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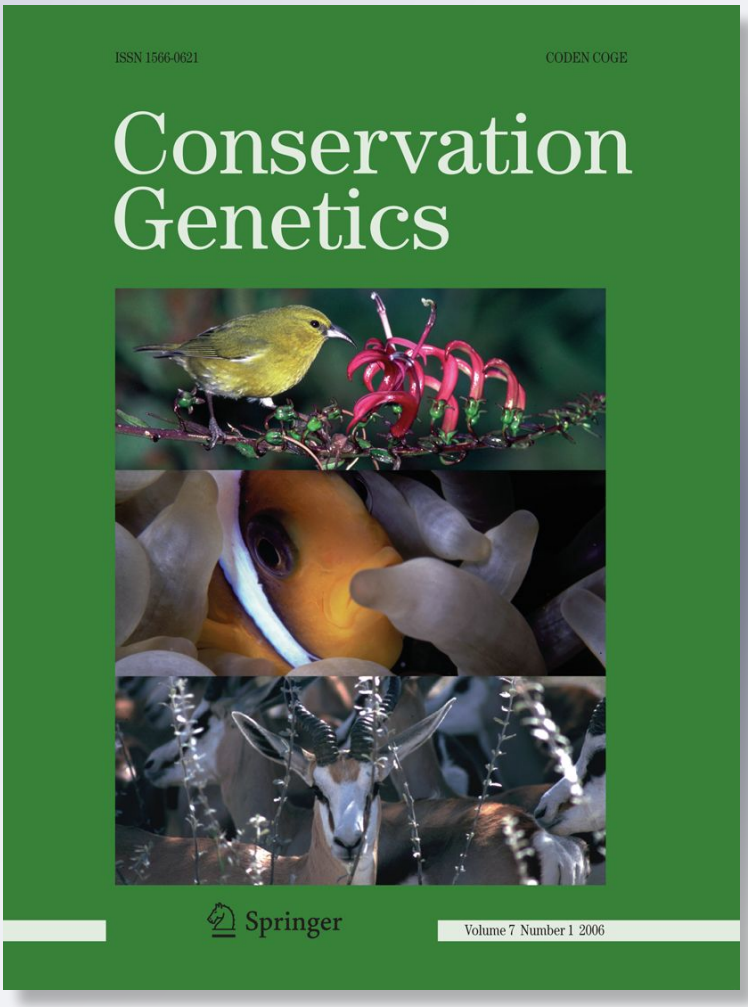

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RESEARCH ARTICLE

Connectivity and population subdivision at the fringe of a large brown bear (*Ursus arctos*) population in North Western Europe

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Abstract Loss of connectivity and habitat destruction may lead to genetic depletion of wild animal populations, especially in species requiring large, connected territories as the brown bear (*Ursus arctos*). Brown bear populations of North Western Russia, Finland and Northern Norway have been assumed to form one large, continuous population; however this hypothesis has not been tested sufficiently. We have genotyped 1,887 samples from 2005 to 2008 from four distinct areas and used the resulting DNA profiles from 146 different individuals to analyze the genetic diversity, population structure, and the migration rates among groups. In addition, we have tested for traces of previous genetic bottlenecks. Individuals from Eastern Finland and Russian Karelia were grouped in the same cluster ("Karelia"), while distinctive subpopulations of brown bears were detected in the north ("Pasvik"), and the east ("Pinega"). All three subpopulations displayed high genetic variation, with expected heterozygosities (H_E) of 0.77–0.81, but differentiation among the clusters was relatively low (average $F_{ST} = 0.051$, $P < 0.001$). No evidence of genetic bottlenecks in the past was found. We detected a highly significant isolation-by-distance (IBD) pattern. For Pasvik, self-recruitment was found to be very high (96%), pointing to the possibility of genetic isolation. In contrast, between Karelia and Pinega we detected high, bi-directional migration rates (~30%), indicating genetic exchange. Conclusively, despite of a substantial influence of IBD on the genetic structure in the region, we detected considerable variation in connectivity among the identified clusters that could not be explained solely by the distance between them.

Keywords Gene flow · Isolation-by-distance · Non-invasive genetic sampling · Microsatellites · Migration · Population structure

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Introduction

Wild animal populations worldwide are increasingly faced by the threat of fragmentation, isolation, and loss of connectivity following habitat discontinuity and anthropogenic disturbance. Due to their large home ranges, long generation time, roaming dispersal, and possible conflict with

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humans, large predators are prone to habitat fragmentation (Crooks 2002; Miller and Waits 2003). After being almost extirpated in most parts of Europe and North America, some populations have recovered and we have just began to understand the importance of large predators in terrestrial ecosystems (Smith et al. 2003).

The connectivity, measured by the degree of differentiation and the amount of migration events that take place among populations, is expected to be strongly linked to the long-term viability of a population (Long et al. 2005; Schwartz et al. 2002). A stable connection among populations, i.e. migration of individuals, will ensure gene flow and thus counteract genetic drift, which leads to genetic depletion (Mills et al. 2003). However, direct measurement of migrating brown bears using GPS equipped animals is expensive and time consuming (Whitlock and McCauley 1999; Spong and Creel 2001; Solberg et al. 2006; Swenson et al. 2011). Capture and handling of wild bears may also have harmful long-term effects on the handled individuals (Cattet et al. 2008). Thus, as an alternative approach, genetic methods based on non-invasive sampling of hairs and faeces have been applied to study movement of individuals and gene flow among populations in both restricted and larger areas (Bellemain et al. 2005; Proctor et al. 2005; Kendall et al. 2009; De Barba et al. 2010).

