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Preliminary results on genetic tracking of the Brown Bear (*Ursus arctos*) individuals in the Malá Fatra National Park (Slovakia)

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Abstract. In this study, we extended the analysis of genetic diversity of the Slovakian brown bear using data from four mitochondrial microsatellite loci. Relatively high levels of genetic heterozygosity was found in the West Carpathian bears. The levels of observed heteroyzgosity ranged from 0.63 to 0.73. Mitochondrial DNA diversity revealed relatively high levels of genetic variation comparable to Scandinavian and North America brown bears. Non-invasive tracking of bears in North Slovakia revealed that current censuses of bears are probably overestimated.

Key words: genetic tracking, diversity, microsatellites, mtDNA, *Ursus arctos*

Introduction

Since 1989, the increase of hunters started to be a very serious problem in Slovakia, which continues until now. Hunter organisations declare that the populations of large carnivores are overgrown in the West Carpathians. The press of hunter organisations to the Ministry of Environment is continually increasing, The public is, for example, informed on 1,200 individuals of wolves (2000) or 1,040 lynxes (2001) at the territory of Slovakia. From the point of view of potential habitat size and concerning ecology of the species, the numbers are clear ecological non-senses and fictions (Hell and Garaj 2002, Ciberej 2002). The main aim of hunting intererests in the current days is brown bear. Month by month, the people may hear or read in media that the number of bears is very high and that population is overgrown.

The genetic approach may significantly contribute to the knowledge of population size and movement of bears in Slovakia. This may, for example, explain the differences between earlier field data of hunters which suggest many individuals, and the current and realistic estimate of the population size.

Microsatellite analysis has often been used to estimate genetic diversity in bear populations (Paetkau *et al.* 1998, Ruiz-Garcia *et al.* 2005), and to examine their paternity, reproductive success and individual features in brown bears (Craighead *et al.* 1995, Taberlet *et al.* 1997). We initiated a study of the mitochondrial DNA diversity and tracking in the Slovakian brown bear by collecting microsatellite data for 23 samples at 4 loci. The goals of this study were: (1) to quantify and compare the levels of mitochondrial DNA diversity to some other European populations, and (2) to track some individuals and possess the data on their individual movement.

Material and Methods

Between 2005 and 2006, 76 samples (mainly hairs) were collected in the Malá Fatra and High Tatra National Parks. Only 23 of the samples (8 soft tissues, one feces, and 14 hair) provided enough DNA for a complete genetic traping of selected polymorphic loci. Because the different results may be obtained from a single extract (Taberlet *et al.* 1997), we performed double genetic analysis of the same samples at two most important loci. Genetic data were compared with the corresponding field data on tracks and hair remnants.

Soft tissues were kept frozen, and hairs were preserved either in 70% alcohol or dry in paper envelopes. Majority of hairs samples originated from the Malá Fatra National park. Two samples of soft tissues and one of faeces from the High Tatra National Park were also analysed to provide an outgroup for the allele comparisons. The distance between two parks is approximately 50 – 70 kilometres.

The DNA from the tissue samples and bear faeces were isolated using the QIAamp DNA Mini Kit and the QIAamp DNA Stool Kit (QIAGEN). The bear hairs were initially microscopied to check the presence of dry cells. The part of hair roots (cca 5 mm) was cut and were placed in the Eppendorf tubes. DNA extraction from hairs was carried out using the Chelex method (Walsh *et al.* 1991).

Four microsattelite loci (G1D, G10X, UarMU26, UarMU64) were amplified using nested PCR. We used the primers described Paetkau *et al.* (1995) and Taberlet *et al.* (1997). PCR reactions of 25 ml were performed using 50 – 100 ng of DNA, 1´ PCR buffer (Tris-Cl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, pH 8.7), 1´ Q-Solution, 200 mM of each dNTP, 0.5mM of each primer and 2.5 U of HotStarTaq Plus DNA Polymerase (QIAGEN). The PCR amplification consisted of 35 cycles (94˚ C for 30 s, 55˚ C for 30 s, 72˚ C for 1 min). The amplification products were

denaturated at 94 ° C for 3 min and loaded on a 6% acrylamide gel. The separation of PCR products by electrophoresis run 2.5 h.

Genetic polymorphism was measured as the number of alleles per locus (A), observed heterozygosity (Ho), and expected heterozygosity (He) were calculated according the standard genetic Nei´ s formula for multiallelic loci (e.g. Gálová *et al.* 2006).

Results

We found a relatively high genetic polymorphism in the population (Table 1). The observed and expected levels of heterozygosity indicate that Slovakian bears still have high levels of genetic diversity. We found from four to nine alleles at one locus. All alleles found in the High Tatra National Park samples were also identified in the Malá Fatra.

Because we used only four loci in our study, we were not able to individually identify all samples. But movement of some bears was clearly presented (Fig. 1). The different samples from the same individuals were found at the distance of 15 – 20 kms.

Locus (number of samples)		A	Ho	He
G10X (23)	5	0.65	0.47	
UarMU26 (23)		4	0.65	0.44
UarMU64 (23)		9	0.74	0.46
G1D (11)		4	0.63	0.43

Table 1. Observed number of alleles (A), observed heterozygosity (Ho), expected heterozygosity (He) by locus for Slovakian individuals of bears with sample size in parentheses.

