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Consequences of a demographic bottleneck on genetic structure and variation in the Scandinavian brown bear

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Abstract

The Scandinavian brown bear went through a major decline in population size approximately 100 years ago, due to intense hunting. After being protected, the population subsequently recovered and today numbers in the thousands. The genetic diversity in the contemporary population has been investigated in considerable detail, and it has been shown that the population consists of several subpopulations that display relatively high levels of genetic variation. However, previous studies have been unable to resolve the degree to which the demographic bottleneck impacted the contemporary genetic structure and diversity. In this study, we used mitochondrial and microsatellite DNA markers from pre- and postbottleneck Scandinavian brown bear samples to investigate the effect of the bottleneck. Simulation and multivariate analysis suggested the same genetic structure for the historical and modern samples, which are clustered into three subpopulations in southern, central and northern Scandinavia. However, the southern subpopulation appears to have gone through a marked change in allele frequencies. When comparing the mitochondrial DNA diversity in the whole population, we found a major decline in haplotype numbers across the bottleneck. However, the loss of autosomal genetic diversity was less pronounced, although a significant decline in allelic richness was observed in the southern subpopulation. Approximate Bayesian computations provided clear support for a decline in effective population size during the bottleneck, in both the southern and northern subpopulations. These results have implications for the future management of the Scandinavian brown bear because they indicate a recent loss in genetic diversity and also that the current genetic structure may have been caused by historical ecological processes rather than recent anthropogenic persecution.

Keywords: bottleneck, decline, microsatellites, mtDNA, *Ursus arctos*

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Introduction

In conservation biology, the maintenance of genetic variation and subpopulation connectivity are of major importance with respect to population viability (Lacy 1997; Bech *et al.* 2009; Lee *et al.* 2010; Bush *et al.* 2011;

Proctor *et al.* 2012). A genetically variable population has a higher potential to cope with environmental changes (Lande & Shannon 1996). Additionally, gene flow between subpopulations can maintain high levels of diversity within each subpopulation. Demographic bottlenecks may reduce genetic diversity and can lead to population fragmentation in wild populations (Nei *et al.* 1975; Glenn *et al.* 1999; Weber *et al.* 2000; Charlier *et al.* 2008; Ferreira *et al.* 2009; Wójcik *et al.* 2009). Given sufficient time, bottlenecked populations that remain small are likely to lose further genetic variation due to genetic drift and have a higher chance of inbreeding (Lacy 1987; Lynch *et al.* 1995; Keller & Waller 2002). Moreover, if population structure is pronounced, declines in the respective subpopulations can lead to increased differentiation, as well as further declines in overall population viability (Segelbacher *et al.* 2003). Therefore, exploring the impact of rapid population declines is crucial when issues of species management and conservation emerge.

A major population decline occurred in the history of Scandinavian brown bear (*Ursus arctos*) (Swenson *et al.* 1994, 1995). Before and during the 19th century, predator extermination programmes greatly reduced the number of brown bears in Norway and Sweden. Estimates suggest that by the 1930s, approximately 130 individuals survived in Sweden, whereas in Norway the brown bear was even closer to extinction (Swenson *et al.* 1994, 1995). Actions to protect brown bears that began in the late 1800s in Sweden and later in Norway proved successful for the recovery of the population (Swenson *et al.* 1995). Today, the brown bear population thrives in Sweden and numbers almost 3000 individuals (Kindberg *et al.* 2011). In Norway however, the current population is only estimated to approximately 150 animals, mainly occurring along the eastern border and in the far north (Aarnes *et al.* 2014).

Phylogeographic studies based on mitochondrial DNA (mtDNA) have classified Scandinavian brown bears into two main lineages, the Western and the Eastern (Taberlet & Bouvet 1994). Presumably, members of each lineage survived in different refugia during the last ice age. At the onset of the Holocene, the two lineages colonized Scandinavia through a southern and an eastern route (Taberlet & Bouvet 1994). Today, these lineages are represented by two different mtDNA haplogroups in Scandinavia and comprise a total of four different haplotypes (Taberlet & Bouvet 1994; Norman *et al.* 2013). The two haplogroups are distinctly separated geographically between southern (Western lineage) and northern (Eastern lineage) Scandinavia, with a contact zone located in the middle of Jämtland County separating them with no signs of mtDNA admixture (Taberlet *et al.* 1995). Whereas males have been recorded to cross the contact zone, females seemingly have not (Taberlet *et al.* 1995; Waits *et al.* 2000), and this distinct matrilineal-based population structure is probably explained by strong female philopatric behaviour (Taberlet *et al.* 1995; Stoen *et al.* 2006).

A more detailed population structure comprising four subpopulations was originally proposed, based on the spatial distribution of hunter-killed females (Swenson *et al.* 1994). Microsatellite analysis has shown significant differences among these putative subpopulations and that each subpopulation has higher heterozygosity than would be expected for a bottlenecked population (Waits *et al.* 2000). However, a subsequent study based on clustering methods with no a priori predefined populations challenged the notion of four subpopulations and instead suggested a division into three subpopulations (Manel *et al.* 2004). This has been further supported by a recent study on single nucleotide polymorphisms (Norman *et al.* 2013).

The causes for the limited number of haplotypes and the genetic structure in Scandinavian brown bears have not yet been clarified. An early phylogeographical study suggested that the mtDNA contact zone was caused by the postglacial recolonization of Scandinavia (Taberlet & Bouvet 1994). This was recently supported by Bray *et al.* (2013), who concluded that the distribution of haplotypes and lack of mtDNA diversity likely are due to the manner in which Scandinavia was colonized by bears, rather than the outcome of anthropogenic hunting during the 19th century. On the other hand, Waits *et al.* (2000) pointed out that it is possible that both the mtDNA and autosomal genetic structures in Scandinavia are emergent patterns of lineage sorting driven by genetic drift following the demographic bottleneck.

These previous studies have thus raised two important questions concerning the consequences of the demographic bottleneck: (i) To what extent, if any, did the demographic bottleneck contribute to loss in mtDNA and autosomal variation? and (ii) Is the genetic structure in the contemporary population an ancient pattern that predates the bottleneck, or is it a consequence of genetic drift following the bottleneck? One way to address questions related to the genetic consequences of past events is to analyse genetic variation in museum samples of known age, because these can be used to study genetic changes directly through time (Miller & Waits 2003; Miller *et al.* 2006; Wandeler *et al.* 2007). The aim of this study was to do this, through analysis of mtDNA and microsatellite genetic variation in contemporary samples, as well as museum specimens from the historical Scandinavian brown bear population dating back to the 19th and early 20th centuries.

Materials and methods

Samples

A historical sample set, dating from before the 1940s, as well as a modern sample set was used in this study. Sixty-five bone samples, seven skin samples and one molar tooth from Scandinavian brown bears were obtained mainly from museum collections (Table S5). Sample age and location were based on museum records. Our sample set mainly covered a time span from 1830 to 1940. However, we also included five samples from archaeological and palaeontological excavations. Although not radiocarbon dated, we concluded that these samples most likely predated the bottleneck 100 years ago. Additionally, 48 modern brown bear muscle samples were acquired from the Swedish Museum of Natural History in Stockholm for mtDNA analyses (Table S6). We also collated previously published mtDNA sequences from Taberlet & Bouvet (1994) and Bray *et al.* (2013), as well as microsatellite data from the 361 individuals genotyped by Waits *et al.* (2000). For calibration of our microsatellite results, five bear muscle samples originally used in Waits *et al.* (2000) were obtained from the National Veterinary Institute (SVA) in Uppsala.

DNA extraction

We collected approximately 100 mg of bone or tooth powder from the historical samples using a Dremel rotary tool with sterilized drill bits. We then extracted total DNA using 10–20 mg of powder (Brace *et al.* 2012). Skin samples and modern muscle samples were extracted using the QIAamp DNA Mini Kit and DNeasy Blood & Tissue Kit, respectively, following the manufacturer's instructions.

