

Genetic structure of the northwestern Russian wolf populations and gene flow between Russia and Finland

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Abstract We examined the genetic diversity and structure of wolf populations in northwestern Russia. Populations in Republic of Karelia and Arkhangelsk Oblast were sampled during 1995–2000, and 43 individuals were genotyped with 10 microsatellite markers. Moreover, 118 previously genotyped wolves from the neighbouring Finnish population were used as a reference population. A relatively large amount of genetic variation was found in the Russian populations, and the Karelian wolf population tended to be slightly more polymorphic than the Arkhangelsk population. We found significant inbreeding ($F = 0.094$) in the Karelian, but not in the Arkhangelsk population. The effective size estimates of the Karelian wolf population based on the approximate Bayesian computation and linkage disequilibrium methods were 39.9 and 46.7 individuals, respectively. AMOVA-analysis and exact test of population differentiation suggested clear differentiation between the Karelian, Arkhangelsk and Finnish wolf populations. Indirect estimates of gene flow based on the level of population differentiation ($\phi_{ST} = 0.152$) and frequency of private alleles (0.029) both suggested a low level of gene flow between the populations ($Nm = 1.4$ and

$Nm = 3.7$, respectively). Assignment analysis of Karelian and Finnish populations suggested an even lower number of recent migrants (less than 0.03) between populations, with a larger amount of migration from Finland to Karelia than vice versa. Our findings emphasise the role of physical obstacles and territorial behaviour in creating barriers to gene flow between populations in relatively limited geographical areas, even in large-bodied mammalian species with long-distance dispersal capabilities and an apparently continuous population structure.

Keywords Large carnivore · Bottleneck · Genetic differentiation · Migration · Barrier to gene flow · Effective population size

Introduction

As in most of the Russian populations of grey wolf (e.g. Boitani 2003), the northwestern populations of the species have also gone through extreme population bottlenecks (Danilov 2005). In the first half of 20th century, wolves were practically extirpated from the remote taiga territories of northwestern Russia. As recently as the 1920s–1930s, wolves were hardly ever encountered in the taiga wilderness. In the late 1940s, the timber industry started to clearcut the forests of northwestern Russia and the resulting young stands provided suitable habitat for moose—the main prey species of wolves in the area. The growth of the moose population formed a stable food resource for the wolf population, and wolves began to recover in the area during the late 1950s. In the mid-1970s, wolves inhabited all parts of northwestern Russia again, and it has been estimated that at that time the number of wolves in the Republic of Karelia was 600–700 individuals (Danilov

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2005). In the early 1980s, the number of moose began to decline (Fig. 1a) because of exploitative hunting and ageing of the forest stands. As a consequence, the number of wolves started to decrease again, and subsequently it has been following the fluctuations of the moose population (Fig. 1a). In addition to prey availability, increased hunting has also reduced the number of wolves during the last decade. From the mid-1960s to the mid-1970s, the number of wolves killed yearly in the Republic of Karelia was about 50 per year, whereas after the mid-1990s it has been over 170 wolves per year (Fig. 1b). Consequently, after the initial recovery, the wolf population has been declining and at present there are only 300–350 wolves in the Republic of Karelia (Danilov 2005).

The closest wolf population to the northwestern Russian populations is the Finnish wolf population, which is presumed to be a fringe population of the larger Russian populations (e.g. Pulliainen 1965, 1980; Boitani 2003). Fluctuations in wolf numbers in Finland have mirrored fluctuations in the neighbouring Russian populations until the late 1990s (Pulliainen 1965, 1980; Boitani 2003). In contrast to the Russian populations, the size of the Finnish wolf population has increased over the last decade as a result of conservation strategies and hunting control (Kojola and Määttä 2004), and in 2006 there were 250–260 wolves in Finland. Since the 1990s, the numbers of wolves

in Finland have not any more been following the numbers of the larger Russian Karelian population (Kojola and Määttä 2004) suggesting that the Finnish population may be in the process of isolation from the larger Russian-East-European population.

Grey wolves have the ability to disperse over long distances even in the absence of suitable corridors across habitats characterised by human activities. Wabakken et al. (2007) have recently documented a straight-line dispersal distance of 1,092 km of a female wolf from southeast Norway to northeast Finland, with a multi-stage actual travel distance of >10,000 km. Individuals typically disperse much shorter distances before establishing territories. Using both genetic and telemetry approaches, Aspi et al. (2006) and Kojola et al. (2006) have shown that the average dispersal distance among Finnish wolves is less than 100 km.

Despite both the long-distance dispersal capabilities and the ability to occupy a variety of habitats, restricted migration between wolf populations has been described even within a relatively small region. Often, restricted migration seems to be associated with the presence of topographical (Carmichael et al. 2001; Geffen et al. 2004; Weckworth et al. 2005; Seddon et al. 2006) or human induced (Seddon et al. 2006) barriers. However, several recent studies have shown that ecological or behavioural processes also may influence the amount of migration between wolf populations (Carmichael et al. 2001; Geffen et al. 2004; Pilot et al. 2007; Musiani et al. 2007).

Genetic methods may be in many cases more useful than the traditional ecological methods in estimating gene flow between natural populations. Two different approaches have been used to estimate the amount of gene flow on the basis of genetic data (e.g. Neigel 1997). Indirect methods of gene flow estimation characterise the spatial distribution of genotypes by some parameter and then apply a population genetic model to determine the level of gene flow which would produce a distribution with the same parameter value (e.g. Neigel 1997). These methods assume equilibrium between drift and migration, Hardy–Weinberg equilibrium within populations, and symmetry of migration rates between populations. These assumptions are not necessarily fulfilled in natural sets of populations (e.g. Whitlock and McCauley 1999), and hence these models are often inappropriate. Because of limitations of indirect methods, many researchers have switched to a more direct approach to estimate gene flow. Individual-based assignment tests, which assign individuals probabilistically to candidate populations by their multilocus genotype, may be used to identify possible migrants (Berry et al. 2004; Paetkau et al. 2004; Manel et al. 2005).

