Taberlet, P., J. E. Swenson, F. Sandegren, and A. Bjärvall. 1995. Conservation genetics of the brown bear (Ursus arctos): localization of a contact zone between two highly divrgent mitochondrial DNA lineages in Scandinavia. Conserv. Biol. 9:1255-1261.

Localization of a Contact Zone between Two Highly Divergent Mitochondrial DNA Lineages of the Brown Bear *Ursus arctos* in Scandinavia

PIERRE TABERLET, * JON E. SWENSON,† FINN SANDEGREN,‡
AND ANDERS BJÄRVALL§

*Laboratoire de Biologie des Populations d'Altitude, CNRS URA 1946, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France
†Norwegian Institute for Nature Research, Tungasletta 2, N-7005 Trondheim, Norway
‡Swedish Hunters' Association, Box 7002, S-750 07 Upssala, Sweden
§Swedish Environmental Protection Agency, S-106 48 Stockholm, Sweden

---

Abstract: *In Europe the brown bear (*Ursus arctos*) is represented by two different mitochondrial DNA (mtDNA) lineages, which probably diverged about 0.85 million years ago. Scandinavia has been colonized by representatives of both lineages, from the north (eastern lineage) and from the south (western lineage), and now bears occur primarily in four main regions called female concentration areas. For management purposes the localization of the contact zone between these two genotypes is important. Using hairs as a source of DNA, 127 individual brown bears from throughout the Scandinavian populations were assayed for lineage assignment. A part of the mtDNA control region was amplified via the polymerase chain reaction, and the product was either sequenced (14 individuals) or digested with two diagnostic restriction endonucleases (113 individuals). Fifty-six and 71 bears were assigned to the western and eastern lineages, respectively. The geographic distribution of the two genotypes allowed precise localization of the contact zone. Only two males from each lineage had crossed the border between the two lineages. We used dispersal data from bears radiomarked as yearlings to determine whether potential mtDNA introgressions agreed with the dispersal behavior of bears. The males in the "wrong" areas were all within the 95th-percentile dispersal distance from the "correct" area. Females were more philopatric than males, and none were found in the wrong areas. The two female concentration areas flanking the contact zone were 134 km apart. Thus, radiotelemetry results on dispersal distances could explain the occurrence of the males in the wrong genetic area. In the absence of information concerning possible male-mediated gene flow, a conservative management approach would be to consider the southern and the three northern female concentration areas as two distinct conservation units.*

Localización de una zona de contacto entre dos linajes de ADN mitocondrial muy divergentes del oso pardo *Ursus arctos* en Escandinavia

Resumen: *En Europa, el oso pardo (*Ursus arctos*) está representado por dos linajes de ADN mitocondrial (mtADN) diferentes, que problablemente divergieron hace unos 0.85 millones de años. Escandinavia ha sido colonizada por representantes de ambos linajes, uno del norte (linaje oriental) y uno del sur (linaje occidental), y en la actualidad, los osos se encuentran en cuatro regiones principales denominadas áreas de concentración de hembras. La localización de la zona de contacto entre estos dos genotipos es importante para el manejo. Se analizaron 127 osos pardos de problaciones a lo largo de Escandinavia para determinar su linaje usando pelos como fuente de ADN. Se amplificó una parte de la región control del mtADN usando la cadena de reacción de polimerasas y el producto fue secuenciado (14 individuos) o digerido con dos endonucleasas de restricción diagnóstica (113 individuos). Se asignaron 56 osos al linaje occidental y 71 al linaje oriental.*

Introduction

The brown bear (*Ursus arctos*) has a Holarctic distribution, from Spain to the United States. Its present range has been dramatically reduced, however, since the mid-1800s by habitat loss caused by human activity and by excessive hunting in the past (Servheen 1990). In western Europe, this species now exhibits an extremely patchy geographic distribution (Sørensen 1990), and several populations face the threat of extinction in the near future. One possible solution to avoid these extinctions is to reinforce the small isolates with bears from larger and non-endangered populations. Because the genetic makeup of conspecific populations may be structured geographically (Avise 1992), identification of the potential units of conservation (or evolutionary significant units) (Ryder 1986; Woodruff 1989) should precede any interpopulation transfer of individuals. Such conservation units correspond to sets of populations that have a common recent history and among which population transfers could be performed.