Mitochondrial DNA

We used two previously described primer pairs designed to target the hypervariable mtDNA control region in brown bears; L16164, H16299 and URSUSF1_136_156, URSUSR1_273_290 (Hänni *et al.* 1994; Valdiosera *et al.* 2007). We used a combination of the forward and reverse primers (URSUSF1_136_156 and H16299) to obtain a final sequence of approximately 300 bp. When this combination proved unsuccessful, we amplified the samples according to the method described in Valdiosera *et al.* (2007), which is more appropriate for fragmented DNA and which produced two shorter fragments (111bp and 135 bp) covering the variable sections of the same mtDNA sequence discussed above.

We used polymerase chain reaction (PCR) for DNA amplification, conducted in 25 μL reactions using illustra PuReTaq Ready-To-Go PCR beads. The solution contained 21 μL dH_2O, 2 μL DNA extract and 1 μL of each forward and reverse primer (0.4 μM). Samples that were not successfully amplified using PCR beads were amplified using an alternative PCR at 25 μL total volume, composed of 2.5 μL PCR buffer, 15.1 μL dH_2O, 0.5 μL of each primer (0.2 μM), 0.5 μL dNTPs (0.2 mM), 1 μL MgCl (1 mM), 2.5 μL BSA (0.1 mg/mL), 0.4 μL HotStarTaq DNA polymerase and 2.0 μL of DNA extract. All historical samples were amplified using the following settings: 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 50–52 °C for 30 s and elongation at 72 °C for 30 s, followed by a final extension stage at 72 °C for 7 min. Modern samples were amplified for 35 cycles using the same settings. We ran the resulting amplicons on agarose gels (1%) to check for successful amplification and contamination during the previous steps. All amplified products were purified by means of exonuclease 1 and FastAP alkaline phosphatase. We performed sequencing reactions using the ABI BigDye Terminator kit (version 1.1, Applied Biosystems), and the resulting products were scored on an automated sequencer (ABI3130XL; Applied Biosystems) at the Department of Bioinformatics and Genetics at the Swedish Museum of Natural History. The resulting forward and reverse chromatograms were analysed in GENEIOUS (ver. 7.1). We excluded a specific sequence region, containing a stretch of T's followed by a stretch of C's, from the alignment, because of numerous insertions/deletions (following Bray *et al.* 2013).

Microsatellites

We used 19 primer pairs described in Waits *et al.* (2000) for microsatellite amplification. Four fluorescent dye groups (6-FAM, PET, NED and VIC) were used for labelling the forward primer of each primer pair. We mixed primer pairs in four different multiplexes (Table S7) using the QIAGEN Multiplex PCR Kit. A reaction solution of 12 μL total volume per sample was used for each multiplex. Amplification was conducted by setting the solution in 95 °C for 15 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s and elongation at 72 °C for 60 s, followed by a final 30 min extension stage at 60 °C. We analysed amplified products on an ABI3130XL (Applied Biosystems) after dilution by 50%. Peak calling was performed using the size standard LIZ-500 in GENEIOUS microsatellite plug-in (ver.1.3). Modern samples used for the calibration of our data were amplified and analysed by employing the same methods, except that the number of PCR cycles was reduced to 35 cycles.

Authenticity of the results

Historical sample extractions were conducted in a laboratory dedicated to the analysis of museum samples, physically separated from the laboratory where the contemporary samples were extracted. During every extraction and amplification procedure, negative blanks were used for detecting possible contamination (one per eight samples). At least two PCR amplifications were sequenced for each targeted mtDNA fragment to resolve sequence errors caused by misincorporations during the PCRs. During microsatellite genotyping, we assigned heterozygous genotypes only when two alleles had appeared at least twice in replicated PCRs for a particular locus. To assign homozygous genotypes, we replicated each individual locus three times, that is until the probability of obtaining a false homozygote due to allelic dropout was <1% both within and across loci. The rate of allelic dropout was calculated accordingly (Gagneux *et al.* 1997).

Data analysis

We estimated mitochondrial genetic diversity in terms of total number of haplotypes, haplotype diversity ($h \pm SE$) and nucleotide diversity (π) using the Tamura and Nei substitution model ($a = 0.1$) (Tamura & Nei 1993) in ARLEQUIN (ver.3.5) (Excoffier & Lischer 2010). A minimum-spanning network was created using the POPART software (available on http://popart.otago.ac.nz). For diversity calculations and network construction, we also included sequences recovered in previous studies on Scandinavian bears (Taberlet & Bouvet 1994; Bray *et al.* 2013).

For the microsatellite data, a Bayesian clustering method was performed using the software STRUCTURE to investigate population structure (Pritchard *et al.* 2000; Falush *et al.* 2003). Parameters were set to default with a burn-in length of 500 000 and 1 000 000 iterations. We ran ten independent replicates testing for 2–6 clusters (K) using both historical and modern samples. A second structure analysis was also performed with the same settings yet assuming independent allele frequencies (see also Appendix S1). CLUMPP ver.1.1.2 (Jakobsson & Rosenberg 2007) was used to align and summarize the results of the independent replicates obtained from STRUCTURE. We estimated pairwise differentiation among the subpopulations inferred from the Bayesian clustering analysis using F_{ST}-statistics calculated in GENALEX using 999 permutations (Peakall & Smouse 2012). GENALEX was also used to perform a principal coordinate analysis (PCoA) on the microsatellite data using the nonstandardized covariance matrix of the genetic distances.

We used the software ARLEQUIN ver.3.5 (Excoffier & Lischer 2010) to estimate the average expected heterozygosity (Ha) and to calculate the observed (H_O) and expected (H_E) heterozygosities for each locus for each subpopulation (Table 1). Deviations from expectations under Hardy–Weinberg equilibrium were calculated using exact tests based on 500 000 dememorization steps and an MCMC length of 1 000 000 steps using Arlequin (Excoffier & Lischer 2010). We also performed a linkage disequilibrium test with 20 000 permutations and 10 initial conditions (Slatkin 1994) for testing the associations between loci, using Bonferroni correction for multiple testing (Rice 1989).

Allelic richness in the historical and contemporary populations was calculated by implementing rarefaction methods using the software ADZE (Szpiech *et al.* 2008). To maintain statistical power during the rarefaction analysis, samples from the central and northern subpopulations were combined for each time period.

To further infer the demographic history of the Scandinavian brown, bear we used approximate Bayesian computation (ABC) methods implemented in DIYABC 2.0 (Cornuet *et al.* 2014). Three different demographic scenarios were compared and evaluated. Briefly, we modelled a first scenario of a bottleneck in both the southern and central+northern subpopulations, a second one with a bottleneck only in the south and a third one without population bottlenecks. In all models, we assume admixture following the bottleneck with gene flow into the southern subpopulation (Fig. 6, see also Tables S1–S4 and Figs S3–S4). The generation time was set to 10 years (Cahill *et al.* 2013), and the ratio of census to effective population size (N_e/N_c) used was 0.06–0.14 (Tallmon *et al.* 2004). The posterior probabilities of the three scenarios were subsequently estimated based on (i) a direct estimate, in which the 500 data sets with summary statistics closest to the target values were extracted, and (ii) a polychotomous logistic approach that recovers the 1% closest to the simulated data sets. For the scenario with the highest support, posterior distributions for the demographic parameters were estimated, using a local linear regression on the closest 1% of the million simulated data set, after the application of a logit transformation.