Aspi et al. (2006) used the latter approach to estimate the amount of migrants from Russian populations to

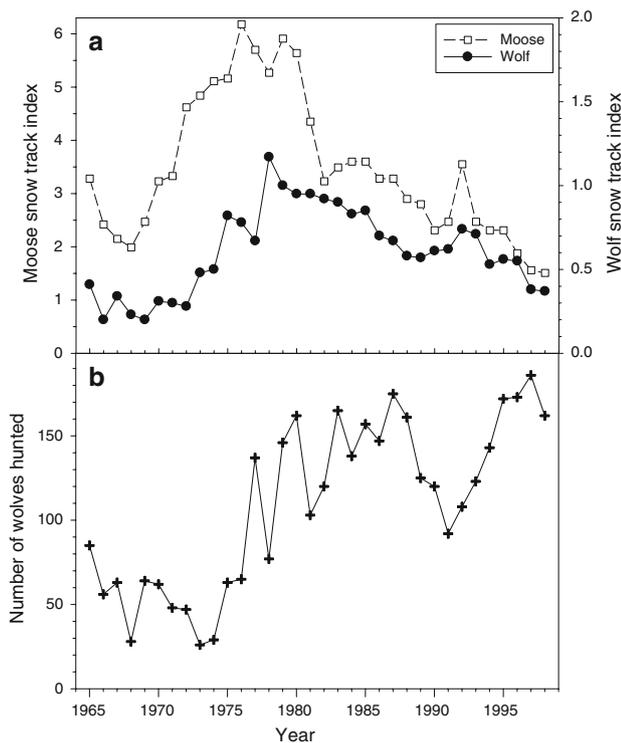


Fig. 1 (a) Moose and wolf track index (number of tracks per 10 km route), and (b) Number of wolves hunted in Russian Karelia in 1965–1998 (after Danilov 2005)

Finland. Their self-classification assignment analysis suggests that only 3% of wolves in the Finnish population seem to be possibly first generation immigrants. However, the study of Aspi et al. (2006) did not include samples for comparison from Russia. Thus, the power of their assignment analysis may have been low, and the true number of immigrants may be somewhat larger.

Pilot et al. (2007) have recently investigated the structure of some east-European wolf populations. However, there is no comprehensive investigation of the genetic structure of the northwestern Russian wolf populations. We are here reporting a preliminary analysis of genetic diversity and population structure of the wolf populations in the Republic of Karelia and Arkhangelsk Oblast. We have also estimated the amount of differentiation and gene flow between the Finnish and Russian populations using different population genetic approaches.

Material and methods

DNA extraction and microsatellite analysis

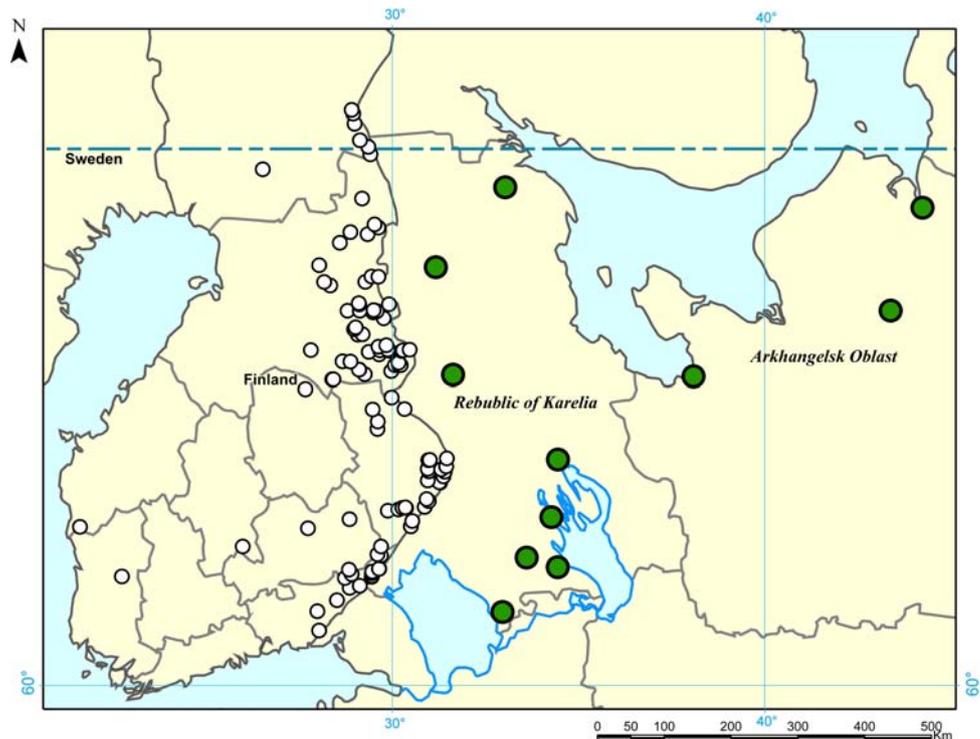
A total of 43 pelt samples were collected from the north-west Russian wolf populations between 1995 and 2000 (Fig. 2). Of the samples, 29 were from Republic of Karelia (Aunus 3, Kalevala 1, Karhumäki 2, Kontupohja 5, Louhi 1, Mujehjärvi 3, Prjázha 6, Pudozhi 2, Prionezhki 2 and 4

from unknown location in the Republic) and 14 from Arkhangelsk Oblast (Mezen 8, Onega 4, Pinega 2). Exact geographic coordinates for the sampling sites were not available. Sexing of the wolves with unknown sex was performed using the method suggested by Seddon (2005), and there were 24 males and 19 females among the samples.