To define these potential conservation units, a phylogeographic study has been carried out. A part of the mitochondrial DNA (mtDNA) control region was sequenced for representatives of the different European populations of the brown bear (Taberlet & Bouvet 1994). Two different mtDNA lineages (eastern and western), differing by more than 7% in their control-region sequences, were found. Based on the evolutionary rate of the homologous human sequence, it has been assumed that the two major lineages could have diverged about 0.85 million years ago, probably during the earlier Quaternary cold periods (Taberlet & Bouvet 1994). In an independent phylogeographic study, a complete revaluation of the fossils of Eurasian bears also suggested a split of *Ursus arctos* into two distinct lineages, one in Europe and one in Asia (Mazza & Rustioni 1994). Furthermore, the western lineage appears to be organized into two clades that originated from two different ancestral refugia (Taberlet & Bouvet 1994). Three potential conservation units have been deduced from the phylogeographic study: (1) populations of the western lineage that originated from the Iberian refugium, (2) populations of the western lineage that originated from the Balkan refugium, and (3) populations of the eastern lineage (Taberlet & Bouvet 1994). Populations that stem from the Iberian refugium are the most endangered. They are represented only by bears living in the Cantabric Mountains in Spain (about 50 individuals divided into two isolates), in the Pyrenees in France (5 to 8 individuals), and in southern Scandinavia. Indeed, Scandinavia has been colonized from the north by representatives of the eastern lineage and from the south by representatives of the western lineage (Fig. 1). This pattern of colonization in Scandinavia, both from the north and from the south, is well documented for some other mammalian species: *Sorex araneus* (Fredga & Nawrin 1977), *Microtus agrestis* (Fredga & Jaarola 1989), and *Clethrionomys glareolus* (Tegelström 1987).

The population size of bears in Norway and Sweden was about 130 in the populations that subsequently survived at the low point in numbers around 1930 (Swenson et al. 1995). Due to a successful management policy, the population is now large and expanding. The Scandinavian brown bear population currently numbers almost 700, 98% of which are in Sweden (Swenson et al. 1995). Almost all females are confined to four geographically separated zones called female concentration areas (Fig. 1) (Swenson et al. 1994*a*).

The precise location of the contact zone between the divergent eastern and western mtDNA lineages in Scandinavia would allow us (1) to estimate the number of bears belonging to the western lineage and (2) to evaluate the possibility of reinforcing the endangered Pyrenean population with genetically similar bears from Scandinavia.

We documented demarcation of the geographic distribution of each mtDNA lineage of brown bear in Scandi-

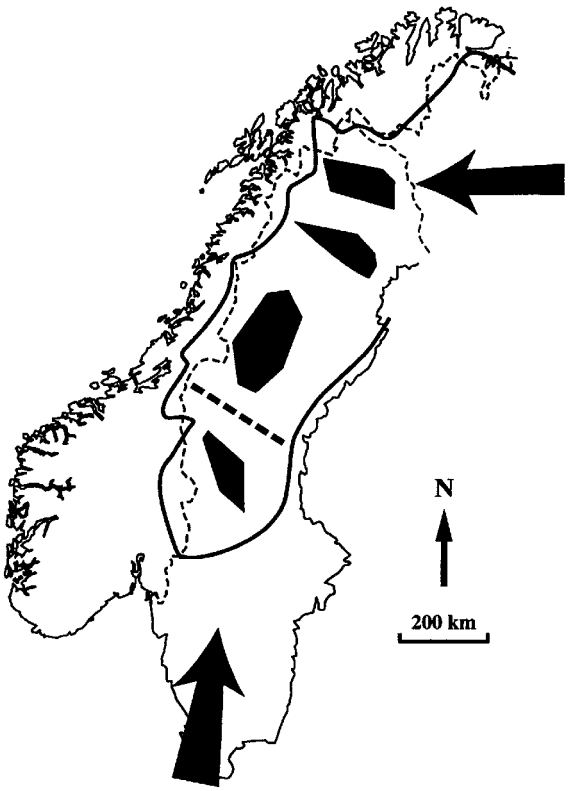

Figure 1. Present geographic distribution of bears in Scandinavia. The two arrows indicate the colonization routes for the eastern lineage (north) and for the western lineage (south). Dark polygons represent female concentration areas, and the heavy dashed line indicates the location of the contact zone between the two mtDNA lineages.

navia, based on extensive sampling, corresponding to about 20% of the actual population size. Both data on mtDNA and radiotracking of bears during dispersal have been used to examine mixing along the contact zone.

