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Limited gene flow among brown bear populations in far Northern Europe? Genetic analysis of the east–west border population in the Pasvik Valley

JULIA SCHREGEL,*† ALEXANDER KOPATZ,*‡ SNORRE B. HAGEN,* HENRIK BRØSETH,§ MARTIN E. SMITH,* STEINAR WIKAN,* INGVILD WARTIAINEN,* PAUL E. ASPHOLM,* JOUNI ASPI,‡ JON E. SWENSON,†§ OLGA MAKAROVA,¶ NATALIA POLIKARPOVA,¶ MICHAEL SCHNEIDER,** PER M. KNAPPSKOG,* MINNA RUOKONEN,‡ ILPO KOJOLA,†† KONSTANTIN F. TIRRONEN,‡‡ PJOTR I. DANILOV‡‡ and HANS GEIR EIKEN*

**Bioforsk Soil and Environment, Svanhovd, Norwegian Institute for Agricultural and Environmental Research, NO-9925 Svanvik, Norway, †Department of Ecology and Natural Resources Management, University of Life Sciences, NO-1432 Ås, Norway, ‡Department of Biology, University of Oulu, PO Box 3000, FIN-90014 Oulu, Finland, §Norwegian Institute for Nature Research, NO-7485 Trondheim, Norway, ¶Pasvik Strict Nature Reserve, 184424 Rajakoski, Murmansk Region, Russia, **Swedish Environmental Protection Agency, SE-106 48 Stockholm, Sweden, ††Finnish Game and Fisheries Research Institute, Oulu Game and Fisheries Research, Rakentajantie 33, FIN-90014 Oulu, Finland, ‡‡Institute of Biology, Karelian Research Centre of the Russian Academy of Science, 185910 Petrozavodsk, Russia*

Abstract

Noninvasively collected genetic data can be used to analyse large-scale connectivity patterns among populations of large predators without disturbing them, which may contribute to unravel the species' roles in natural ecosystems and their requirements for long-term survival. The demographic history of brown bears (*Ursus arctos*) in Northern Europe indicates several extinction and recolonization events, but little is known about present gene flow between populations of the east and west. We used 12 validated microsatellite markers to analyse 1580 hair and faecal samples collected during six consecutive years (2005–2010) in the Pasvik Valley at 70°N on the border of Norway, Finland and Russia. Our results showed an overall high correlation between the annual estimates of population size (N_c), density (D), effective size (N_e) and N_e/N_c ratio. Furthermore, we observed a genetic heterogeneity of ~0.8 and high N_e/N_c ratios of ~0.6, which suggests gene flow from the east. Thus, we expanded the population genetic study to include Karelia (Russia, Finland), Västerbotten (Sweden) and Troms (Norway) (477 individuals in total) and detected four distinct genetic clusters with low migration rates among the regions. More specifically, we found that differentiation was relatively low from the Pasvik Valley towards the south and east, whereas, in contrast, moderately high pairwise F_{ST} values (0.91–0.12) were detected between the east and the west. Our results indicate ongoing limits to gene flow towards the west, and the existence of barriers to migration between eastern and western brown bear populations in Northern Europe.

Keywords: capture–mark–recapture, DNA, effective population size, microsatellites, migration rates, N_e/N_c ratio, noninvasive genetic sampling, population structure

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Correspondence: Julia Schregel, Fax: +47 78 99 56 00; E-mail: julia.schregel@bioforsk.no

Introduction

Noninvasive genetic methods, based on the analysis of hair and faecal samples, are increasingly used in wildlife biology as a feasible and cost-effective tool to monitor large carnivore populations (Bellemain *et al.* 2005; Proctor *et al.* 2010). The approach has great potential value in the conservation and the management of large carnivores, as it might enable the estimation of important population parameters from genetic data, that is, census and effective population sizes, population density, genetic diversity, degree of inbreeding and gene flow among populations (Quéméré *et al.* 2010; Roberts *et al.* 2011; Wang *et al.* 2011). In particular, knowledge about the connectivity among populations assessed by estimating the degree of genetic differentiation and gene flow among populations is important as it is believed to counteract the effects of genetic drift (Mills *et al.* 2003) and be strongly linked to the long-term viability of populations (Schwartz *et al.* 2002; Long *et al.* 2005).

There is little knowledge about the diversity and connectivity of large carnivore populations, especially across national borders and on larger scales (Dalerum *et al.* 2009). One reason is that large predators were almost extirpated in Western Europe and much of North America (Enserink & Vogel 2006; Dalerum *et al.* 2009). Conflicts with humans and the resulting persecution and habitat destruction, combined with life history traits, such as large home ranges, long dispersal distances and long generation times, make large predators vulnerable (Crooks 2002) as has been shown recently for brown bears (*Ursus arctos*) (Miller & Waits 2003; Proctor *et al.* 2005; Kendall *et al.* 2009). Large predators are now recovered in many places. Elucidating their functions in natural ecosystems and requirements for long-term survival has become a major research interest (Smith *et al.* 2003; Estes *et al.* 2011). In this context, noninvasive genetic data on large-scale connectivity patterns among populations of large predators may contribute to the conservation and management of these species without disturbing them. This is especially important as invasive methods, like capturing and equipping animals with GPS-collars, have several drawbacks. It has been shown for brown and black bears (*Ursus americanus*) that trapping may have long-term negative effects, such as reduced body condition (Cattet *et al.* 2008). GPS-tagging is also expensive and cannot reveal large-scale biological patterns involving numerous individuals and populations.

The demographic history of brown bears in Northern Europe indicates several extinction and recolonization events (Swenson *et al.* 1995; Danilov 2005). In Norway and Sweden, the brown bear population nearly went extinct during the 19th and 20th centuries due primarily to state-financed persecution. The species was functionally extirpated in Norway, whereas three to four small and separate relict populations survived in Sweden (Swenson *et al.* 1995). This historical population fragmentation also is evident in the current genetic population structure (Waits *et al.* 2000; Manel *et al.* 2004). In Finland and northwestern Russia, similar bottlenecks have been recorded for brown bears from observations and hunting statistics (Pulliainen 1990; Ermala 2003; Danilov 2005). The genetic connectivity among these and other brown bear populations in Northern Europe is not clear. In particular, we lack information about the gene flow between the westernmost brown bear populations of Norway and Sweden and the eastern ones of Russia and Finland. A recent genetic study of brown bear populations from six different geographical areas in Finland, Estonia and Russia suggested large-scale gene flow from Finland far into southeastern European Russia, whereas the more southern populations formed three distinct genetic clusters (Tammeleht *et al.* 2010). Moreover, a phylogenetic study of mitochondrial DNA determined a common maternal lineage among four different brown bear haplotypes in northern Eurasia, indicating the historical existence of a large, genetically uniform group throughout the area (Korsten *et al.* 2009). In a recent study, we found a more restricted pattern of effective migration and gene flow among the populations in the region (Kopatz *et al.* 2012). However, the gene flow between the western and eastern parts of the Northern European brown bear populations still remains to be understood.