Genomic DNA from pelts was extracted employing the Chelex[®] method of Walsh et al. (1991). Because 10 of the samples were not amplifying adequately in the PCR-reactions, they were extracted a second time using the DNeasy[®] Tissue Kit (QIAGEN). The samples were genotyped for allelic variation at ten autosomal microsatellite loci (Ostrander et al. 1993; Fredholm and Winterø 1995; Francisco et al. 1996) including six dinucleotide (C20.253, C09.173, CXX.225, CPH2, CPH8, CPH12) and four tetranucleotide repeats (CPH4, C2001, C2088, C2096). Each microsatellite loci is located in a different chromosome in the genome of domestic dogs. To minimise scoring errors, some samples were amplified twice. In the few samples where an ambiguous result still occurred a half-locus was recorded (Miller et al. 2002). Negative extraction and polymerase chain reaction (PCR) controls were used throughout the study to monitor possible contaminations.

Amplification of DNA extracts was performed using a Peltier Thermal Cycler-200 (MJ Research) in 10- μ l reactions containing 20 ng of template DNA, 1 \times PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 2.0 mM MgCl₂,

Fig. 2 Collection localities of the wolf samples in the Republic of Karelia and Arkhangelsk Oblast (large grey circles), and collection sites of the Finnish wolf samples (small circles)



0.2 mM dNTP, 3.2 pmol of each primer, 0.5 U of DNA polymerase (AmpliTaQ GOLD[®]), and sterile water. For C2088 the amount of template DNA used was 35 ng. The PCR profile was identical across all markers and included an initial denaturation step of 95°C for 10 min, 11 touchdown cycles with 94°C for 30 s, 58°C for 30 s decreasing by 0.5°C in each cycle and 72°C for 1 min, 28 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 1 min and a final extension of 72°C for 10 min. All PCR microsatellite products were run on an ABI 3730 instrument (PerkinElmer Applied Biosystems) and gel analysis was performed using the software packages GENEMAPPER 3.7 (Applied Biosystems). The programme MICROCHECKER, version 2.2.3 (van Oosterhout et al. 2004) was used to identify possible null alleles, large allele dropout, scoring errors due to stutter peaks, and possible typographic errors. The analysis suggested signs of null alleles in CPH8 and CXX.205 and C2001 in the Karelian wolf population. However, because the binomial tests were not significant and no signs of null alleles were found in the other two populations, these loci were kept in the data set. No signs of large allele dropout and scoring errors due to stutter peaks were seen at any loci either in the Karelian or the Arkhangelsk sample.

To estimate differentiation between Russian and Finnish populations and to estimate the amount of gene flow between these wolf populations, a further sample of 118 Finnish wolves was collected between 1996 and 2004 (Fig. 2). These individuals have been genotyped for allelic variation at the same microsatellite loci than the Russian samples, and were previously used to investigate the genetic structure and demographic history of the Finnish wolf population by Aspi et al. (2006).

Genetic diversity and inbreeding

We used the software GENETIX (Belkhir et al. 2004) to estimate observed and expected heterozygosities, number of alleles and inbreeding coefficients for both Russian populations. The programme provides the distribution of the parameter values by the appropriate resampling scheme of the relevant objects. We also estimated the allelic richness which would be obtained, if all samples were to be of equal size using the rarefaction method of Petit et al. (1998) as implemented in the program FSTAT (Goudet 2001). We tested for linkage disequilibrium between all pairs of loci in both populations according to the method of Black and Kraftsur (1985) implemented in GENETIX.

Population bottlenecks

When a population experiences a reduction of its effective size, it generally develops excess gene diversity at

selectively neutral loci, i.e. the gene diversity computed from a sample of genes is larger than the gene diversity expected from the number of alleles found in the sample of a constant-size population. This condition occurs because the rare alleles that were lost contributed little to the overall heterozygosity (Cornuet and Luikart 1996). Both Russian wolf populations were assessed for a deficiency of low frequency allele classes by examining the overall distribution of allele frequency classes ('mode shift' test) and using the Wilcoxon test as implemented in the programme bottleneck (Cornuet and Luikart 1996) under the two-phase mutation model with 95% single-step mutations. Population bottlenecks may also initiate gaps in the size distribution of microsatellite alleles (Garza and Williamson 2001). The gaps in distributions can be quantified as the *M* ratio, the mean ratio of the number of alleles to the allele size range across all loci (Garza and Williamson 2001). Means of *M* ratios were calculated for each temporal sample using AGARST (Harley 2004).

Effective population size of the Karelian population

Because the survey size of the Russian wolf populations is not well known, the study included two genetic methods that require only a single distinct genotypic sample from a population for estimating the effective size (N_e) of the Karelian population. First, we estimated N_e 's (and their 95% credible limits) using the approximate Bayesian computation method (see Beaumont et al. 2002) implemented in the programme ONESAMP (Tallmon et al. 2008). When running the programme, we used priors of 2 to 1,000 for N_e 's. Secondly, we also used the programme LDNE (Waples and Do 2008) to estimate the linkage disequilibrium based estimator of N_e 's. LDNE implements a recently developed bias correction (Waples 2006) for estimates of effective population size. We assumed a monogamous mating system, and excluded all alleles with frequencies less than 0.05 (see Waples and Do 2008). Given the small sample size of the Arkhangelsk population, we did not estimate N_e for that population (cf. Tallmon et al. 2008; Waples and Do 2008).

