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CONSERVATION GENETICS: NEW TOOL FOR WILDLIFE MANAGEMENT AND NATURE CONSERVATION

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Summary: Conservation genetics became in the last two decade a useful tool for all decision making in nature conservation. Correct estimation of population size, gene diversity and differentiation of populations and population structures are necessary for all decisions concerning species conservation and/or their management.

The Carpathians and a big part of the Balkan Peninsula are for many species considered to be the refuge for their further migration in postglacial period. This is the case of *Rupicapra rupicapra*, which is the species with three different subspecies, *Ursus arctos* and *Cervus elaphus* with two different genetic lines. Similar pattern is expected also in other large mammals and/or animal species (fish, birds, and insects).

Comparative studies of genetic diversity and differentiation of protected and managed wildlife species within the larger areas of Carpathians should serve for decisions concerning their management and conservation practices. Such comparison is necessary especially for brown bear and chamois populations in Slovakia and Romania) where populations of both species with different population sizes and densities occur.

Key words: conservation genetics, *Ursus arctos, Rupicapra rupicapra,* pylogeography, genetic diversity

Introduction

Conservation genetics is an interdisciplinary science that aims to apply genetic methods to the conservation and restoration of biodiversity. Interdisciplinary of conservation genetics is based on the interaction of several fields including population genetics, molecular ecology, biology, evolutionary biology, and systematic. Genetic diversity is one of the three fundamental levels of biodiversity with direct impact on conservation of species and ecosystem diversity [1], [2].

Conservation genetics is a new scientific field, broader applications that have been introduced to conservation biology after the advent of molecular methods in 1990. With regard to sampling of experimental material, plant conservation genetics is methodically simpler, however, while wildlife conservation genetics must rely on more sophisticated sampling methods, e.g. non-invasive sampling.

Phylogeography is the study of the historical processes that may be responsible for the contemporary geographic distributions of individuals. This term was introduced to describe geographically structured genetic signals within and among species. An explicit focus on a species biogeographic past sets phylogeography apart from classical population genetics and phylogenetics. Past events include population expansion, population bottlenecks, vicariance and migration [2].

Study of phylogeography and large-scale population differentiation may play an important role in resolving the conservation genetic issues in defining evolutionary significant units (ESU) or conservation units and/or understanding of the intra specific taxonomical classification. Although phylogeography is a rather young branch of biological science (it was coined in 1987), the recent two decades of its applications contributed significantly to understanding the processes of the establishing present ranges of numerous wildlife species as a result of postglacial migration and other evolutionary processes.

The study of mitochondrial markers is considered as a principal tool of the phylogeography studies. The advent of the polymerase chain reaction (PCR), the process where millions of copies of a DNA segment can be replicated, was crucial in the development of phylogeography. Thanks to this breakthrough, the information contained in mitochondrial DNA sequences was much more accessible. Advances in both laboratory methods (e.g. capillary DNA sequencing technology) that allowed easier sequencing of DNA and computational methods make better use of the data.

Nevertheless, markers of the nuclear DNA, which due to their biparental inheritance also depict recent events, e.g. the influence of selection, mutual mating and the gene flow should be also considered as a parallel tool for phylogeography studies. The application of both, mitochondrial and nuclear DNA markers for phylogeographic studies requires genetic inventories with a large-scale sampling of biological material for analyses. The advantage of the DNA analyses is the possibility to collect samples by both invasive and non-invasive methods and since many of

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the wildlife species are at the same time game species, even historical samples with well-preserved DNA (museum and trophy specimens) can be used as a source of biological material for comparative studies.

Among the practical applications of the conservation, genetic studies in wildlife populations there are many aims in resolving the taxonomy (systematic) questions e.g. position of the lower taxonomic units, the studies aimed at the investigation of the genetic differentiation of the endangered and managed wildlife populations, identification of the evolutionary significant units (ESU) and the consequences of population fragmentation due to the human impact. Study of all of these problems in wildlife populations would not have been possible before the discovery of PCR techniques and subsequent elaboration of non-invasive sampling for numerous wildlife species [9].

Recent advances of conservation genetics are methodologically linked with molecular ecology and large-scale genetic sampling on one hand and with sophisticated statistical analyses based on Bayesian approach and methods of landscape genetics on the other hand.

The aim of our research activities were large-scale inventories of genetic differentiation of several wildlife species:

- red deer (*Cervus elaphus*) – intensive study of genetic differentiation of red deer populations within Carpathians and adjacent territories,
- wild boar (*Sus scrofa*) – Europe-wide study of genetic differentiation with special attention to the intraspecific taxonomical units,
- brown bear (*Ursus arctos*) – genetic differentiation of brown bear populations along the Carpathians and Eastern Balkan, and
- chamois (*Rupicapra rupicapra*) – genetic differentiation of chamois populations within three subspecies.

