

## INVITED REVIEW

# Genetics and conservation of European brown bears *Ursus arctos*

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## ABSTRACT

1. We review the genetics research that has been conducted on the European brown bear *Ursus arctos*, one of the genetically best-studied mammalian species.
2. The first genetics studies on European brown bears were on phylogeography, as a basis for proposed population augmentations. Two major mitochondrial DNA lineages, western and eastern, and two clades within the western lineage were found. This led to a hypothesis that brown bears had contracted to southern refugia during the last glacial maximum. More recent results suggest that gene flow among brown bears blurred this structure and they survived north of these putative refugia. Thus, today's structure might be a result of population fragmentation caused by humans.
3. The nuclear diversity of European brown bears is similar in range to that in North American bears: low levels occur in the small populations and high levels in the large populations.
4. Many non-invasive genetic methods, developed during research on brown bears, have been used for individual identification, censusing populations, monitoring migration and gene flow, and testing methods that are easier to use in endangered populations and over large areas.
5. Genetics has been used to study many behavioural and population ecological questions that have relevance for the conservation and management of brown bears.
6. The European brown bear has served, and will continue to serve, as a model for the development of methods, analyses and hypotheses in conservation genetics.

**Keywords:** Brown bear, conservation genetics, Europe, phylogeography, *Ursus arctos*

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## INTRODUCTION

The brown bear *Ursus arctos* played an important cultural and mythological role in ancient Europe (Shepard & Sanders 1985, Pentikäinen 2007). However, it was also an important predator on domestic livestock, and state-financed and state-supported campaigns were conducted throughout Europe to eradicate the species (Swenson et al. 1995, Breitenmoser 1998); they were successful in most of Western Europe. However, many populations survived, and now large populations exist in Russia, the Carpathian Mountains, the Dinaric Mountains and the Nordic countries. In addition, some extremely small populations survived in Spain, France, Greece, Italy and Austria (Zedrosser et al. 2001). The bear's endangered status and probably also its charismatic status resulted in projects to augment the size and genetic status of small populations in Austria, France, Italy and Finland (Clark et al. 2002, Saarma & Kojola 2007). Prior to moving bears into these small populations, genetic studies were conducted to determine the most appropriate donor populations (Randi et al. 1994, Taberlet & Bouvet 1994, Kohn et al. 1995). The results of these studies have inspired research in phylogeography, conservation genetics, landscape genetics, wildlife biology and behavioural ecology. Thus, the genetics of the European brown bear is especially well known. Several new methods developed during this research have contributed to the conservation of other brown bear populations and other species. Here, we review the role that genetics has played in the conservation, management and understanding of the ecology of the brown bear in Europe.

## MITOCHONDRIAL DNA PHYLOGEOGRAPHY

The first genetic studies of the phylogeny of European brown bears had a clear conservation biology goal: to sequence a portion of the mtDNA (mitochondrial DNA) control region in order to identify appropriate population units for planning translocations to augment endangered populations (Randi et al. 1994, Taberlet & Bouvet 1994, Kohn et al. 1995). Taberlet and Bouvet (1994) found two major mtDNA lineages, the eastern (bears in northern Scandinavia, Russia, Eastern Europe and part of Romania) and western, differing by more than 7% of the sequence, suggesting that they separated about 0.85 million years ago, based on a human molecular clock. The western lineage was divided into two groups, which suggested two refugia during the Pleistocene glaciations: the Iberian (bears now in Spain, France and southern Scandinavia) and Balkan/Italian refugia (bears now there and in part of Romania). In their study of Holarctic brown bear populations, Leonard et al. (2000) called the western lineage Clade I and the eastern lineage Clade IIIa. A zone of contact between these lineages is sharply delineated in Scandinavia, but the lineages are sympatric in eastern Romania (Kohn et al. 1995, Zachos et al. 2008), which is rare for such divergent clades (Avice 2000). Throughout northern Eurasia, brown bears belong to Clade IIIa (Korsten et al. 2009). These results have been corroborated using nuclear data (Tammeleht et al. 2010) and suggest large-scale gene flow that is unparalleled in other wild mammals studied so far, although part of the population is structured, probably by a combination of demographic history and landscape barriers (Tammeleht et al. 2010).