Population differentiation and gene flow

A Bayesian MCMC approach (Pritchard et al. 2000; Falush et al. 2003) was used to infer the number of subpopulations most appropriate for interpreting the microsatellite data without prior information of the number of locations at which the individuals were sampled and into which location each individual belongs. For this analysis, we used only a subset of the Finnish wolf data. Previous Bayesian analysis has shown the existence of more than one cluster

in the population because of close relatedness among neighbouring wolf packs (Aspi et al. 2006). To avoid spurious results due to the family structure, we only used 60 random individuals from the Finnish sample for this analysis. We used several different subsamples, but because each one gave very consistent results, we report only the results of one sample here. We used the programme STRUCTURE, version 2.0 (Pritchard et al. 2000; Falush et al. 2003) to infer the number of populations, and assumed a model with population admixture and that the allele frequencies were correlated within populations (Falush et al. 2003). We conducted 10 independent runs for each value of *K* (the number of subpopulations) between 1 and 4. After conducting numerous runs to investigate the behaviour of the programme, we chose to use a burn-in period of 10⁴ iterations and collect data for 10⁵ iterations. We ran 10 independent simulations of this length for each *K*, and averaged the parameters given by the runs. We ran the programme also with the same parameters separately for both Karelian and Arkhangelsk samples.

The presence of genetic structure among the wolf populations was investigated also by an analysis of variance framework using analysis of molecular variance (AMOVA). We used the programme ARLEQUIN (version 3.11; Excoffier et al. 2005) to perform the AMOVA analysis. We used this software to estimate genetic distances (ϕ_{ST}) and corresponding estimate of the average effective number of migrants (*Nm*) exchanged per generation among the Karelian, Arkhangelsk and Finnish populations. Exact tests of population differentiation between the wolf populations were conducted as described by Raymond and Rousset (1995a) using ARLEQUIN. We estimated the average number of migrants (*Nm*) also using the private allele method of Slatkin (1985) as implemented in GENEPOP (Raymond and Rousset 1995b). We used a recently developed Bayesian method for estimating migration rates (Wilson and Rannala 2003) to calculate the proportion of migrants (*m*) between populations. Contrary to indirect estimators of long-term gene flow, this nonequilibrium approach does not assume Hardy–Weinberg equilibrium within populations. The method calculates separate inbreeding coefficients for each population, the joint probabilities of which are used to estimate recent migration rates. The programme BAYESASS, version 1.3 (Wilson and Rannala 2003) was run for 3 × 10⁶ iterations, sampled every 2000, and the first 1 × 10⁶ iterations were omitted as suggested by the authors. The first run used default settings, with subsequent runs incorporating different random seed and delta values. The data set was run four times to check for consistency of results, and the values were averaged. We did not include the Arkhangelsk population in this analysis because the simulations of Wilson and Rannala (2003) have shown that the number of individuals sampled in this population was

too low to allow reliable estimates of migration rates when this method is applied.

We conducted an assignment analysis to identify directly possible migrants. We performed assignment runs for the pooled Karelian and Finnish samples using the Rannala and Mountain (1997) Bayesian individual assignment method as implemented in the programme GENECLASS 2 (Piry et al. 2004) to estimate the likelihood that a wolf originated from a given population. The marginal probability of a given individual multilocus genotype was compared to the distribution of marginal probabilities of randomly generated multilocus genotypes (10⁴ replicates) using the resampling method of Paetkau et al. (2004) recommended for first generation migrant detection. Because reliable assignment of individuals is dependent on number of individuals sampled (e.g. Paetkau et al. 2004), we chose to omit the small sample from the Arkhangelsk Oblast from this analysis.

Results

Genetic diversity and inbreeding

Genetic diversity appeared to be slightly higher in the Karelian than in the Arkhangelsk wolf population. The average number of alleles (Table 1) was higher (Wilcoxon test: $Z = -2.640$, $P = 0.008$) in the Karelian (5.7) compared to the Arkhangelsk sample (4.7). However, the number of alleles is highly dependent on sample size, and when average number of alleles was estimated for a sample of similar size using a rarefaction method then the average number of alleles was not any more

Table 1 Expected (H_E) and observed (H_O) heterozygosities, number of alleles (*A*) and inbreeding coefficient (*F*) in the studied microsatellite loci in the wolf populations of Republic of Karelia and Archangelsk Oblast

Locus	Karelia (<i>N</i> = 29)				Archangelsk (<i>N</i> = 14)			
	H_E	H_O	<i>A</i>	<i>F</i>	H_E	H_O	<i>A</i>	<i>F</i>
C20.253	0.785	0.747	7	0.076	0.754	0.923	6	-0.185*
C2001	0.762	0.592	6	0.240	0.719	0.769	5	-0.030
C2088	0.822	0.846	7	-0.010	0.605	0.500	5	0.209
C2096	0.687	0.815	5	-0.169	0.618	0.615	4	0.045
C09.173	0.706	0.680	5	0.057	0.700	0.600	4	0.194
CXX.225	0.588	0.333	4	0.448*	0.455	0.333	3	0.307
CPH2	0.764	0.696	5	0.111	0.722	0.778	5	-0.018
CPH4	0.746	0.760	6	0.001	0.701	0.750	6	-0.026
CPH8	0.820	0.600	8	0.287*	0.650	0.500	6	0.280
CPH12	0.409	0.500	4	-0.203*	0.439	0.571	3	-0.231
Mean	0.709	0.656	5.7	0.094*	0.636	0.634	4.7	0.051

* $P < 0.05$

significantly ($Z = -1.889$, $P = 0.059$) higher in the Karelian (4.6) than in the Arkhangelsk (4.2) population.

