the data set. Replication of genotyping experiments—the multiple-tube approach (Taberlet et al. 1996)—reduces the prominence of genotyping errors. To explore the nature of these errors, an appropriately designed pilot project is highly recommended (Taberlet et al. 1999). A pilot study may indicate whether reliable genotyping can be achieved with a reasonable number of replicates and whether the questions of interest can be answered through a purely noninvasive approach.

The Scandinavian wolverine (*Gulo gulo*) inhabits remote uplands of Norway and Sweden; in these areas, conventional research and monitoring approaches, including capturing and tracking of the animals, are expensive and time-consuming. Reliable estimates of population size require large sample sizes, which may be hard to obtain through conventional approaches (Mills et al. 2000). Thus, the species belongs to a category for which noninvasive genetic sampling may be particularly useful. Previously, the wolverine was numerous and widely distributed across the Scandinavian Peninsula (Fig. 1). Because of intense persecution, however, its distribution range and population size declined markedly during the 1900s (Fig. 1). A particularly dramatic decline took place in southern Norway, and this population was considered functionally extinct by the 1960s (Landa & Skogland 1995, Landa et al. 1999). However, the area was recolonized a few years later (Fig. 1), an event that apparently was correlated with the enactment of protection in 1973 (Heggberget & Myrberget 1980). The return of the species led to conflict with sheep husbandry, and control measures have been practiced in selected areas since wolverines reappeared in southern Norway. In 1994 a 13,500-km^2

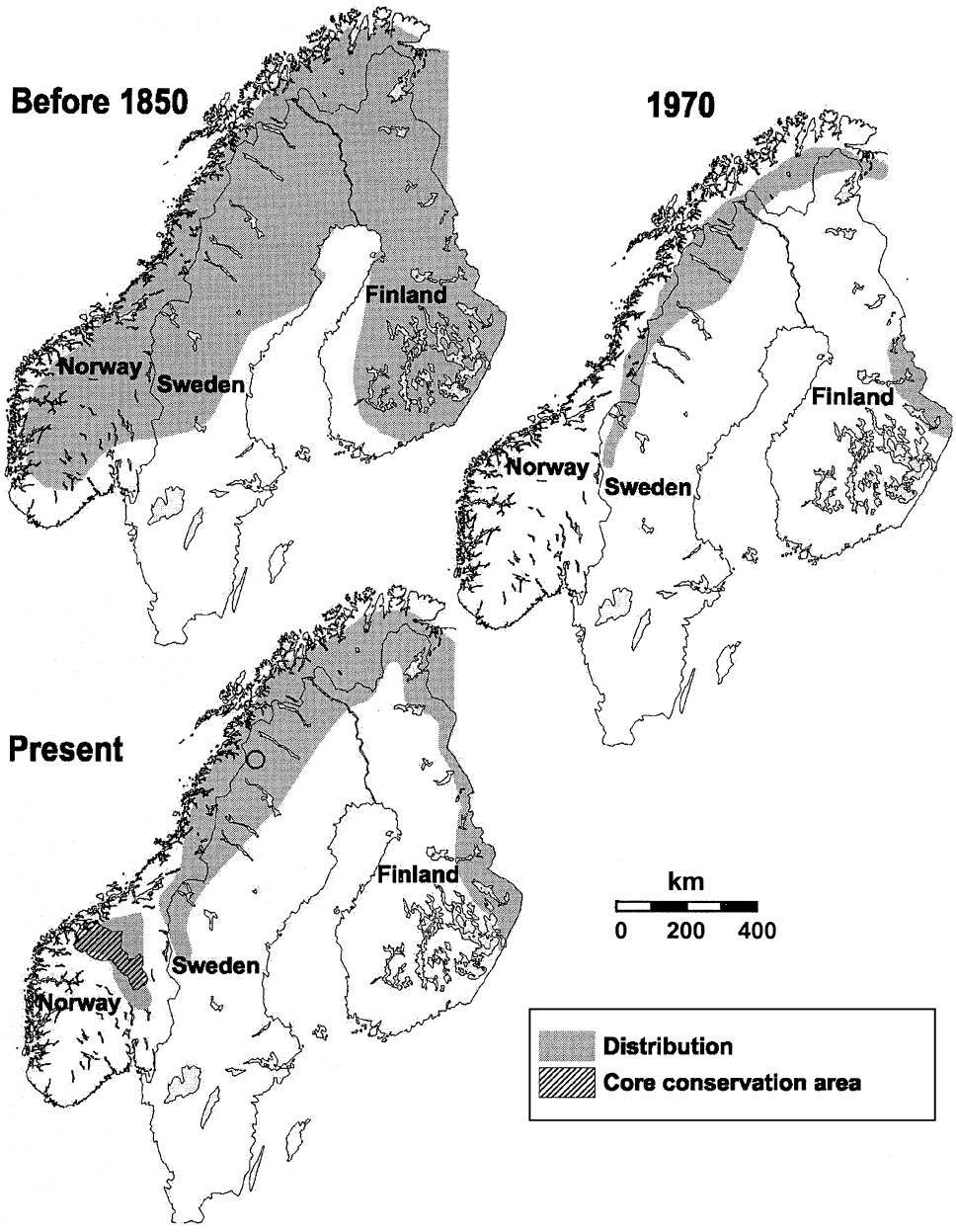

Figure 1. Distribution of wolverines in Scandinavia around 1850, 1970, and at present. Southern Norwegian animals were sampled over the entire distribution range in southern Norway. Northern Scandinavian animals were sampled centrally (the circle) within the northern distribution range.

core conservation area, embracing the main southern Norwegian distribution, was established. However, increased conflict with the sheep-farming industry led to reinstatement of legal hunting outside this area in 1998, and eventually, in 2002, the core conservation area was abolished. Because large harvesting quotas may compromise the persistence of this population, reliable estimates of critical population parameters are crucial for proper management.