Results

Mitochondrial DNA

No contamination was observed during amplification or sequencing. We successfully retrieved 285-bp mtDNA sequences (after exclusion of the poly-T/C site) from 60 of the 73 historical samples and 47 of the 48 contemporary samples. We found 12 distinct haplotypes in the historical sample set, 9 of which corresponded to the

Table 1 Number of observed alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He) of historical and modern samples of Scandinavian brown bears. Average expected heterozygosity (Ha) given with standard deviation values (SD) and allelic richness with standard error values (SE). Numbers marked with asterisks indicate deviations from Hardy–Weinberg equilibrium

	Historical southern (n = 30)			Modern southern (n = 145)			Historical central (n = 5)			Historical northern (n = 11)			Modern central (n = 84)			Modern northern (n = 132)		
Locus	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
G10C	8	0.67*	0.78	5	0.68	0.67	2	0.80	0.53	4	0.82	0.70	5	0.68*	0.67	5	0.73	0.67
Mu15	6	0.52*	0.73	4	0.68	0.65	2	0.40	0.53	4	0.60	0.49	5	0.43	0.47	5	0.55	0.51
G1D	7	0.63	0.78	3	0.56	0.60	4	0.80	0.64	5	0.67	0.75	6	0.64	0.64	7	0.80	0.74
G10L	6	0.83	0.79	7	0.81	0.78	4	0.80	0.71	6	0.82	0.79	7	0.57*	0.70	8	0.73*	0.80
G10B	9	0.73	0.84	6	0.68	0.69	3	0.80	0.60	4	0.82	0.70	6	0.71	0.64	10	0.65	0.68
Mu05	8	0.63	0.82	7	0.66	0.62	2	0.00	0.36	5	0.80	0.75	6	0.67	0.66	6	0.69	0.73
Mu50	6	0.74	0.74	5	0.76	0.73	5	0.80	0.80	5	0.90	0.83	7	0.73	0.79	7	0.64	0.68
Mu59	10	0.73*	0.89	8	0.82	0.77	5	1.00	0.87	8	0.91	0.88	8	0.82*	0.84	10	0.80	0.83
Mu10	7	0.66*	0.78	7	0.86	0.81	5	1.00	0.84	7	0.82	0.84	9	0.80	0.77	8	0.77	0.77
G10X	3	0.66	0.65	4	0.58	0.57	2	0.60	0.47	3	0.50	0.67	4	0.62	0.61	6	0.54	0.51
G1A	7	0.71	0.81	5	0.64	0.63	3	0.80	0.69	5	0.70	0.77	6	0.73	0.71	7	0.61	0.67
G10J	8	0.67	0.76	6	0.57	0.59	3	0.80	0.73	4	0.45	0.65	5	0.68	0.65	7	0.80	0.74
Mu61	4	0.52*	0.72	3	0.52	0.54	3	0.80	0.64	4	0.88	0.65	3	0.63	0.63	4	0.52	0.49
G10P	8	0.85	0.85	6	0.79	0.77	3	1.00	0.68	3	0.91	0.65	6	0.77	0.75	6	0.74*	0.78
Mu51	8	0.77	0.81	6	0.81	0.76	5	0.80	0.80	4	1.00	0.77	8	0.78	0.77	6	0.75	0.75
Mu23	8	0.73	0.84	7	0.77	0.69	4	0.40	0.53	3	0.64	0.67	6	0.86	0.82	7	0.67	0.71
Ha (±SD)	0.78 ± 0.40			0.68 ± 0.34			0.65 ± 0.37			0.74 ± 0.40			0.69 ± 0.35			0.69 ± 0.35		
AR (±SE)	5.45 ± 0.27			4.09 ± 0.23					4.43 ± 0.32						4.58 ± 0.25			

Western lineage and 3 to the Eastern lineage. Moreover, two additional historical haplotypes belonging to the Eastern lineage had previously been observed in a study on Holocene bear samples from northern Norway (Bray *et al.* 2013). In the modern samples, when our results were combined with sequences from previous studies (Taberlet & Bouvet 1994), only four haplotypes were confirmed, two in southern and two in northern Scandinavia. Thus, the total number of haplotypes before the bottleneck was 14, whereas there are only four haplotypes in the contemporary population (Fig. 1).

In the minimum-spanning network (Fig. 2), we observed two distinct haplogroups that were distributed in southern and northern Scandinavia, respectively (from here on referred to as the southern and northern haplogroups). Each of the haplogroups displayed a star-like pattern, where haplotypes S3 and N1 constituted the modal haplotypes (Fig. 2). All haplotypes, with the exception of S1, S5, S7 and S9 in the southern haplogroup, were only one substitution distant from the modal haplotypes. The haplotype diversity estimated for the historical samples (h = 0.82, SE = ±0.003) was significantly higher (t_{139} = 29.5, P < 0.0001) than in the modern population (h = 0.66, SE = ±0.003). However, we found no significant differences in nucleotide diversity (t_{139} = 0.9, P = 0.37) between the historical and modern samples ($\pi \pm$ SE was 0.111 ± 0.006 and 0.102 ± 0.003, respectively).

Microsatellites

Forty-six of 61 tested historical samples were successfully genotyped for 16 of the 19 loci (with 3.1% missing data in the final data set). After three replications, we estimated the probability of falsely scoring a heterozygote as a homozygote due to allelic dropout to be <1% within and across loci.

In the Bayesian clustering analysis, the ln-likelihood values for the assumed number of clusters (K) increased with each assumed K-value without any plateau being reached (Fig. S1). Using the method proposed by Evanno *et al.* (2005) on the results of the first structure analysis (default settings), the suggested number of clusters was five (ΔK = 5). However, when examining the barplot outputs during analyses for the different K-values, the results were biologically meaningful until K = 4, where samples were grouped according to time period or geography (for further details, see Appendix S1). At higher K-values, individuals within the northern subpopulation were assigned to additional clusters in a way that did not correspond to time period or geography (Fig. S2). At K = 4, it should also be noted that the modern samples were clustered in agreement with previous studies (Manel *et al.* 2004), with the contemporary population being divided into three geographically distinct clusters. We therefore consider K = 4 to be the most likely number of clusters in the total data set (Fig. 3).