The Northern European brown bear (*Ursus arctos*), which was once widespread, has experienced severe reductions, caused mainly by anthropogenic factors such as habitat destruction and unrestrained hunting (Swenson et al. 1994, 1995; Chestin 1999; Kojola et al. 2003; Swenson et al. 2000; Danilov 2005). At the beginning of the twentieth century the brown bear nearly disappeared from Northern Europe. The Fennoscandian brown bear populations reached their minimum at around the first half of the last century. With the exception of areas in North Western Russia, Eastern Finland and North Eastern Norway, the brown bear was functionally extirpated in the area (Kolstad et al. 1986; Elgmork 1990; Pulliainen 1990; Nyholm and Nyholm 1999; Sørensen et al. 1999). During the 1970s, brown bear populations in Fennoscandia and Eastern Europe started to recover and to expand again towards the west into areas where they had been extirpated (Pulliainen 1983a, b, 1990; Swenson et al. 1994; Nyholm 1990; Nyholm and Nyholm 1999). In Sweden, effort-corrected field observations combined with genetic studies have shown that the population has recovered from an estimated extreme bottleneck in the 1970s ($N < 50$, Tallmon et al. 2004) to a population size of approximately 3,200 individuals in 2009 (Kindberg et al. 2009). Based on field observations, the population size in Finland is estimated to be between 900 and 1,000 brown bears (Wikman 2009), while at same time in Norway, 166 brown bears were detected using non-invasive sampling and DNA-methods (Wartiainen et al. 2010).

Russia is home to the probably largest brown bear population in the world. Based on hunting and observations of bears, Kolesnikov (2009) has estimated the population in European Russia to be around 40,000 brown bears. North Western Russia has been assumed to be the major reservoir for large carnivores migrating into areas of Finland and Northern Norway (Pulliainen 1990; Swenson and Wikan 1996). The respective border areas of both countries with Russia include protected and unprotected zones which are relatively pristine, and have been referred to as the "Fennoscandian Green Belt" as part of the European Green Belt initiative (Karivalo and Butorin 2006). Trans-border brown bear movements in both directions have been recorded previously at these borders, but nevertheless population numbers, densities as well as connectivity between the populations remained obscure (Pulliainen 1990; Swenson and Wikan 1996; Kojola et al. 2003).

Previous studies of brown bear mitochondrial genetic variation suggested that the bears of Northern Eurasia form a lineage distinct from other bear populations in Western and Southern Europe (Taberlet and Bouvet 1994; Kohn et al. 1995; Saarma et al. 2007). However, these results represent only the maternal lineages and phylogeographic connections on a broad time scale, which may not reflect the current population structure and gene flow that are important for ongoing conservation and management actions (Waits et al. 2000). A recent study applying autosomal microsatellites to a number of bears in North Western Eurasia was suggestive of large-scale gene flow in northernmost parts of the region (Tammeleht et al. 2010).

Our general assumption and hypothesis is that the brown bears in North Eastern Europe represent one unified genetic population. To test this we have used samples from different monitoring projects across Fennoscandia and North Western Russia and applied genetic methods to investigate the population structure, connectivity and migration rates of brown bear populations in the region. We also evaluated the assumption that the region of Russian Karelia acts as a source population for the neighbouring Finnish and Norwegian bear populations, and we have tested if the dramatic decline in bears during the nineteenth and twentieth centuries within these populations left any signs to their genetic composition.

Materials and methods

Sampling

Genetic sampling of brown bears was carried out between 2005 and 2008 in four areas in North Western Europe with

previous and ongoing brown bear monitoring (Swenson and Wikan 1996; Kojola et al. 2003; Danilov 2005): (1) The Pasvik area including parts of Norway, Finland and Russia at ~67–70°N, ~25–30°E, (2) the Kainuu area in Finland and in Northern Karelia in Russia at ~63–65°N, ~29–30°E, (3) in Southern Karelia in Russia at ~60–62°N, ~31–37°E and (4) the Pinega Strict Nature Reserve in Archangelsk, Russia at ~64°N, ~43°E. (Fig. 1a). Distance between the research areas of Pasvik and Kainuu/Northern Karelia as well as between Southern Karelia and Pinega was about 600 km. Distance between the North and the South Karelian sampling area was about 150 km. Pasvik and Pinega sampling areas were furthest from each other with an approximate terrestrial distance of 1,200 km. Faeces and hairs were collected opportunistically in the field. Scats were stored either in stool collection tubes with DNA stabiliser (Invitek) or in plastic bags and kept at −20°C until DNA extraction. In the Pasvik study area, we also collected hair samples systematically within a geographic grid using hair snares following a method modified from Woods et al. (1999). The hair samples were stored dry and dark in paper envelopes until DNA-extraction. In addition, for the study areas of Northern and Southern Karelia in Russia, we obtained 34 tissue samples originating from bears legally harvested from 2005 to 2007, which were stored in ethanol at −20°C until DNA extraction.

DNA extraction and microsatellite genotyping

We extracted DNA from faeces using the PSP Spin Stool DNA Plus Kit (Invitek) and from hairs and tissues we used the DNeasy Tissue Kit (Qiagen) by following the manufacturers' instructions. We ran all samples with selected markers in order to check for successful extraction and quality of the sample, followed by the genotyping using 13 different dinucleotide markers (Short-tandem-repeats, STRs) originally developed for bears: G1A, G1D, G10B, G10L (Paetkau and Strobeck 1994, 1995; Paetkau et al. 1995); Mu05, Mu09, Mu10, Mu15, Mu23, Mu26, Mu50, Mu51, and Mu59 (Taberlet et al. 1997) plus one marker for sex determination using the primers SE47 (Yamamoto et al. 2002) and R143 (5′-AGGTGGCTGTGGCGGCA-3′). PCR and fragment analysis were performed as previously described by Eiken et al. (2009).