Based on the guidelines of Taberlet et al. (1999), we recently performed a pilot study to assess the reliability of genetic analysis of nuclear DNA (nDNA) extracted from wolverine feces (Hedmark et al. 2004). This study showed that reliable genotypes could be obtained after three replicates. Given these promising results, we initiated a large-scale noninvasive study of the southern Norwegian wolverine population. A central goal was to evaluate the minimum estimate of the current population size based on the number of active natal dens (mean 10; range 8–12) in the area (Landa et al. 1998), which suggests that the population comprises approximately 64 individuals 1 year old and older (Brøseth & Andersen 2001; document available at http://www.dirnat.no/archive/images/01/15/rovte051.pdf). Based on the results from molecular tagging of individual samples (Paalsbøll et al. 1997), we used an approach derived from capture-recapture methodology (Kohn et al. 1999; Levy 1999; Mills et al. 2000). Another central question was whether

immigration occurs from the potential source population in northern Scandinavia. Finally, we conducted relationship analysis to provide data on dispersal distances and to see whether possible immigrants have contributed to reproduction. As a background to the current status of the population, we assessed the recolonization event in the 1970s.

Methods

Laboratory Procedures

SAMPLES AND DNA EXTRACTION

We collected 211 feces samples of presumed wolverine origin from the entire southern Norwegian distribution range (Fig. 1) during 2000 and 2001. Most of the samples (89%) were collected during tracking of wolverines on snow between February and June. All these samples were presumably fresh. The rest of the samples were collected on bare ground between July and October around carcasses used by wolverines. During transportation, samples were packed in ice and kept frozen in the laboratory until DNA extraction could be performed. The total time from sample collection to DNA extraction varied from 2 to 12 months, depending on the collection date.

Tissue samples from the southern Norwegian population were available for animals born between 1980 and 1987 ($n = 11$), between 1988 and 1995 ($n = 10$), and after 1995 ($n = 20$). Finally, we included a sample of 47 individuals from a central site in the northern Scandinavian population (Fig. 1) collected between 1998 and 2000.

We extracted DNA from feces with the QIA amp DNA stool mini kit (Qiagen, GmbH, Hilden, Germany). Each round of extraction included 8–12 samples and a negative control, each of which gave final extracts of 150 μL. To reduce the risk of contamination, we used a special room for low-copy-number DNA research during extractions and PCR setup. We extracted DNA from tissue samples in other localities with a standard phenol:chloroform protocol (Sambrook et al. 1989).

MICROSATELLITE GENOTYPING, SEX DETERMINATION, AND DNA SEQUENCING

As an initial test of the performance of the samples, we amplified nDNA with one marker (*Ggu14*; Walker et al. 2001) that gives strong amplification products that are clearly visible on an agarose gel. Amplifications were performed in 10-μL reactions containing 3.0 mM $MgCl_2$, 0.2 mM of each dNTP, 3.2 pmol of each primer, 0.5 μg of bovine serum albumine (BSA), 0.45 units of HotStar DNA polymerase (Qiagen), and 2 μL of template. A 15-minute predenaturation step at 95° C was followed by 45 cycles of amplification, with 30 seconds at 94° C, 30 seconds at 52° C, and 1 minute at 72° C. A final 10-minute extension step was added at the end. In this initial test we ran all samples in two replicates and visualized amplification products on a 2% agarose gel.

Table 1. Amplification details of the microsatellite markers used for genotyping of wolverines.

Marker	*Annealing temperature (°C)*	*$MgCl_2$ concentration*	*Reference*
Ggu10	52	4.0 mM	Walker et al. 2001
Ggu14	52	1.5 mM	Walker et al. 2001
Ggu25	58	3.0 mM	Walker et al. 2001
Ggu42	52	4.0 mM	Walker et al. 2001
Gg443	58	4.0 mM	Walker et al. 2001
Gg452	55	3.0 mM	Walker et al. 2001
Gg454	55	3.0 mM	Walker et al. 2001
Gg465	55	3.0 mM	Walker et al. 2001
Gg216	52	3.0 mM	Duffy et al. 1998
Gg101B	58	2.5 mM	Duffy et al. 1998
Gg234	52	1.5 mM	Duffy et al. 1998
Gg470	58	1.5 mM	Walker et al. 2001
Gg471	55	1.5 mM	Walker et al. 2001
Ggu 7	55	3.0 mM	Davis & Strobeck 1998
Mvis57	58	2.5 mM	O'Connell et al. 1996
Mvis72	55	3.0 mM	O'Connell et al. 1996
Mvis75	55	3.0 mM	O'Connell et al. 1996
Lut604	touchdown*	2.5 mM	Dallas & Piertney 1998

**For this marker, we used a modification of the touchdown protocol as originally described. The initial denaturation step at 95° C for 15 minutes was followed by 20 touchdown cycles with 94° C for 30 seconds; 60° C for 30 seconds, decreasing 0.5° C each cycle; and 72° C for 1 minute, followed by 23 cycles of 94° C for 30 seconds, 50° C for 30 seconds, and 72° C for 1 minute. An extension step of 72° C for 10 minutes was added at the end.*

We amplified samples visible from the initial test with fluorescence-labeled primers for the first nine microsatellite loci listed in Table 1, using the same conditions and PCR profile as described for *Ggu14*, except for the number of cycles, which in these cases was 37. We amplified all samples at least twice for each locus and did not accept a single-locus genotype before it had shown at least three identical homozygote profiles or two identical heterozygote profiles.

