

SHORT COMMUNICATION

Assumed and inferred spatial structure of populations: the Scandinavian brown bears revisited

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Abstract

We reanalysed the spatial structure of the Scandinavian brown bear (*Ursus arctos*) population based on multilocus genotypes. We used data from a former study that had presumed *a priori* a specific population subdivision based on four subpopulations. Using two independent methods (neighbour-joining trees and Bayesian assignment tests), we analysed the data without any prior presumption about the spatial structure. A subdivision of the population into three subpopulations emerged from our study. The genetic pattern of these subpopulations matched the three geographical clusters of individuals present in the population. We recommend considering the Scandinavian brown bear population as consisting of three (instead of four) subpopulations. Our results underline the importance of determining genetic structure from the data, without presupposing a structure, even when there seems to be good reason to do so.

Keywords: Bayesian assignment test, genetic diversity, microsatellites, neighbour joining tree, spatial pattern, *Ursus arctos*

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Introduction

A phylogeographical study using fossils of Eurasian brown bears (*Ursus arctos*) suggested a split of the species into two distinct lineages: one in Europe and one in Asia (Mazza & Rustioni 1994). Analyses of the geographical structure of mitochondrial DNA (mtDNA) also suggested two major lineages, one in the east and one in the west. Furthermore, the western lineage appeared to be organized into two clades that originated from two ancestral refuges (Taberlet & Bouvet 1994). Three potential conservation units were deduced from the phylogeography study: (i) populations of the western lineage that originated from the Iberian refugium, (ii) populations of the western lineage that originated from the Balkan refugium, and (iii) populations of the eastern lineage.

Based on mtDNA analysis of 127 individuals, Taberlet *et al.* (1995) proposed two separate brown bear colonizations of Scandinavia, one from the north by representatives of the eastern lineage and one from the south by represent-

atives of the Iberian clade from the western lineage (Taberlet & Bouvet 1994). The contact zone for the two mtDNA haplotypes was situated between the South (S) and the Middle (M) female concentration areas (see Swenson *et al.* 1998 and Fig. 1 for the definition of M and S female concentration areas). However, because of the maternal mode of inheritance, mtDNA can only provide information concerning female-mediated gene flow, and Taberlet *et al.* (1995) found no evidence of female-mediated gene flow between the S and M areas.

Scandinavian brown bears were almost exterminated by the end of the nineteenth century, but efforts to protect them in Sweden, from eliminating bounties in 1893 to making killed bears State property in 1927, were successful. The near extinction and recovery of bears in Scandinavia has been well documented and thus provides an excellent record of a population bottleneck and subsequent population expansion (Swenson *et al.* 1994, 1995, 1998). The population currently consists of more than 1000 individuals (unpublished results). Using hunting data, four female concentration areas were identified as North North (NN), North South (NS), Middle (M) and South (S) (Swenson