In this study, we have used noninvasively obtained genetic data from the brown bear population in the Pasvik Valley at the border of Norway, Finland and Russia to investigate the degree of genetic connectivity between western and eastern brown bear populations in Northern Europe. To address this issue, we have studied the Pasvik bear population's genetic structure, connectivity and variability in relation to a regional area, including the bear populations of Karelia (Russia, Finland), Västerbotten (Sweden) and Troms (Norway). Thus, our study includes individuals of both the westernmost brown bear populations in Northern Europe as well as the eastern brown bear populations.

Population size (N_c), effective population size (N_e) and the ratio between them are important indicators of population viability (Luikart *et al.* 2010). We used data from the Pasvik Valley during 6 years (2005–2010) to determine the magnitude and between-year variation in the N_e/N_c ratio. The N_e/N_c ratio might allow us to infer N_e from N_c (and vice versa) and be useful for planning management actions to increase N_e (Ficetola *et al.* 2010; Brekke *et al.* 2011).

Material and methods

Study areas

Samples were collected at four different locations in Northern Europe (Fig. 1). The focus population was located in the Pasvik Valley at the border between Norway, Finland and Russia (~70°N, 30°E) and the study area encompassed ~5000 km^2. The three other sampling areas were located to the west and south of Pasvik Valley: (i) Troms County Norway, ~420 km to the west, ~70°N, 20°E, encompassing ~5000 km^2; (ii) Västerbotten County, Sweden, ~725 km to the southwest, ~65°N, 17°E, encompassing ~45 000 km^2; and (iii) the transboundary area in Karelia (Finland and Russia), ~600 km to the south, ~64–60°N, 30–37°E and encompassing ~130 000 km^2. The airline distances between the study areas Troms, Västerbotten and Karelia are as follows: Troms-Västerbotten: ~460 km, Troms—Karelia: ~830 km, Västerbotten—Karelia ~680 km.

Sampling

Hair and faecal samples were collected opportunistically in the field (Pasvik from 2005 to 2010, Troms in 2006, 2008 and 2009; Västerbotten in 2009; Karelia in 2005 and 2007, Table 1). In 2007 and 2008, additional hair samples were obtained from the Pasvik population, using hair snares placed systematically in geographical grids, with trap design, collection protocol and lure composition adapted from previous studies (Kendall 1999; Woods *et al.* 1999; Romain-Bondi *et al.* 2004). In 2007, we used 56 traps for 2 months in a 5 km × 5 km grid, and in 2008, we used 20 traps for 1 month in a 2.5 km × 2.5 km grid. Additionally, to further increase the coverage, we included tissue samples from legally harvested bears (Table 1). Brown bear monitoring in the Pasvik Valley is included in both the Norwegian Large Predator Monitoring Program and as a part of the management of a certified transboundary park (Europarc Federation). In Västerbotten, a county-wide brown bear faecal collection programme was conducted for population estimation, during which a large number of individuals (N = 270) was detected. This population was recently estimated to consist of around 300 individuals (Kindberg *et al.* 2011), and thus, we sampled ~90% of the population. To minimize the risk of family over-representation, which can bias the results of the algorithms used for the population structure analyses (Anderson & Dunham 2008), we used a subset of 84 individuals for statistical testing. To avoid large families and at the same time ensure sufficient geographical and gender distribution, we selected randomly three males and three females from each municipality in Västerbotten in this subset.

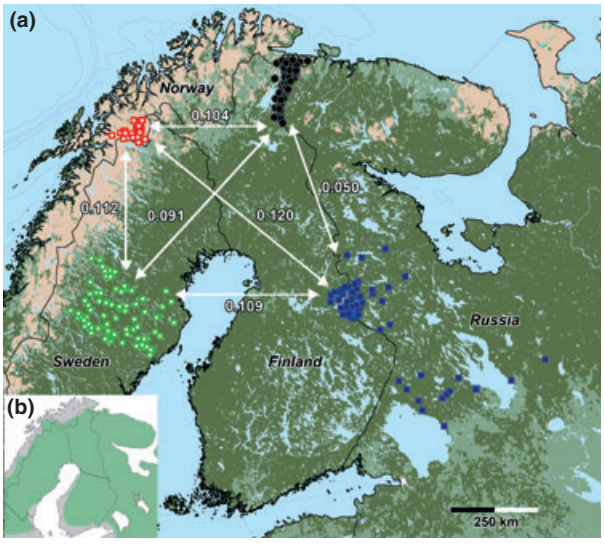

Fig. 1 (a) The four sampling locations in Northern Europe and pairwise F_{ST} values among them. Each mark represents the average position of a genotyped brown bear. Black filled circles: Pasvik (n = 94), red open squares: Troms (n = 34), green open circles: Västerbotten (n = 84) and blue filled squares: Karelia (n = 79). The map legend is as follows: blue = water bodies; dark green = forest cover; light green = brush/scrub/grassland; light brown = tundra. All F_{ST} values are significant, the arrows indicate the pairs of populations compared. (b) Map showing brown bear distribution across Northern Europe. Green = area with possible brown bear occurrence (see also http://www.lcie.org), dashed line = southern border of the reindeer husbandry area in the three Nordic countries.

Molecular analysis

Faecal samples were stored in stool collection tubes with DNA stabilizer (Invitek) or in plastic bags and kept at minus 20 °C until DNA extraction. The hair samples were stored dry and dark in paper envelopes until DNA extraction. To extract DNA, we used the PSP Spin Stool DNA Plus Kit (Invitek) for the faecal samples and the DNeasy Tissue Kit (Qiagen) for the hair and tissue samples, following the manufacturers' instructions. We used the following 12 dinucleotide markers (short tandem repeats, STRs) to genotype the DNA samples: G1A, G1D, G10B, G10L (developed for the black bear; Paetkau & Strobeck 1994; Paetkau *et al.* 1995; Paetkau & Strobeck 1995); Mu05, Mu09, Mu10, Mu15, Mu23, Mu50, Mu51 and Mu59 (developed for the brown bear; Taberlet *et al.* 1997). All of the STRs used here have been validated with respect to species specificity, sensitivity, accuracy and probability of identity (Eiken *et al.* 2009; Andreassen *et al.* 2012). Sex was determined as described by Kopatz *et al.* (2012).