Materials and Methods

Genetic Analysis

The use of hairs as a source of DNA (Taberlet & Bouvet 1991, 1992) greatly facilitates the sampling procedure. Hair samples were obtained from 106 bears shot legally during the Swedish bear hunting season since 1990, when hunters were required to provide a hair sample from shot bears. An additional 22 samples were collected from bears captured for radiotracking purposes since 1989. The hair samples were preserved (dry) in paper envelopes until the analyses were performed.

Lineage assignment was based on two diagnostic restriction sites. These two sites were deduced from the sequences of a portion of the mtDNA control region for seven individuals from the eastern lineage and for seven individuals from the western lineage (sequence assession numbers in EMBL database X75874 and X75868 respectively).

Total DNA was extracted from the root of hairs via a protocol widely used in forensic laboratories (Walsh et al. 1991). This method involves the use of Chelex 100 resin (Biorad) at 5% (w/v) for the preparation of DNA before amplification. The presence of dry cells was first confirmed by microscopy, and the root part (2-3 mm) was cut and put into an Eppendorf tube containing a mixture of Chelex 100 and water (200 µl). The tube was incubated with intermittent shaking at 56°C for 6-8 hours, thoroughly vortexed, left in boiling water for 8 minutes, and then centrifuged at 12,000 g.

Five microliters of this DNA extract were used subsequently as a template for the polymerase chain reaction (PCR; Mullis & Faloona 1987; Saiki et al. 1988). The primers used to amplify a portion of the mtDNA control region were 5′-GCCCCATGCATATAAGCATG-3′ (forward) and 5′-GGAGCGAGAAGAGGTACACGT-3′ (reverse).

The amplification reaction was performed in a final volume of 10 µl (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 50 µM each dNTP, 1.5 mM $MgCl_2$, 0.2 units of Amplitaq [Perkin-Elmer/Cetus], 1 µM each primer) and consisted of 40-45 cycles of amplification (denaturation at 93°C for 1 minute, annealing at 50°C for 1 minute, extension at 72°C for 1 minute with a Perkin Elmer/Cetus DNA thermal cycler 480).

At the end of the amplification 2.5 µl of the appropriate digestion buffer and about 1 unit of restriction endonuclease was added to the same tube. The digestion was then carried out for 2-3 hours. An aliquot of 5 µl from each digest was run on a 4% agarose gel (1% SeaKem, 3% NuSieve, FMC) to visualize the DNA fragments by ethidium bromide staining.

For each individual two PCR reactions were performed, followed by digestions with either AluI or BclI. These two restriction enzymes produce typical patterns for each lineage (Fig. 2). The digestion of the PCR product by AluI gives two bands (114bp, 62bp) for the western lineage and three bands (64bp, 62bp, 50bp) for the eastern lineage. The digestion by BclI gives two bands (116bp, 60bp) for the eastern lineage and a fragment corresponding to the entire amplicon (176bp) for the western lineage (no restriction site).

Field Data on Juvenile Dispersal

Juvenile dispersal distances were obtained from 10 male and 11 female bears radiomarked as yearlings and followed until three or four years of age. We measured the distance from the site of marking to the farthest re-

```
                          10        20        30        40        50        60        70        80
Western lineage  gccccatgcatataagcatgTACATATTGTGCTTGGTCTTACATGAGGACTTACGTTCCAAAAGTTTGTTTCAGGCGTATAGTCTGTA
Eastern lineage  gccccatgcatataagcatgTACATATTACGCTTGGTCTTACATAAGGACTTACGTTCCGAAAGCTTATTTCAGGCGTATGGTCTGTA
                                                                               AluI

                 90        100       110       120       130       140       150       160       170
Western lineage  AGCATGTATTTCACTTAGTCCGGGAGCTTAGTCACCAGGCCTCGAGAAACCAGCAATCCTTGCGAGTacgtgtacctcttctcgctcc
Eastern lineage  AGCATGTATTTCACTTGATCCGGGAGCTTAGTCACCAGGCCTCGAGAAACCAGCAACCCTTGCGAGTacgtgtacctcttctcgctcc
                                 BclI    AluI
```