Based on the obtained allele frequencies in the southern Norwegian wolverine population, the probability of identity across these nine loci was estimated to be as low as 1.29×10^{-5} for unrelated individuals and 7.8×10^{-3} for siblings (Waits et al. 2001). Samples that were identical across all 9 loci were therefore interpreted as representing the same individual. To increase resolution power for subsequent data analysis (levels of genetic variability, assessment of immigration, and relationship analysis; see below), we genotyped each of the identified individuals across nine additional autosomal microsatellite loci (Table 1). To reduce genotyping effort, only one sample per individual was used for genotyping of these additional loci. It is important, though, that we consistently applied

the same strict criteria as described above for accepting a consensus genotype. We amplified tissue extracts for the same 18 loci, with identical protocols as described above but with a reduced number of cycles (28, 32, or 34). The PCR products were run on an ABI 377 instrument, and the subsequent determination of allele lengths was performed with GENESCAN and GENOTYPER (Applied Biosystems, Foster City, California).

We used a mustelid-specific Y-chromosome marker (*DBY7*, i.e., intron 7 of the *DBY* gene; Hedmark et al. 2004) to determine the sex of all detected individuals. At least four replicates were run per individual, and samples that gave Y-chromosome-specific amplification were interpreted as representing males.

Feces extracts that did not amplify nDNA when initially tested with *Ggu14* were sequenced for a 330 bp cytochrome *b* (*cyt b*) fragment from mitochondrial DNA (Irwin et al. 1991). We performed 42 cycles of amplification in 25-μL volumes with identical profile (annealing temperature, 50° C) and conditions ($MgCl_2$ concentration, 2.5mM) as described for the microsatellite markers. The PCR products were purified enzymatically with ExoSAP-IT (Amersham Pharmacia, Uppsala, Sweden) and sequenced with BigDye terminator cycle sequencing chemistry on an ABI 377 instrument (Applied Biosystems), following the protocol provided by the manufacturer.

Data Analysis

We assessed genotyping error (allelic dropout and false alleles) for all analyzed feces samples. Allelic dropout was interpreted in cases where at least one replicate showed a homozygous pattern, whereas the others were considered heterozygous. We considered alleles that occurred in only one of the independent replicates' false alleles. In such cases, we ran at least six replicates to ensure that the presumed spurious allele was indeed an amplification artifact and not a true allele that did not amplify in several successive replicates.

Levels of genetic variability were described across all 18 loci by the number of alleles, and heterozygosity (Nei 1987). Population differentiation was measured in terms of θ, an estimator of F_{ST} (Weir & Cockerham 1984). To detect possible immigrants in the southern Norwegian population, we used the Bayesian clustering approach implemented in the program Structure (Pritchard et al. 2001). This approach uses allele frequencies to infer the posterior probability (q value) that a given multilocus genotype originates from either of the populations under study. A genotype sampled in one population but with a higher posterior probability of originating from another may be considered a migrant. We included prior information on population origin and used a q-value threshold of 0.75 to identify putative migrants or individuals that had a mixed ancestry. We applied a burn-in period of 100,000 Markov Chain Monte Carlo (MCMC) cycles to reach the stationary phase and 1,000,000 additional cycles from which the results were extracted. To visualize the presence of likely immigrants, we performed a factorial correspondence analysis (Benzecri 1973), as implemented in GENETIX 4.0 (Belkhir et al. 1999). This approach clusters individual microsatellite profiles in a multidimensional space.

For capture-recapture estimation of the current population size, we initially performed a model-selection procedure implemented in the program Capture (Otis et al. 1978, White et al. 1978). A model allowing heterogeneity in capture probabilities fitted the data significantly better than a null model assuming constant capture probabilities ($p < 0.001$). Given that several groups of field collectors were involved in sampling and that sampling effort probably varied among localities, heterogenous capture probabilities were expected. Among estimators of different heterogeneity types, the selection procedure suggested that the jacknife estimator was the most appropriate. We used the jacknife approach described by Burnham and Overton (1979) for subsequent estimation of the population size. Although this approach was originally developed for sighting and resighting data recorded during a definite number of successive trapping events within a strictly defined grid, the authors also describe how the method can be used in special cases where the temporal dimension becomes less important and the number of trapping events becomes very large or even approaches infinity. In our case, we treated a successfully analyzed feces sample as one trapping event and simply recorded how many individuals were trapped once, twice, three times, four times, and so on. One important assumption of the chosen model is population closure, which is obviously violated in our case, in which immigration from the north seems possible (Fig. 1). This could potentially lead to an upward bias of the population size estimate. Results from previous studies (Walker et al. 2001) suggest, however, that southern Norwegian wolverines to some extent are genetically isolated from the northern Scandinavian population (i.e., immigration rate is low). For estimation of population size, moreover, we used only individuals represented at least once in 2001, a year in which >95% of the samples was collected within a period of 3 months (March-May). Thus, it is not likely that rare immigration events during the short sampling period can pose a significant upward bias to our estimate.

We performed relationship analysis using the likelihood-based approach as implemented in Cervus (Marshall et al. 1998). Because of the limited levels of variability in the population (Walker et al. 2001), we accepted a relaxed confidence level of 0.80 for parent-offspring relationships. We tested the detected parent-offspring and sibling relationships through simulations in the program Kinship (Goodnight & Queller 1999). When age of the analyzed individuals is not known, it is difficult if not

impossible to distinguish between parent-offspring relationships and those between full siblings. Therefore, we accepted parent-offspring relationships only when both putative parents had been sampled. The detected parent-offspring relationships were used to indirectly estimate dispersal distances by measuring the geographic distance between the sampling sites of mother and offspring (Spong & Creel 2001). This approach is based on the assumption that reproducing females show strong home-range fidelity throughout life (Vangen et al. 2001).

To carefully evaluate observations in our data set, we carried out population genetic simulations of mutation and genetic drift with the programs Geneloss (England 2001) and Easypop (Balloux 2001). First, we tested the possibility of two independent mutations arising and being kept in a small founder population during a time interval of just a few generations. Simulations were run across 1000 loci. To be conservative, we applied a high mutation rate of 0.001 and a relatively large effective founder population of 10 individuals, stable in size. Also, we determined whether immigration at a low rate is sufficient to maintain levels of genetic variability in a population of similar size, as the lower 95% confidence interval (CI) inferred from our data. This was assessed by simulation of genetic drift across 1000 loci under two different scenarios: (1) loss of genetic variability in a population receiving immigrants at a low rate (the observed immigration rate) and (2) loss of variability in a closed population with no immigration. Mutation was ignored under both scenarios.