A detailed description of PCR protocols and the fragment analysis as well as protocols for individual

Table 1 Brown bear sample collection and genetic analyses* from four locations in Northern Europe

	Pasvik (2005–2010)	Troms (2006, 2008–2009)	Västerbotten (2009)	Karelia (2005–2007)	Total
No. of samples	1580	307	1355	123	3365
Faeces	1180	239	1346	89	2854
Hair	92	67	3	0	162
Hair from hair traps†	281	0	0	0	281
Tissue	27	1	6	34	68
No. of samples genotyped*	901	178	914	113	2106
No. of males	54	19	138	49	260
No. of females	37	15	131	29	212
n.d.	3	0	1	1	5
No. of bears	94	34	270‡	79	477

n.d., not determined.
*Genotyping was performed using 12 different STRs and an amelogenin gene XY-assay (see Materials and methods).
†Only for 2007 and 2008.
‡From Västerbotten, only a subset of 84 individuals was used in the population genetic analyses (see Materials and methods), while the remaining individuals were typed for only 8 STRs and gender in this study.

identification can be found in Andreassen *et al.* (2012). In this study, the genetic analysis was performed as follows. PCR mixes were set up with 10 μl reaction volumes and contained 1× PCR Gold buffer (ABI), 200 μM dNTP (Eurogentec), 1.5 mM MgCl2 (ABI), 0.5 μM of each primer (MedProbe Inc.), 1 U AmpliTaq-Gold DNA polymerase (ABI), 1× BSA (NEB) and 1 μl template DNA. The conditions for PCRs for the loci G1A, MU10, MU05, MU09, MU23, MU50, MU51, MU59 and G10L were 10 min at 95 °C, 35 cycles of 30 s at 94 °C, 30 s at 58 °C and 1 min at 72 °C. A final extension phase was set for 15 min at 72 °C on an ABI 2720. PCR conditions for loci G1D, G10B and MU15 were similar, except for a higher annealing temperature of 60 °C, and a shorter final extension of 5 min. PCR products were run on an ABI 3730, and the PCR fragments were analysed with GeneMapper 4.0 (Applied Biosystems).

The first and the last four samples on every 96-well plate were positive controls, and every eighth sample was a negative control. The positive controls functioned also as a control for between-run variation; all genotypes were assigned manually. The samples were genotyped independently twice if allele designation showed a heterozygote and three times if it showed a homozygous genotype for the specific markers (peak height threshold values >300 RFU). A sample was only assigned an identity if all runs across all markers were consistent. If not, an identity was not assigned and the sample was discarded from further analyses, and, accordingly, we did not construct consensus DNA profiles. We only accepted a single negative result for STRs if the sample showed consistent results for the overall DNA profile. PCRs for 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 with the requirements published by Linacre *et al.* (2011). The laboratory procedures, that is, the extraction of samples and the analysis of the STRs, were accredited according to the EN ISO/IEC 17025 standard. 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).

Statistical analysis

Genetic diversity, inbreeding and linkage disequilibrium (LD). We used the software Genetix 4.05.2 (Belkhir *et al.* 1996–2004) to calculate observed and expected heterozygosities, allele numbers, inbreeding coefficients and LD for all sampled locations. As implemented by Genetix, we tested for LD between pairs of loci for all areas using the method of Black & Kraftsur (1985).

We used Genepop version 4.0.11 (Rousset 2008) to run the exact test for deviations from Hardy–Weinberg equilibrium (HWE) for all loci and geographical locations. All combinations of locations were tested with unbiased *P* values by a Markov chain method of 1000

burn-in iterations, 500 batches and 1000 iterations per batch.

Population bottlenecks. We used the software BOTTLENECK v. 1.2.02 (Cornuet & Luikart 1997; Luikart *et al.* 1998; Piry *et al.* 1999) to test for genetic signatures of a demographic bottleneck, that is, whether the heterozygosity in the studied populations was larger than the heterozygosity expected from the number of alleles found in the sample if the population were 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 was tested using the Wilcoxon test.

Population structure. We analysed population structure using both population- and individual-based approaches. First, we utilized the Bayesian approach to detect the number of genetic clusters (K) using the software STRUCTURE version 2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2003; Hubisz *et al.* 2009). For this analysis, we assumed population admixture and correlated allele frequencies within the population. To achieve consistency of results, we performed ten independent runs for each K value (number of genetic clusters) between one and ten. For each run, we set a burn-in period of 100 000 Markov Chain Monte Carlo (MCMC) iterations, followed by sampling of 1 000 000 iterations. Because the log-likelihood estimated with the STRUCTURE software often displays higher variance between runs for the higher K values, we calculated the rate of change in the log probability of data between successive K values (ΔK) to determine the most likely number of clusters (Evanno *et al.* 2005).

In a second step, we used an individually based spatially explicit model 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 10 000 000, with a thinning of 100. The maximum rate of Poisson process was set to 100, and the maximum number of nuclei was 300.

Finally, to visualize the extent of regional differentiation, we ran a factorial correspondence analysis (FCA) with GENETIX 4.05.2 (Belkhir *et al.* 1996–2004). We also used the software ARLEQUIN version 3.5.1.2 (Excoffier & Lischer 2010) to calculate pairwise F_{ST} values (Weir & Cockerham 1984) among detected populations with 10 000 burn-in iterations, 100 batches and 500 iterations per batch. We also ran an analysis of molecular variance (AMOVA) to identify genetic structure among and within populations, using 10 000 permutations.

Migration rates among populations. To estimate migration rates among the four populations, we used a Bayesian approach implemented in the software BAYESASS 1.3 (Wilson & Rannala 2003). Contrary to the classical methods (Paetkau *et al.* 1995; Rannala & Mountain 1997; Cornuet *et al.* 1999), this approach may provide rates of recent migration among populations. The number of burn-in iterations was set to 6 000 000 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. As recommended, we adjusted the delta values to 0.07 (allele frequency), 0.05 (inbreeding coefficient) and 0.15 (migration), so that acceptance rates for changes in these parameters would be between 40 and 60% (Faubet *et al.* 2007). We carried out three independent runs to confirm the consistency of results. To examine differences between the sexes, the same analysis with the same settings was run with data sets split according to sex. Individual membership values q_i, estimated in the population structure analysis with the program Structure, can indicate possible migrants. Therefore, individuals with a q_i value >0.7 for a different population than the one it was sampled in was recorded to identify possible migrants.