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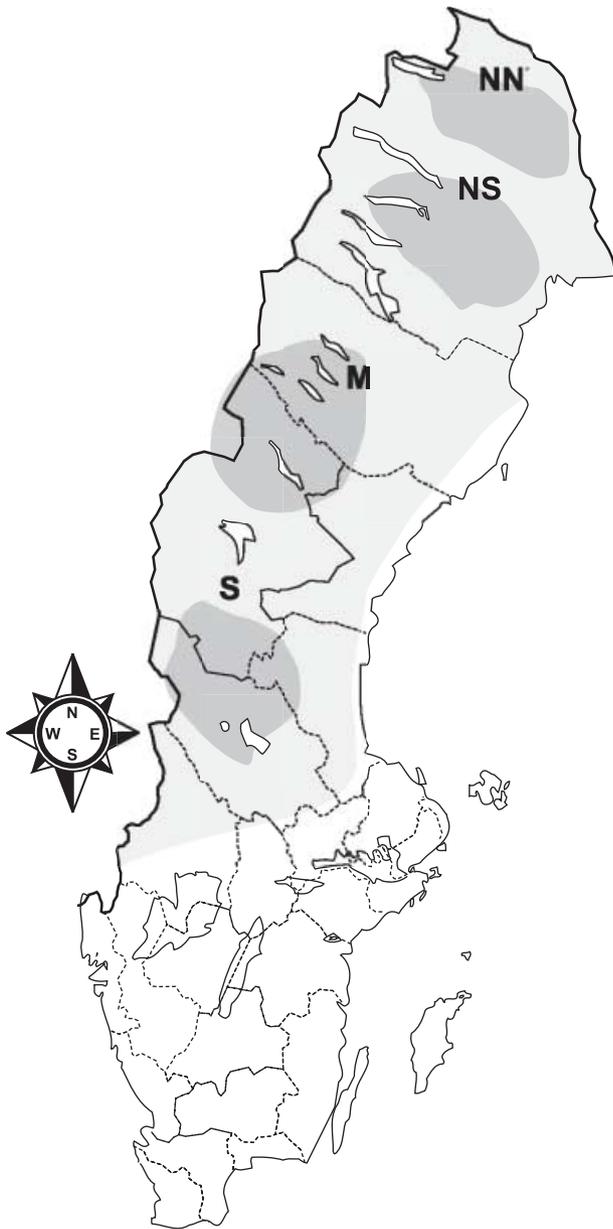


Fig. 1 The primary distribution of female brown bears (dark areas) and all bears (lines) in Scandinavia. The four study subpopulations (N, NS, M, S) are defined as areas of female concentration (adapted from Swenson *et al.* 1998).

et al. 1998; Fig. 1). These areas were thought to represent the surviving relict subpopulations, maintained separately because of the strong philopatry of females. The structuring of this population into subpopulations has been analysed both from mtDNA data (Taberlet *et al.* 1995) and nuclear DNA markers, which give further characterization of the population genetic status (Waits *et al.* 2000). Waits *et al.* (2000) used 19 microsatellite markers collected from 380 bears in this population and assignment tests to quan-

tify and compare the levels of nuclear DNA diversity for the total population and for each of the four predefined subpopulations. They also estimated the degree of genetic differentiation and the level of gene flow among these four subpopulations. Using *F*-statistics, Waits *et al.* (2000) were unable to confirm the existence of the contact zone identified from mtDNA (Taberlet *et al.* 1995).

In this paper, the persistence of the four relict geographical areas was investigated using genetic data only. Using two independent methods [neighbour-joining (NJ) trees and a Bayesian assignment tests], a new subdivision of the population was identified. The conservation biology of Scandinavian brown bears is discussed in the light of these new insights.

Materials and methods

Data

The data consist of 366 brown bears, sampled in Scandinavia from 1986 to 1996 and genotyped for 19 microsatellites. Sampling, amplification and analysis of microsatellites have already been described in Waits *et al.* (2000) and are not reported here. In addition, for this study, the individual geographical locations of the 366 bears were used.

Distance and trees

Shared allele distances (Chakraborty & Jin 1993) were computed between individuals and used to build a NJ tree (Satou & Nei 1987). This method has been shown to be successful in allocating unknown individuals to their population of origin (Estoup *et al.* 1995a,b), and efficient at revealing cryptic structures (Bowcock *et al.* 1994). The Chakraborty–Jin (CJ) distance, $d_{CJ}(i, j)$, between individuals i and j was computed by averaging the number of distinct alleles, $n_l(i, j)$, at locus l over the $m = 19$ different loci:

$$d_{CJ}(i, j) = \sum_{l=1}^m n_l(i, j) / 2m$$

Bootstrap values for NJ trees based on CJ distances were computed over 2000 replications by re-sampling loci within the population. Subpopulations were obtained from the main branches of the NJ trees, and were mapped using the geographical information system ARCVIEW (version 3.1). In addition, the probability $p(k/x)$ that a bear located at location x belonged to subpopulation k was estimated with a nonlinear flexible regression model (neural network) using the Venable & Ripley R package (Ripley 1996).