Results

Performance and Reliability of the Applied Methodology

Of the 211 feces samples collected in 2000 and 2001 (70%), 147 gave DNA of sufficient quality for microsatellite amplification (Table 2). When we removed putatively misidentified samples, apparently originating from other species, the success rate was 77% (Table 2), which is high compared with most previous studies in which feces were used as the source of DNA (e.g., Reed et al. 1997; Taberlet et al. 1997; Ernest et al. 2000; Lucchini et al. 2002). We hypothesize that the relatively good performance may largely be due to three critical factors. First, most samples were presumably very fresh and collected on snow. A large proportion of these samples amplied well (75%). In contrast, only 6 out of 23 samples (26%) collected on bare ground between July and October gave DNA of sufficient quality to be genotyped. Second, the use of inhibitor-binding substances during DNA extraction (the anti-inhibitor provided in the applied kit) and amplification (BSA) may efficiently remove PCR inhibitors. Finally, we used the HotStar DNA polymerase (Qiagen), which in our experience is particularly efficient in amplifying low-copy-number DNA samples.

Table 2. Amplification success of wolverine-specific nDNA and source of origin for the 211 wolverine feces samples collected in 2000 and 2001.

Feces origin	Cyt b *sequence*	*Number of samples*	*Amplification success*[a]
Ascertained wolverine	not sequenced	147	147/161 (91%)
	wolverine	14	
Potential wolverine	rodents	5	147/190 (77%)
	hares	5	
	sheep	1	
	reindeer	6	
	ptarmigan and pipits	8	
	cyt b amplification failed	4	
Different species[b]	arctic fox	5	147/209 (71%)
	red fox	2	
	other mustelids	2	
	Raven	8	
	pig	2	
Contaminated sample	human	2	147/211 (70%)

[a] *The denominator of the ratio refers to the cumulative number of samples obtained after samples were added from each of the categories defined in the left-most column.*
[b] *These feces samples were probably misidentified by the collector.*

Importantly, the obtained single-locus genotypes were generally consistent across independent replicates, although allelic dropout was detected in 9.8% of all independent replicates of inferred heterozygotes. False alleles were much less common than allelic dropout and occurred in <0.5% of the PCR amplifications. These genotyping error rates were comparable to those obtained in a pilot study on wolverine feces carried out in our laboratory, where we compared data obtained from feces samples to those obtained from blood or tissue samples of the same individuals (Hedmark et al. 2004). In that study, which included >200 single-locus genotypes, three replicates were always sufficient for deriving the correct genotype. The comparable error rates and the same rigid use of predefined criteria for accepting single-locus consensus genotypes strongly suggest that most—if not all—errors were eliminated from the present data set prior to formal analysis. This was a critical task in our study, because even a small error rate in single-locus consensus genotypes would lead to severe upward bias of a capture-recapture estimate of population size based on more than a few loci (Waits & Leberg 2000).

Patterns of Genetic Variability and Differentiation through Time

No significant differences in genetic variability were detected at any time interval between northern Scandinavian and southern Norwegian wolverines for level of heterozygosity or number of alleles (Table 3). Notably

Table 3. Levels of genetic variability for all 18 microsatellite markers in southern Norway at three different time intervals and in northern Scandinavia at present.[a]

	Southern Norway (individuals born 1980–1987)				*Southern Norway (individuals born 1988–1995)*				*Southern Norway (present population)*				*Northern Scandinavia (present population)*			
Locus	n	*A*	H_{obs}	H_{exp}	n	*A*	H_{obs}	H_{exp}	n[b]	*A*	H_{obs}	H_{exp}	n	*A*	H_{obs}	H_{exp}
Gg10	11	4	0.45	0.40	10	3	0.70	0.58	79	4	0.43	0.45	47	4	0.57	0.55
Gg14	11	3	0.82	0.57	10	3	0.50	0.63	83	3	0.63	0.57	47	3	0.64	0.57
Gg25	11	2	0.45	0.37	8	3	0.50	0.58	84	3	0.64	0.58	47	3	0.53	0.50
Gg42	11	3	0.18	0.18	10	3	0.30	0.51	84	3	0.37	0.35	47	3	0.38	0.48
Gg101	9	3	0.44	0.39	10	3	0.20	0.36	83	3	0.47	0.40	46	3	0.37	0.35
Gg216	10	3	0.10	0.28	10	3	0.40	0.47	80	3	0.49	0.60	47	5*	0.87	0.70
Gg234	11	3	0.64	0.65	10	3	0.60	0.57	83	4	0.65	0.56	47	4	0.68	0.58
Gg443	11	2	0.45	0.37	10	2	0.60	0.44	81	2	0.37	0.41	47	2	0.45	0.41
Gg452	11	3	0.73	0.69	10	4	0.70	0.54	82	4	0.56	0.53	47	4	0.51	0.58
Gg454	10	4	0.70	0.70	10	3	0.70	0.57	80	5	0.65	0.66	47	5	0.74	0.60
Gg465	11	2	0.55	0.42	10	3	0.60	0.61	84	3	0.71	0.61	47	3	0.51	0.56
Gg470	11	2	0.09	0.45	9	2	0.22	0.52	82	2	0.37	0.50	47	2	0.23	0.27
Gg471	11	2	0.55	0.42	9	2	0.56	0.53	81	2	0.56	0.50	46	2	0.39	0.34
Ggu7	11	3*	0.64	0.67	10	3*	0.50	0.69	82	3*	0.65	0.66	47	2	0.55	0.50
Lut604	11	2	0.09	0.09	9	2	0.44	0.37	81	2	0.46	0.43	47	2	0.49	0.39
Mvis57	10	4*	0.70	0.68	10	4*	0.70	0.72	84	4*	0.52	0.48	41	4*	0.59	0.48
Mvis72	10	3	0.60	0.57	9	3	0.67	0.63	77	3	0.58	0.56	46	3	0.63	0.65
Mvis75	11	4	0.73	0.77	10	4	1.00	0.74	82	4	0.61	0.65	47	4	0.77	0.72
Mean	10.7	2.89	0.50	0.48	9.7	2.94	0.55	0.56	81.8	3.17	0.54	0.53	46.5	3.22	0.55	0.51
SE	0.14	0.18	0.06	0.04	0.14	0.15	0.05	0.03	0.45	0.20	0.03	0.02	0.33	0.23	0.04	0.03