Annual estimates of population size (N_c), density (D) and effective population size (N_e) for Pasvik 2005–2010. We used the DNA-based single session capture–mark–recapture (CMR) method to estimate N_c and N_e, as it has been shown to work well with capture heterogeneity and small population sizes (Miller *et al.* 2005) and has also been compared with and found more efficient than other field-based methods (Solberg *et al.* 2006). To avoid biased estimates and to maximize both the detection and sampling frequencies of individuals, we used the combined data of the opportunistic and systematic sampling approaches to estimate N_c and N_e (Boulanger *et al.* 2008; Gervasi *et al.* 2008; De Barba *et al.* 2010). The annual estimates of N_c were made using both *Capwire* (Miller *et al.* 2005), based on the two innate rates model (TIRM) and using ordered samples (Miller *et al.* 2005, 2007; Bromaghin 2007), and CAPTURE (Otis *et al.* 1978), based on the M_h Chao (a closed-population heterogeneity estimator). To estimate population density (D), we first estimated annual effective sampling areas to correct for geographical closure violation by creating a concave buffer around each sample location. As no home-range estimates were available for bears in the Pasvik population, we applied both an upper and a lower buffer: (i) a wide buffer of 15 km around the samples, equivalent to a circular home-range size of 707 km^2; and (ii) a narrow buffer of 7.5 km, equivalent to a circular home-range

size of 177 km^2. The upper and lower buffers were based on home-range sizes estimated from telemetry data of males and females, respectively, from neighbouring populations in Sweden (Dahle & Swenson 2003; Støen *et al.* 2006). In addition, the mean maximum distance (MMD) between resampling events (Obbard *et al.* 2010) and the equivalent circular home-range sizes of individual bears were determined for individuals with at least five resampling events during a year in Pasvik.

The effective population size N_e is an indicator of the factors affecting the strength of inbreeding and genetic drift processes (Wright 1931, 1938). N_e was estimated annually with the software LDN$_e$ (Waples & Do 2008), which is based on LD data. The method uses the principle that, with declining N_e, LD is generated by genetic drift and thus LD can be used to calculate N_e (Hill 1981). We also calculated N_e with the online software OneSamp (Tallmon *et al.* 2008), which utilizes approximate Bayesian computation and allows user-specified priors. We tested for consistency using differing priors (minimum and maximum effective population size) in the analysis settings (Tallmon *et al.* 2008).

To determine the magnitude and stability of the N_e/N_c ratio across years, we calculated the N_e/N_c ratio for all 6 years for the Pasvik population. In this context, we also tested for a correlation between N_e and N_c across years, to test the hypothesis that N_e may be estimated from N_c (and vice versa), using the Pearson's product-moment correlation implemented in the software R (R Development Core Team 2011). We also used the same function to test for correlation between the different estimators used for the estimation of N_c and N_e across the years.

Results

Sampling and genetic analysis

In total, 3365 samples were collected for genetic analyses in the four regions (Fig. 1 and Table 1). In each region, the vast majority of samples were faecal samples collected opportunistically, followed by hair and tissue samples. Systematic hair trapping was performed only in the Pasvik Valley. Successful genotyping with 12 different STRs was obtained for 2106 samples from 477 different bears: Pasvik, n = 94; Troms, n = 34; Västerbotten, n = 270; Karelia, n = 79 (Fig. 1a and Table 1). The number of bears identified annually in Pasvik in 2005–2010 ranged from 27 in 2005 to 44 in 2007 (Table 2). In 2007 and 2008 in Pasvik, several individuals (2007: 19 and 2008: 3) were detected with the hair traps only. The effect was most pronounced in 2007, when the hair trap area was largest (1400 km^2).

Genetic diversity, inbreeding and LD

We determined the expected and observed heterozygosities, the number of different alleles and the inbreeding coefficient F_{IS} for all 12 STRs for 290 individuals (Table 3). Deviations from HWE ($P < 0.05$) were observed in 8 of 48 tests, although after Bonferroni correction, only one marker (G10B) in the Karelia population deviated significantly from HWE (Table 3). Mean H_{exp} ranged from 0.68 (Troms) to 0.82 (Karelia), and mean H_{obs} from 0.69 (Västerbotten) to 0.80 (Pasvik). Mean F_{IS} values ranged from −0.02 in Pasvik to 0.04 in Västerbotten, whereas the only significant F_{IS} value was

Table 2 Annual estimates of census population size (N_c) and density of brown bears in the Pasvik Valley (2005–2010). Estimates of census population size (N_c) using both the two innate rates model (TIRM) and the M_h Chao estimator are shown. For population density (D) estimates, the N_c estimates were corrected for geographical closure by first estimating the effective sampling area with two different buffer widths around each sample: Buff7.5 ≈ 177 km^2; Buff15 ≈ 707 km^2 (see Materials and Methods) Obs./ind. = - mean number of observations per individual bear; No. of ind. = number of individuals

				Census populations size N_c		Population Density Ind./1000 km^2			
				TIRM	M_h Chao	TIRM		M_h Chao	
Year	No. of samples	Obs./ind.	No. of ind.	N_c (CI 95%)	N_c (CI 95%)	Buff7.5	Buff15	Buff7.5	Buff15
2005	68	2.52	27	36 (27–49)	39 (31–70)	11.1	4.8	12.1	5.2
2006	50	2.08	24	39 (25–57)	41 (29–86)	12.3	4.5	12.9	4.8
2007	141	3.20	44	56 (46–66)	67 (52–112)	12.3	6.2	14.7	7.4
2008	144	3.89	37	46 (37–53)	53 (43–80)	14.5	7.2	16.7	8.3
2009	137	4.42	31	33 (31–36)	43 (35–79)	9.9	4.7	12.8	6.1
2010	80	3.48	23*	27 (23–33)	29 (25–54)	8.6	4.9	9.3	5.2
Mean	103	3.27	31	39.5	45.3	11.5	5.4	13.1	6.2

*One individual only represented by a tissue sample was deleted from the data set.

Table 3 Expected, (H_{exp}) and observed (H_{obs}) heterozygosities, number of different alleles (A) and inbreeding values (F_{IS}) calculated for the 12 short tandem repeats in four Northern European brown bear populations