The observed and expected heterozygosities also were somewhat higher in the Karelian population (Table 1). The average expected heterozygosity in the Karelian population (0.709 ± 0.126) was significantly higher (Wilcoxon test: $Z = -2.599$, $P = 0.009$) than in the Arkhangelsk wolf population (0.636 ± 0.110). However, the observed heterozygosity among the Karelian wolves (0.656 ± 0.155) was not significantly higher (Wilcoxon test: $Z = -0.415$, $P = 0.678$) than among the Arkhangelsk wolves (0.634 ± 0.173). The overall inbreeding coefficient among the Arkhangelsk wolves was not significant ($F = 0.051$; 95% confidence limits -0.162 – 0.170), whereas in the Karelian population the inbreeding coefficient was relatively high ($F = 0.094$; 95% confidence limits: 0.003 – 0.139). In the latter population, both 95% bootstrapped (1000 permutations) confidence limits of the inbreeding coefficient were positive indicating significant inbreeding.

We found significant linkage disequilibrium between some pairs of loci in both populations. After Bonferroni correction, there was significant (at level $P < 0.05$) linkage disequilibrium between three pairs of loci in the Arkhangelsk population and between 11 pairs in the Karelian population. The pairs of loci with significant linkage disequilibrium were totally different in the two populations, suggesting that disequilibrium was due to different demographic histories of the populations.

Population bottlenecks

Little evidence was found of past population bottlenecks in the allele frequency distributions. The allele frequency distribution of the Arkhangelsk population suggested loss of rare alleles, whereas the allele frequencies had a normal L-shaped distribution in the Karelian population (Fig. 3). On the other hand, we did not detect significant heterozygote excess either in the Karelian (Wilcoxon test; $P = 0.084$) or in the Arkhangelsk population (Wilcoxon test; $P = 0.492$). The M -ratio test did not suggest gaps in the allele size distribution. Garza and Williamson (2001) suggest that values of M lower than 0.7 would indicate evidence of a bottleneck, whereas values greater than 0.8 would denote no bottleneck history. In our data set, the M value in the Karelian population was $0.850 (\pm 0.023)$ and in the Arkhangelsk population $0.900 (\pm 0.023)$, suggesting no gaps in allele size distributions in these populations.

Effective population size of the Karelian population

The estimated mean effective size using the approximate Bayesian computation method for the Karelian wolf population was 39.9 (95% CL = 24.8–80.0). The linkage

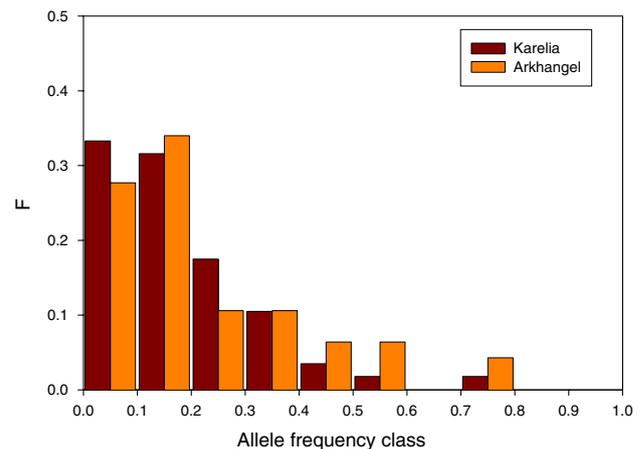


Fig. 3 Allele frequency distributions of the microsatellite loci in the wolf populations of the Republic of Karelia and Arkhangelsk Oblast

disequilibrium based estimate of N_e was rather similar, 46.7 (95% jack-knifed CI = 38.2–115.8).

Population differentiation and gene flow

In the Bayesian analysis of population structure (Pritchard et al. 2000; Falush et al. 2003), the most probable number of clusters was three (Fig 4). The probability of three clusters was ≈ 1.0 and for all other number of clusters ($K = 1$ – 2 and $K = 4$) the probability was < 0.001 in each case. Accordingly, the data did not contain one panmictic population only, and the most probable number of populations was three. The STRUCTURE analyses with only either Karelian or Arkhangelsk samples did not suggest any further substructuring in these populations. In both cases, the most probable number of clusters was one ($P \approx 1.0$ in both cases).

The AMOVA analysis suggested also a clear differentiation between the three populations (Table 2). The

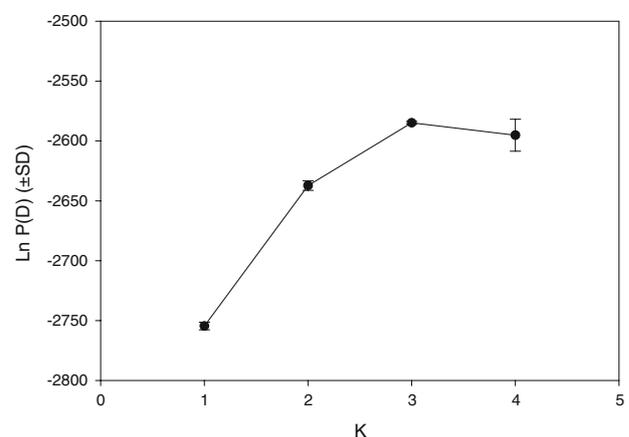


Fig. 4 The model choice criterion $\ln P(D) (\pm SD)$ of the STRUCTURE-analysis over 10 runs for each k (number of clusters) value

Table 2 Analysis of molecular variance (AMOVA) for Russian and Finnish wolf populations

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	2	8.621	0.059	15.20
Within populations	319	104.130	0.326	84.80
Total	321	112.752	0.385	

overall genetic differences (Raymond and Rousset 1995a) between the Karelian, Arkhangelsk and Finnish populations were highly significant (exact differentiation: $P = 1.0$). Most of the variation (85%) appeared to be within populations. However, the variation between populations was also highly significant ($P < 0.001$; 1023 permutations) and explained 15% of the total variation. The ϕ_{ST} —estimate of 0.152 would suggest $Nm = 1.4$ as the average effective number of migrants exchanged per generation between the populations. The pairwise estimates of ϕ_{ST} between populations suggested that the two Russian populations are genetically closer to each other ($\phi_{ST} = 0.051$; $Nm = 4.7$; $P = 0.048$, 1023 permutations) than to the Finnish population. The genetic composition of the Finnish population was more similar to the Karelian population ($\phi_{ST} = 0.151$; $Nm = 1.4$; $P < 0.001$; 1023 permutations) than to the Arkhangelsk population ($\phi_{ST} = 0.176$; $Nm = 1.2$; $P < 0.001$; 1023 permutations).

