Genetic analysis for geographic isolation comparison of brown bears living in the periphery of the Western Carpathians Mountains with bears living in other areas

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ABSTRACT

Populations of the European brown bear (Ursus arctos L.) differ substantially in size, degree of geographic isolation and level of genetic diversity. Present patterns result from phylegenegetic processes and profound human intervention. We assessed the genetic variability of a subpopulation of brown bears near the periphery of their range in the Western Carpathian Mountains and compared their genetic properties with those of bears in the core of the same population and elsewhere. Samples were collected non-invasively in 2007–2008 and 2010 in Štrbské Pleso protected landscape area (PLA) in Slovakia (included to the NATURA 2000 networking program). Seven polymorphic microsatellite loci (UaMU26, UaMU50, UaMU51, G10, G16, G18, UaMU50 and UaMU51) were amplified using a nested PCR in order to assess the following parameters: variability, allelic combinations, heterozygosity, number of alleles and inbreeding coefficient. Sufficient brown bear DNA for analysis was obtained from 57 out of 94 samples (61%), among which 45 different genotypes were identified. Loci had a mean of 2.7 ± 0.76 alleles. Average observed heterozygosity was 0.59. The inbreeding coefficient was negative for all but one of the analyzed loci (2007–2008). In the year 2010 was negative of three seven loci. These results imply that gene flow with other parts of the population has been maintained in the reduced level and the isolation level of bears in the study area was not so low. Nevertheless, the genetic variability of bears in Štrbské Pleso PLA was lower than that reported from other localities in the Carpathian Mountains. The results are discussed in the context of behavioural ecology and conservation genetics.

Keywords: Carpathian Mountains; European Brown Bear; Ursus arctos L.; Genetic Diversity; Microsatellite Markers; Non-invasive Sampling

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MATERIAL AND METHODS

1. Sample Collection and DNA Isolation

A total of samples 57 (34 of which, out of 94 different sites of Štrbské Pleso PLA's were collected. A 37 samples collected during the year 2007 (frozen and 8 samples) and 20 samples from Štrbské Pleso PLA territory during the period 2007–2008 were examined. Sufficient brown bear DNA for analysis was obtained from 37 (2007–2008) out of 46 samples and 37 (2010) out of 48 samples. A total of 46 different genotypes were identified among 28 samples from 2007 and 28 from 2010. DNA extraction from hair was performed using 10% Chelex according to Kuckrova et al. 4]. Depending on quality and quantity of the hair samples, with visible roots were used, DNA extractions from non-invasive samples were performed with the QIAGEN DNA Kit (QIAprep Spin) with a final elution volume of 20 µl.

2. Microsatellite Analysis and Gender Identification

Seven microsatellite Markers, UaMU26, UaMU50, UaMU51, G10, G16, G18 and UaMU51 were amplified using polymerase chain reaction (PCR) and fragment length (alleles) analyses were carried out by an eight-capillary sequencer (Genome Lab, USA, Beckman Coulter). Analyses were repeated in order to verify the accuracy of the analysis. DNA samples from adult (those genotypes that were extracted from fur using 10% Chelex) and adult animals, in microsatellite loci (UaMU26, UaMU50, G10, G16, G18, UaMU51 and UaMU51) were amplified in a nested polymerase chain reaction (PCRs); a longer fragment of each locus was amplified prior to amplifying a more specific area. Two-step PCR procedure improved amplification of the non-amplifying genotypes 5]. Observed (H0) and expected (H0) heterozygosity were calculated using GenAlex 6.3 software (Field genetics). Results were compared with genetic data from brown bears in core ranges of the Carpathian Mountains in Slovenia (Krausová et al. 7] and Catalonia [8] as well as in central Austria [9].

3. Statistical Methods

Observed (H0) and expected (H0) heterozygosity were calculated with GenAlex software. Descriptive statistics for each locus (mean number of alleles, heterozygosity, percentage of private alleles, percentage of polymorphic loci and percentage of polymorphic bands) were calculated (Table 1). Descriptive statistics from non-invasive samples were calculated using the software program FSTAT (2004) 10, and the observed and expected heterozygosity (H0) were used to evaluate the level of genetic differentiation. H0 was calculated with the six methods.