	Pasvik 2005–2010 (n = 93)					Troms 2006, 2008–2009 (n = 34)					Västerbotten 2009 (n = 84)					Karelia 2005–2007(n = 79)				
Marker	A	H_{exp}	H_{obs}	P	F_{IS}	A	H_{exp}	H_{obs}	P	F_{IS}	A	H_{exp}	H_{obs}	P	F_{IS}	A	H_{exp}	H_{obs}	P	F_{IS}
G1D	9	0.83	0.87	0.411	−0.04	5	0.70	0.71	0.166	0.00	6	0.67	0.64	0.152	0.05	9	0.82	0.81	0.069	0.01
G10B	10	0.76	0.76	**0.022**	0.00	6	0.68	0.76	0.244	−0.10	7	0.62	0.64	0.141	−0.03	11	0.85	0.65	**0.001***	**0.25****
Mu05	8	0.82	0.89	**0.035**	−0.08	6	0.70	0.71	0.560	0.01	6	0.64	0.62	0.589	0.04	8	0.79	0.77	0.128	0.04
Mu09	13	0.84	0.85	0.427	0.00	6	0.68	0.76	0.942	−0.11	9	0.82	0.77	0.509	0.07	9	0.87	0.85	0.453	0.04
Mu15	6	0.76	0.82	0.492	−0.07	4	0.39	0.38	0.828	0.04	5	0.62	0.64	**0.016**	−0.04	9	0.80	0.85	0.486	−0.05
G1A	8	0.80	0.80	0.665	0.01	5	0.66	0.76	0.769	−0.15	5	0.70	0.63	0.206	0.10	9	0.80	0.80	0.094	0.01
G10L	9	0.64	0.63	0.284	0.02	6	0.80	0.82	0.154	−0.02	7	0.64	0.60	**0.033**	0.07	10	0.78	0.75	0.339	0.05
Mu10	8	0.73	0.75	0.416	−0.01	4	0.67	0.74	0.900	−0.09	7	0.74	0.67	0.535	0.11	10	0.79	0.76	0.279	0.05
Mu23	9	0.69	0.73	**0.028**	−0.05	5	0.73	0.76	0.654	−0.03	8	0.81	0.77	0.737	0.06	9	0.84	0.68	**0.014**	0.19
Mu50	8	0.84	0.86	0.484	−0.02	6	0.73	0.85	0.333	−0.16	7	0.81	0.80	0.861	0.02	8	0.74	0.71	0.583	0.05
Mu51	8	0.82	0.84	0.066	−0.01	6	0.77	0.82	0.405	−0.05	7	0.76	0.80	0.632	−0.05	10	0.83	0.77	0.510	0.07
Mu59	9	0.82	0.80	0.116	0.03	5	0.70	0.76	0.844	−0.07	9	0.82	0.75	**0.006**	0.10	13	0.90	0.92	0.465	−0.01
Mean	8.75	0.78	0.80		−0.02	5.33	0.68	0.74		−0.06	6.92	0.72	0.69		0.04	9.58	0.82	0.78		0.02

Significant deviations from Hardy–Weinberg equilibrium ($P < 0.05$) are marked in bold.
*The only significant deviation after Bonferroni correction.
**The only significant F_{IS} value. $P < 0.05$.

detected for the marker G10B in the Karelian population (Table 3). The highest number of alleles for a single STR was 13 (MU09 in Pasvik and MU59 in Karelia), and the mean numbers of alleles for all STRs were highest in Karelia (9.6) and lowest in Troms (5.3). After sequential Bonferroni correction, significant LD was found in 52 of 66 marker pairs, with 37 of these observed in Pasvik. None of the remaining 15 marker pairs showed significant LD in more than two populations and were not the same in all of the sampled populations.

Population bottlenecks

Allele frequencies showed no signs of a genetic bottleneck in any of the tested populations. All tests for heterozygote excess were negative (Wilcoxon test; $P > 0.190$ for all populations).

Population structure

The four methods we used to test for genetic differentiation resulted in the same four genetic clusters. First, the Bayesian approach in the program Structure found the highest mean likelihood [lnP(D)] for $K = 4$ (Fig. 2a,b), after correction using Evanno's ad-hoc approach (Evanno *et al.* 2005), as did the software GENELAND with geographical coordinates and a priori correlated allele frequencies (results not shown). Similarly, the visualization of the extent of regional differentiation with FCA suggested four clusters, with the first axis explaining 5.7% and the second axis explaining 3.8% of the variation (Fig. 3). Pairwise F_{ST} values between populations ranged from 0.050 (between Pasvik and Karelia) and 0.120 (between Karelia and Troms), and the overall average substructuring was 0.1 (Table 4). All these comparisons were statistically significant ($P \leq 0.01$) (Table 4). AMOVA analysis revealed that 9.18% of the genetic variation was among, and 90.82% was within, the populations ($P < 0.001$).

Migration

We estimated high rates of self-recruitment in each population and low migration rates among the four locations using the Bayesian method (Table 5). The estimated rates of self-recruitment were high, ranging from 94.1% to 98.9%. Our results indicated that 96.3% of the bears sampled in Pasvik originated from the same population, and only 3.7% of the individuals originated from the other three populations. The highest estimated migration rates were found from Västerbotten to Troms (4.7%), from Karelia to Pasvik (2.3%), and from Västerbotten to Pasvik (1.2%). The lowest migration rates were found from Karelia to Västerbotten (0.1%), from Troms to Pasvik (0.2%) and Karelia (0.2%) and from Västerbotten to Karelia (0.2%). However, these differences in migration rates were not significant. Running these same analyses for males and females separately showed no signs of differences in migration rates between the sexes (data not shown). In total, eight individuals were identified as