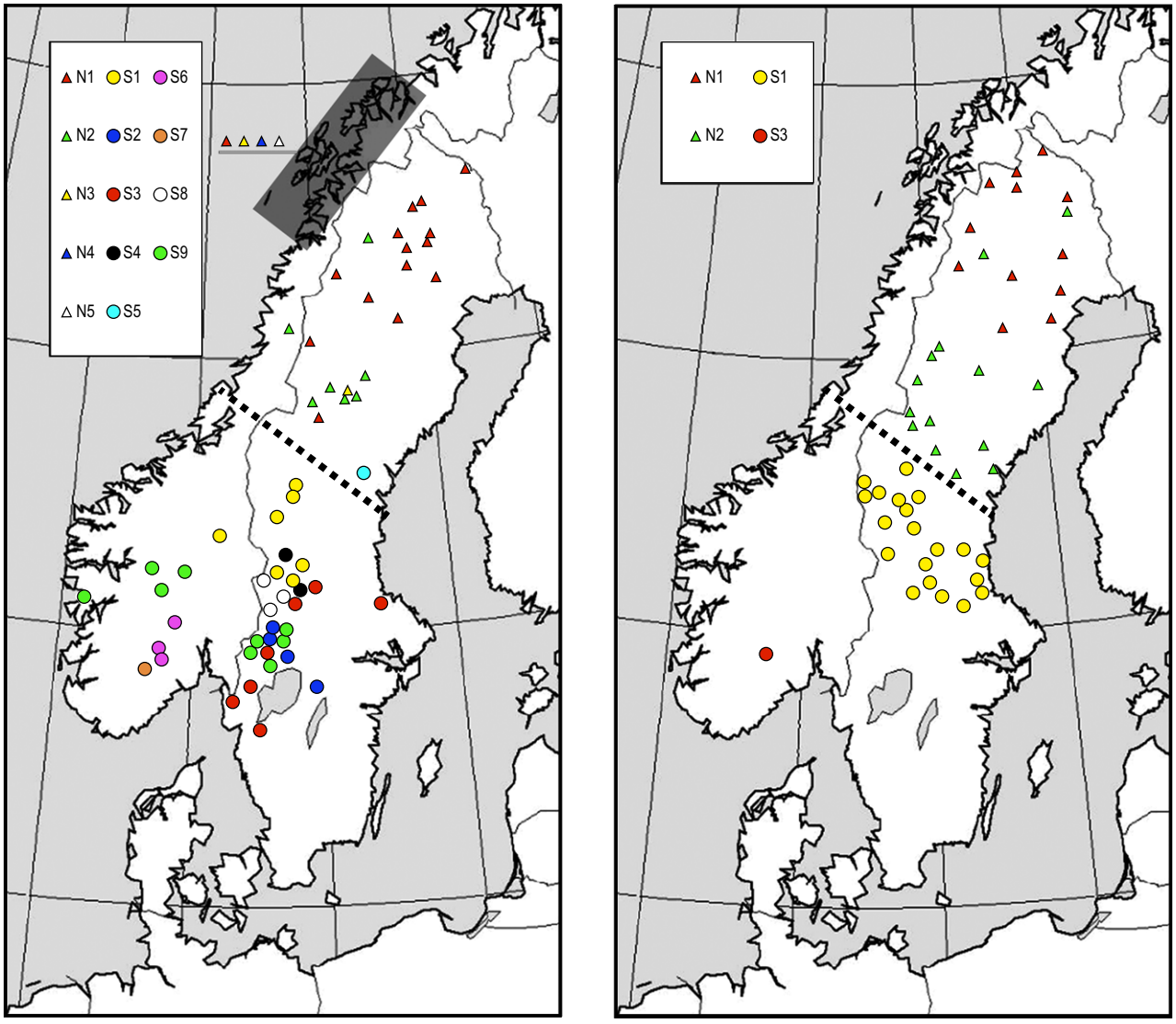

Fig. 1 Geographical distribution of mtDNA haplotypes. (a) From 1830 to 1940 including Holocene bear samples from Bray *et al.* (2013) (shaded area). (b) From 1990 to present including Norwegian sample (S3) from Taberlet & Bouvet (1994). Colours correspond to different haplotypes and shape corresponds to Western (circle) and Eastern (triangle) lineages (Taberlet *et al.* 1995). The dashed line shows the geographical location of the contact zone between the two lineages.

The historical samples from the central and northern subpopulations were generally assigned to the same clusters as the modern samples, although the historical northern subpopulation appeared admixed, with some individuals displaying a certain amount of assignment to other clusters (Fig. 3). In contrast, almost all of the historical samples from southern Scandinavia were assigned to a fourth cluster, not observed in any of the modern samples (Fig. 3). One exception to this was that one of the historical samples from the southern subpopulation had a 91% probability of assignment to the cluster observed in the contemporary southern subpopulation (Fig. 3).

Analysis of the 1st and 2nd principal coordinates in the PCoA identified four groups (Fig. 4). These groups corresponded well to the four clusters observed in the Bayesian Structure analysis, with historical and modern samples from the southern subpopulation being differentiated from each other, while those from the central and northern subpopulations were grouped together.

All pairwise comparisons showed statistically significant ($P < 0.05$) differentiation among groups, except between historical and modern samples from the central subpopulation (Table. 2). Temporal differentiation between historical and modern samples was higher for

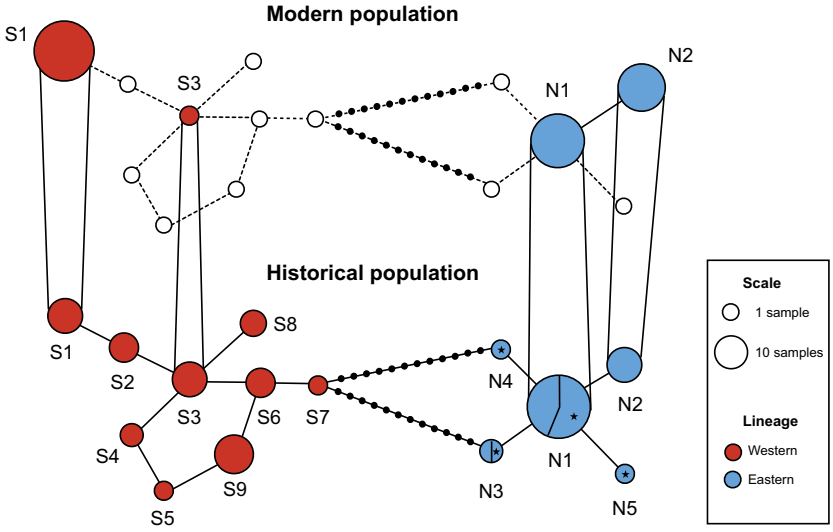

Fig. 2 Minimum-spanning networks. Associations between different haplotypes in the historical and modern sample set are shown. Colours correspond to the Western (red) and Eastern (blue) lineages (Taberlet & Bouvet 1994). Empty circles depict unobserved haplotypes in the modern population. Black dots represent missing haplotypes in the overall data set. Bears from the Holocene period that likely predate the 19th century (Bray *et al.* 2013) are marked with a star.

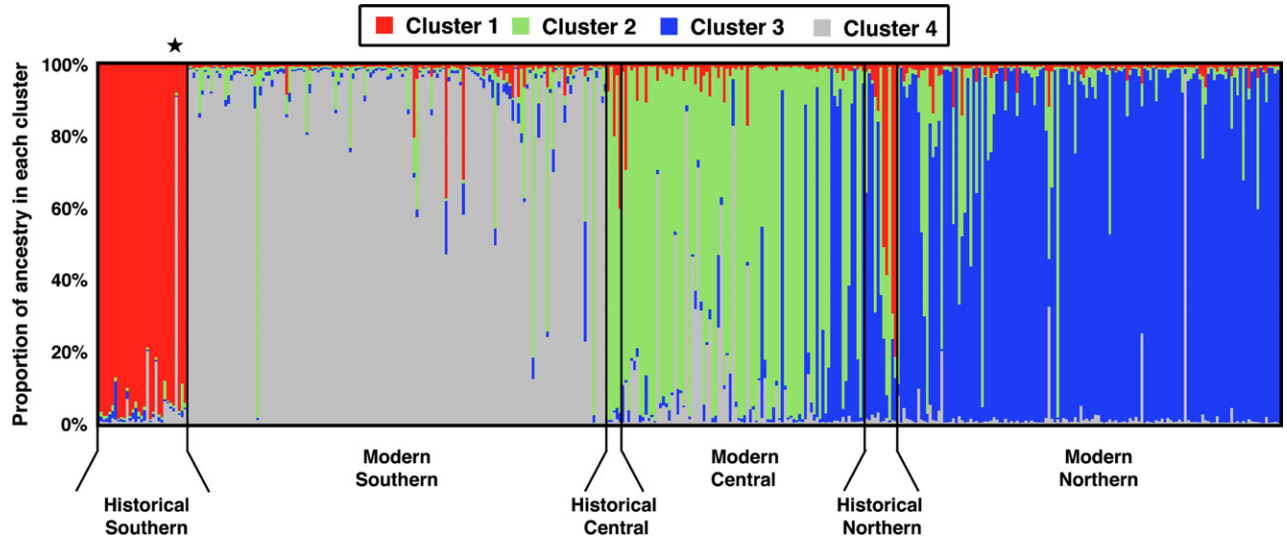

Fig. 3 Bayesian clustering analysis. Assignment of Scandinavian brown bear individuals after performing five replicates under the assumption of four clusters ($K = 4$). The *y*-axis shows the individual proportion of ancestry assigned to each cluster. Samples are sorted on the *x*-axis from left to right according to time period (past to present) and latitude (south to north). The star indicates a historical sample from the southern subpopulation that was assigned with 91% probability to the modern southern subpopulation.

the southern subpopulation ($F_{ST} = 0.084$) than for the northern subpopulation ($F_{ST} = 0.029$). A significant spatial differentiation was also found among the southern and central subpopulations, in both the historical ($F_{ST} = 0.087$) and modern ($F_{ST} = 0.089$) samples.