The documentation of this genetic structure has inspired considerable research in conservation genetics and the role that glacial refugia have played in current haplotype distributions in Europe. Taberlet et al. (1998) hypothesized that there were three general glacial refugia in Europe, the Iberian, Italian and Balkan, based on

intraspecific genetic variation in 10 taxa of plants and animals, including the brown bear, but with little data from Eastern Europe and Fennoscandia. The western lineage was found also in North Africa and Lebanon, where bears are now extinct (Calvignac et al. 2008, 2009), and the eastern lineage is found from Eastern and Northern Europe to the border of Canada, 12000km away, as well as in Japan (Korsten et al. 2009). Korsten et al. (2009) concluded that the founder population of this lineage underwent a severe demographic bottleneck, followed by a sudden demographic expansion from a single glacial refugium throughout most of continental Eurasia following the last glacial maximum, approximately 22000–17000 years BP (before present), and that this was a common pattern for several species of mammals. Saarma et al. (2007) studied the mtDNA structure of bears from Eastern Europe and Finland, and suggested that the western Carpathians, present day Slovakia, probably formed an important glacial refugium for the brown bears in the eastern lineage. However, the eastern lineage (Clade IIIa) has also been found in Alaska, suggesting the existence of a more eastern refugium, such as eastern Beringia, which was a major refugium for arctic plants (Abbott et al. 2000).

Molecular ecological researchers often employ deep fossil calibrations or canonical substitution rates (e.g. 1% per million years for birds and mammals) to estimate divergence times. Saarma et al. (2007) and Ho et al. (2008) addressed the impact of calibration problems when estimating molecular time-scales, using brown bears and other taxa such as birds and whales. Saarma et al. (2007) calibrated the molecular clock to calculate formation times for European brown bear lineages using Beringian brown bear sequences from radiocarbon-dated ancient samples. The brown bear mutation rate was similar to that of the cave bear *Ursus spelaeus*; both were higher than Taberlet and Bouvet (1994) assumed from mutation rates in humans. Thus, the most recent common ancestor of all European bears lived about 175000 years BP and the western and eastern lineages were established about 70000 and 25000 years BP, respectively (Saarma et al. 2007).

The results of ancient mtDNA studies have been used to question the hypothesis that brown bears, and other taxa, followed this proposed model of expansion and contraction to southern refugia in response to Quaternary climatic fluctuations. Valdiosera et al. (2007) found that western European brown bears were markedly more genetically diverse during the last glacial maximum than today, and showed a complex glacial and postglacial phylogeographic structure with low levels of population differentiation that made it difficult to separate haplotypes geographically, suggesting gene flow among populations during the last glacial maximum. Also, Hofreiter et al. (2004) found that two Austrian brown bears from before the last glacial maximum did not match the modern-day phylogeographical structure. Valdiosera et al. (2007) proposed an alternative hypothesis: that brown bears were not restricted to the previously suggested southern refugia during the last glacial maximum, but survived in the cold tundra-steppe of central Europe, which was probably similar to the arctic habitats inhabited by brown bears today in North America. European brown bear subfossils show that brown bears did inhabit tundra-steppes, recolonized central Europe after the glacial maximum fastest of the Holocene carnivores and were restricted to glacial refugia, including the Carpathians, for a maximum of 10000 years (Sommer & Benecke 2005). Another study of ancient mtDNA from brown bears from the Iberian Peninsula suggested that their genetic structure was formed by population bottlenecks and gene flow from other populations

(valdiosera et al. 2008). These authors explain today's phylogeographical structure as a result of strong female philopatry (Støen et al. 2006) and the recent severe reduction in population size and distribution (Zedrosser et al. 2001). This is important for conservation genetics, because the hypothesis suggests that the brown bear lineages in Europe are not evolutionarily significant units, but the result of recent population fragmentation caused by humans (Valdiosera et al. 2007). Understanding the origin of lineages is relevant for many European taxa. Hopefully, future conservation genetic research on brown bears will help us better understand and resolve this issue.