The samples were genotyped independently two times if heterozygous and three times if homozygous for the specific markers (peak height threshold values >300 RFU). For a sample to be assigned an identity, all runs across all markers had to be consistent. If this was not the case, the

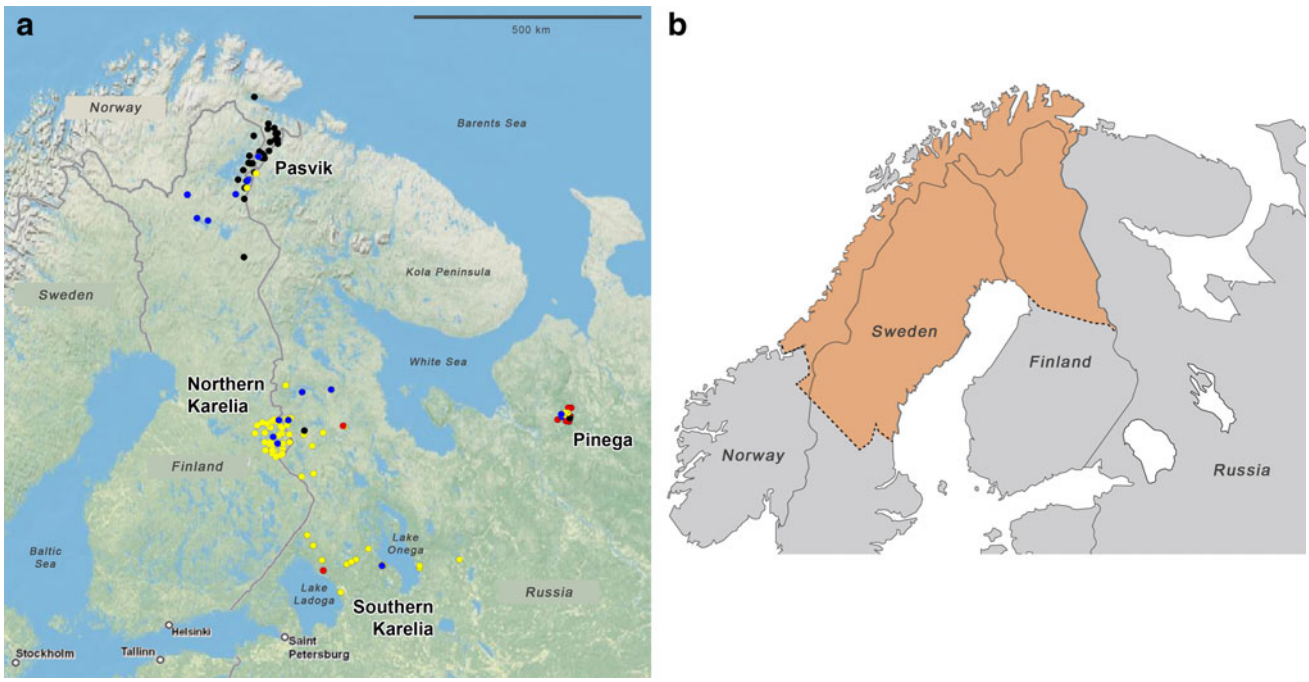

Fig. 1 **a** The sampling location of the 146 different brown bears, represented by a *circle*. Genotyping with 13 different STRs identified individuals from the research areas in Pasvik: $n = 41$ (Norway, Finland and Russia); Kainuu/Northern Karelia: $n = 60$ (Finland and Russia); Southern Karelia: $n = 18$ (Russia); and Pinega: $n = 27$ (Russia). The membership (coefficient ≥0.7) of the genotypes of each of the individuals were assigned with the program Structure (Pritchard et al. 2000), and the cluster assigned to each genotype is indicated by different *colours*: Pasvik (*black*), Karelia (*yellow*) and Pinega (*red*). Genotypes with a probability of cluster membership coefficient <0.7, ($n = 20$; *blue circles*) could not be assigned to any of the detected clusters. **b** The reindeer husbandry area in Northern Europe (*orange*)

sample was not assigned an identity and discarded from further analyses. Evaluations leading to consensus DNA profiles were not used in our study. We only accepted single negative result for STRs if the sample showed consistent results for the overall DNA profile. Negative controls were run for every eight samples, two positive controls were run first and last on the 96-well plates. PCRs for the sex determination were run twice with positive controls. Our procedures followed the strict guidelines for forensic examination of animal DNA material, which are in accordance to the requirements published recently by Linacre et al. (2011). The uniqueness of the DNA profiles was verified by calculating the probability of identity of each sample using the software Gimlet version 1.3.3 (Valiere 2002). Tests for allelic dropout, presence of null alleles, and scoring errors caused by stutter peaks were performed with Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004).

Population structure

In order to detect population structure and thus possible areas of genetic discontinuity, we used the spatial explicit model as implemented in the software Geneland version 3.2.4 (Guillot et al. 2005). We ran five independent runs, where the parameters for possible populations were $K = 1$–10, and the number of MCMC iterations was 1,000,000, saving every 100th. The maximum rate of Poisson process was set to 100.

Additionally, we studied possible population structure using the Bayesian approach implemented in program Structure version 2.3.3 (Falush et al. 2003; Hubisz et al. 2009; Pritchard et al. 2000), which allows detection of modest population differentiation. For this analysis we assumed population admixture and correlated allele frequencies within the populations. We carried out 10 independent runs for each value of K (number of subpopulations) between one and ten. Program parameters included a burn-in period of 100,000 Markov Chain Monte Carlo (MCMC) iterations, followed by sampling of 1,000,000 iterations. If there is hierarchical structure in the studied population, the log-likelihoods estimated with the Structure program does not necessary reflect the real number of clusters (Evanno et al. 2005). Therefore, we estimated the rate of change in the log probability of data between successive K values (ΔK) as described by Evanno et al. (2005) to determine the most likely number of clusters.

The factorial correspondence analysis (FCA) in the program Genetix 4.05.2 (Belkhir et al. 1996–2004) was used to visualize the relative similarity among samples and possible genetic structure within each region in a multivariate space. We used program Arlequin (version 3.5.1.2; Excoffier and Lischer 2010) to perform the AMOVA analysis to reveal genetic structure among regions, among populations and within populations using 10,000 permutations. We also used the program Arlequin to estimate pairwise F_{ST} values (Weir and Cockerham 1984) among each population (10,000 dememorization steps, 100 batches and 500 iterations per batch).