The private allele method (Slatkin 1985) gave a slightly higher estimate of migrants. The mean frequency of private alleles among the populations was 0.029, and based on this frequency the private allele approach would suggest an overall estimate of $Nm = 3.7$ between all populations. The pairwise estimates suggested that the mean number of wolf migrants between Finland and Karelia would be 3.0 and between Finland and Arkhangel 1.8 individuals in a generation.

The Bayesian approach to estimate asymmetrical migration rates between the populations suggested that the self recruitment is high in both Karelian ($m = 0.906$; $SD = 0.053$) and Finnish ($m = 0.931$; $SD = 0.032$) wolf populations reflecting low immigration rates between the populations. The estimate of the migration rate from the Karelian to the Finnish population ($m = 0.069$; $SD = 0.032$) was lower than the estimate in the opposite direction ($m = 0.094$; $SD = 0.053$). However, because the SDs of both estimates overlap with the estimate in the opposing direction, the migration rates between the populations may not be very asymmetrical.

In the assignment analysis performed with the GENECLASS2-programme (Piry et al. 2004), 97.3% of the wolves were assigned to the population from which they were sampled. In the Karelian population, 26 (89.7%) of the individuals were assigned correctly, and three (10.3%) of

the individuals originated probably from the Finnish population (with probabilities of 0.003, 0.014 and 0.024 being residents). In the Finnish population, 117 (99.1%) of the wolves were assigned to the population from which they were sampled and only one (0.8%) individual was not assigned to the Finnish population (with a probability of 0.004). Accordingly, the analysis suggested that the overall proportion of recent migrants between the populations was only 2.7%, which is compatible with the earlier observations (Aspi et al. 2006). In practice, estimation of a migration rate could be obtained by dividing [the total number of individuals falling past the critical value minus the number of expected type I errors] by [the total number of sampled individuals] (Paetkau et al. 2004). Accordingly, the assignment method suggests that the migration level between the Karelian and Finnish population would have been only less than $m = 0.03$.

Discussion

Despite the historically documented bottlenecks in the northwestern Russian wolf population, we found relatively high amounts of genetic variation. However, genetic diversity tended to be slightly lower in eastern part of the study area. Both the average expected heterozygosity and allele number was significantly higher in the Karelian population (0.709 and 5.7) compared to the respective estimates in the Arkhangelsk population (0.636 and 4.7).

The genetic diversity of the northwestern Russian wolves seems to be similar to other eastern European wolf populations. In the closest wolf population in Finland, the level of heterozygosity has been at a similar level as in the Karelian population. According to Aspi et al. (2006), the observed heterozygosities in different temporal samples vary between 0.680 and 0.706, and the expected heterozygosities between 0.663 and 0.691 in the Finnish wolf population. Lucchini et al. (2004) have investigated several eastern European wolf populations and the expected heterozygosities in those populations varied between 0.69 and 0.71.

The expected heterozygosity was higher than the observed one, and the inbreeding coefficient slightly positive in both studied Russian populations ($F = 0.094$ in Karelian and $F = 0.051$ in Arkhangelsk population). In most wild wolf populations inbreeding coefficients tended to be negative because of active inbreeding avoidance, and the coefficient for the northwestern Russian population are among the highest reported. Only Lucchini et al. (2004) and Ramirez et al. (2006) have reported higher inbreeding coefficients in the isolated Italian and Iberian populations (0.10 and 0.15, respectively).

We did not find very much evidence of past bottlenecks in size of the northwestern Russian wolf populations. Even

though the allele frequency distribution in the Arkhangelsk population was typical for a bottlenecked population (Fig. 3), the level of heterozygosity was not higher than theoretically expected. Neither did the allele frequency distributions and observed versus expected heterozygosities suggest past population bottlenecks in the Karelian wolf population. Cornuet and Luikart (1996) estimated that a bottleneck of $N_e = 50$ is likely to be detectable with the heterozygote excess method for 25–250 generations (0.25–2.5 times $2N_e$) after the initiation of a population reduction, and M -ratios should also achieve equilibrium anew, even after a few hundred generations (Garza and Williamson 2001). Accordingly, the assumed population bottlenecks in the Arkhangelsk and Karelian populations should still be detectable. On the other hand, both tests rely on the assumption that there have been no migration between the populations, and this assumption is obviously violated in wolf populations rendering the power of the analysis when searching past bottlenecks using these methods.

The northwestern Russian and Finnish wolf populations were previously assumed to form a uniform population (e.g. Pulliainen 1965, 1980; Boitani 2003). However, genetic differentiation between the Russian and Finnish wolf populations appears to be much more substantial than formerly thought. Scandinavian and Finnish wolf populations are geographically more than 600 km apart from each other, and connected by a limited dispersal corridor. Seddon et al. (2006) has estimated that the amount of differentiation between the Scandinavian and Finnish wolf populations is $F_{ST} = 0.177$. This is only slightly higher than the estimate between the Finnish and Karelian populations ($\phi_{ST} = 0.151$), even though these population are closer to each other, and there are no obvious geographic barriers between them. However, it is known that during the Soviet Era there were tall fences 5–40 km east of the Finnish–Russian border and at least some of these fences still exist. Information on the status of these fences at this moment is not easy to obtain. However, at least in some cases, these fences seem to prevent wolf migration between Finland and Karelia. As an example, Fig. 5 shows a track of a GPS-collared young male wolf dispersing from Finland to Russia. The wolf was collared on the 11th of April in 2003, and the transmitter provided 1,439 locations before it closed down on the 26th of August, probably because the wolf was killed and the transmitter destroyed. The path, straight as an arrow and partially going back and forth, demonstrates that the above-mentioned fences have prevented the wolf individual from moving directly eastward. There is also another human-induced migratory barrier in the northern part of the study area. Hunting is allowed in the reindeer herding area in Finland, and hunting and poaching effectively prevent wolf dispersal and keep wolves away from northern Finland (Fig. 6).