[a] *Asterisks symbolize private alleles, meaning alleles found in only one of the two examined populations. Abbrevations: A, number of alleles;* H_{exp} *expected heterozygosity;* H_{obs} *observed heterozygosity.*
[b] *Both feces and tissue samples are included.*

though, our data may indicate increased variability in southern Norway between time intervals 1980–1987 and 1988–1995, expressed through a significant increase in expected heterozygosity ($p < 0.05$, pair-wise t test). Two population-specific alleles were detected in southern Norway, present at frequencies of approximately 15% and 25%, respectively, in all three time intervals.

Southern Norwegian wolverines born during 1980–1988 showed evidence of significant differentiation from the northern Scandinavian population ($\theta = 0.085$, $p < 0.001$). For the time period 1988–1995, differentiation was less pronounced, although still significant ($\theta = 0.049$, $p < 0.01$). The reduced differentiation can be illustrated in a factorial correspondence analysis (Fig. 2a & 2b): most individuals born between 1980 and 1987 appeared distinct from northern wolverines, whereas individuals born between 1988 and 1995 tended to cluster closer to or within the distribution of northern animals. Apparently, the level of differentiation between northern Scandinavia and southern Norway did not change after 1995 ($\theta = 0.045$, $p < 0.01$).

The Bayesian clustering analysis suggested that animals with a recent northern ancestry—either pure immigrants or animals with a presumed mixed ancestry—were present in southern Norway in all three time intervals. We illustrated an immigration origin with factorial correspondence analyses. Animals with a presumed northern ancestry were found within the distribution of northern Scandinavian animals (Fig. 2).

Estimates of Current Population Size

Sixty-eight different individuals were detected among the 147 genotyped samples collected in 2000 and 2001. Eight of the detected animals were sampled only in 2000 ($n = 37$), 5 were represented by samples from both years, and the remaining 55 individuals were sampled only in 2001 ($n = 110$). Therefore, there were at least 60 wolverines in the population in 2001 (Fig. 3a). The sex ratio was uniform, with 31 females and 29 males. Moreover, the two sexes appeared to be uniformly distributed in the densely populated core area and in the more peripheral areas to the north and the east. Only males were represented among the few individuals collected toward the southern edge of the distribution range. Multiple samples representing the same individual (32 animals) were in all but four cases collected <25 km away from one another (Fig. 3b).

The 60 individuals known to be alive in 2001 can be seen as a minimum estimate of the population size. Almost 50% of these animals were sampled only once, however, and only 3 individuals were represented among 5 adults legally killed after the sampling period in 2001, indicating

