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 phylogeographic 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 Carpathians 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 Strážovské Hory Protected Landscape Area (PLA) in Slovakia (included to the NATURE 3000 networking program). Seven polymorphic microsatellite loci (UaMU26, UaMU40, G10B, G10D, G10L, UaMU50 and UaMU51) were amplified using a nested PCR in order to assess the following parameters: variability, allelic combinations, number of alleles and inbreeding coefficient. Sufficient brown bear DNA was obtained from 57 out of 94 samples (61%), among which 45 different genotypes were identified. I was had a mean of 2.7 ± 0.75 alleles. Average observed heterozygosity was 0.59. The inbreeding coefficient was negative for all but one of the analysed loci (2007–2008). In the year 2010 was negative three of 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 in the study area was not so low. Nevertheless, the genetic variability of bears in Strážovské Hory 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

MATERIAL AND METHODS

1. Sample Collection and DNA isolation

A total of 94 samples (47 females and 47 males) were collected. The samples were collected during the years 2007 and 2010 from 4 and 45 samples from Strážovské Hory protected landscape area (PLA) in Slovakia (included to the NATURE 3000 networking program). Seven polymorphic microsatellite loci (UaMU26, UaMU40, G10B, G10D, G10L, UaMU50 and UaMU51) were amplified using a nested PCR in order to assess the following parameters: variability, allelic combinations, number of alleles and inbreeding coefficient. Sufficient brown bear DNA was obtained from 57 out of 94 samples (61%), among which 45 different genotypes were identified. I was had a mean of 2.7 ± 0.75 alleles. Average observed heterozygosity was 0.59. The inbreeding coefficient was negative for all but one of the analysed loci (2007–2008). In the year 2010 was negative three of 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 in the study area was not so low. Nevertheless, the genetic variability of bears in Strážovské Hory 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.

2. Microsatellites Analysis and Gender Identification

Seven microsatellite loci (UaMU26, G10D, G10B, G10L, UaMU51 and UaMU50) were amplified using polymerase chain reaction [2] and fragment length [3] obtained were carried out on eight capillary sequencer (GeneScan Lab Edit Beckman Coulter). Analyses were repeated in order to verify the reproducibility of sizing. All genetic analyses were made using GENEPOP (version 2.00) [4]. The obtained data were used to calculate allelic richness (Ar) for individuals, mean microsatellite loci (G10B, G10D, G10L, UaMU50 and UaMU51) were amplified using a nested polymerase chain reaction (PCR) [2]: a longer fragment of each locus was amplified prior to amplifying a more specific area. Twenty-Four PCR procedure improved the accuracy of the analysis new genotyping errors [5]. Observed (A) and expected (E) heterozygosities were calculated using GenoDive 2.0 software (Field Genetics). Results were compiled with genetic data from brown bears in core ranges of the Carpathian Mountains in Slovakia (A) and Romania (B) as well as central Austria (C).