Figure 2. Nucleotide sequence of the PCR products (part of the left domain of the mtDNA control region) obtained for the western and the eastern lineages of the brown bear in Scandinavia. The parts corresponding to the primers are indicated in lower case. The restriction sites for AluI and BclI restriction endonucleases are underlined.

corded location from the marking site as a three- to four-year-old, or, in the case of a three-year-old male that had lost his transmitter, to the location of kill by a hunter. In our analysis, we used the 95th-percentile dispersal distance. Males generally disperse as two-year-olds and females as three-year-olds, if they disperse (Swenson et al. 1994*b*). Our estimates of dispersal distances are conservative, because maximum cumulative home ranges are reached at about four years of age in females and probably beyond that in males (Swenson et al. 1994*b*). At this time, we have too few data from bears marked as yearlings that are five years or older to analyze. Our purpose, however, was to test if the genetic results could be due to dispersal, not to fully document dispersal distances in this population.

Results

Among the 114 individual brown bears sampled, we were able to extract DNA from 113. In the single unsuccessful case, no DNA was available because of the absence of hair roots in the sample.

Figure 3 illustrates typical results obtained after the restriction enzyme digestion. The lineage assignment was unambiguous for all individuals, and the two restriction sites were always associated in the same way as in the 14 individuals analyzed previously by amplification and direct sequencing (Taberlet & Bouvet 1994). Forty-nine and 64 individuals were assigned to the western and eastern lineages, respectively.

Including the 14 individuals for which sequence data were available, a total of 127 individual brown bears of known lineage (56 of the western and 71 of the eastern lineage) were located on the map of Sweden (Fig. 4). From this map, the contact zone can be localized without ambiguity. Only four male individuals, two of each lineage, were found on the wrong side of the contact zone and therefore do not fit in with the general pattern of geographic distributions of the two mtDNA genotypes.

Because mtDNA is maternally inherited, juvenile dispersal is one mechanism that can explain the presence of male bears of the wrong lineage. To evaluate whether the four bears of the wrong lineage could have been dispersing individuals, we compared the assigned lineages of bears within a radius corresponding to the 95th-percentile dispersal distance from the nearest portion of the other lineage's female concentration area with those outside this radius. Up to 1991, 96% of all female bears were shot within these concentration areas (Swenson et al. 1994*a*).

Mean maximum dispersal distance for males marked as yearlings was 158 km, and the 95th-percentile dispersal distance was 195 km ($n = 10$). Females were much more philopatric; mean maximum dispersal distance was 29 km, and the 95th-percentile dispersal distance was 34 km ($n = 11$). Dispersal data from a northern and a southern study area in Scandinavia (Swenson et al. 1994*b*) were combined because they were not statistically different (males, Mann-Whitney $U = 19$, df = 9 $p = 0.14$; females, $U = 23$, df = 10, $p = 0.09$).

The closest distance between the two female concentration areas on either side of the contact zone was 134

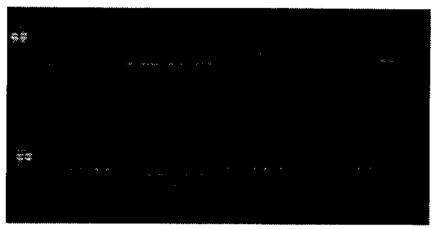

Figure 3. Agarose gel showing how the lineage assignment was inferred: lane 1, molecular weight marker VIII (Boehringer, Mannheim); lanes 2 to 13, PCR products digested with AluI (top) and BclI (below) corresponding to bears of unknown lineage; lanes 14 and 15, PCR products digested with AluI (top) and BclI (below) corresponding to a control individual from the western and the eastern lineage respectively; lane 16, PCR negative control (amplification without template). The comparison with the control individuals from lanes 14 and 15 allows assignment of the western lineage to the individuals of lanes 3, 4, 9, 10, 11, 12, and 13, and the eastern lineage to the individuals of lanes 2, 5, 6, 7, and 8.