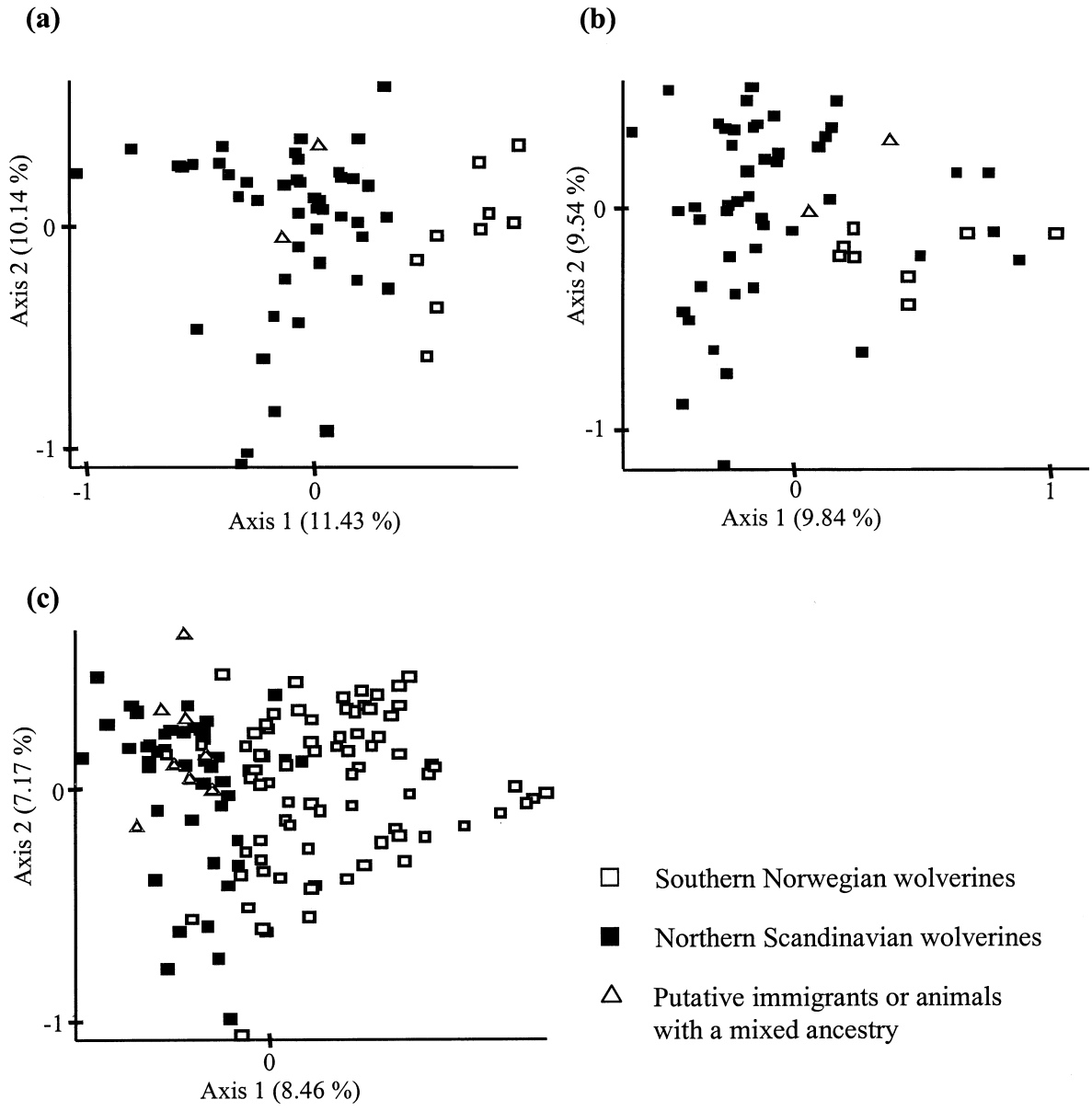

Figure 2. Factorial correspondence analyses (FCA) showing the distribution of multilocus genotypes of southern Norwegian wolverines (a) born before 1988, (b) born between 1988 and 1995, and (c) sampled after 1995. Animals with a putative northern ancestry were inferred from the Bayesian clustering approach. The proportion of the total variation explained by the two first dimensions in the FCA is indicated on the axes labels.

that the true population size may be markedly higher. Accordingly, a capture-recapture estimate based on the observed resampling rates suggested a population size of 89 wolverines (95% CI = 74–104).

Relationship Analysis and Dispersal Distances

We inferred from the likelihood-based relationship analysis (Table 4) 46 likely parent-offspring associations, distributed across 16 different family groups. Some of the litters had known mothers (legally shot families), and one litter had an inferred mother (from the location of the feces sample of this female, 31 m from the den). A likely father was successfully assigned for all these litters. Also, in several cases where the mother was not known, several likely parent-offspring relationships (both mother and father) were detected. None of the assigned parent-offspring and sibling relationships was rejected through simulations in Kinship. Nevertheless, the inferred relationships showing a relaxed confidence level (0.80) should be interpreted with some caution.

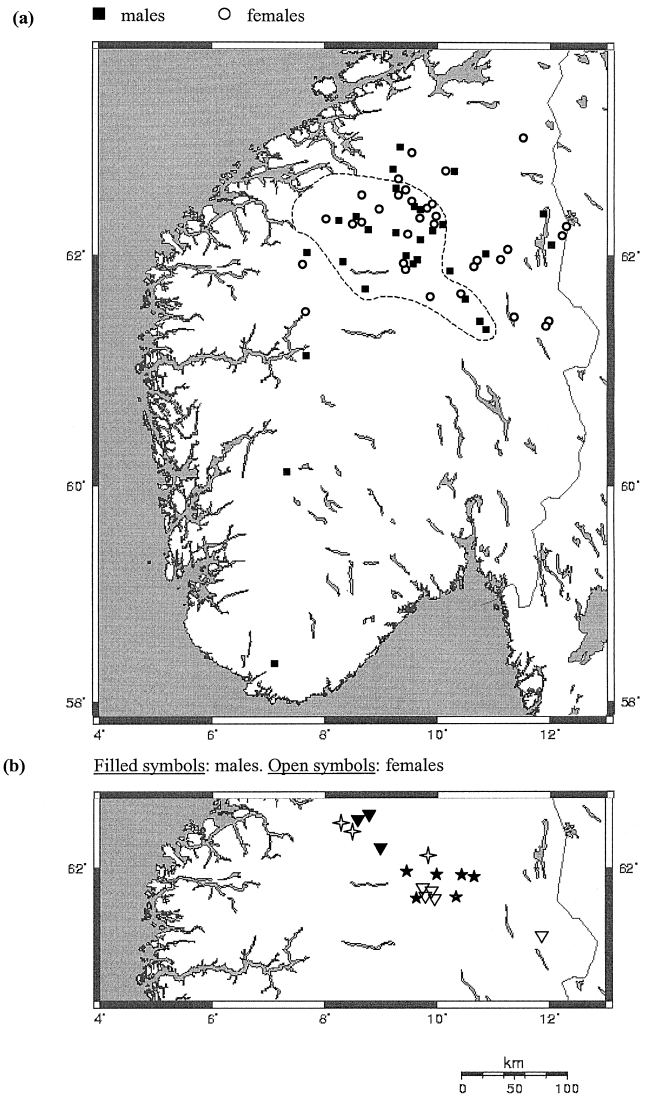

Figure 3. (a) Geographic distribution of southern Norwegian wolverines alive in 2001. For simplicity, individuals represented by more than one feces sample are indicated only once. The dotted line is the border of the old core conservation area (area of special protection), which was abolished by the Norwegian government in 2002. (b) Four individuals (different symbols) represented by more than one sample where the distance between individual samples was >25 km.

Assigned parents were generally sampled at moderate distances from each other (mean ± SE = 35 ± 4; median = 29 km). In contrast, a large variance was observed in the sampling distances between assigned offspring and their mother (indirect estimates of dispersal distances: mean ± SE = 125 ± 32 km, median = 83 km). The variance was significantly higher for males (mean ± SE = 164 ± 64 km, median = 85 km) than for females (mean ± SE = 78 ± 18 km, median = 75 km) ($p < 0.01$, *F*-test for variances).