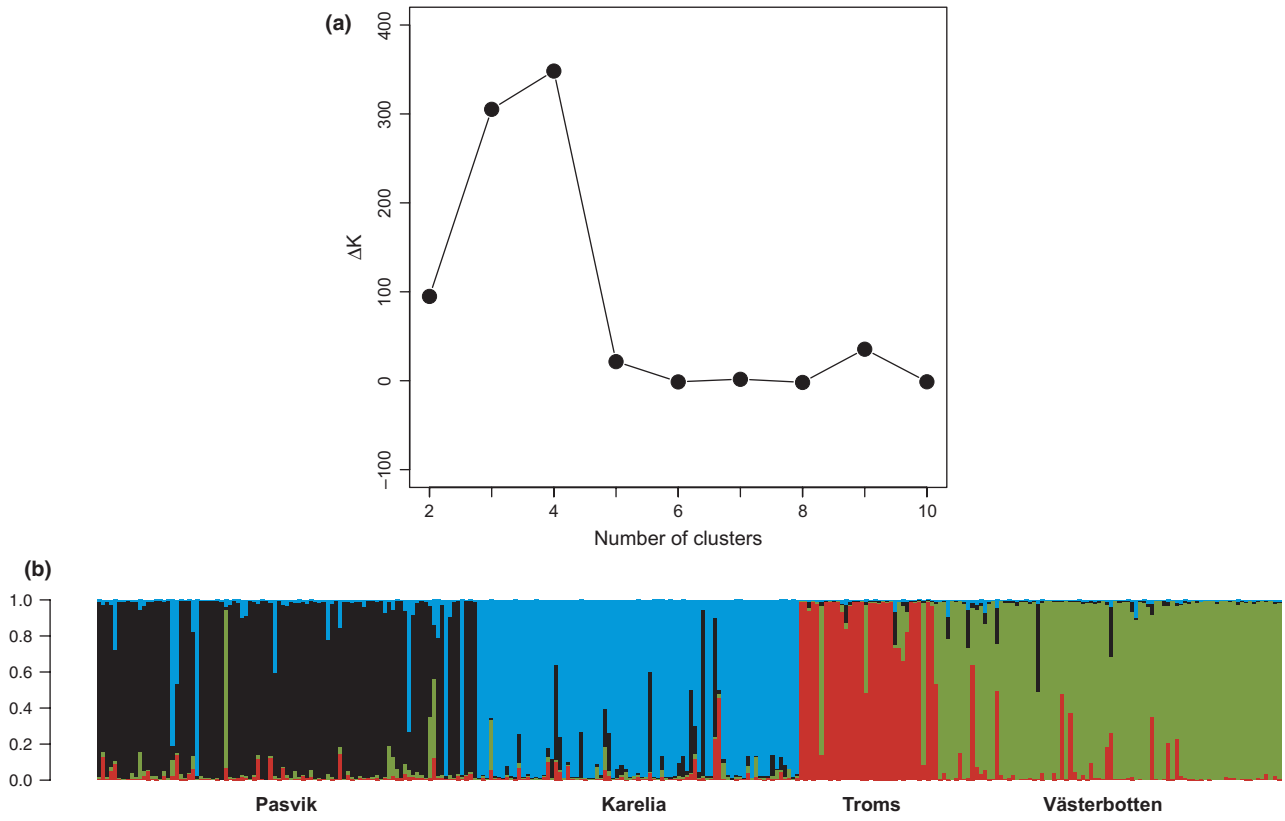

Fig. 2 (a) Population structure analysis of individual brown bear genotypes (12 STRs) from four locations in Northern Europe using the program Structure. Results were processed with the Evanno approach, *x*-axis: No. of clusters, *y*-axis: Δ*K*. (b) Population structure analysis of individual brown bear genotypes (12 STRs) from four locations in Northern Europe using the program Structure, individual admixture for $K = 4$, each bar represents one individual partitioned into segments, the length of each segment corresponds to the individual membership value (q_i).

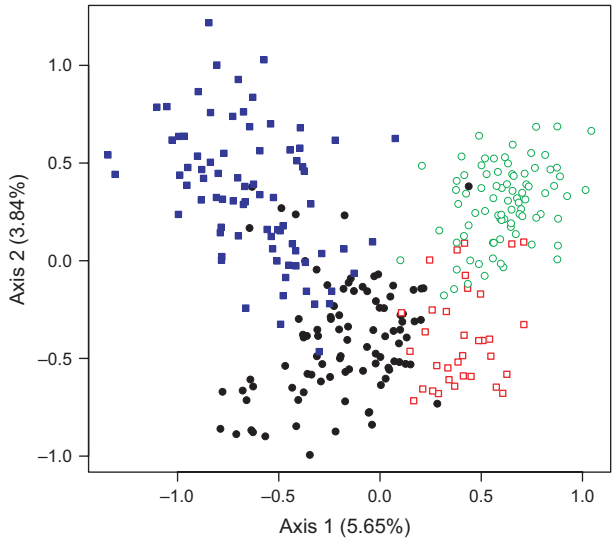

Fig. 3 Factorial correspondence analysis of individual brown bear genotypes (12 short tandem repeats) from four different geographical locations in Northern Europe; blue filled squares = Karelia, black filled circles = Pasvik, red open square = Troms, green open circle = Västerbotten.

possible migrants by having an estimated membership value $q_i > 0.7$ in Structure. In Pasvik, six individuals had $q_i > 0.7$. Five of these were assigned to Karelia (three males, one female and one of unknown sex). The last one was assigned to Västerbotten (a male). In addition, a male from Troms was assigned to Västerbotten, and a male from Karelia was assigned to Pasvik.

Annual estimated population size (N_c), density and effective population size (N_e) for Pasvik 2005–2010

The CMR estimates of N_c from the TIRM model and the M_h Chao were similar, with a correlation value of $r = 0.969$ ($P = 0.001$) between the two estimators across the years (Table 2). Both methods showed the largest N_c value in 2007 and the lowest value in 2010. Mean estimated population size in Pasvik was 39.5 (TIRM) and 45.3 (M_h Chao), ranging from 27 to 56 (TIRM) and from 29 to 67 (M_h Chao) between years (Table 2). The mean number of observations per individual ranged from 2.1 in 2006 to 4.4 in 2009.

Table 4 Pairwise F_{ST} values (ARLEQUIN 3.11) for brown bears from four locations in Northern Europe

	Pasvik	Troms	Västerbotten
Troms	0.104*		
Västerbotten	0.091*	0.112*	
Karelia	0.050*	0.120*	0.109*

*$P < 0.01$.

The estimated mean maximum distance (MMD) between resampling events was 21.7 km ($n = 46$), which corresponds with a circular home-range size of 370 km^2. Thus, this was slightly lower than the mean of the upper and lower buffers (442 km^2) that refer to previous telemetry-determined home-range sizes from Sweden.

The estimated effective sampling areas in the Pasvik Valley were used to estimate densities (Table 2). We found overall mean population densities between 5.4 and 13.1 individuals/1000 km^2. Annual population density estimates (D) using the N_c TIRM results ranged from 4.5 to 7.2 (Buff7.5) and from 8.6 to 14.5 (Buff15) individuals/1000 km^2, whereas densities ranged from 4.8 to 8.3 (Buff15) and from 9.3 to 16.7 (Buff7.5) individuals/1000 km^2, using the N_c M_h Chao results.

The results obtained with differing priors were consistent in the ONESAMP model, so we proceeded using only the results obtained with the a priori information of N_e min = 2 and N_e max = 100. The LDN_e estimates of N_e ranged from 9.1 (in 2006) to 21.1 (in 2007), with a mean of 13.5 individuals, and the ONESAMP estimates of N_e were from 18.1 (in 2009) to 36.9 (in 2007), with a mean of 25.1 individuals (Table 6). The correlation value between the LDN_e and the ONESAMP methods across all years was $r = 0.520$ ($P = 0.290$).

We found a significant correlation across years between the N_c estimates and the ONESAMP estimates of N_e ($r = 0.858$; $P = 0.029$ (TIRM) and 0.815; $P = 0.048$ [M_h Chao)], but there was no significant correlation between N_c and LDN_e estimates ($r = 0.618$; $P = 0.191$ (TIRM) and 0.561; $P = 0.247$ [M_h Chao)]. However, we have detected high LD in this population sample, and the LDN_e method is not recommended when closely related individuals are sampled. Therefore, annual N_e/N_c ratios were calculated only with the N_e estimate based on the ONESAMP method. The annual N_e/N_c ratios ranged from 0.53 to 0.82, with a mean of 0.64 (N_c from TIRM), and from 0.42 to 0.76, with a mean of 0.57 (N_c from M_h Chao) (Table 6). Note, however, that the corresponding estimates of the N_e/N_c ratio based on LDN_e, rather than the ONESAMP, would have been considerably smaller, as the LDN_e estimates of N_e were always considerably smaller than the ONESAMP estimates of N_e (Table 6).