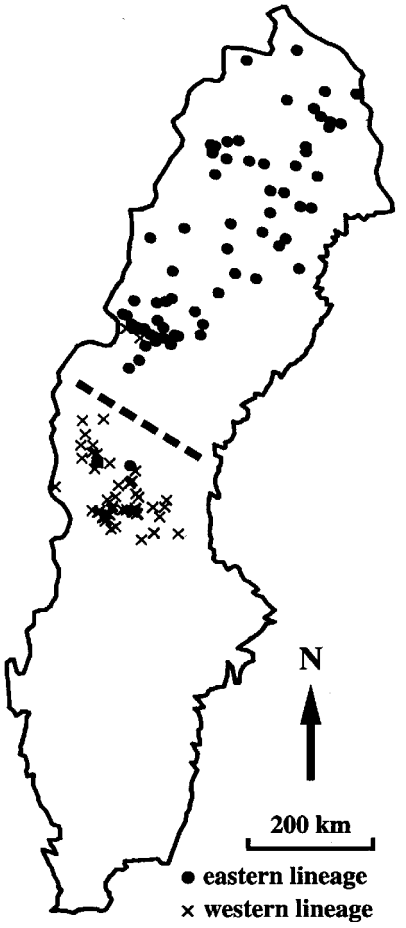

Figure 4. Geographic distributions of the western and eastern lineages of brown bear in Sweden. The dashed line indicates the location of the contact zone between these two mtDNA lineages.

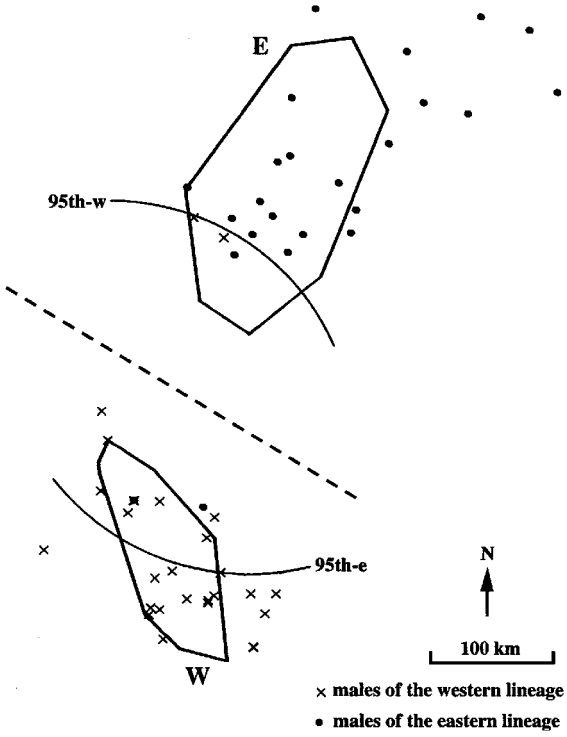

Figure 5. Location of male brown bears of the western and eastern lineages in relation to 95th-percentile dispersal distance from their respective female concentration areas on either side of the contact zone (dotted line). E and W identify female concentration areas corresponding to the eastern and western lineages, respectively, and 95th-e and 95th-w identify limits of the 95th-percentile dispersal distance of males from the eastern and western female concentration areas, respectively.

km. This was more than four times the 95th-percentile dispersal distance for females, and no females of the 53 examined were in the wrong area. In both areas, the wrong males were within 95th-percentile dispersal distance from the correct area (Fig. 5). In the southernmost area, 2 of 13 males within this distance were wrong, compared with 0 of 16 beyond it. In the other areas 2 of 3 males within 95th-percentile dispersal distance from the southern area were of western lineage, compared with 0 of 37 beyond it.

Discussion

The mtDNA lineages of 127 brown bear individuals of known geographic origin were identified, either by amplification and direct sequencing (14 samples) or by restriction analysis of PCR products (113 samples). The localization of the contact zone between two highly divergent mtDNA lineages was deduced from these data. The current population size of brown bears in Scandinavia has been estimated recently to be almost 700 individuals (Swenson et al. 1995). Our sample corresponds to about one fifth of the current population size and therefore gives a reliable picture of the geographic distribution of the two genotypes. A large number of samples were analyzed because of the use of hair roots preserved dry in paper envelopes as a source of DNA. Such a sampling procedure greatly facilitates field work and demonstrates that the recent development of molecular techniques in relation to the polymerase chain reaction also can greatly improve studies in conservation genetics.