### **NUCLEAR GENETIC DIVERSITY OF POPULATIONS**

European brown bear populations vary greatly in size, from large populations in and near Russia, the Carpathian Mountains, the Dinaric-Pindos area on and north of the Balkan Peninsula, and Scandinavia, to extremely small and isolated populations in the Cantabrian Mountains of northern Spain, the Pyrenees on the French-Spanish-Andorran border, and in the Apennine Mountains and Alps of Italy (Zedrosser et al. 2001). Nuclear DNA diversity has been studied in several of these populations, using microsatellite markers. Although researchers used different sets of microsatellite loci, reducing the comparability of the values, the results show a clear dichotomy, in which higher expected heterozygosity and allelic diversity occurs in the largest populations and low heterozygosity occurs in the smallest populations (Table 1). Heterozygosity in the large populations is similar to that found in large North American populations, and heterozygosity in the small populations is lower than in most North American populations, except those on some isolated Alaskan islands (Paetkau et al. 1998). The small populations may suffer from genetic drift and inbreeding (Taberlet et al. 1997, Lorenzini et al. 2004, Karamanlidis 2009, Pérez et al. 2009). Inbreeding depression has not been documented in wild brown bear populations, but captive brown bears in Nordic zoos show a reduction in litter size and increased incidence of albinism due to inbreeding (Laikre et al. 1996). Low genetic diversity is one of the many factors endangering small European brown bear populations. Brown bear populations in the Pyrenees and Alpine Italy were augmented with Slovenian bears to increase their numbers and genetic diversity (Clark et al. 2002). Small, recently reintroduced populations have relatively high genetic diversity (Table 1).

### **NON-INVASIVE GENETICS IN BEAR CONSERVATION**

Bears are difficult to monitor because they are secretive, solitary and occur at low densities. Non-invasive genetic methods are useful for monitoring, allowing an increased understanding of population ecology, behaviour and genetic dynamics, and helping to design conservation strategies. Non-invasive genetic sampling (NGS) by the collection and analysis of, e.g. faeces or hairs, aims to minimize human impact when gathering biological information. This method is especially relevant for small, endangered populations to address questions that could not be answered using conventional methods (Gervasi et al. 2008, Pérez et al. 2009). Even in larger, healthy bear populations, NGS has many advantages over traditional genetic sampling and has provided valuable biological information (Kohn & Wayne 1997, Bellemain et al. 2005). A much larger proportion of the population can potentially be surveyed, usually at a lower cost than by using traditional field methods (Solberg et al. 2006).

An experimental study of the small bear population in the Italian Alps (De Barba et al. 2010b) showed that the optimal sampling strategy combined systematic hair

**Table 1.** Summary of the nuclear genetic diversity of several European brown bear populations, based on microsatellite loci

Population (area sampled)	N samples	N loci	H <sub>e</sub>	Alleles per locus	Population size	Source (G = genetics, P = population size)
Russia; Kirov Oblast	13	17	0.83	8.1	4900	GP <sup>4</sup>
Finland; North	26	17	0.82	9.2	920 <sup>1</sup>	GP <sup>4</sup>
Romania	16	9	0.81	7.8	6600	G <sup>5</sup> , P <sup>6</sup>
Western European Russia	32	17	0.80	9.1	5900	GP <sup>3</sup>
Russia; Arkhangelsk Oblast	16	17	0.79	7.6	8700	GP <sup>3</sup>
Finland; South	44	17	0.77	8.5	920 <sup>1</sup>	GP <sup>4</sup>
Albania, Greece, Macedonia	49 (NI)	6	0.76	6.3	250 <sup>2</sup>	G <sup>5</sup> , P <sup>7</sup>
Italian Alps	2781 (NI) and 12	8 & 10	0.74	4.5	>27 (introduced)	GP <sup>8</sup>
Sweden	380	19	0.71	6.8	3300	G <sup>9</sup> , P <sup>10</sup>
Estonia	62	17	0.68	7.4	700 <sup>3</sup>	GP <sup>4</sup>
Austria	379 (NI)	8	0.61	2.9	5–8 detected (introduced)	GP <sup>11</sup>
Spain; Western Cantabrians	39 (NI)	18	0.45	3.3	119 detected	GP <sup>12</sup>
Italy; Appenines	30 (NI)	12	0.44	2.2	45	G <sup>13</sup> , P <sup>14</sup>
France; Western Pyrenees	5 (NI)	6	0.40	1.5	6	GP <sup>15</sup>
Spain; Eastern Cantabrians	71 (NI)	18	0.25	1.6	21 detected	GP <sup>16</sup>