Pritchard's assignment method

A Bayesian clustering method, STRUCTURE (available at <http://pritch.bsd.uchicago.edu>; Pritchard *et al.* 2000) was

used to examine population structure and assign individuals to inferred subpopulation clusters based upon multilocus genotypes. STRUCTURE calculated (i) the log likelihood value of the data; and (ii) the probability of individual assignment to each subpopulation cluster without prior information on the origin of individuals. A series of tests was conducted considering successively two, three, four and five subpopulations. Each individual was then assigned to one of the subpopulations according to the highest posterior probability and represented on a map using the geographical information system ARCVIEW (version 3.1).

Results

Analysis of spatial structure

In this study, UPGMA (unweighted pair group method with arithmetic average) and NJ trees built from distances based on the stepwise mutation model (Goldstein *et al.* 1995) mixed the S and the M subpopulations (result not shown). This result was therefore not consistent with our present knowledge about the history of *Ursus arctos* in Scandinavia (Taberlet *et al.* 1995) and justified the choice of distances based on the infinite allele model to compute the NJ tree. In the NJ tree the top branches separated the bear population into two groups (Fig. 2), which matched with the mtDNA partition between the south and the other subpopulations (Taberlet *et al.* 1995). The bootstrap values for those two groups were estimated as 0.81 (S) and 0.89 (other subpopulations). The tree clearly allowed the definition of three subpopulations (Figs 2 and 3a). The bootstrap values for the three subtrees were equal to 0.82 for subpopulation S, 0.75 for subpopulation M and 0.88 for subpopulation N. The values were lower than those usually obtained in phylogenetic studies using DNA sequence data, but similar to

those obtained with microsatellites (Estoup *et al.* 1995a,b). The probability that a bear belonged to its most probable group was lower than 70% for the M group, which was the most difficult to characterize. We were unable to characterize the NN subpopulation from the nuclear data and the northern subpopulation (N) combined the NN and NS subpopulations, but this was not in contradiction with Waits *et al.* (2000). Using assignment tests, they found that the majority of wrong assignments was between NN and NS, and implicitly suggested that the data from NN and NS should be combined. A potential fourth subpopulation composed of 34 individuals was detected in the North-West-North area (NWN, Fig. 2a). However, individuals located in this area had an assignment probability to this cluster, NWN, lower than 70% and the bootstrap value for this cluster was lower than that obtained for the other subpopulations (0.62) (Fig. 2).

The spatial structure obtained from STRUCTURE was examined for the 366 bears in the case of two, three, four and five subpopulations. Again, the structure into two subpopulations confirmed the mitochondrial boundary (Taberlet *et al.* 1995). Only results obtained with three and four subpopulations are shown (Fig. 3b).

Both the NJ tree and STRUCTURE gave no particular reason for distinguishing the NN and NS subpopulations since the existence of a fourth subpopulation would consist of the NWN cluster, which is unrelated to any of the four maternal founding subpopulations. Finally, the structure was considered as three subpopulations. Based on mean assignment values of the three subpopulations, 91% of bears were classified in the same way by the two methods (means assignment values were normalized by the subpopulation sizes computed with the NJ tree method).

To evaluate the dispersal of animals within the three subpopulations, the sum of the squared eigenvalues of the covariance matrix was computed in each subpopulation based on spatial coordinates. Sums with small values usually indicate that a population is spatially concentrated around a central point whereas high values reflect that the population is more widely dispersed. These values were calculated for the two clustering methods to compare them. These sums were found to be 1.27×10^{12} (S), 4.95×10^{12} (M) and 10.75×10^{12} (N) for STRUCTURE, and 2.68×10^{12} (S), 2.78×10^{12} (M) and 16.88×10^{12} (N) for the subpopulations obtained with the NJ tree. These sums are of the same magnitude. Nevertheless, the genetic structure identified by STRUCTURE was closer to the geographical S and N clusters and the NJ tree led to the less dispersed M subpopulation.