Genetic diversity and inbreeding

Observed and expected heterozygosities as well as allele numbers, inbreeding coefficients and linkage disequilibrium for all sampled populations were estimated using the software Genetix 4.05.2 (Belkhir et al. 1996–2004). We used the method of Black and Kraftsur (1985) to test for linkage disequilibrium among pairs of loci in each population. Genepop version 4.0 (Rousset 2008) was used for a global test for deviations from Hardy–Weinberg equilibrium (HWE) using Fisher's method (Rousset and Raymond 1995) across all loci and populations. All combinations of populations were tested with unbiased P values by a Markov chain method of 1,000 dememorization steps, 500 batches and 1,000 iterations per batch.

Migration and isolation-by-distance

We estimated migration rates between the populations using the private allele method (Barton and Slatkin 1986). In addition we estimated recent migration rates among the detected subpopulations with the program BayesAss 1.3 (Wilson and Rannala 2003). The program uses a Bayesian approach to calculate asymmetric proportion of non-migrants and inter-population migration rates. We performed 6,000,000 burn-in iterations followed by 3,000,000 iterations and a thinning of 2000. Initial input parameters of allele frequencies, migration and inbreeding coefficient were set at 0.15 for each respectively. Three independent runs were carried out to confirm consistency of results. Differences between migration rates were considered significant in cases where the 95% confidence intervals from the posterior distribution did not overlap.

When analyzing possible population subdivision of a far ranging mammal, as the brown bear, an effect of isolation-by-distance (IBD) has to be considered (Forbes and Hogg 1999). We used the software Spagedi version 1.3 (Hardy and Vekemans 2002) to estimate the correlation between geographical distance and relatedness among pairs of individual brown bears (Hardy 2003) using the kinship coefficient by Loiselle et al. (1995). This estimator weights the allele contribution and is not influenced by bias in the presence of low frequency alleles. Estimates for standard errors for average multilocus statistics were obtained by jackknifing over loci.

Population bottlenecks

For all subpopulations, we used the program Bottleneck 1.2.02 (Cornuet and Luikart 1997; Luikart et al. 1998; Piry et al. 1999) to detect whether the heterozygosity was larger than the heterozygosity expected from the number of alleles found in the sample given that the population was at mutation drift equilibrium. We applied the two-phase mutation model using 95% single-step mutations to estimate the expected heterozygosities (20,000 iterations). Significance of the differences between observed and expected heterozygosities were tested using the Wilcoxon test. Because the heterozygosity excess persists only a certain number of generations until a new equilibrium is established (0.2–4N_e generations; Luikart and Cornuet 1998), the detection of a past bottleneck is limited using the method above. The ratio of the number of alleles with respect to allele size range decreases after a population reduction and can be detected after up to approximately 125 generations following a severe population bottleneck (Garza and Williamson 2001). We calculated such ratios (hereafter Garza-Williamson indices) with the software Arlequin 3.5.1.2 (Excoffier and Lischer 2010).

Results

Sampling and molecular analysis

A total of 1,887 bear samples were collected throughout the four areas between 2005 and 2008 (Fig. 1a; Table 1). The distance between the research areas are given in "Materials and methods". From these 1,887 samples, 854 samples could be successfully genotyped, 906 did not contain enough DNA, and 127 samples were discarded because of inconsistent genotyping results. We identified in total 215 different individuals (Table 1). In two of the four areas (Pasvik and Pinega) sampling was carried out within a relatively restricted area (see Fig. 1a), and in order to avoid possible overrepresentation of family members (Anderson and Dunham 2008) a subset of bears was selected randomly for further genetic analysis. In the resulting dataset of 146 individuals, we observed consistent DNA typing results in 13 STRs for 124 of the individuals, while 15 individuals lacked data for one STR, six individuals lacked data for two STRs and one individual lacked data for four STRs. We chose to also include these 22 individuals that were lacking results for single STRs in the following genetic analysis as their DNA profiles were consistent for all other markers. Analysis using Micro-Checker did not suggest allelic dropout, presence of null alleles, or scoring errors in our microsatellite data of the 146 individuals. Our dataset is accessible from supplementary Table S1.

Table 1 Brown bear samples collected in four different geographical regions in North Western Europe between 2005 and 2008

	Pasvik	Northern Karelia	Southern Karelia	Pinega
Samples in total	1,339	104	18	426
Faeces	827	89	0	426
Hair	499	0	0	0
Tissue	13	15	18	0
Samples with assigned ID	644	93	18	99
Samples with no ID	70	0	0	57
Negative samples	625	11	0	270
Number of males	48	33	15	28
Number of females	28	26	3	15
Unidentified sex	5	1	0	13
Numbers of bears in total	81[a]	60	18	56[a]

The DNA profiles and individual identity (ID) were determined using 13 different STR markers, and gender was determined using an amelogenine XY-assay (see "Materials and methods")

[a] From Pasvik and Pinega a subset of 41 and 27 individuals respectively were used in the population genetic analyses (see "Materials and methods")

Population structure

The FCA-analysis suggested geographic structuring among the sampled regions (Fig. 2). Bears from Pasvik and Pinega regions tended to belong to specific and separate clusters. However, there was considerable overlap between bears from North and South Karelian regions. The Bayesian program Structure, with correction using Evanno's ad-hoc approach (Evanno et al. 2005), showed the highest ΔK for $K = 3$ clusters (Figs. 3, 4a), as did the analysis with geographic coordinates and correlated allele frequencies used as prior with Geneland (Fig. 4b). Lower membership coefficient of a few genotypes could indicate admixture between the populations and may point to possible migrants. Across all individuals, 26 (17.8%) genotypes showed an estimated membership coefficient of <0.7 for their original sampling area. Out of these 26 genotypes, nine individual genotypes could be assigned to one of the other two clusters i.e. for Pasvik two such genotypes (4.8%) could be assigned to Karelia; for Karelia, two genotypes were assigned to Pasvik (3.3%) and one to Pinega (1.7%); and in Pinega, one genotype could be assigned to Pasvik (3.7%) and three to Karelia (11.1%). 17 (11.6%) genotypes with a membership coefficient <0.7 could not be assigned to one of the detected clusters. These specific assignments of individual genotypes are illustrated by different colors for all of the clusters in Figs. 1a, 4a. Additional analyses with Bayesian algorithms for each of the four sampled groups did not reveal further sub-structure. Thus, all subsequent analyses involving population substructure were conducted in accordance to the