The contact zone between the two mtDNA of brown bears bisects the province of Jämtland. Bears in the

southern female concentration area in southcentral Sweden are almost all from the western lineage and probably originated from an Iberian refugium. The three more northern female concentration areas correspond to the eastern lineage and originated from Karelia (Russia), where the same genotypes have been found (Taberlet & Bouvet 1994). The contact zone is localized in a low-density area between the two female core areas. Such a location is quite common for a hybrid zone. Indeed, when two different genotypes meet, the location of the hybrid zone does not move considerably over time and is usually stabilized in low-density areas (Hewitt 1988). An alterative explanation is that bears were previously exterminated from this contact zone. This area, near the city of Östersund, has a higher human population density than do the female concentration areas to the north and south. Swenson et al. (1994*a*) suggested that the female concentration areas are relicts of populations that survived the near extermination of bears at the beginning of this century.

The contact zone we have identified corresponds well with those of three other mammals that colonized Scandinavia from both the north and the south: *Sorex araneus* (Fredga & Nawrin 1977), *Microtus agrestis* (Fredga & Jaarola 1989), and *Clethrionomys glareolus* (Tegelström 1987). This occurrence of contact zones in the same region for four different species suggests that a common biogeographic event is responsible.

Only four individuals, all males, had crossed the border between the two lineages. This observation is fully congruent with results concerning the pattern of dispersal as obtained using radiotelemetry. Females are more philopatric than males, and the 95th-percentile dispersal distance was less than one-tenth of the distance separating the two female concentration areas nearest the contact zone. All males in the wrong area were within the 95th-percentile dispersal distance for males from the correct area. Thus, juvenile dispersal data are consistent with our lineage-assignment data and can explain the occurrence of the males in wrong areas close to their correct areas. Because as mtDNA is maternally inherited, we can deduce that there is probably no mtDNA introgression in this contact zone. The high level of female philopatry also can explain the strong link between mtDNA genotypes and geographic distribution that previously was obtained on a European level (Taberlet & Bouvet 1994).

However, the likely absence of mtDNA introgression throughout the contact zone does not mean that there is no introgression at all; for example, if the two males of the eastern lineage reproduce with females of the southern female concentration area (western lineage), then an introgression of nuclear genes will occur. Four of the 16 males within the 95th-percentile dispersal distances for males were from the other lineage. Thus, we can estimate that 25% of the matings within this 250-km-wide area on both sides of the contact zone will be with males of the other lineage if both groups have equal opportunities to mate. The gene flow between the two lineages could be detected by studying nuclear loci such as microsatellites (Tautz 1989; Bruford & Wayne 1993; Queller et al. 1993).

Due to the fact that some transplantations can lead to the irretrievable loss of the historical genetic record (Avise 1992), management guidelines can be proposed based on knowledge of the location of the contact zone between the eastern and the western lineage. In the absence of information concerning possible male-mediated gene flow, a conservative approach would be to consider the southern and the three northern female concentration areas as two distinct conservation units. Therefore the artificial mixing of individuals between these two entities should be avoided, because brown bears associated with the southern area may have a unique genetic value. They number about 150 individuals (Swenson et al. 1995) and represent the largest surviving legacy of bears from the Iberian refugium. Other Scandinavian populations from this group are now extinct (Swenson et al. 1995), and the remaining populations in France and Spain are endangered. If further studies indicate a low level of male-mediated gene flow, then individuals from the southern female concentration area could be used as a ready source of bears to reinforce the other endangered populations that originated from the Iberian refugium, particularly the Pyrenean population, which is threatened by extinction in the short term.

The localization of such a contact zone between two highly divergent lineages within a species under intensive management can help us answer some fundamental questions in conservation biology. The two brown-bear lineages probably diverged about 0.85 million years ago (Taberlet & Bouvet 1994) and may have met in Scandinavia only about 5000–9000 years ago following the melting of ice from the last Ice Age (Siivonen 1982; Liljegren & Lagerås 1993). We do not know what happens when they hybridize. Do the hybrids suffer a decline in fertility? Do females of one lineage prefer males from the same lineage? Do females avoid dispersing into areas with an established high-density bear population, thus maintaining the distinct border between the two lineages? A long-term study of this contact zone, including both field and genetic research, can answer these questions and could be of prime importance for future management programs, including the reinforcement of endangered populations.