The studies are listed according to expected heterozygosity (H<sub>e</sub>). N = sample size, NI = non-invasive samples. Population estimates made using NGS (non-invasive genetic sampling) are shown in bold.

<sup>1</sup>The population estimate is for all of Finland.

<sup>2</sup>This population is continuous with the bears to the north in the Alps-Dinara-Pindos population (Zedrosser et al. 2001).

<sup>3</sup>This population is continuous with the bears in Russia.

<sup>4</sup>Tammela et al. 2010.

<sup>5</sup>Zachos et al. 2008.

<sup>6</sup>Karamanlidis et al. 2010.

<sup>7</sup>Zedrosser et al. 2001.

<sup>8</sup>De Barba et al. 2010a.

<sup>9</sup>Waits et al. 2000.

<sup>10</sup>Kindberg 2010.

<sup>11</sup>Kruckenhauser et al. 2009.

<sup>12</sup>Pérez et al. 2010.

<sup>13</sup>Lorenzini et al. 2004.

<sup>14</sup>Gervasi et al. 2008.

<sup>15</sup>Taberlet et al. 1997.

<sup>16</sup>Pérez et al. 2009.

trapping and opportunistic sampling of faeces and hairs, as the pooled data allowed increased bear identification and detection, and were suitable for population size estimation. Hair samples can be collected at marking or rubbing sites, by using barbed-wire around trees or power poles (Karamanlidis et al. 2010), or baits can be used to attract bears.

The limited amount and often degraded nature of DNA in non-invasive genetic samples causes technical challenges such as extracting only host DNA from faeces (i.e. avoiding prey DNA) and avoiding genotyping errors (Taberlet et al. 1999). Brown bear research has provided field precautions and laboratory methods to deal with these challenges (Bellemain & Taberlet 2004, Bonin et al. 2004, Piggott et al. 2004, Murphy et al. 2007, De Barba & Waits 2009). It is now common to calculate the genotyping error rate to indicate data reliability, or to associate a quality index to each genotype (Miquel et al. 2006).

NGS is used for individual identification in forensic studies (Eiken et al. 2009), identifying problem bears or monitoring individuals in small populations. For instance, the Austrian population has been monitored since 2000 using >1000 hair and faecal samples in a 3000-km<sup>2</sup> area, and only five to eight individuals were detected annually (Kruckenhauser et al. 2009). All identified individuals were descendants of the founder individuals, indicating no effective immigration. Sub-adult bear migrations from the core area, increased natural mortality and illegal hunting were proposed as potential explanations for the absence of bear occurrences in this region.

Non-invasive samples can be used to estimate population size reliably, which is important for the management and conservation of brown bears. Available estimators include capture-mark-recapture methods (Seber 1982) and rarefaction analysis (Kohn et al. 1999). A large number of brown bear censuses have been carried out in Europe using NGS (Table 1). Brown bear densities determined from DNA-based censuses from faeces collections have also been used to verify the accuracy of relative density estimates based on effort-corrected observations by moose *Alces alces* hunters in Sweden (Kindberg et al. 2009).

NGS can also document migration, such as between the two Cantabrian populations (Pérez et al. 2010). Of 76 genetically identified individuals, three males sampled in the eastern subpopulation were assigned to the western subpopulation, suggesting connectivity and potential gene flow between these subpopulations (Pérez et al. 2010). One of them was even sampled repeatedly along his route to the neighbouring subpopulation. Such information may help identify natural corridors that could be improved through restoration management.