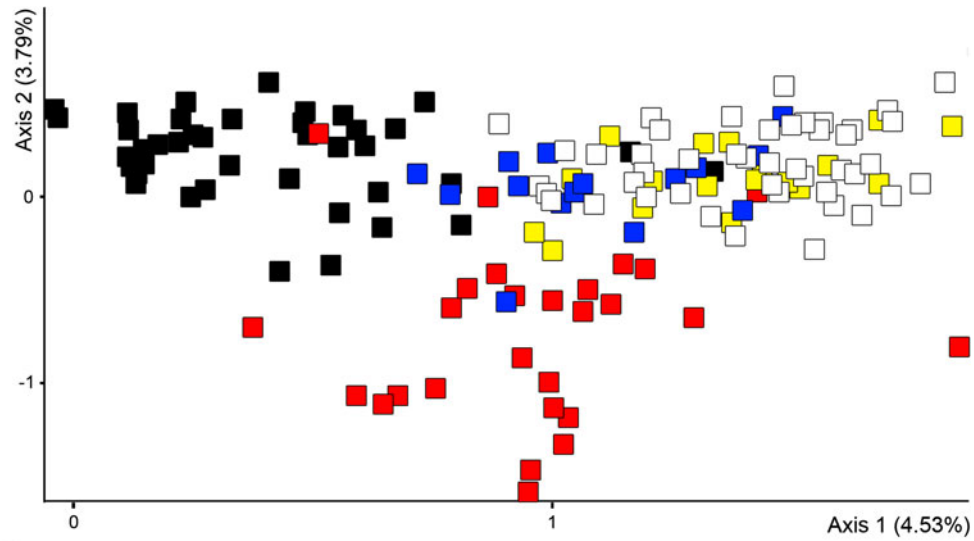

Fig. 2 Two-dimensional factorial correspondence plot using Genetix 4.05.2 (Belkhir et al. 1996–2004) for allele frequencies at 13 microsatellite loci from brown bears sampled in: Pasvik (*black squares*), Kainuu (*white*), Northern Karelia (*blue*), Southern Karelia (*yellow*) and Pinega (*red*)

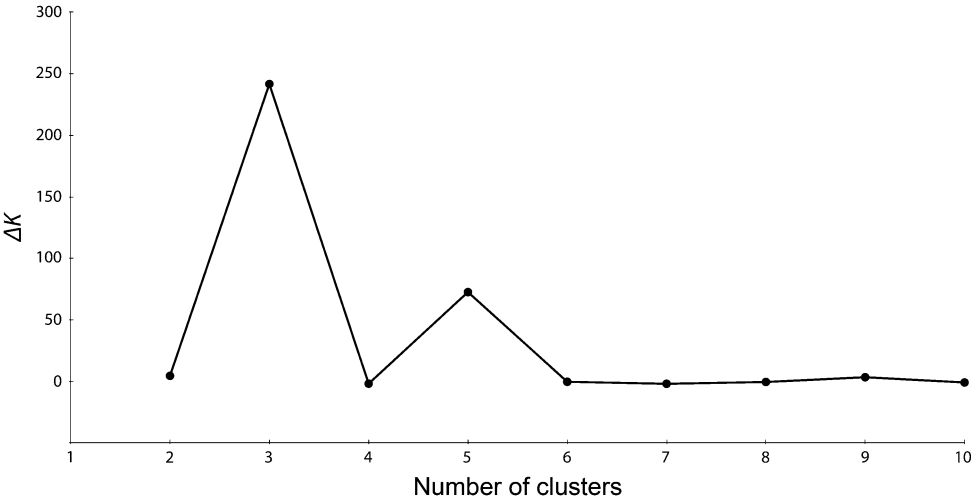

Fig. 3 Rate of log-likelihood values (ΔK) for samples from all the individual bears for different number of clusters from the program Structure (Pritchard et al. 2000) post-processed with Evanno's approach (Evanno et al. 2005)

detected three clusters Pasvik, Karelia and Pinega. AMOVA analysis revealed significant geographic structuring of these three populations. Most of the variation (95%) appeared to be within populations, and the variation between populations was also highly significant ($P < 0.001$; 1,023 permutations) and explained 5% of the total variation. The population pairwise F_{ST} values were significant for all population pairs ($P < 0.001$): Pasvik versus Karelia = 0.049, Pasvik versus Pinega = 0.064 and Karelia versus Pinega = 0.048. The overall F_{ST}-value was 0.051 ($P < 0.001$).

Genetic diversity and inbreeding

Among the genetic groups, we found that Pasvik and Pinega conformed to HW expectations. However, the population of bears from Karelia showed significant deviations from HWE associated with significant and positive F_{IS}-values at the loci MU23, MU26 and G10B. Table 2 summarises the results for the three clusters showing the number of alleles and values for expected and observed heterozygosities. Mean expected and observed heterozygosities were between 0.76 and 0.81 in all three groups. Only two loci, MU26 and MU23, showed observed heterozygosities lower than 0.6. After sequential Bonferroni correction, we found 11 pairs of loci (14.1%, $P < 0.05$) with significant linkage disequilibrium. These pairs of loci were different in all sampled areas and none of the pairs of loci showed linkage disequilibrium in samples from more than two areas.