Acknowledgments

We thank Robert Franzén and Arne Söderberg for their help in sampling and data organization, and Christiane Dubois-Paganon for her precious technical help during

the genetic analysis. This work was financially supported by the University Joseph Fourier, the Centre National de la Recherche Scientifique, the French Ministry of the Environment (DRAEI-EGPN, DNP), the Swedish Environmental Protection Agency, the Norwegian Directorate for Nature Management, the Norwegian Institute for Nature Research, the Swedish Hunters' Association, and the World Wildlife Fund (Sweden).

Literature Cited

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oïkos **63**:62–76.

Bruford, M. W., and R. K. Wayne. 1993. Microsatellites and their applications to population genetic studies. Current Opinion in Genetics and Development **3**:939–943.

Fredga, K., and M. Jaarola. 1989. Åkersorkens invandring och utbredning i Skandinavien. Pages 73–78 in Genetik och Humaniora I (Annals of the Erik Philip-Sörensen's Foundation). Lund University Press, Lund, Sweden.

Fredga, K., and J. Nawrin. 1977. Karyotype variability in *Sorex araneus* L. (Insectivora, Mammalia). Pages 153–161 in A. DeLaChapelle, and M. Sorsa, editors. Chromosome today. Elsevier/North Holland Biomedical Press, Amsterdam.

Hewitt, G. M. 1988. Hybrid zones. Natural laboratories for evolutionary studies. Trends in Ecology and Evolution **3**:158–167.

Liljegren, R., and P. Lagerås. 1993. Fran mammutstäpp till kohage, djurens historia i Sverige. Wallin & Dalholm Boktryckeri, Lund, Sweden.

Mazza, P., and M. Rustioni. 1994. On the phylogeny of Eurasian bears. Palaeontographica Abt. A, **230**:1–38.

Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalysed chain reaction. Methods in Enzymology **155**:335–350.

Queller, D. C., J. E. Strassman, and C. R. Hughes. 1993. Microsatellites and kinship. Trends in Ecology and Evolution **8**:285–288.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution **1**:9–10.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.

Servheen, C. 1990. The status and conservation of the bears of the world. International Conference of Bear Research and Management Monography Series **2**:1–32, International Association for Bear Research and Management, University of Tennessee, Knoxville.

Siivonen, L. 1982. The history of the Fennoscandian mammal fauna. Acta Zoologica Fennica **169**:7–10.

Sørensen, O. J. 1990. The brown bear in Europe in the mid 1980's. Aquilo Ser. Zoologica **27**:3–16.

Swenson, J. E., F. Sandegren, A. Bjärvall, A. Söderberg, P. Wabakken, and R. Franzén. 1994*a*. Size, trend, distribution and conservation of the brown bear *Ursus arctos* population in Sweden. Biological Conservation **70**:9–17.

Swenson, J. E., F. Sandergren, P. Wabakken, A. Bjäruall, A. Söderberg, and R. Franzén. 1994*b*. The historic and present status of the brown bear (*Ursus arctos L.*) in Scandinavia. (In Norwegian with English summary.) NINA Forskningsrapport **53**:1–23, Norwegian Institute for Nature Research, Trondheim.

Swenson, J. E., P. Wabakken, F. Sandegren, A. Bjäruall, R. Franzén, and A. Söderberg. 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.

Taberlet, P., and J. Bouvet. 1991. Génétique de l'Ours brun des Pyrénées (*Ursus arctos*): premiers résultats. Comptes Rendus de l'Académie des Sciences Série III, **314**:15–21.

Taberlet, P., and J. Bouvet. 1992. Bear conservation genetics. Nature **358**:197.

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

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research **17**: 6463–6471.

Tegelström, H. 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). Journal of Molecular Evolution **24**:218–227.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques **10**:506–513.

Woodruff, D. S. 1989. The problems of conserving genes and species. Pages 76–88 in D. Western, and M. C. Pearl, editors. Conservation for the twenty-first century. Oxford University Press, New York.