Discussion

The West Carpathian bears form a part of the Eastern lineage of brown bears in Europe which is mainly represented by large populations of Russia and Romania (Taberlet and Bouvet (1994). Two European lineages diverged approximately 850,000 years ago. The split probably occurred in the long, cold period that arose before the warm climatic period. The evolution of bears and their radiation in various independent lineages were shaped by the cuccession of contrasted climatic periods that took place during the Quarternary. (Loreille *et al.* 2001). The molecular phylogeny suggests that the cave bears (*Ursus spaeleus*) were then responsible for long diversification of two lineages of brown bears which lasted more than 800.000 years (Hänni *et al.* 1994). The cave bears diverged before the split of the eastern and western lineages of the brown bear. Between the split of these two lineages 850,000 years ago and the extinction of the cave bear about 20,000 years ago, the cave bear could have been responsible for the isolation of the two brown bear populations, the first in Asia, which corresponds to the eastern lineage, and the second in Europe, which corresponds to the western lineage in an area where the cave bear fossils have never been found (i.e., in the south of Spain). It seems reasonable to hypothesize that the two brown bear lineages became neighbors when the cave bear disappeared 20,000 years ago (Hänni *et al.* 1994). Such a hypothesis may explain the relatively high degree of divergence existing between the two brown bear populations, which seems surprising given their present geographical proximity (Taberlet and Bouvet 1994).

The results of this microsatellite analysis revealed relatively high levels of genetic variability of Fatra and Tatra bears. No visible differences may be found in

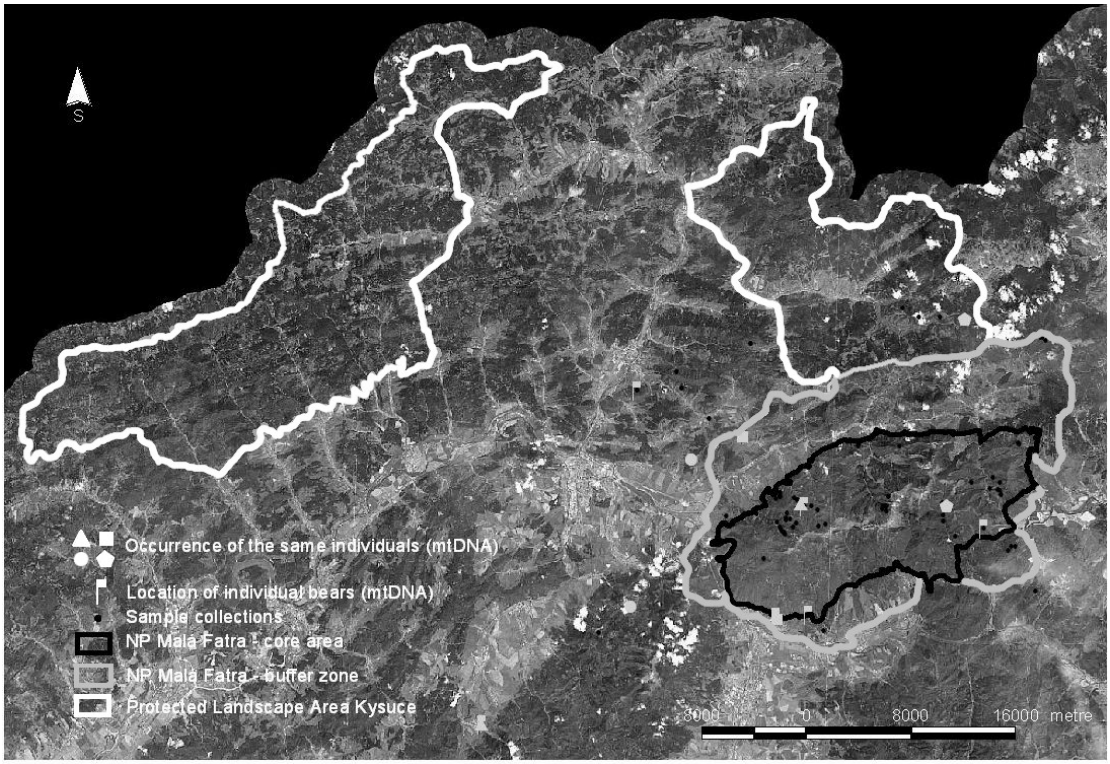

Fig. 1. Occurence of the same individuals (mtDNA).

genetic diversity among the Scandinavian and Slovakian bears. Data from two loci used in this study (G10X, G1D) can be directly compared with levels of microsatellite DNA diversity observed in the surveys of North American and Scandinavian brown bear populations (Paetkau *et al.* 1998). The observed heterozygosity for these loci in the Scandinavian and Slovakian population tended to be in the higher range of genetic diversities observed in North America (Waits *et al.* 2000). These results are particularly relevant to the conservation biology debates concerning the desired size and distribution of conservation reserves. Our results suggest one way in which the continuos – not industrially fragmented – habitat must be kept for large carnivores in the North Slovakia. It is the only way how to protect the genetic diversity of West Carpathian bears.

The analysis of a large number of loci would increase the power of detecting population substructure because each locus may contain an independent history of the population depending on the amounts of random drift, mutation, and migration that have occurred. Current work extends the application of molecular methods in conservation biology by demonstrating that individual identification of bears (Fig. 1) and identification of home ranges is possible by non-invasive simple technique. However, attention must be paid as the very low quantities of DNA obtained from field samples make accurate genetic typing difficult. The autenticity of results obtained must be supported by an appropriate methodology (Taberlet *et al.* 1997). Our preliminary data indicate that individual bears may translocate for many kilometres in the Malá Fatra NP. From this point of view we feel that current censuses of bears may be highly overestimated in the state and private hunter organisations in the Slovak Republic.

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