Trans-boundary bears, i.e. bears with a home range of over two or more management jurisdictions, can cause a number of conservation and management challenges. Double counting can inflate the total population estimate, and management decisions that do not acknowledge the trans-boundary status of a population can be flawed. Monitoring those populations using NGS allows researchers to assess the extent of these potential problems. However, this requires that all of the relevant jurisdictions cooperate closely and standardize their choice of genetic markers, which may prove difficult. The management agencies in Norway and Sweden have begun to standardize their genetic data sets for brown bears (Aarnes et al. 2009), cooperative genetic research on brown bears recently has been conducted over vast areas of European Russia and adjacent countries (Tammeleht et al. 2010), and preliminary

efforts have been started to agree on common guidelines for genetic studies of brown bears in south-eastern Europe (Karamanlidis 2009). Nevertheless, more trans-boundary cooperation is needed to manage most European brown bear populations properly. We encourage the appropriate management agencies and research groups to begin such cooperation as soon as possible.

NGS can also be used to monitor reintroduced populations, allowing documentation of their demography, ecology and genetics and thus, a better understanding of the processes influencing their viability, evolution and successful restoration. A recent study on the demographic and geographical expansion and changes in genetic composition of the reintroduced brown bear population in northern Italy was based on >2700 hair and faecal samples, and 12 samples from captured or dead bears (De Barba et al. 2010a). The data showed that the population increased rapidly, from nine founders in 1999–2002 to >27 individuals in 2008, and that most bears were distributed in the region of the translocation site. In spite of a relatively high genetic diversity, De Barba et al. (2010a) documented a small effective population size ( $N_e = 3.03$ ), an elevated mortality rate and a lack of immigration, raising concerns about the conservation of this population. Such data are critical for implementing future conservation plans for reintroduced brown bear populations.

In summary, NGS has aided the conservation of European brown bears in many ways. However, due to inherent constraints, the range of questions that can be answered is limited. For instance, individual attributes, such as exact age, body condition or reproductive status, cannot be determined from non-invasive genetic samples alone. Therefore, whenever possible, a combination of non-invasive genetics data and traditional intensive monitoring data should be used to provide a wider understanding of the species' conservation biology.

#### **GENETICS AS A METHOD TO STUDY CONSERVATION-ORIENTED BIOLOGY**

Humans influence bear populations in many ways, particularly through hunting and effectively reducing the amount of habitat available to bears (Nellemann et al. 2007, Bischof et al. 2009). Genetic methods have been used to document some effects of human-induced mortality in European brown bears that are relevant for conservation biology. For example, paternity analyses, based on 18 microsatellite loci, showed that among the males that were close enough to be mating partners, female brown bears in Sweden selected males to father their young that were the largest, most heterozygous and least inbred, and also the geographically closest (Bellemain et al. 2006b). In Sweden, of two studied populations, the southern was hunted and had a rather normal age and sex ratio, whereas the northern was both hunted and suffered a higher rate of illegal killing, resulting in a relative lack of older males (Swenson et al. 2001). Legal hunters show little selection for age or sex in Sweden (Bischof et al. 2008). Zedrosser et al. (2007a) found that older and larger males were more successful fathers, as determined by the minimum known number of offspring surviving up to the age of 1 year; however, in the northern area, with fewer older males, size was more important in determining paternal success than age. There, with few males  $\geq 9$  years old, females were apparently forced to mate with the larger of the available young males (3–4 years old). Thus, high human-caused mortality of older males may have forced the females to mate with younger males than they otherwise would have selected. Although we do not know the genetic implications

of this, hunting that disrupts the mating system and counteracts sexual selection may have evolutionary consequences (Festa-Bianchet 2003).

Swenson et al. (1997) proposed that killing older, dominant male bears could promote high cub mortality through sexually selected infanticide (SSI). Prerequisites of SSI are that: (i) infanticide shortens the time to the mother's next oestrus; (ii) the perpetrator is not the father of the infants he kills; and (iii) perpetrators have a higher probability of siring the female's next young (Hrdy & Hausfater 1984). The second requirement has been supported by paternity analyses in both Sweden and Spain (Bellemain et al. 2006a, Fernández-Gil et al. 2010), and the third requirement has been supported by genetic results from Sweden (Bellemain et al. 2006a). The finding that the killing of adult males can lead to increased juvenile mortality has important ramifications for the conservation of small populations of brown bears and other species showing SSI, as well as for predicting the impact of hunting on populations of these species (Swenson 2003).