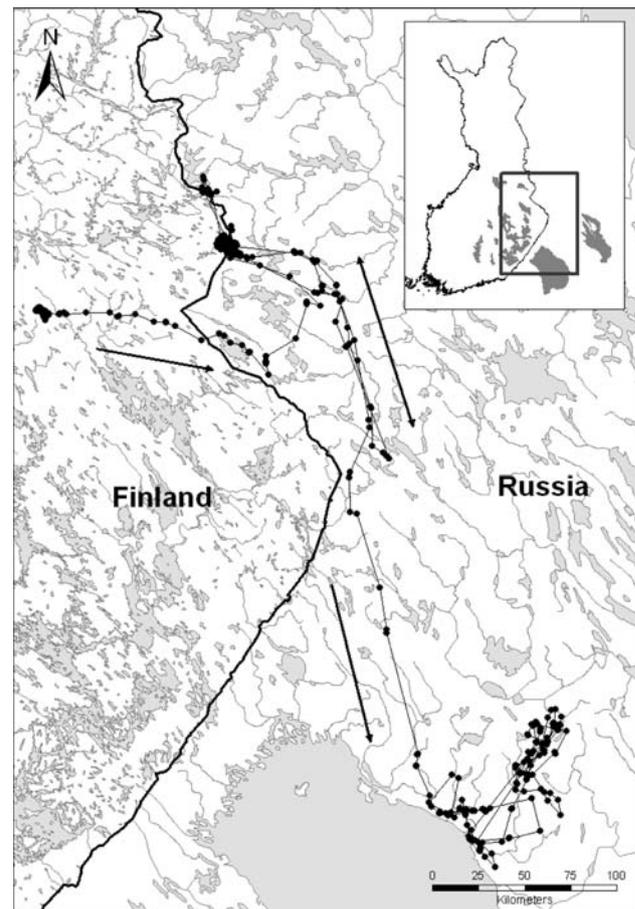


Fig. 5 The track of a GPS-collared male wolf dispersing from Finland to Russia between April and August in 2003

Even though these human-induced barriers may decrease the basic level of migration, they do not totally prevent movement between the Finnish and Russian populations (Kojola et al. 2006). Neither can the barriers contribute to the suggested decline in the amount of gene flow.

All indirect approaches based on population differentiation to estimate the amount of migration between the Finnish and Karelian populations gave very similar long term estimates. The F_{ST} -based method suggested an average number of 1.4 and the private allele approach 3.0 migrants between the populations per generation. A Bayesian model based on MCMC simulations suggested a migration rate (m) of 0.094 from Finland to Karelia and $m = 0.069$ from Karelia to Finland. Aspi et al. (2006) estimates that the effective size (N_e) of the Finnish wolf population is approximately 40 individuals. These estimates together would suggest that the actual number of migrating individuals based on m and N estimates would be 3.8 individuals (40×0.094) from Finland to Karelia. The effective population size of the Karelian population based on Bayesian method was 39.9 and based on linkage disequilibrium method 46.7 individuals. If we are using the

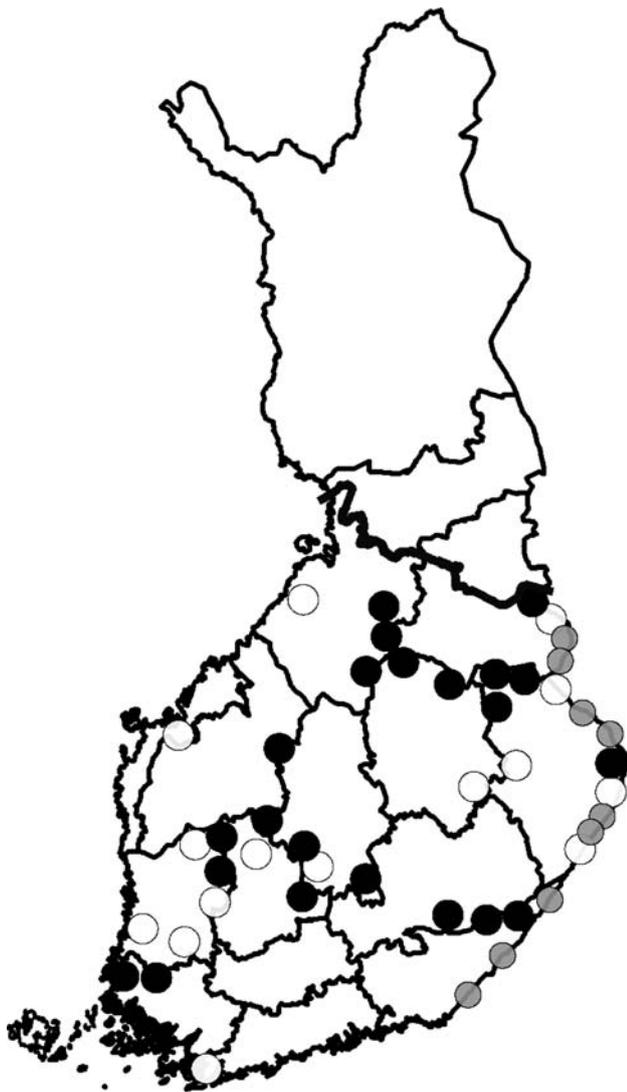


Fig. 6 The position of the wolf territories in Finland in 2007. White circles represent wolf pairs, black circles wolf packs with their territories totally within the borders of Finland, and grey circles wolf packs extending their territories over the national frontier to Russia. The thick black line is showing the border of the reindeer management area

harmonic mean of these estimates (43.0) as an estimate of N_e , the number of migrating individuals from Karelia to Finland would be 3.0 individuals (43×0.069) per generation, which seems to be consistent with the estimate given by the private allele method.