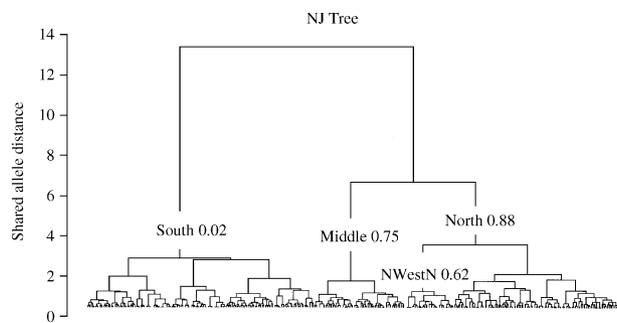


Fig. 2 Neighbour-joining tree computed using the shared allele distance for brown bears in Scandinavia. Bootstrap values, calculated over 2000 replications by resampling loci within the population, were indicated near the branch. Subpopulations were obtained from the main branches of the neighbour-joining trees. Spatial coordinates allow identification of north, middle and south groups.

Discussion

This study investigated the assumption that the Scandinavian brown bear population was structured into four subpopulations, as defined by female concentration areas

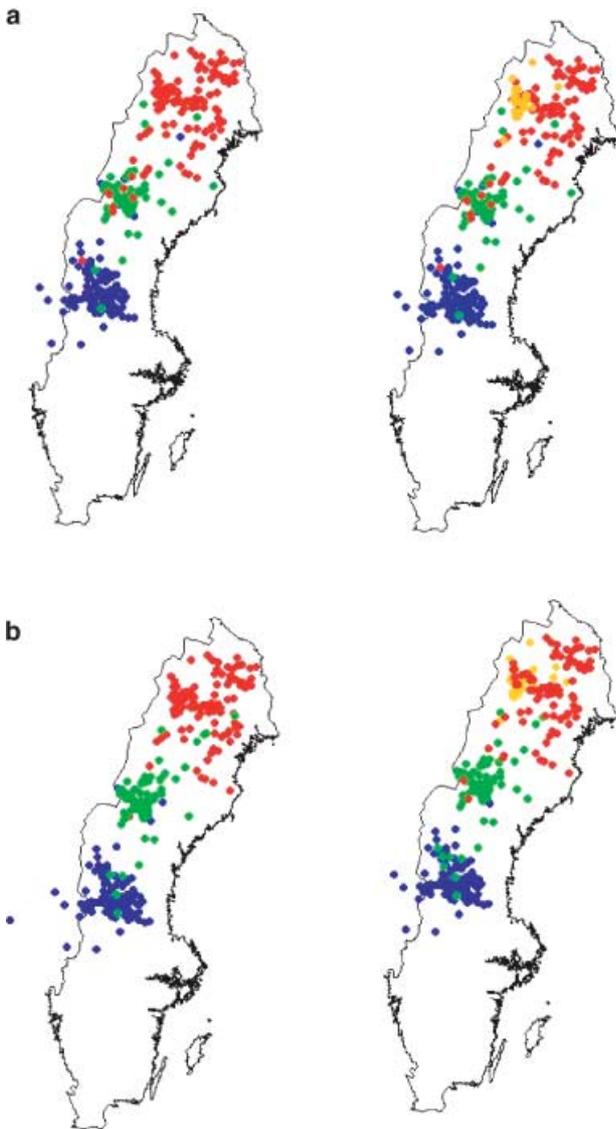


Fig. 3 Three (right) and four (left) subpopulations of Scandinavian brown bears obtained from (a) neighbour-joining tree and (b) assignment methods. Subpopulations were represented using the geographic information system ARCVIEW (version 3.1). Colours are specific to subpopulation.

based on locations of hunter-killed individuals. Using NJ trees and assignment methods, the existence of three instead four subpopulations was documented.