Støen et al. (2005) documented spatial structure in the relationships of female brown bears in Sweden: related females apparently defend common 'territories' against unrelated females, and home range overlap is positively correlated with relatedness. The implications of this for conservation biology are unclear, but females that disperse from their matriline have an earlier age of primiparity than those who are philopatric (Zedrosser et al. 2007b).

Changes in observed and expected heterozygotes and heterozygote excess have been used to estimate effective immigration in brown bear populations (Tallmon et al. 2004): effective immigration into the southernmost subpopulation of brown bears in Sweden was very low, and the genetic effective population size was estimated to be 6–14% of the census population size.

## EUROPEAN BROWN BEARS AS A MODEL IN CONSERVATION GENETICS

Research on European brown bear genetics has had not only consequences for the conservation of this species, but also a strong general impact on conservation genetics. First, NGS was initially developed using European brown bears. Taberlet and Bouvet (1992a, 1992b) were the first to use hair samples to analyze the genetics of an endangered species. Höss et al. (1992) subsequently demonstrated that faeces represent a valuable source of DNA, both for studying bear genetics and for analysing the diet of bears. Taberlet et al. (1996) were able to develop a new methodology for producing reliable genotypes using hair and faeces, with the brown bear as a model. This represents the standard for genotyping in limiting conditions today, and is used extensively in almost all non-invasive studies of birds and mammals.

Second, the three early phylogeographic studies on European brown bears (Randi et al. 1994, Taberlet & Bouvet 1994, Kohn et al. 1995) were among the first phylogeographic studies in Europe. They clearly stimulated this type of research both for plants and animals, and resulted in the brown bear being called a 'paradigm species' (Hewitt 2004) and 'an important model species' (Valdiosera et al. 2007).

Third, the early availability of a large microsatellite dataset for Scandinavian brown bears (19 loci, 380 individuals; Waits et al. 2000) contributed to the estimation of the probability that two randomly chosen genotypes in a natural population are identical (Waits et al. 2001). Previously, theoretical estimates of this probability of identity ( $P_{ID}$ ) were based on allele frequency data and Hardy–Weinberg expectation for randomly mating individuals. It appeared that the theoretical  $P_{ID}$  were consis-

tently lower than the observed ones in natural populations, and could differ by as much as three orders of magnitude. As a consequence, a genotyping system based on the theoretical  $P_{ID}$  would not be able to distinguish all individuals. Waits et al. (2001) also proposed to estimate the probability of identity among sibs ( $P_{SIBS}$ ) as a conservative upper bound for the probability of observing identical multi-locus genotypes between two individuals sampled from a natural population. The computer software PARENTE was developed to conduct analyses of maternity and paternity in this dataset (Cercueil et al. 2003).

Finally, the large microsatellite dataset of geolocalized samples continuously distributed over Sweden (18 loci, 964 individual bears; Manel et al. 2004) stimulated the development of new individual-centred concepts and methodologies, replacing the classical population-centred approach that actually was not applicable to Scandinavian bears. The concept of landscape genetics was launched (Manel et al. 2004), as well as different methodologies for assessing population structure without any *a priori* information about the limits of the populations (Francois et al. 2006, Manel et al. 2007, Jombart et al. 2008), identifying the geographical location of genetic discontinuities, and determining whether an individual is a resident or a putative migrant (Manel et al. 2007).

Genetic studies on European brown bears will certainly continue to stimulate the field of conservation genetics in the future. The excellent knowledge of bear genetics in Europe, and especially of their biology in Scandinavia (references in this review), coupled with both the recent development of next-generation sequencing (Shendure & Ji 2008), and the improvements in global positioning system and physiological logger technology, will probably lead to a better understanding of the mechanisms underlying the impressive diversity of adaptive strategies exhibited by European brown bear populations.

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