The assignment-based approach suggested an even lower number of recent migrants between the populations (less than 0.03) than the indirect estimates. Discrepancies between indirect and direct assignment-based estimates of Nm suggest that the relative role of migration between Finnish and northwestern Russian populations was earlier more important as suggested by the higher indirect long-term estimates of migration. There may be several reasons

for the decreased migration rate between the populations. Most importantly, it is associated with the density dependence of dispersal. Wolff (1997) has suggested that in territorial species such as wolf dispersal should be inversely density-dependent because territoriality at high densities may impede immigration and make it difficult for juveniles to leave their natal area. Since wolf packs can be highly territorial and often kill lone wolves within their territories (e.g. Packard 2003), dispersal into occupied areas may be difficult. A recent study of Seddon et al. (2006) reports that the survival of migrants among wolves may often be poor. Using assignment methods, they identified four wolves immigrating from Finland to the Scandinavian population, and only one of these immigrants survived. The mean territory size of the Finnish wolves is about 1,000 km². Because of the recent population growth in Finland, the area used by occupied territories has increased, and at present they are effectively filling all available space along the eastern border preventing immigration to the population (Fig. 6).

The assignment-based approach suggested that the migration rates may be asymmetric with more migration from Finland to Karelia than vice versa. Asymmetric migration rates have been observed earlier between other wolf (Wilson and Rannala 2003) and coyote populations (Sacks et al. 2005). For example, Wilson and Rannala (2003) present migration estimates between nine wolf populations in North America utilizing the data collected by Carmichael et al. (2001). Most of these populations did not have symmetrical migration rates, suggesting that movement of animals between these regions is predominantly unidirectional. The total pattern given by the long-term and short-term estimates for migration between Finland and Karelia suggests that the migration in the past has been more symmetric, but nowadays there is more migration from west to east than vice versa. One obvious reason for possible shift in the direction of migration, is the decline in the population size of the wolf in Russian Karelia (Danilov 2005) and increase in Finland (Kojola and Määttä 2004).

A few recent studies have suggested that the amount of gene flow between populations could be correlated with climate, habitat type, and wolf diet composition Carmichael et al. 2001; Geffen et al. 2004; Pilot et al. 2007; Musiani et al. 2007). These results imply that behavioural and ecological processes may strongly influence the amount of gene flow among wolf populations. However, the diet composition and habitat of Finnish and Karelian wolf populations seems to be very similar, and it is questionable whether the genetic differentiation could be caused by prey-habitat specialization. In addition to human induced barriers, the mechanism behind the differentiation is more probably restricted dispersal together with territorial behaviour. These findings emphasise the role of

physical obstacles and territorial behaviour in creating barriers to gene flow between populations in relatively limited geographical areas especially in large-bodied mammalian species with long-distance dispersal capabilities and apparently continuous population structure.

Conservation implications

Understanding connectivity between populations is an essential component of managing populations for conservation. Lack of gene flow from neighbouring populations together with a small population size could lead to inbreeding, loss of genetic variability and increased risk of extinction. Grey wolf populations seem to be vulnerable to harmful effects of inbreeding. Inbreeding depression has been documented in wolves held in captivity (Pulliainen 1965; Laikre 1999). Negative effects of inbreeding have been documented also in natural populations of wolf. Vilà et al. (2003) provide evidence that the Scandinavian population of wolf was for a long time limited in size by a lack of genetic diversity. In the same population, Rääkkönen et al. (2006) report an overall higher incidence of vertebral malformations, and Liberg et al. (2005) strong correlation between the inbreeding coefficient of parents and pup mortality: each increase of 0.1 in inbreeding coefficient would decrease the litter size by 1.15 pups, corresponding to 6.04 lethal equivalents (2B).

On the other hand, it seems that an inbred wolf population may be to some degree able to avoid the deleterious consequences of inbreeding. Bensch et al. (2007) show that among the Scandinavian wolf population the most heterozygous wolves seem to establish themselves as breeders, and this process has decelerated the loss of heterozygosity in the population despite a steady increase of inbreeding. It also seems that among wolves a very low amount of gene flow may be extremely effective in restoring genetic diversity and reducing the risk of extinction through inbreeding. Vilà et al. (2003) demonstrates that the steady increase in the Scandinavian wolf population started with the arrival of a single immigrant wolf.

In a recent study, Aspi et al. (2006) estimate that despite the increased numbers, the effective population size of the Finnish wolf population is still too low to maintain a self-sufficient population, and that migration from Russia is essential for the long-term survival of the population. According to the Management Plan for the Wolf Population in Finland (Anonymous 2005), 1–2 wolves arriving in the area per wolf generation ensure adequate genetic variation to maintain the viability of the wolf population in the long term (cf. Liberg 2005). Our present analysis suggests that immigration of wolves from the population in the Republic of Karelia into Finland has declined recently. It seems that the level of immigration is probably not any more sufficient

enough to maintain an adequate level of genetic variation in the Finnish wolf population. On the other hand, our results also suggest that despite the suggested larger survey size the effective population size of the Karelian wolf population may not be very much larger than that of the Finnish population and may also be too small to avoid the harmful effects of inbreeding. If this population continues to decline, it might also need migrants from the Finnish or from the other surrounding populations to avoid harmful effects of inbreeding in the long run. For the conservation of these populations, increasing the level of migration between them seems to be essential.

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