Discussion

Colonization History

Two scenarios for the origin of the reestablished southern Norwegian population that are not mutually exclusive have been proposed: survival of a few animals from the historical population or immigration of wolverines from northern Scandinavia. The pronounced population differentiation present in the early 1980s may indicate that a few animals, differentiated from northern wolverines, survived the bottleneck in southern Norway and contributed to reproduction when the population slowly started to grow in the 1970s. However, large differentiation per se does not exclude the possibility that the population was founded by northern wolverines only. The effects of genetic drift can be dramatic in a small population founded by a handful of individuals (Barton & Charlesworth 1984; Merilä et al. 1996; Tarr et al. 1998, Vila et al. 2003), potentially leading to highly divergent populations in just a few generations.

Nevertheless, the presence of two population-specific alleles in southern Norway, at frequencies of 15% and 23% among individuals born before 1988, may contradict a pure northern origin. It seems unlikely that these alleles arose by mutation among a few northern founders in about five generations (1970–1985). To formally test this possibility, we simulated mutations in 1000 loci through five generations by using a high mutation rate of 0.001 in a founding population of 10 individuals (N_e) that was stable in size. With these relatively conservative assumptions, the probability that a mutation arose in one locus and was kept in the population is only 0.028. Accordingly, the probability for surviving mutations at two independent loci is <0.001. This suggests that a few southern Norwegian individuals survived the bottleneck in situ and contributed to reproduction when the population started to grow. On the other hand, because we do not have data for the northern Scandinavian population from this period, we cannot formally exclude the possibility that these two alleles actually were present in the population at low frequencies. It follows that a pure northern origin of the southern Norwegian population in the 1970s cannot be conclusively rejected, although the probability of such a scenario appears low from the available data.

Despite the uncertainty over the origin of the southern Norwegian population, it seems evident from our data that northern migrants were present in the population at least from the 1980s onward (Fig. 2a & 2b). This interpretation is supported by results of the Bayesian approach that assigned four individuals born between 1980 and 1995 as likely immigrants (Fig. 2a & 2b). Moreover, there was a significant increase in expected heterozygosity (Table 3) between 1980–1987 and 1988–1995, accompanied by reduced differentiation (Fig. 2a & 2b). This may

Table 4. Likely parent-offspring relationships in the southern Norwegian wolverine population.

Family group	*Father*	*Mother*	*Offspring*[a]	*Confidence level*[b]	*Distance between parents (km)*	*Inferred dispersal distances of assigned offspring (km)*[c]
I	M67	F61	M76, M118	*	66	85, 303
II	M67	F219	M138	*	50	496
III	M67	F86	F183	*	65	118
IV	M217	F44	F97, M104	+	14	—
V	M217	F44[d]	F505, F506	*	14	—
VI	M40	F150	F58	*	45	50
VII	M177	F510[d]	F511	+	63	—
VIII	M201	F90	M188	*	18	54
IX	M106	F19	F211, F158	+	29	28, 29
X	M106	F29	M113	+	19	109
XI	M188	F29	F194	+	29	83
XII	M101	F140[e]	F133	*	28	75
XIII	M513	F140[e,f]	M501, M502, M503	*	23	—
XIV	M208	F155	F50, M64, M88	+	27	161 km, 27 km, 76 km
XV	M69	F509[d]	F507, F508	*	15	—
XVI	M175	F191[e]	F154	*	58	—

[a] *Males and females are represented by F and M, respectively.*
[b] *Confidence levels are 0.80 (+) and 0.95 (*), as inferred from the program Cervus.*
[c] *Dispersal distances were not inferred (—) when the offspring were sampled close to the sampling site of their mother (<10 km).*
[d] *Mother known (cubs killed together with their mother in or near the den).*
[e] *Detected immigrants.*
[f] *Mother inferred from location of her feces.*

indicate that northern migrants actively contributed to reproduction during the 1980s and early 1990s.

Current Population Size

Our capture-recapture estimate of population size suggests that the southern Norwegian wolverine population is composed of approximately 90 animals (95% CI = 74–104). This is about 35% higher than the 64 individuals estimated from the number of observed active natal dens (95% CI = 46–95; $p = 0.08$, one-tailed t test). The discrepancy between the two point estimates has a number of explanations. First, the latter estimate is a conservative minimum estimate based on the recorded number of active natal dens, and some dens may have been missed during the surveys (Landa et al. 1998). Second, the demographic parameters included in the model (age at first reproduction, age and sex distribution) might underestimate the actual population size. Third, this model is designed to capture the population structured around reproducing females and does not capture solitary individuals in peripheral areas. Several of the sampled individuals detected by our method were located outside the distribution area as set by recorded denning females (functional population).

We also acknowledge that our own method may be biased. As pointed out by Waits and Leberg (2000), a genetically based capture-recapture approach is highly sensitive to genotyping error. As discussed above, however, we anticipate that errors in consensus genotypes are negligible and that genotyping error should not be an important source of bias. Finally, capture-recapture approaches as such may be sensitive to sampling design (e.g., Burnham & Overton 1979). However, to reduce the importance of varying sampling effort and design, we used an approach that accounts for variance in sampling probability among different individuals. Consequently, sampling design may not be too critical when this approach is used. Our approach may thus give the best estimate of the total population, whereas the field-based model is designed to cover the functional population and therefore might underestimate the true population size. We conclude that the southern Norwegian wolverine population is likely composed of some 90 animals with a lower 95% CI of 74.