Inference of the Scandinavian brown bear population structure from genetic data

The NJ tree used a phylogenetic analysis based on a specific model of mutation. In our case, the infinite allele model gave the best results, although this is a very rough model of microsatellite evolution. The second method was based

on assignment tests and did not assume any particular model of mutation process. It relied however, on the hypothesis that the subpopulations were under Hardy–Weinberg equilibrium, which was not the case in these data (Waits *et al.* 2000).

Finally, both methods had their own limitations, as the hypotheses on which they depend could not be fully checked (this is generally the case for field data). Nevertheless, the fact that both methods were in agreement with the structure is an indication of the robustness of these methods when their assumptions are violated, although this might require more specific investigations. Because they were obtained from very different methods, the results provided good evidence of the structure of the Scandinavian brown bear population.

How many subpopulations?

Waits *et al.* (2000) recommended that the Scandinavian brown bear population should be viewed as four subpopulations connected by male-mediated gene flow and increasing with distance. Their work was based on the four female concentration areas (NN, NS, M, S) defined from female bears killed by hunters during 1981–93 (Swenson *et al.* 1998). These female concentration areas corresponded to location of bears during the population bottleneck. As a consequence, all statistical analyses conducted by Waits *et al.* (2000) relied on the existence of these four subpopulations.

In contrast, the present study did not assume a pre-defined structure but sought spatial patterns from genetic data only. Our study provided evidence for three subpopulations rather than four. The three ‘genetic’ subpopulations were consistent with the three geographical clusters that were apparent in the data (M, S, N). This suggests that it is important to deduce genetic structure from the genetic data, and not from a presumed structure, even when there seems to be a good reason to do so. Nevertheless, Waits *et al.* (2000) did observe a lower degree of differentiation between subpopulations NN and NS, separated by only 19 km. In fact the apparent fusion of the NN and NS subpopulations in our work could be explained in two ways. Either, (i) these two groups had never existed, and were an artefact of sampling based on hunter-killed bears in the northern area; or (ii) there was a possible admixture of the two groups facilitated by the small distances between them. A small group located in the NWN (about 34 individuals) was identified by both NJ tree and STRUCTURE. This group could be explained by a matriarchal structure. Matriarchal structure is known to occur in Scandinavian brown bears (unpublished results) as suggested in American black bears (*Ursus americanus*) (Rogers 1987), although it was not found in that species by Schenk *et al.* (1998). Moreover, we ran a parentage analysis for the 34 individuals

of this group (unpublished results) and concluded that all those bears were closely related. Actually, one male was responsible for 88% of the descendants in the group (he was the father of 70% of them, grandfather of 12% and great-grandfather for 6% of them, and probably the uncle for 9% of them). A closer look at the NJ tree (Fig. 2) indicated that similar familial structures in the southern subpopulation probably also occur.

Implications for conservation and management

The implications for conservation and management were well discussed in Waits *et al.* (2000). Our results show that there is no particular reason for distinguishing NS and NN subpopulations. As a consequence it led us to define only three (N, M, S) management units, where management units help to define the appropriate geographical scale for current monitoring and management of populations (Moritz 1994). Moreover, more recent data on the location of hunter-killed females show that the NS and NN areas have grown together. As a consequence, from genetic and distributional data, we recommended that the Scandinavian brown bear population be viewed as three subpopulations connected by male-mediated gene flow.

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This study was performed at the laboratory of Ecology Alpine. Stéphanie Manel conduct geographical analysis of genetic variability. Eva Bellemain is PhD student in this laboratory and works on population genetics of the Scandinavian brown bear. Jon Swenson is a Professor of Ecology and Natural Resources Management. Olivier François research interests are in probability and statistics applied to Biology.