Migration and isolation-by-distance

The mean frequency of private alleles among the populations was 0.039, suggesting reasonable amount of migrants among them (Nm = 2.2). The results of the Bayesian approach of detecting asymmetrical migration rates showed that in the Pasvik population the self-recruitment was about 96% (CI 0.915–0.994; Table 3). We found

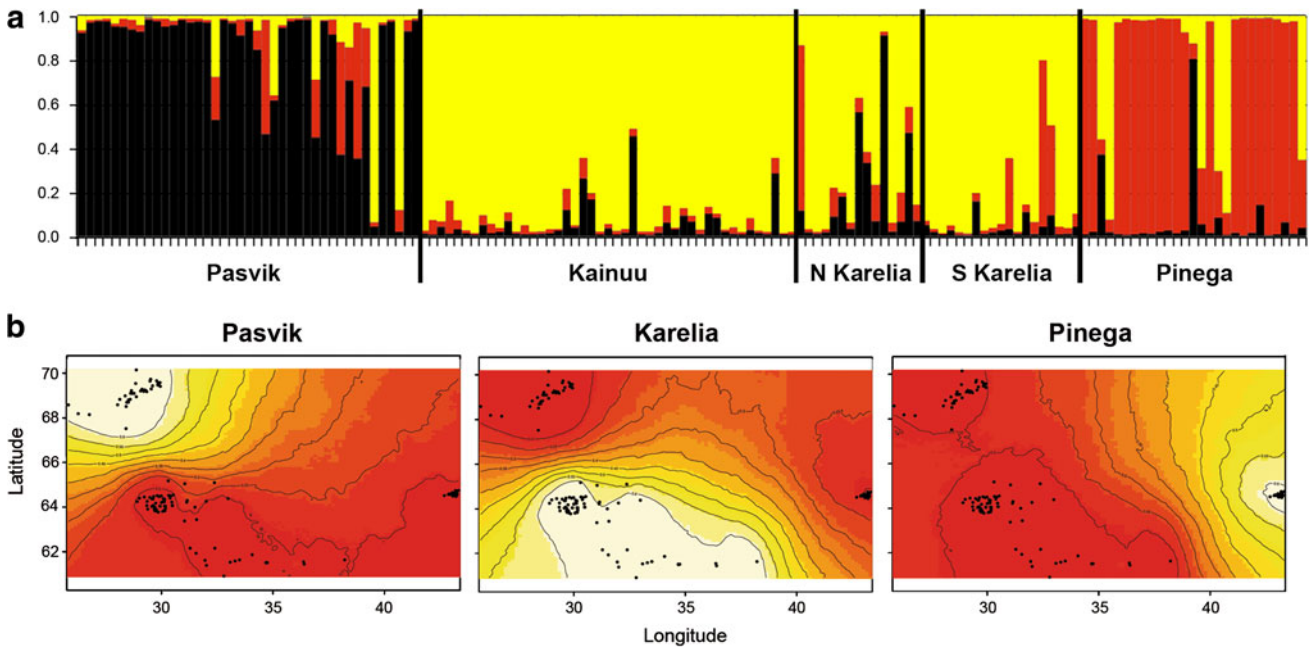

Fig. 4 **a** The individual *Q*-matrix based on the probabilities of the results using Structure. The *colours* represent the different populations (Pasvik, Karelia (Kainuu, North Karelia, South Karelia) and Pinega) detected with $K = 3$ clusters and each *bar* correspond with a single individual, in which the height of the *bar* represents the probability of that individual bear belonging to the particular cluster. The *black colour bars* show cluster one, *yellow* represents cluster two and cluster three is marked in *red colour*. **b** Map and probabilities of population membership calculated with Geneland. The dots represent individuals sampled in Pasvik, Karelia (Kainuu, North Karelia, and South Karelia) and Pinega. Bears belonging to the same cluster are shown in the *lighter colored area*

Table 2 Number of alleles (A) and observed (H_O) and expected heterozygosity (H_E) as well as the inbreeding coefficients (F_{IS}) of the sampled regions

Locus	Pasvik ($n = 41$)				Karelia ($n = 78$)				Pinega ($n = 27$)			
	A	H_E	H_O	F_{IS}	A	H_E	H_O	F_{IS}	A	H_E	H_O	F_{IS}
MU05	7	0.82	0.90	−0.088	9	0.79	0.77	0.039	10	0.84	0.82	0.043
MU09	12	0.86	0.81	0.072	9	0.87	0.85	0.038	9	0.82	0.93	−0.105
MU10	6	0.75	0.68	0.098	10	0.79	0.76	0.046	8	0.81	0.80	0.032
MU15	6	0.76	0.81	−0.045	9	0.80	0.85	−0.054	6	0.70	0.82	−0.138
MU23	7	0.71	0.68	0.048	9	0.84	0.69	0.188*	8	0.66	0.58	0.141
MU26	5	0.57	0.50	0.130	8	0.67	0.56	0.169*	7	0.74	0.56	0.264*
MU50	8	0.84	0.88	−0.031	8	0.74	0.71	0.047	10	0.79	0.89	−0.111
MU51	7	0.82	0.83	0.002	10	0.83	0.77	0.071	8	0.78	0.78	0.020
MU59	10	0.81	0.85	−0.046	15	0.90	0.92	−0.013	12	0.89	0.92	−0.018
G1A	8	0.78	0.81	−0.015	9	0.80	0.80	0.008	8	0.84	0.96	−0.127
G1D	9	0.86	0.98	−0.122	9	0.82	0.81	0.014	8	0.83	0.89	−0.059
G10B	9	0.75	0.73	0.035	11	0.85	0.65	0.246*	8	0.82	0.85	−0.016
G10L	9	0.69	0.71	−0.007	10	0.78	0.75	0.046	8	0.82	0.81	0.039
Mean	7.9	0.77	0.78	−0.002	9.7	0.81	0.76	0.064*	8.5	0.80	0.81	−0.005
SD	1.9	0.08	0.12		1.8	0.06	0.09		1.5	0.06	0.12	

* $P < 0.05$

migration rates of about 30% (CI 0.256–0.328) from Karelia to Pinega, whereas the rate from Pinega into Karelia was about 32% (CI 0.299–0.332).

