Mammalian Biology 76 (2011) 615–621

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.de/mambio

Original Investigation

Genetic diversity of Dinaric brown bears (*Ursus arctos*) in Croatia with implications for bear conservation in Europe

Ivna Kocijan[a,*], Ana Galov[a], Helena Ćetković[b], Josip Kusak[c], Tomislav Gomerčić[c], Đuro Huber[c]

[a] *Division of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10 000 Zagreb, Croatia*
[b] *Laboratory of Molecular Genetics, Division of Molecular Biology, Ruđer Bošković Institute, Bijenička 54, HR-10 000 Zagreb, Croatia*
[c] *Department of Biology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, HR-10 000 Zagreb, Croatia*

ARTICLE INFO

Article history:
Received 18 August 2010
Accepted 10 December 2010

Keywords:
Ursus arctos
Dinaric brown bears
Genetic diversity
Management
Conservation

ABSTRACT

Brown bears have lost most of their range on the European continent. The remaining western populations are small, isolated and highly endangered. The Dinaric-Pindos brown bear population is the western-most stable population and the fourth largest in Europe. It has been recognized as a potential source for recolonization of populations whose survival is at risk. Indeed, several translocations of Dinaric bears to Italy, Austria and France have recently been made. Despite the importance of the Dinaric bear population, its genetic status remains poorly understood. Using tissue samples from 156 hunted or accidentally killed Dinaric bears in Croatia, this study analysed genetic diversity at 12 microsatellite loci, as well as population structure and past reductions in size. In addition, a subset of 59 samples was used to assess diversity of the mitochondrial DNA control region. The results indicate that Dinaric bears have high nuclear genetic diversity, as compared to other extant brown bear populations, despite genetic evidence of a bottleneck caused by past persecutions. However, haplotype diversity was low, probably as a result of male-biased dispersal and female philopatry. Not surprisingly, no evidence of population sub-structure was found using nuclear markers, as the bear habitat has remained continuous and the highway network has been built only recently. Management should focus on maintaining habitat connectivity and keeping the effective population size as large as possible. In addition, when removing individuals, care should be taken not to further deplete the population of rare haplotypes. A coordinated transboundary management of the entire Dinaric-Pindos brown bear population should be a priority for its long-term conservation.

Introduction

Brown bears (*Ursus arctos*) once inhabited nearly the entire European continent. Today, large and stable populations inhabit northeastern Europe, Scandinavia, the Carpathian Mountains and the Dinaric-Pindos region, whereas the populations in western Europe are small, isolated and under threat of extinction, numbering from just a few individuals up to several dozen (Zedrosser et al. 2001; Linnell et al. 2007). Their patchy geographical distribution today is the consequence of prolonged human impact on the species through extensive hunting and habitat destruction (Zedrosser et al. 2001).

The Dinaric-Pindos brown bear population spans the territories of Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Kosovo, Macedonia, Albania and Greece, and its size is estimated at 2100–2500 bears (Linnell et al. 2007). Indeed, Dinaric bears get their name from the Dinarides, the mountain range stretching from Slovenia in the northwest to Albania in the southeast. In Croatia, the bear population is estimated at 600–1000 individuals (Huber et al. 2008a). The population size has recovered from centuries of uncontrolled hunting and persecution, which lasted until the introduction of the country's first Hunting Act in the mid-1940s. At that time, fewer than roughly 100 bears remained (Huber and Frković 1993; Huber et al. 2008b). Since then, the number has steadily increased, reaching approximately 400 in the 1980s and coming close to the habitat's ecological and social capacity in the last official estimate in 2008 (Huber et al. 2008a,b). Today, the bear territory occupies 11 824 km^2, with the highest concentrations in the central part of the range, in the forested counties of Lika and Gorski Kotar, where densities may reach over 1 bear/10 km^2. The brown bear is managed as a game species in Croatia. Several national laws and documents regulate hunting and population management, the annual removal quota and duration of the hunting season (Huber et al. 2008b).

As the western-most stable brown bear population in Europe, Dinaric bears have been recognized as a potential source of animals for recolonization or reinforcement in western European areas where bears have become extinct or populations are too small to

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* Corresponding author. Tel.: +385 1 4877742; fax: +385 1 4826260.
E-mail address: ikocijan@biol.pmf.hr (I. Kocijan).

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doi:10.1016/j.mambio.2010.12.003

survive independently (Taberlet and Bouvet 1994). In fact, on several occasions over the last two decades Dinaric bears have been translocated to reintroduce the species in Austria (Rauer 1997; Kruckenhauser et al. 2009) and the central Pyrenees of France (Quenette et al. 2001; Chapron et al. 2003), and to augment a remnant wild population in the western Pyrenees of France (Chapron et al. 2003) and in the Italian Alps (Clark et al. 2002). A female brown bear translocated in 1989 from Gorski Kotar (Croatia) to Austria was the first brown bear ever captured in the wild and hard released for the purposes of reintroduction (Clark et al. 2002).