Territories, Exploratory Movements, and Dispersal Distances

Multiple samples representing the same individual may provide information on territory size and movement patterns. In our case, almost all samples representing the same individual were collected close to one another (<25 km). However, four cases where individual samples were distributed farther apart were detected (Fig. 3b). These cases may represent exploratory movements (e.g., Vangen et al. 2001), dispersal events, or individuals occupying large territories. For example, the individual represented by filled stars in Fig. 3b could be a male occupying a territory of >1000 km^2. Paternity analysis showed that this animal had left offspring in the population (family group XII; Table 4) and thus likely held a territory. The

distribution of samples from the female represented by open circles (Fig. 3b) could be interpreted as a dispersal event, but one of the four aggregated samples (within approximately 200 km^2) was collected more than 1 month later than the single sample about 100 km away, which may indicate that the samples represent two different individuals. Additional genotyping showed, however, that all five samples were still identical across 12 loci ($PI_{sibs} = 1.7 \times 10^{-3}$), making this possibility less likely. Rather, these samples may be an example of a long exploratory journey of approximately 200 km total (Fig. 3b). Exploratory movements are common among wolverines (Vangen et al. 2001), although such a large total distance as indicated here has not been reported from radiotracking studies.

Assessment of individual relationships showed that pairs of parents were sampled within limited distances from each other (35 ± 4 km). Because wolverine territories can extend over considerable areas (e.g., Magoun 1985), this observation is concordant with the view that reproducing individuals of the opposite sex inhabit neighboring or partly overlapping territories (e.g., Powell 1979). We also used relationship analysis for indirect inference of dispersal distances, under the assumption that reproducing females show strong home-range fidelity throughout life (Vangen et al. 2001). The variance in dispersal distances was significantly higher for males than for females. Two males that dispersed exceptional distances (303 and 496 km) could largely explain this difference. A dispersal distance of almost 500 km has to our knowledge never been reported in the literature, probably because radio contact with long-distance dispersers is readily lost (Vangen et al. 2001). Dispersal distances of >100 km were also detected for females. These results suggest that both sexes have dispersal capacities enabling them to contribute to genetic exchange between the northern population and the population in southern Norway, which are separated by 100–200 km (Fig. 1), a gap characterized by good wolverine habitat and low human density.

Current Gene Flow from Northern Scandinavia

The Bayesian clustering approach suggested that eight individuals, of which three were cubs in a natal den, likely have a recent northern ancestry (Fig. 2c). Importantly, subsequent relationship analysis showed that two presumed female immigrants reproduced successfully, one of them twice (family groups XII, XIII, and XVI; Table 4). Both females occupied territories in the eastern parts of the population (i.e., close to Sweden). Our observation of no loss of genetic variability since the founding of the population in the 1970s (Table 3) is consistent with reproductive contribution from immigrants, which is of major importance in counteracting genetic erosion and reducing the risks of inbreeding depression (Spielman & Frankham 1992). The evidence of reproductive

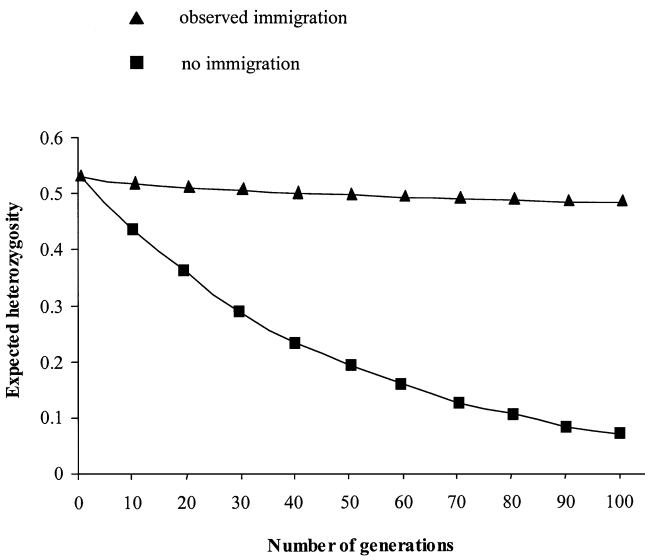

Figure 4. Simulation of loss of genetic variability in a small wolverine population over 100 generations, assuming a constant population size of 75 individuals (probably corresponding to $N_e \approx 25$ [Nunney & Elam 1994; Frankham 1995]). Two simulation scenarios were carried out: the first applies to a population that receives immigrants at a rate of 0.062 (observed immigration rate), and the second applies to a closed population with no immigration.

events involving migrants suggests that there is a considerable potential for immigrants to breed successfully in the southern Norwegian population, particularly in the eastern parts, where population density is still low.

Implications for Conservation

Our results show that noninvasive genetic sampling can provide estimates of a number of critical population parameters, such as population size, dispersal distance, immigration rate, and reproductive contribution from immigrants. It should be emphasized, though, that full integration of noninvasive molecular techniques in the management of small, endangered populations requires strict criteria for accepting a genotype (e.g., the multiple-tubes approach).

From a management point of view, the observation that northern immigrants have contributed, and still seem to contribute, to reproduction in southern Norway may be particularly relevant. To determine whether the current migration rate suggested by the Bayesian approach (0.062) is sufficient to maintain levels of genetic variability in the population, we simulated genetic drift in a population of 75 individuals for 100 generations (Fig. 4). Genetic erosion was dramatic under a scenario of no migration, in which virtually all variation was lost after 100 generations. In contrast, when the observed migration rate was maintained, only a moderate proportion

(approximately 10%) of the variability was lost after 100 generations. These results suggest that maintenance of gene flow should be a central issue when future management strategies are discussed.

Southern Norwegian wolverines are currently being harvested at a rate of >10% per year, and it is of considerable importance to obtain data that can be used to predict the immediate and long-term consequences of such a harvesting quota. Additional sampling efforts will therefore be undertaken during the coming years to allow for observations of population trends, immigration rate, and reproductive variance among individuals, the latter parameter being an important factor in population viability analysis (Shaffer 1981; Miller & Lacy 1999). Such data will provide an important basis for the design of an appropriate conservation plan for this small and vulnerable population.

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