Eleven of 96 tests gave significant ($P < 0.05$) deviations from Hardy–Weinberg equilibrium (Table 1). However, there was no consistent pattern among subpopulations and time periods, suggesting that these deviations from Hardy–Weinberg equilibrium were not caused by null alleles. Tests for linkage disequilibrium in each of the respective subpopulations were significant in only 3 of 120 comparisons in the historical samples. However, in line with previous studies (Waits *et al.* 2000), we found a high degree of linkage disequilibrium in the modern samples, with 93 of 120 comparisons being statistically significant. The observation of a much higher amount of linkage disequilibrium in the contemporary subpopulations compared to the historical subpopulations suggests that the former is a consequence of the demographic bottleneck, a Wahlund effect or a recent mixing of divergent groups (Nei & Li 1973; Slatkin 2008; Tenaillon *et al.* 2008), rather than an actual physical linkage among loci.

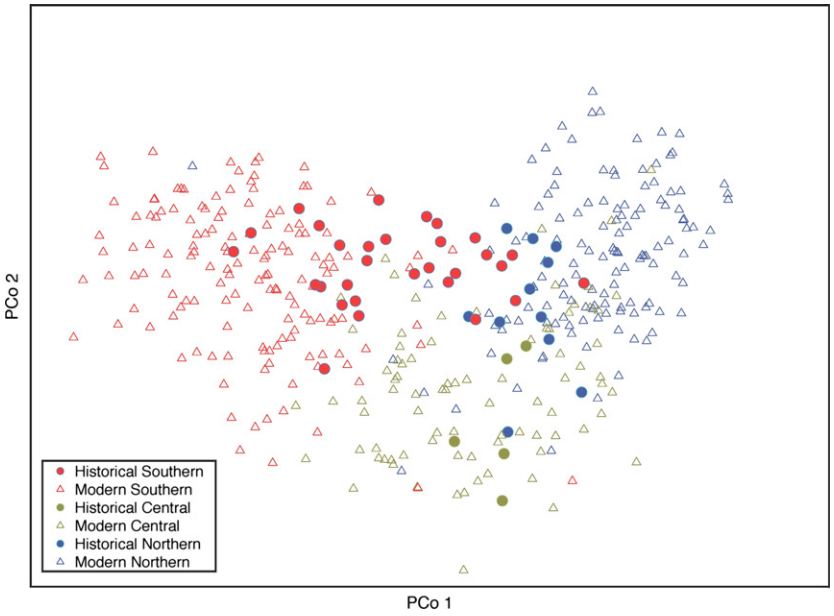

Fig. 4 Principal coordinate analysis for the historical and modern Scandinavian brown bear populations. Unfilled triangles correspond to the modern subpopulations. Filled circles designate the historical subpopulations. Different colours designate the subpopulations in the historical and modern sample sets (red: southern historical/modern, green: central historical/modern, blue: northern historical/modern subpopulation).

	Historical southern	Modern southern	Historical central	Modern central	Historical northern	Modern northern
Historical southern	–	***	***	***	***	***
Modern southern	0.084	–	***	***	***	***
Historical central	0.087	0.130	–	n.s.	*	***
Modern central	0.089	0.089	0.022	–	***	***
Historical northern	0.049	0.107	0.037	0.034	–	**
Modern northern	0.097	0.134	0.084	0.060	0.029	–

Table 2 Pairwise differentiation values; by means of *F*st (below diagonal) between all subpopulations in the historical and modern populations of Scandinavian brown bears. Above diagonal, the level of significance is indicated (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n.s.: nonsignificant)

We found no significant differences ($P > 0.05$) when comparing the average expected heterozygosity (Ha) among historical and modern subpopulations (Table 1), although there was a trend for significance when comparing the historical and modern southern subpopulations ($P = 0.053$). However, allelic richness was significantly higher in the historical southern subpopulation compared to the contemporary southern subpopulation ($t_{173} = 2.64$, $P < 0.01$), whereas no significant difference ($t_{230} = 0.15$, $P = 0.88$) was detected among the historical and contemporary samples from the central/northern subpopulations (Fig. 5).

The ABC simulations showed high support for model 1 (bottlenecks occurring in both the southern and central+northern subpopulations) and clearly favoured this as the most probable scenario (Fig. 6 and Table S2). Although not optimal, the confidence in the model choice estimated as rates of type I and type II errors demonstrated a high ability to differentiate between the three scenarios investigated (Table S3). The median estimates of effective population sizes during the bottleneck, as estimated from posterior distributions, were around 23 individuals for both the southern and central+northern subpopulations (90% highest posterior density interval, HPDI: 10.5–29.6 for the southern and HPDI: 4.6–54.8 for the central+northern). Despite the fact that some of the estimated parameters presented low robustness (Factor 2 statistic, Table S4), the ABC analyses conclusively support a recent bottleneck in the Scandinavian bear populations. Posteriors of the effective population sizes, time of population splits and rate of admixture are given in Table 3.

Discussion

The brown bear is one of many species that has suffered recent declines as a consequence of human encroachment and persecution, something which is especially evident among large carnivores (Ripple *et al.* 2014). However, the consequences of these declines can only partially be explained by studying the genetic diversity in present-day populations. By including data from historical museum samples, a more detailed record can be established, and this approach has been

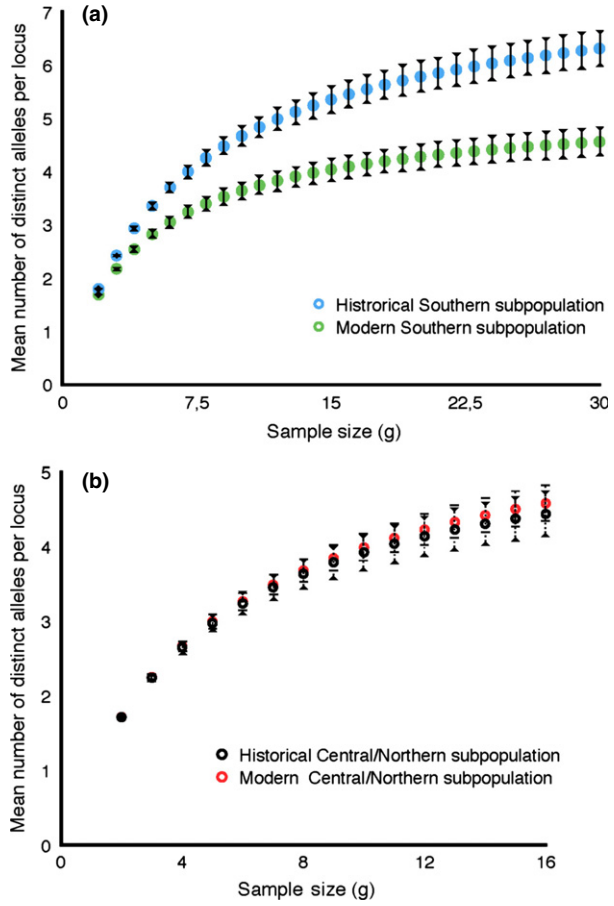

Fig. 5 Mean number of distinct alleles per locus against number of rarefied samples. (a) Historical and modern southern subpopulations and (b) Historical and modern central/northern subpopulations. Bars indicate the standard error (SE) for the mean estimate. Standardized maximum sample size was set to 16.

successfully applied to a range of species throughout the world, from large carnivores (Mondol *et al.* 2013) to small rodents (La Haye *et al.* 2012). Historical samples of brown bear have also been analysed in a similar fashion. In North America, such a study revealed a pattern with a severe range contraction accompanied by a dramatic decline in mitochondrial haplotype diversity (Miller *et al.* 2006), which in many ways mirror what our results suggest for the brown bear population in Scandinavia, although on a larger scale.