Discussion

Using the Pasvik and three surrounding populations, we investigated the genetic diversity and the gene flow among western and eastern brown bear populations in Northern Europe using mainly noninvasive genetic sampling. We found four distinct genetic clusters with low migration rates among the populations. The overall results of the study indicate present limitations to gene flow between the eastern and western populations. The high genetic heterogeneity we found among bears in the Pasvik Valley, on the border between east and west, is comparable with results from Kirov in central Russia (Tammeleht *et al.* 2010), Pinega in Archangelsk in northwestern Russia (Kopatz *et al.* 2012), and Karelia in southwestern Russia (this study). Thus, we suggest that the Pasvik population has genetic contact with other bear populations to the east. In contrast, we detected substantial substructuring among our study populations with moderately high F_{ST} values and separate genetic clusters between east and west. F_{ST} values have been found to be substantially lower between populations that show some degree of bidirectional migration (Waits *et al.* 2000; Proctor *et al.* 2005; Kendall *et al.* 2009) than between subpopulations separated by direct barriers to migration (Proctor *et al.* 2005). Our results are somewhere between these two extremes, and both, the geographical distances and the degree of genetic differentiation, may be comparable to studies on the two most distant subpopulations in Sweden more than 10 years ago (Waits *et al.* 2000). At that time, the authors proposed that the observed population substructuring was

Table 5 Bayesian analysis (BAYESASS 1.3) of migration rates and self-recruitment among four brown bear populations in Northern Europe

	Pasvik	Troms	Västerbotten	Karelia
From Pasvik to	0.963 (0.93–0.98)	0.006 (0.00–0.03)	0.005 (0.00–0.01)	0.006 (0.00–0.02)
From Troms to	0.002 (0.00–0.01)	0.941 (0.87–0.98)	0.005 (0.00–0.02)	0.002 (0.00–0.01)
From Västerbotten to	0.012 (0.00–0.03)	0.047 (0.01–0.10)	0.989 (0.96–1.00)	0.002 (0.00–0.01)
From Karelia to	0.023 (0.01–0.05)	0.006 (0.00–0.02)	0.001 (0.00–0.01)	0.990 (0.96–1.00)

The 95% CIs are given in brackets.

Table 6 Annual estimates of effective population size (N_e) and the N_e/N_c ratio in brown bears in the Pasvik Valley, 2005–2010. The estimates for N_e using the OneSamp method were applied to calculate N_e/N_c ratios using the two innate rates model (N_{c1}) and the M_h Chao (N_{c2}) N_c estimates

Year	n	LDNe (P_{crit} = 0.05) N_e (CI 95%)	OneSamp N_e (CI 95%)	N_e/N_{c1}	N_e/N_{c2}
2005	27	9.1 (7.2–11.6)	25.3 (21.5–33.9)	0.703	0.649
2006	24	18 (13.1–26.1)	20.5 (18.5–24.4)	0.526	0.500
2007	44	21.1 (17.4–26.0)	36.9 (33.5–42.0)	0.659	0.551
2008	36*	10.2 (8.3–12.3)	27.4 (24.4–33.4)	0.596	0.517
2009	31	10.7 (8.1–14.4)	18.1 (16.1–22.8)	0.548	0.421
2010	24	12 (9.2–15.9)	22.1 (19.1–28.3)	0.819	0.762
Mean	31	13.5	25.1	0.642	0.567

n, number of detected individuals by DNA analysis.
*One individual with a partial genotype was deleted from the data set.

because of a pattern of isolation by distance (IBD) and residual genetic differentiation caused by the 19th and 20th century bottleneck event and subsequent population fragmentation. Recently, Tammeleht *et al.* (2010) proposed that brown bears in northeastern Europe also are structured by IBD. Thus, the observed substructuring in this study may have resulted from a combination of IBD and the demographic history of the northern European bear populations. However, our results do not indicate any bottlenecks and are clearly suggestive of limited gene flow in the region, especially towards the west. A high rate of self-recruitment in all of our study areas suggests that barriers hinder migration. The geographical distances between the Pasvik bears and the other bear populations are similar, but substructuring is definitely more pronounced towards the west than towards the south. In comparison, we detected a relatively low degree of substructuring with the bears in Pinega, which are twice as far to the east (see Kopatz *et al.* 2012). Thus, apparently, additional mechanisms than merely spatial distance are necessary to fully explain the genetic differentiation among the bear populations in Northern Europe.

Migration between Karelia and Pasvik might be aided by the relatively undisturbed area along the Russian border. In this area, the 'Fennoscandian Green Belt' (Karivalo & Butorin 2006), transborder movements of bears have been recorded previously (Pulliainen 1990; Swenson & Wikan 1996; Kojola *et al.* 2003), but more precise, recent information about these movements is not available. In comparison, to move between Pasvik and Västerbotten, migrating bears would have to cross an area with reindeer husbandry. The reindeer husbandry area (Fig. 1b) has been suggested to constitute a migration barrier for the northern European wolf population (*Canis lupus*), because of illegal hunting (Wabakken *et al.* 2001; Vilà *et al.* 2003; Kojola *et al.* 2006), which also may be the case for brown bears. Recent studies have shown that illegal killing has a substantial effect on the large carnivore populations in the region and occurs more frequently in northern than in southern Scandinavia (Andrén *et al.* 2006; Persson *et al.* 2009; Liberg *et al.* 2012). Also, in some areas, migration to the east may be hindered by border fences from Soviet times. They are located all along the Russian border and are believed to act as barriers to large carnivore migration (e.g. for wolves, Aspi *et al.* 2009).

The limited availability of suitable habitat also might reduce gene flow among the populations. Generally, brown bears are adaptable to a wide range of habitat types, although in northern Europe, they seem to prefer rugged, forested terrain (Nellemann *et al.* 2007) at lower elevations (May *et al.* 2008). Thus, the relatively open areas of high-elevation tundra, scrub and brush west of the Pasvik Valley might impede migration between western and eastern populations. Also, human disturbance seems to be a major factor influencing home-range selection (Nellemann *et al.* 2007), and although the density of human settlements is generally low in the study area, it is possible that other human activities, such as forest industry (logging, forest roads, etc.), may have a negative impact on migration in some areas. However, the impact of these factors is not known, and we suggest that such possible barriers to migration and gene flow may be investigated in future studies.