We detected a significant, negative relationship between kinship and spatial distance between pairs of individuals ($b = -0.015$; $P < 0.001$; Fig. 5), pointing to IBD. All ten

Table 3 Bayesian analysis of migration with the program BayesAss resulted in this matrix on the high rate of self-recruitment within the population of Pasvik and migration rates between the bear populations of Karelia and Pinega

	Pasvik	CI	Karelia	CI	Pinega	CI
From Pasvik to	0.96	0.915–0.994	0.01	0.000–0.029	0.02	0.001–0.062
From Karelia to	0.01	0.000–0.050	0.67	0.667–0.682	0.30	0.256–0.328
From Pinega to	0.02	0.003–0.062	0.32	0.299–0.332	0.68	0.667–0.710

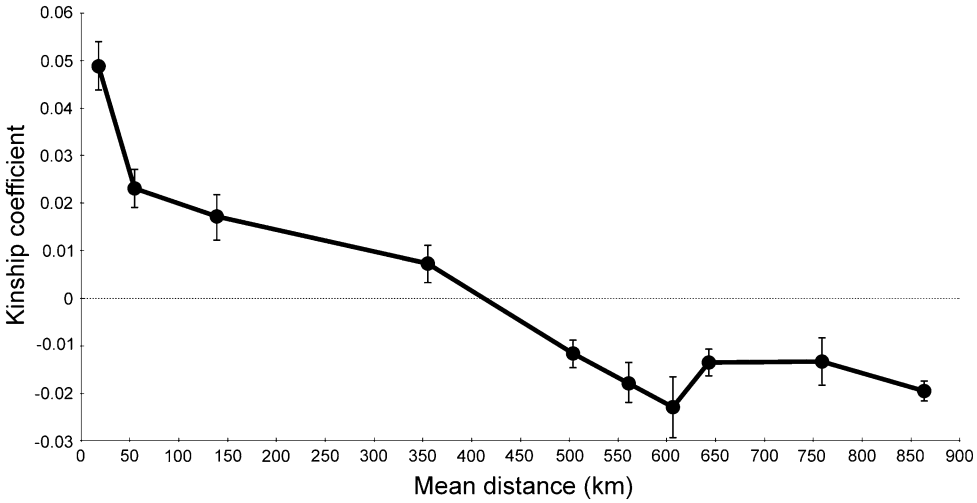

Fig. 5 Kinship coefficient plotted against the mean distance between the pairs of individuals of the sampled brown bears with the software Spagedi 1.3 (see "Materials and methods"). All ten distance classes differ significantly ($P < 0.001$) from the mean kinship of the population

distance classes showed highly significant deviation from the population mean.

Population bottlenecks

The allele frequency distribution of the three distinctive genetic groups of bears (Pasvik, Karelia and Pinega) showed no signs of population bottlenecks. All tests for heterozygote excess were negative (Wilcoxon test; $P > 0.340$ for all populations). Similarly, the Garza-Williamson-indices represented values typically found in stable populations (Pasvik $M = 0.79$ (± 0.12), Karelia $M = 0.83$ (± 0.14), and Pinega $M = 0.81$ (± 0.13), with the average value $M = 0.81$ (± 0.13).

Discussion

Previous research assumed that the brown bears of Northern Norway, Eastern Finland and North Western Russia form one, unified population (Pulliainen 1990; Swenson and Wikan 1996), and, moreover, that the region of Russian Karelia acts as a source population for the Finnish and Norwegian bear populations. We have used genetic methods to investigate these assumptions by determining the degree of differentiation and migration among areas with high brown bear densities in the region. Our results indicate a substantial influence of IBD on the genetic structure as well as the existence of at least three separate genetic clusters in Pasvik, Karelia and Pinega, respectively. However, these three subpopulations were not completely isolated from each other. Specifically, we found that the northernmost cluster in the area of Pasvik showed restricted connectivity with the clusters of Karelia and Pinega, while we detected substantial bidirectional gene flow between Karelia and Pinega. In addition, we found that brown bears from Eastern Finland (Kainuu) indeed belong to the population of Russian Karelia.

Differentiation caused by IBD in continuous populations of large predators is expected from previous studies (Forbes and Hogg 1999; Aspi et al. 2006). Recent empirical studies as well as simulations on data of wild animal populations have also suggested that more than one Bayesian algorithm on the genetic data should be employed to avoid false results caused by sampling design and IBD (Pritchard et al. 2000; Latch et al. 2006; Robinson et al. 2007; Rowe and Beebee 2007; Schwartz and McKelvey 2009; Frantz et al. 2009). The interpretation of genetic clusters deduced from different Bayesian assignment tests can be challenging and should be done cautiously, especially when IBD is a likely underlying mechanism and when samples from the areas between the detected clusters are missing (Robinson et al. 2007; Rowe and Beebee 2007; Frantz et al. 2009). Moreover, sampling should be conducted in a short temporal scale (Anderson et al. 2010).

In this study, we have collected samples discontinuously from four regions in a timeframe of four years and used Bayesian algorithms widely applied in population genetic studies, both with and without spatial information. The pairwise F_{ST}-values (0.48–0.64) were significant and in accordance to the threshold of Pritchard et al. (2000), suggested for correct assignment with their program Structure. Latch et al. (2006) reached 97% assignment accuracy with an F_{ST}-value above 0.05. The relatively low F_{ST}-values in our study could indicate high current gene flow or recent common ancestry (Wright 1969; Schwartz et al. 2002). In the light of our results and the history of these populations, the latter may be the case for the population of Pasvik bears according to sub structuring and migration rates, while high gene flow seems to occur between the clusters of Karelia and Pinega.