In Scandinavia, the ecological conditions have changed dramatically over time, from full glaciation in the late Pleistocene to ameliorating conditions during the Holocene along with expanding human populations. Over the last centuries, anthropogenic impact has further intensified, and all these changes have in different ways shaped the distribution and abundance of the Scandinavian fauna, which in turn has led to losses in

Table 3 Posterior statistics for demographic parameters estimated under the best supported scenario (model 1). The median, mode, 90% highest density probability interval (HDPI) and factor 2 for the median are given for each parameter. Population size estimates are effective population sizes ($N_{\#}$), and time estimates are in number of generations ($t_{\#}$)

Parameter	Median	Mode	90% HDPI	Factor 2 (median)
Demographic parameters posteriors				
N_1 – postbottleneck, southern	45.4	35.8	32.6–152	0.882
N_4 – postbottleneck, central+northern	172	139	84.8–283	0.932
N_{2b} – bottleneck, southern	23.4	30.0	10.5–29.6	0.856
N_{4b} – bottleneck central+northern	23.3	30.0	4.57–54.8	0.692
N_{2c} – prebottleneck, southern	246	183	100–592	0.940
N_{3c} – prebottleneck, central+northern	652	786	373–787	0.916
t_7 – splitting time south–central +northern	31.9	23.8	19.4–63.1	0.976
R – admixture rate	0.31	0.31	0.11–0.64	0.904

genetic variation and population structure. This has most evidently been observed in species targeted for hunting or extermination over the last centuries (Walker *et al.* 2001; Flagstad *et al.* 2003; Nyström *et al.* 2006). For some animals, like the Scandinavian otter (*Lutra lutra*), declines have been observed on both a long- and short-term scale (Pertoldi *et al.* 2001; Tison *et al.* 2014), and similar patterns have also been recognized in other European species (Niedziałkowska *et al.* 2014; Rodríguez-Varela *et al.* 2015).

In contrast to previous studies, which have suggested that the demographic bottleneck had little effect on the genetic diversity of the Scandinavian brown bear (Bray *et al.* 2013), our results indicate that the bottleneck had a considerable impact on genetic diversity. However, we also found that the mtDNA genetic structure in Scandinavia, as well as the location of the mtDNA contact zone, predates the demographic decline (Fig. 1). Moreover, our results based on autosomal microsatellite markers suggest that the historical population was composed of three genetically discrete subpopulations, which correspond to the three subpopulations identified in the contemporary brown bear population. Taken together, these results imply that the genetic structure in the contemporary population is not a result of fragmentation and genetic drift during the bottleneck (Waits *et al.* 2000).

Both mitochondrial and autosomal markers indicated declines in genetic diversity caused by the demographic

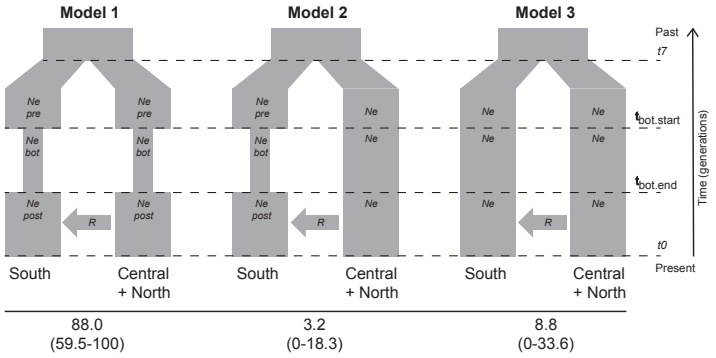

Fig. 6 Demographic scenarios tested with approximate Bayesian computation (DIYABC). Direct estimates of the posterior probabilities are presented below each scenario along with 90% highest posterior density intervals in parentheses. Letters indicate the parameters sampled from prior distributions; Ne = effective population size, Ne pre = effective population size before bottleneck, Ne bot = effective population size during bottleneck, Ne post = effective population size after bottleneck. Dashed lines represent the timing (t_x) of changes in population sizes, and R represents admixture rate.

bottleneck in the Scandinavian brown bear population. The decline in genetic diversity was strongest in the southern subpopulation, where we estimated a 78% reduction in the number of mtDNA haplotypes and a 27% decline in microsatellite allelic richness. The loss of haplotype diversity is comparable with that of the Indian tigers studied by Mondol *et al.* (2013) although no turnover in haplotype composition was detected through our data. Moreover, in the central and northern subpopulations, we observed a 60% loss in the number of mtDNA haplotypes, whereas there was no change in microsatellite allelic richness. Thus, the bottleneck appears to have had a negative impact on the genetic diversity of the entire brown bear population in Scandinavia, and this decline seems to have been more pronounced in the south. A potential reason for this could be that the population decline was more intense in the southern areas than in the central and northern subpopulations, which thus were able to retain higher levels of diversity. A population decline, with similarly different effects on closely distributed subpopulations, has been shown in a study on the Danish pine marten (*Martes martes*) utilizing autosomal markers in historical and contemporary samples (Pertoldi *et al.* 2008). The greater decrease in genetic diversity in one of the subpopulations was explained by habitat disturbance caused by more intense agricultural activity (Pertoldi *et al.* 2008). For the southern subpopulation of the Scandinavian brown bear, it is more likely that direct factors such as hunting were behind the severe decrease in genetic diversity.

As previously mentioned, temporal changes in population structure as a consequence of recent bottlenecks have been proposed for other large carnivores (Mondol *et al.* 2013). In contrast, our results for the Scandinavian brown bear show that the overall population structure, in terms of distribution of lineages and genetically distinct clusters, seems to be the same for the historical and modern population. The distribution of haplotypes in the historical and modern samples showed that the geographical position of the contact zone between the two linages remained the same (Fig. 1). Moreover, the results from the genetic clustering analysis based on microsatellite markers suggest that the autosomal genetic structure in the contemporary population also existed before the bottleneck, even though there seems to have been a major change in genetic composition in the southern subpopulation.

We find it highly unlikely that this marked change in genetic composition in the southern subpopulation was caused by extinction in the south followed by recolonization from the central subpopulation. This is because we observed a survival of mtDNA haplotypes belonging to the Western lineage across the bottleneck, and no change in the geographic location of the mtDNA contact zone. This implies that bears survived in the southern subpopulation during the peak of the demographic bottleneck. It is therefore likely that the change in autosomal genetic composition in the southern subpopulation was caused either by strong genetic drift, due to a severe decline in effective population size, or by an increase in gene flow from the central subpopulation, possibly driven by a reduced population density in the south. The latter scenario implies that we would have observed a decrease in genetic differentiation when comparing historical and contemporary samples from the southern and central subpopulations. However, we found no such change in the pairwise analyses of genetic differentiation. Thus, it seems more probable that a severe demographic decline in the southern subpopulation, accompanied by strong genetic drift, led to a marked change in the subpopulation's allele frequencies.

Because the Bayesian clustering analysis we used to detect population structure was based on an approach to minimize deviations from Hardy–Weinberg equilibrium (Pritchard *et al.* 2000), such a temporal shift in allele frequencies is likely to lead to a 'temporal Wahlund effect' and thus separation into different genetic clusters (Nyström *et al.* 2012).

It is also interesting to note that one individual in the historical southern subpopulation was assigned, with high probability, to the contemporary southern subpopulation in the Bayesian clustering analysis (Fig. 3). This individual might thus represent one of the surviving animals, whose descendants compose today's southern subpopulation. This fits well with the location and age of this sample, which was collected almost at the height of the bottleneck, in 1928 at Sånfjället, which is the nature reserve where it has previously been suggested that brown bears survived the peak of the bottleneck in southern Scandinavia (Lönnberg 1929). Thus, the time, area and genetic constitution of that individual fit well with the bottleneck hypothesis.