Results from studies on polar bears (*Ursus maritimus*) and wolverine (*Gulo gulo*), equally able to disperse across large distances, have shown similar patterns of reduced gene flow because of barriers to migration (Paetkau *et al.* 1999; Kyle & Strobeck 2001, 2002; Cegelski *et al.* 2006). Although the results are not directly comparable, because of differences in, for example, life history traits and ecological requirements, these

similarities suggest that long-distance dispersal is apparently strongly influenced by the quality of the habitat to be crossed and can be easily disturbed by unfavourable circumstances.

In the Pasvik Valley, we detected bears in densities that were comparable to that found in northern Sweden (~10 bears/1000 km^2), but lower than in southern Sweden (Støen *et al.* 2006). These densities seem to be lower than in North America (Mowat *et al.* 2005; Kendall *et al.* 2008), although these differences may be due to differences in methodology. Danilov (2005) estimated substantially higher densities in Russian Karelia, but these estimates are based only on hunting bag and bear observations. However, densities may vary among the areas. Brown bears are not distributed evenly across Northern Europe, and core and peripheral areas are identifiable (Swenson *et al.* 1998; Kojola & Laitala 2000; Kojola *et al.* 2003; Kojola & Heikkinen 2006; Kindberg *et al.* 2011).

Population size (N_c), effective population size (N_e) and the ratio between these measures may have a practical value in conservation, because they have been suggested to be important indicators of population viability (Ficetola *et al.* 2010; Luikart *et al.* 2010; Brekke *et al.* 2011). Estimates of these parameters in the Pasvik Valley with two different methods during six consecutive years showed little annual variation, but substantial methodological variation. We detected almost a threefold difference between the LDN_e and the ONESAMP estimates for N_e. However, the LDN_e estimate may be biased low in small and extensively sampled populations because of family over-representation (Luikart *et al.* 2010) and the high number of significant linkage disequilibria found in the Pasvik population may indicate the sampling of closely related individuals (Slate & Pemberton 2007). Accordingly, we used the ONESAMP method to calculate the N_e/N_c ratios. Noninvasive genetic sampling data yield no information about the age of individuals and the sampling of overlapping generations may generate a biased N_e estimate (Luikart *et al.* 2010). The result of such an N_e estimate may be somewhere between the number of breeding pairs and the effective population size (Waples 2005). The N_e results achieved by the ONESAMP analysis may be the more accurate, as it uses multiple summary statistics and therefore more information from the data (Luikart *et al.* 2010). Keeping these methodological uncertainties in mind, we found substantial correlations between the census and effective population size estimates and our results indicate that the population's N_e may be estimated directly from N_c (and vice versa) as previously suggested by Brekke *et al.* (2011) and Ficetola *et al.* (2010). This must be tested using other data sets that incorporate geographical variation and potentially confounding factors, because such estimates have important practical implications.

The mean annual N_e/N_c ratio of approximately 0.6 is very high compared with other published ratios for brown bear. For North American grizzly bears, the N_e/N_c ratio ranged from 0.20–0.38 in a simulated population using a demographic estimate (Harris & Allendorf 1989) and from 0.04–0.19 using a genetic estimate (Paetkau *et al.* 1998). In southern Sweden, the ratio ranged from 0.06 to 0.14, also using a genetic estimate (Tallmon *et al.* 2004). The two latter studies were conducted in populations in which isolation and/or bottleneck events led to low heterozygosity, which may explain the very low ratio compared with our estimates. However, the demographic estimate also is lower than in our study in Pasvik. Nunney (2000) and Storz *et al.* (2002) have shown that differences in methods for estimating N_e may produce results that are not necessarily comparable, which could also cause differences in the N_e/N_c ratio. Nevertheless, our estimate does not seem to represent genetic stochastic effects, as it was relatively stable for all 6 years. As it seems that fluctuations in population size, variance in family size and unequal sex ratio have a negative impact on the N_e/N_c ratio (Frankham 1995), the relatively high ratio may indicate that Pasvik is a stable population. In addition, the N_e/N_c ratio has been shown to be generally higher in smaller populations (Palstra & Ruzzante 2008), which might apply to the Pasvik population as well. Pasvik may receive continuous migration from the east, which is indicated by the relatively high genetic heterogeneity, and this may bias the N_e upwards by causing the local N_e to approach the global or metapopulation N_e (Pray *et al.* 1996; Palstra & Ruzzante 2008; England *et al.* 2010; Luikart *et al.* 2010), and thus resulting in a higher N_e/N_c ratio. Although research regarding the usefulness of the N_e/N_c ratio is still ongoing, our results show that in an apparently stable bear population, the ratio seems to be relatively stable and may have the potential to be used in management and conservation actions (Luikart *et al.* 2010).

Noninvasive sampling schemes have been applied to different large carnivore species mostly for monitoring purposes (e.g. wolf, Marucco *et al.* 2009; tiger (*Panthera tigris*), Mondol *et al.* 2009; wolverine, Brøseth *et al.* 2010); however, our study demonstrates that noninvasively obtained genetic data may be used to investigate population genetic structure on a large spatial and temporal scale. To our knowledge, we have also been one of the first to apply this kind of data to study the relationship of N_e and N_c in large carnivores. Thus, there is only a limited amount of comparable studies, although it would be desirable to be able to compare our findings with those from similar species in the future.

Both the genetic substructuring and the N_e/N_c ratios may support the same conclusion of higher genetic variation and gene flow towards the east than the west and the apparent existence of barriers to migration between those areas. In this context, the small population of the Pasvik Valley may represent a genetic border, as the gene flow decreases towards the west and, to some degree, also to the south. Other populations, especially from Northern Norway and Sweden, as well as from the areas between Pasvik and Karelia, should be included in future studies to improve our understanding of migration routes and population structure in Northern European brown bear populations. If the reasons for the lower gene flow are poaching and fragmentation, our findings raise concerns about the future conservation of brown bear populations in Northern Europe.

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The study is part of a larger study on brown bear genetics and ecology carried out at the research section at Bioforsk Svanhovd in Norway, lead by S.B.H and H.G.E. The research work is also part of the PhD studies of J.S. and A.K. J.A., P.M.K. and M.R. are senior geneticists with interests in conservation of animal species. The research work of P.E.A., O.M., N.P. and K.F.T. is focused on northern forest ecosystems. H.B., M.E.S., M.S., S.W., I.W. and I.K. work on monitoring and conservation genetics of large carnivores in Northern Europe. J.E.S. is a Professor of Ecology and Natural Resources Management and P.I.D. is a Professor of Biology.

Data accessibility

For microsatellite genotype data and sampling location per identified individual, please see Table S1 in the supporting materials provided with the online version of this article.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Summary of the microsatellite genotype data and sampling location per identified individual.

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