Recently, studies of the mitochondrial DNA (mtDNA) of brown bears have shown that bears from Finland and Western Russia belong to the Eurasian clade, with a few distinctive haplotypes (Saarma et al. 2007; Korsten et al. 2009). In Finland, only two different haplogroups have been identified (Saarma and Kojola 2007). One of these haplogroup was only found in Southern Finland, while the other was represented throughout the country. However, mtDNA results represent different genetic timescales and our results should rather be compared to a recent study of autosomal STRs that have been analysed in Western Eurasian brown bears (Tammeleht et al. 2010). Results of that study were suggestive of large-scale gene flow among distantly located populations such as Arkhangelsk and Eastern Finland. However, this study was performed over a time span of 11 years and did not include samples from Karelia. Thus, the very long time span of sampling as well as the lack of samples from the substantial population in Karelia may be the reason why the authors could not detect the genetic sub structuring within the region. Both studies found a pattern of IBD, suggesting that IBD must play an important role in shaping bear populations in North Western Europe. Conclusively, our observations may be explained as a result of a combination of IBD and restricted migration and gene flow. However, the factors that may limit the genetic exchange and causing the clusters of the northernmost areas in Fennoscandia and the more southern regions in Finland and Russia are unknown.

All clusters displayed high genetic variation and the heterozygosities were among the highest reported in wild brown bear and similar to the values found previously in Russia (>0.76, Tammeleht et al. 2010) and higher than in Sweden (<0.7, Waits et al. 2000; Stoen et al. 2005). A deviation from HW expectations was found for the Karelian subpopulation and a more detailed analysis showed that it was mainly relevant for bears from the western edge of the population (Kainuu; results not shown). We also found elevated F_{IS}-values for a few STRs in the same population. We did not detect any further population subdivision within Karelia caused by the Wahlund effect in our data. Similar findings have been described earlier in an expanding brown bear population in Sweden (Waits et al. 2000), and population expansion may also explain our results.

Despite the high genetic diversity, we found a low number for overall migrants. However, a few migrants could be enough to keep the populations healthy and genetically diverse (Hedrick 1995; Mills and Allendorf 1996). Connectivity and gene flow between the brown bears from Karelia and Pinega ($\sim$600 km) was detected and we found high bi-directional migration among these populations, opening the possibility that both clusters may belong to a common population shaped by IBD. Conclusively, more sampling in the region is needed. In contrast to this, we found the Pasvik population ($\sim$600 km distance to Karelia; $\sim$1,200 km distance to Pinega) to be quite isolated from the rest of the bear populations, showing very low bi-directional migration. The result from Structure (Fig. 4a) seems to suggest considerable admixture of genotypes sampled in Pasvik with genotypes originating from Pinega. However, only three individuals showed a slightly higher probability of assignment to the cluster of Pinega, than to Pasvik. We do not think these few individuals are enough to lead to a contradictory interpretation. Based on all our results we believe the differentiation between Pasvik and Karelia is unlikely to be solely caused by IBD. The high genetic variation found in Pasvik could still be representative of past connectivity with the Russian Karelian population, or indicate that this population is or was part of another, still not characterized subpopulation of brown bears, which may be located on the Kola Peninsula.

Bears in Northern Fennoscandia and Russia are not distributed uniformly and field observations indicate that population densities may vary (Kojola et al. 2003; Danilov 2005; Wikman 2009). The areas of higher bear abundance are characterized by being concentration areas of females and their densities are estimated to decrease significantly outside the core areas, such as for Karelia (Kojola and Heikkinen 2006) and is represented in our sampling. Climate and habitat barriers to dispersal as well as ecological and behavioral processes might have influenced the amount of migration and connectivity among the bears in the northern parts, as have been described for wolves (Carmichael et al. 2001; Geffen et al. 2004; Pilot et al. 2006; Musiani et al. 2007). Aspi et al. (2009) found patterns of a possible recent reduction of connectivity among populations of grey wolves in Eastern Finland and Russian Karelia. Furthermore, variations in the habitat and landscape due to forestry policies may have caused differences in the distribution and migration of the bears among Russia,

Finland and Norway in the recent past (Gromtsev et al. 2009; Linden et al. 2000). We believe that in the large wilderness areas of North Western Europe more such specified differences may be found when more individuals are sampled at additional locations.

Another factor influencing gene flow between the regions and the isolation of Pasvik bears may be the presence of a border fence and the reindeer husbandry area. While the so called green belt, the forested area along the Finnish-Russian as well as Norwegian-Russian border, may play an important role as pristine retreat area, border fencing may have a negative influence on migration of larger mammals (Aspi et al. 2009). The continuous border fence, originating from Soviet times, is located all along and in close proximity to the state border of Russia. The reindeer husbandry area (Fig. 1b), covering one third of Finland, is fenced and semi-domestic reindeer roam free during summer. Special legislation for large predator removal is keeping the population density for large carnivores to a minimum. The area could be another reason for the lack of connectivity of Pasvik with the other populations further south as it constitutes an obstacle for large carnivores. This is indicated by the fact that, despite no obvious geographical barriers, such as water bodies or mountains, only a few individual wolves have succeeded in migrating through that area (Wabakken et al. 2001; Vila et al. 2003). Substantial data proving that this is the case for brown bears as well are still lacking.

Brown bears of Finland, Northern Norway and North Western Russia share the same history and they may have originated from the same population (Taberlet and Bouvet 1994; Saarma et al. 2007). All three populations have experienced substantial reduction in their sizes (Ermala 2003; Danilov 2005). Despite this recorded demographic bottleneck, we did not find any evidence of a genetic bottleneck having occurred in either of the studied populations in the past. There may be several reasons for the inconsistence between the historical records and our genetic findings. Firstly, the population sizes may have been underestimated, particularly during the period between 1900 until the late 1970s. However, the hunting statistics showed large numbers of harvested bears and affirmed a drastic population decline nearing extirpation for that period (Ermala 2003). And secondly, undetected migration and gene flow with bear populations in the east may have taken place. Even though the estimates for brown bears in North Western Europe and Russia, such as Karelia, indicated a population decline (Swenson et al. 1995; Danilov 2005), the numbers of bears in these or neighbouring regions might have been sufficient enough to maintain a certain degree of gene flow among these areas to avoid a genetic bottleneck.

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