Another way to investigate the effect of genetic drift within each subpopulation is to examine F_{ST} values among historical and contemporary samples within each subpopulation (Table 2). In the southern subpopulation, we observed a considerable genetic differentiation among the historical and contemporary samples (F_{ST} = 0.084). We also observed a similar, albeit less strong, differentiation when we compared historical and contemporary samples from the northern subpopulation (F_{ST} = 0.029). In the central subpopulation, however, we observed no significant differentiation among the historical and contemporary samples. These results indicate that genetic drift has been highest in the southern subpopulation, lower in the northern subpopulation and very low in the central subpopulation. However, it should be noted that the observed lack of temporal differentiation in the central subpopulation also could be due to the small sample size in the historical samples (n = 5).

The ABC analysis provided additional support for the scenario of recent bottlenecks in effective population size in both the southern and the central/northern subpopulations. The results also indicated a stronger recovery in the central/northern subpopulations, as well as substantial gene flow into the southern subpopulation after the bottleneck. The estimated posteriors for the bottleneck effective population sizes (N_e) were somewhat larger than what has been suggested previously based on historical records (Swenson *et al.* 1994, 1995). In addition, the effective population size in the southern subpopulation during the bottleneck is probably underestimated, as the posterior distribution is skewed towards the higher end of the prior distribution. However, based on the median values of the effective population sizes estimated here (N_e = 47 in total; Table 3) and assuming an N_e/N_c ratio of 0.06–0.14 (Tallmon *et al.* 2004), the census population size during the bottleneck was probably at least 300–800 animals in total in Scandinavia. We would like to stress that these figures are highly dependent on the rather low ratio of census to effective population size we have assumed. Moreover, the estimates of effective population size (N_e), timing (t) and rate of admixture (R) should be treated with caution, as they rely on several assumptions and may be affected by limitations in DIYABC. For instance, the estimated split between the southern and central/northern subpopulations (T_7) is surprisingly recent (HPDI: 19.4–63.1), but this estimate is likely affected by the limitation that no gene flow could be modelled prior to the bottleneck. Additional analyses with more data and more flexible models might resolve this and provide more accurate estimates.

In contrast to other large carnivores in Scandinavia (Walker *et al.* 2001; Flagstad *et al.* 2003), our results show that the low mtDNA diversity in the brown bear is not a consequence of a small effective population size during a long time during the Holocene. This implies that the comparatively high mtDNA diversity in the prebottleneck population may allow for a more accurate inference of the postglacial recolonization history in the Scandinavian brown bear, as compared to an analysis based only on the modern-day mtDNA haplotypes. Interestingly, we found almost twice the number of haplotypes and significantly higher allelic richness in the historical samples from southern Scandinavia compared to the central and northern subpopulations. We believe that this is related to the time of colonization in Scandinavia following the retreat of the ice sheet at the end of the last ice age. Glaciological models suggest that the ice sheets in Scandinavia initially retreated from the south towards the north (Björck 1995), and this implies that brown bears may have colonized southern Scandinavia much earlier than northern Scandinavia, which in turn would have provided more time for genetic diversification in the southern subpopulation. An alternative explanation for the comparatively high genetic diversity in the southern prebottleneck subpopulation could be that southern Scandinavia was colonized by a larger number of founders compared to northern Scandinavia. Further genetic analysis of late Pleistocene and Holocene samples from continental Europe could help to resolve this question.

Conclusion

In conclusion, this study shows that demographic bottlenecks are important in shaping the genetic diver-

sity in wild populations. We show that the Scandinavian brown bear experienced population bottlenecks leading to loss of variation. However, the impact of the overall decline approximately 100 years ago varied among the different subpopulations, likely due to differences in the numbers of individuals that survived the peak of the bottleneck. The survival of individuals in different parts of the Scandinavian Peninsula also appears to have maintained the genetic structure that existed prior to the bottleneck, even though genetic drift led to declines in allelic diversity and haplotype numbers, especially in the southern subpopulation. From a conservation perspective, the genetic structure in the Scandinavian brown bear may therefore be a consequence of prebottleneck natural processes, rather than recent anthropogenic impacts, and this should be taken into consideration in the management and conservation of the population.

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References

Aarnes SG, Tobiassen C, Brøseth H *et al.* (2014) Populasjonsovervåking av brunbjørn: DNA-analyse av prøver innsamlet i Norge i 2013. *Bioforsk Rapport*, **48**, 1–54. Bioforsk Jord og miljø.

Bech N, Boissier J, Drovetski SV, Novoa C (2009) Population genetic structure of rock ptarmigan in the 'sky islands' of French Pyrenees: implications for conservation. *Animal Conservation*, **12**, 138–146.

Björck S (1995) A review of the history of the Baltic Sea, 13.0-8.0 ka BP. *Quaternary International*, **27**, 19–40.

Brace S, Palkopoulou E, Dalén L *et al.* (2012) Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability. *Proceedings of the National Academy of Sciences, USA*, **109**, 20532–20536.

Bray SCE, Austin JJ, Metcalf JL *et al.* (2013) Ancient DNA identifies post-glacial recolonisation, not recent bottlenecks, as the primary driver of contemporary mtDNA phylogeography and diversity in Scandinavian brown bears. *Diversity and Distributions*, **19**, 245–256.

Bush KL, Dyte CK, Moynahan BJ *et al.* (2011) Population structure and genetic diversity of greater sage-grouse (Centrocercus urophasianus) in fragmented landscapes at the northern edge of their range. *Conservation Genetics*, **12**, 527–542.

Cahill JA, Green RE, Fulton TL *et al.* (2013) Genomic evidence for Island population conversion resolves conflicting theories of polar bear evolution. *Plos Genetics*, **9**, e1003345.

Charlier J, Laikre L, Ryman N (2008) Genetic structure and evidence of a local bottleneck in moose in Sweden. *Journal of Wildlife Management*, **72**, 411–415.

Cornuet J-M, Pudlo P, Veyssier J *et al.* (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism. DNA sequence and microsatellite data. *Bioinformatics*, **30**, 1187–1189.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Ferreira E, Souto L, Soares AMVM, Fonseca C (2009) Genetic structure of the wild boar population in Portugal: evidence of a recent bottleneck. *Mammalian Biology*, **74**, 274–285.

Flagstad Ø, Walker CW, Vilà C *et al.* (2003) Two centuries of the Scandinavian wolf population: patterns of genetic variability and migration during an era of dramatic decline. *Molecular Ecology*, **12**, 869–880.

Gagneux P, Boesch C, Woodruff DS (1997) Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Molecular Ecology*, **6**, 861–868.

Glenn TC, Stephan W, Braun MJ (1999) Effects of a population bottleneck on Whooping Crane mitochondrial DNA variation. *Conservation Biology*, **13**, 1097–1107.

Hänni C, Laudet V, Stehelin D, Taberlet P (1994) Tracking the origins of the cave bear (*Ursus spelaeus*) by mitochondrial DNA sequencing. *Proceedings of the National Academy of Sciences, USA*, **91**, 12336–12340.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics (Oxford, England)*, **23**, 1801–1806.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, **17**, 230–241.

Kindberg J, Swenson JE, Ericsson G *et al.* (2011) Estimating population size and trends of the Swedish brown bear *Ursus arctos* population. *Wildlife Biology*, **17**, 114–123.

La Haye MJJ, Neumann K, Koelewijn HP (2012) Strong decline of gene diversity in local populations of the highly endangered Common hamster (*Cricetus cricetus*) in the western part of its European range. *Conservation Genetics*, **13**, 311–322.

Lacy RC (1987) Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. *Conservation Biology*, **1**, 143–158.

Lacy RC (1997) Importance of genetic variation to the viability of mammalian populations. *Journal of Mammalogy*, **78**, 320–335.

Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution*, **50**, 434–437.

Lee KE, Seddon JM, Corley SW *et al.* (2010) Genetic variation and structuring in the threatened koala populations of Southeast Queensland. *Conservation Genetics*, **11**, 2091–2103.

Lönnberg E (1929) *Björnen i Sverige 1856–1928*. Almquist & Wiksell, Uppsala.

Lynch M, Conery J, Burger R (1995) Mutational meltdowns in sexual populations. *Evolution*, **49**, 1067–1080.