Recently, recommendations have been made based on published results of animal relocations, that more detailed studies dealing with the species targeted for relocation should be conducted prior to any such actions (Fischer and Lindenmayer 2000). Genetic diversity of a source population is an important factor in achieving a higher probability of a successful reintroduction or boosting of wild populations. Re-established populations are often very small and thus susceptible to stochastic genetic processes, such as founder effects or genetic drift. Therefore, knowledge of genetic variability of the source population is crucial for the success of repopulation efforts. Nevertheless, genetic studies of Dinaric bears remain scarce, unlike some other European brown bear populations that have attracted a significant amount of attention (Hartl and Hell 1994; Waits et al. 2000; Saarma and Kojola 2007; Zachos et al. 2008; Kruckenhauser et al. 2009; Pérez et al. 2009; Karamanlidis et al. 2010). The genetic diversity of Dinaric bears has been described in a small set of samples in several studies of mitochondrial DNA (mtDNA) polymorphism in European brown bears (Randi et al. 1994; Taberlet and Bouvet 1994; Kohn et al. 1995). These studies identified two distinct mtDNA lineages in Europe, called eastern and western, and they showed that Dinaric bears belong to the latter. Together, these studies detected seven mtDNA haplotypes in Dinaric bears. However, no additional information on their genetic diversity exists in the scientific literature.

In the current study, we investigated the genetic diversity of Dinaric brown bears in Croatia. Using samples taken from hunted and accidentally killed bears, we analysed microsatellite loci and the mtDNA control region sequence. Mitochondrial DNA was analysed in an attempt to detect more maternal lineages, since previous studies included only a few samples from Croatia (four, Randi et al. 1994; two, Taberlet and Bouvet 1994). Furthermore, we examined the genetic structure of the population. There are suggestions that the Dinaric-Pindos brown bear population comprises several subpopulations, because the forested habitat is not continuous and in some regions there are isolated sub-areas, albeit with some inter-connectivity (Zedrosser et al. 2001). We hypothesized that no population sub-structuring exists in Croatia, because the habitat is continuous and anthropogenic barriers (i.e. highways) were absent until a decade ago. Finally, since bears have been hunted and persecuted throughout history and especially in the first decades of the 20th century, we examined whether past reductions in size have left a genetic signature. The results of this study will provide a basis for long-term genetic monitoring using tissue and non-invasive samples, and for formulating guidelines for management of Dinaric brown bears.

Material and methods

In the period 2005–2008 we sampled 156 hunted or accidentally killed (mostly by traffic) brown bears across their range in Croatia (Fig. 1). Muscle tissue samples were preserved in 96% ethanol (1:10 volume ratio) until DNA extraction. DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA).

A hypervariable part of the mtDNA control region was amplified from 59 randomly selected samples by the polymerase chain reaction (PCR) using a primer pair described in Taberlet and Bouvet (1994). Amplifications were carried out in 20 μl containing 1× Qiagen Master Mix (Qiagen Multiplex PCR Kit, Qiagen, USA), 0.2 μM of forward and reverse primer and 1 μl template DNA. Cycling conditions were as follows: 95 °C for 15 min, 32 cycles of 30 s at 94 °C, 90 s at 58 °C, 1 min at 72 °C, and 10 min final extension at 72 °C. The amplification products were purified (Wizard SV Gel and PCR Clean-Up System, Promega, USA) and sequenced in both directions. The sequences were aligned using ClustalW (Thompson et al. 1994) implemented in BioEdit software (Hall 1999) and compared to the sequences deposited in the GenBank database. Genetic diversity

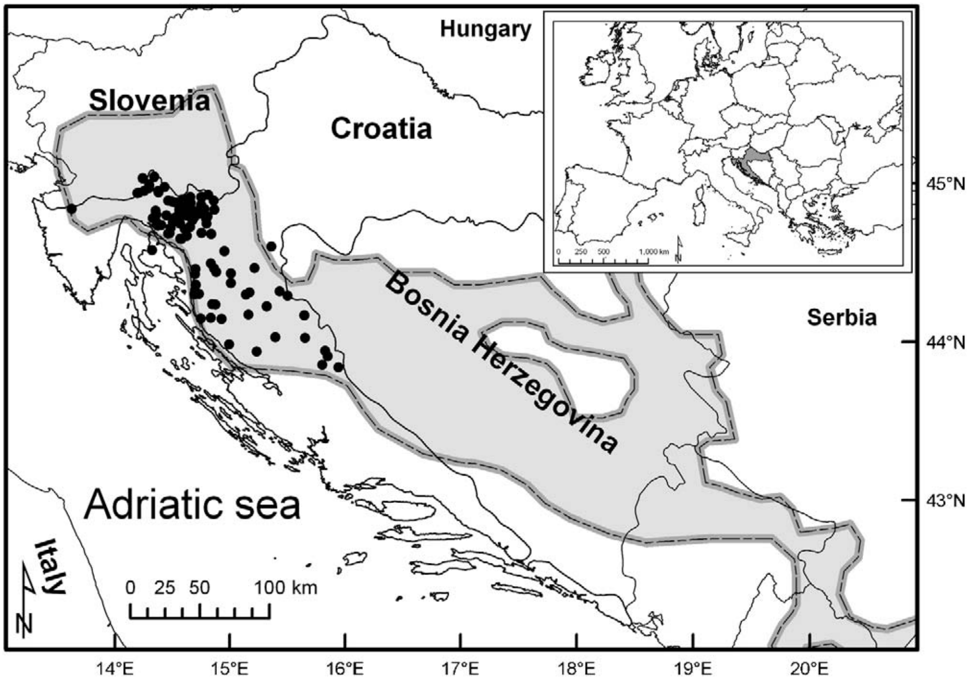

Fig. 1. Range of Dinaric brown bears (shaded) and sampling locations (dots).