Manel S, Bellemain E, Swenson JE, François O (2004) Assumed and inferred spatial structure of populations: the Scandinavian brown bears revisited. *Molecular Ecology*, **13**, 1327–1331.

Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. *Proceedings of the National Academy of Sciences, USA*, **100**, 4334–4339.

Miller CR, Waits LP, Joyce P (2006) Phylogeography and mitochondrial diversity of extirpated brown bear (*Ursus arctos*) populations in the contiguous United States and Mexico. *Molecular ecology*, **15**, 4477–4485.

Mondol S, Bruford MW, Ramakrishnan U (2013) Demographic loss, genetic structure and the conservation implications for Indian tigers. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20130496.

Nei M, Li WH (1973) Linkage disequilibrium in subdivided populations. *Genetics*, **75**, 213–219.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Niedziałkowska M, Hundertmark KJ, Jędrzejewska B *et al.* (2014) Spatial structure in European moose (*Alces alces*): genetic data reveal a complex population history. *Journal of Biogeography*, **41**, 2173–2184.

Norman AJ, Street NR, Spong G (2013) De novo SNP discovery in the Scandinavian brown bear (*Ursus arctos*). *PLoS ONE*, **8**, e81012.

Nyström V, Angerbjörn A, Dalén L (2006) Genetic consequences of a demographic bottleneck in the Scandinavian arctic fox. *Oikos*, **114**, 84–94.

Nyström V, Humphrey J, Skoglund P *et al.* (2012) Microsatellite genotyping reveals end-Pleistocene decline in mammoth autosomal genetic variation. *Molecular Ecology*, **21**, 3391–3402.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. *Bioinformatics (Oxford, England)*, **28**, 2537–2539.

Pertoldi C, Hansen MM, Loeschcke V *et al.* (2001) Genetic consequences of population decline in the European otter (Lutra lutra): an assessment of microsatellite DNA variation in Danish otters from 1883 to 1993. *Proceedings of the Royal Society B-Biological Sciences*, **268**, 1775–1781.

Pertoldi C, Barker SF, Madsen AB *et al.* (2008) Spatio-temporal population genetics of the Danish pine marten (*Martes martes*). *Biological Journal of the Linnean Society*, **93**, 457–464.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Proctor MF, Paetkau D, McLellan BN *et al.* (2012) Population fragmentation and inter-ecosystem movements of grizzly bears in western Canada and the northern United States. *Wildlife Monographs*, **180**, 1–46.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Ripple WJ, Estes JA, Beschta RL *et al.* (2014) Status and ecological effects of the world's largest carnivores. *Science*, **343**, 1241484.

Rodríguez-Varela R, Tagliacozzo A, Ureña I *et al.* (2015) Ancient DNA evidence of Iberian lynx palaeoendemism. *Quaternary Science Reviews*, **112**, 172–180.

Segelbacher G, Höglund J, Storch I (2003) From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. *Molecular Ecology*, **12**, 1773–1780.

Slatkin M (1994) Linkage disequilibrium in growing and stable populations. *Genetics*, **137**, 331–336.

Slatkin M (2008) Linkage disequilibrium – understanding the evolutionary past and mapping the medical future. *Nature Reviews Genetics*, **9**, 477–485.

Stoen OG, Zedrosser A, Saebo S, Swenson JE (2006) Inversely density-dependent natal dispersal in brown bears *Ursus arctos*. *Oecologia*, **148**, 356–364.

Swenson JE, Sandegren F, Bjärvall A *et al.* (1994) Size, trend, distribution and conservation of the brown bear *Ursus arctos* population in Sweden. *Biological Conservation*, **70**, 9–17.

Swenson JE, Wabakken P, Sandegren F *et al.* (1995) The near extinction and recovery of brown bears in Scandinavia in relation to the bear management policies of Norway and Sweden. *Wildlife Biology*, **1**, 11–25.

Szpiech ZA, Jakobsson M, Rosenberg NA (2008) ADZE: a rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics (Oxford, England)*, **24**, 2498–2504.

Taberlet P, Bouvet J (1994) Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear *Ursus arctos* in Europe. *Proceedings of the Royal Society of London – B. Biological Sciences*, **255**, 195–200.

Taberlet P, Swenson JE, Sandegren F, Bjarvall A (1995) Localization of a contact zone between two highly divergent mitochondrial DNA lineages of the brown bear *Ursus arctos* in Scandinavia. *Conservation Biology*, **9**, 1255–1261.

Tallmon DA, Bellemain E, Swenson JE, Taberlet P (2004) Genetic monitoring of Scandinavian brown bear effective population size and immigration. *Journal of Wildlife Management*, **68**, 960–965.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Tenaillon MI, Austerlitz F, Tenaillon O (2008) Apparent mutational hotspots and long distance linkage disequilibrium resulting from a bottleneck. *Journal of Evolutionary Biology*, **21**, 541–550.

Tison J-L, Blennow V, Palkopoulou E *et al.* (2014) Population structure and recent temporal changes in genetic variation in Eurasian otters from Sweden. *Conservation Genetics*, **16**, 371–384.

Valdiosera CE, García N, Anderung C *et al.* (2007) Staying out in the cold: glacial refugia and mitochondrial DNA phylogeography in ancient European brown bears. *Molecular Ecology*, **16**, 5140–5148.

Waits L, Taberlet P, Swenson JE, Sandegren F, Franzén R (2000) Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). *Molecular Ecology*, **9**, 421–431.

Walker CW, Vilà C, Landa A, Lindén M, Ellegren H (2001) Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Molecular Ecology*, **10**, 53–63.

Wandeler P, Hoeck PEA, Keller LF (2007) Back to the future: museum specimens in population genetics. *Trends in Ecology and Evolution*, **22**, 634–642.

Weber DS, Stewart BS, Garza JC, Lehman N (2000) An empirical genetic assessment of the severity of the northern elephant seal population bottleneck. *Current Biology*, **10**, 1287–1290.

Wójcik JM, Kawałko A, Tokarska M *et al.* (2009) Post-bottleneck mtDNA diversity in a free-living population of European bison: implications for conservation. *Journal of Zoology*, **277**, 81–87.

L.D., G.X. and E.E. designed the study. G.X. and E.E. carried out DNA extractions and sequencing. G.X. performed the computational analyses, evaluated the results and drafted the manuscript together with L.D. and E.E. E.E. and J.L.T. performed the Bayesian simulations. All authors contributed to the interpretation of the results and took part in writing the manuscript.

Data accessibility

Sequences of mtDNA produced for this study have been submitted to GenBank (Accession nos. KM886400-KM886459). Microsatellite data for the historical samples have been deposited in Dryad (http://dx.doi.org/10.5061/dryad.dn7b8).

Supporting information

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

Appendix S1 Supplementary methods, figures and tables.

Fig. S1 Mean values for the log probability of the brown bear microsatellite data against the assumed number of cl*usters* (K) assuming (a) correlated allele frequencies and (b) independent allele frequencies.

Fig. S2 Bayesian clustering analysis, showing barplots for the proportion of ancestry assigned to individual Scandinavian brown bears of the historical and contemporary population under the assumption of different numbers of clusters ($K = 2$–6).

Fig. S3 Principal Component Analysis (PCA) for the pre-evaluation of the scenario-prior combination.

Fig. S4 Principal Component Analysis (PCA) performed from the model checking in the ABC analyses.

Table S1 Prior distributions of parameters used in the ABC analysis.

Table S2 Relative posterior probability of the demographic scenarios expressed as a percentage.

Table S3 Confidence in the model choice of model 1 as the best-fitting model in the ABC analysis.

Table S4 Model checking analysis for model 1.

Table S5 Historical samples used for mtDNA and microsatellite analyses of Scandinavian brown bears.

Table S6 Modern brown bear samples from Sweden used for mtDNA analysis.

Table S7 Multiplex mixes used in the present study.