Material and Method

Samples of all four species were collected in the period between 2004 and 2010 within the Carpathian Mountains and Balkan Peninsula. In total, 564 samples of red deer, 900 samples of wild boar, 300 samples of brown bear and 700 samples of chamois were collected. DNA from tissues and blood was isolated either using the modified method of Sambrook [10] involving overnight digestion with K followed by phenol-chloroform extraction or by Chelex 100 Resin (Biorad) with 20 minutes at 99 °C in 10 % Chelex solution. Bone samples were first ground then decalcified in EDTA and finally DNA was isolated using NucleoSpin® Tissue kit (Macherey-Nagel). Faecal and hair samples were processed in a laboratory used exclusively for non-invasive samples. DNA from faeces was isolated using the QIAamp DNA Stool Mini Kit (Qiagen) according to the producer's manual. Hair DNA was extracted with Chelex, using the same protocol as for tissues and blood. One or two negative controls were used in each batch of extractions to detect possible contamination.

For all four studies, a set of microsatellites of nuclear DNA (14, 11, 13 or 24, respectively) optimized for sets of multiplexes was used. Bayesian analyses (STRUCTURE) for attributing individuals into pre-defined populations were used as a principal tool of the statistical evaluation that was later completed by several methods of landscape genetics (BARRIER, GENELAND).

Results and Discussion

For the case study of genetic differentiation of red deer populations, 564 samples (soft tissues and antlers) have been used in total and 14 microsatellite loci have been optimized into four multiplexes. The sample set covered the area of Central and South-Eastern Europe with a special emphasis to enlighten the genetic differentiation of red deer from Carpathians and the adjacent territories with the aim to study the position of Carpathian red deer, *Cervus elaphus montanus* Botezat.

Taxonomical position of the Carpathian red deer was first described by Botezat [4], and since that time it has been considered as the subspecies *Cervus elaphus montanus* Botezat (however, according to Grubb [8], the name *montanus* is taxonomically invalid). Dobroruka (1960) gives the geographic distribution of the *C. elaphus montanus* as the one reaching from the Eastern Carpathians up to Krym [5], and on the other side, according to Groves and Grubb (1987) it reaches to Baltics and southern Hungary [7].

Besides the body size, zoologists and hunters paid much attention to the trophy size and shape, which were at the end of the 19th and the beginning of the 20th centuries considered the largest in Europe. Similarly, the skull size of red deer originating from the Eastern Carpathians was larger than that from Western Europe. According to Philipowicz (1961), the skull length varied between 47 and 50 cm and zygomatic width varied between 15 and 18

cm. In contrast, the skull length of the western European red deer varied between 42 and 43 cm, the skull lengths of the Carpathian red deer were larger by 15–20 % [11].

Craniological analyses have revealed a differentiation of the Carpathian and western European red deer in the skull size (as expressed in the skull length and width) and some other characteristics. The presence of convex nasal bones, as claimed earlier by several authors to be typical for Carpathian red deer, has not been proven. This type of skulls occurs but it is not very frequent.

Genetic studies based on a set of 311 individuals and 12 microsatellite loci have shown a rather good differentiation of the Carpathian populations (including the adjacent territories i.e. Slovakia, Hungary southern Poland) from the populations originating from the Czech Republic (Krkonoše) and Poland (Sztralowo). These differences were proved statistically significant using the BARRIER as well as the STRUCTURE software (Fig. 1). A high proportion of Carpathian genes are depicted in red colour, while the western European ones in yellow and all transition populations in blue colour.

The Hungarian population originating from Zala has shown a more pronounced similarity with the Carpathian red deer than the population from Baja, because many red deer have been moved to the region of Zala during the 19th century [14].

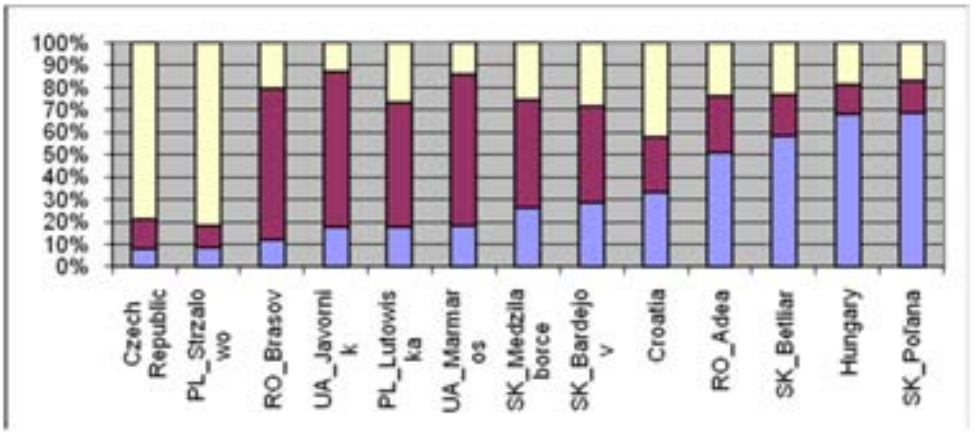

Fig. 1 Results of the STRUCTURE analysis (number of groups = 3). All 311 individuals were clustered into groups according to their geographic origin; different colors represent the proportion of genes attributed to individual groups (yellow – western European; red – Carpathian and blue – transition group).

The second case study was aimed at intra specific structuring of wild boar populations within the European range of the species. So far, we have used 900 samples and 11 microsatellites loci optimized into four multiplex PCR reactions. The overall evaluation has shown the pattern of large-scale genetic structuring of populations that might resemble the presence of subspecies as they were originally defined, however, the borders of ranges do not coincide with ranges described in the literature. The expected border between the occurrence of *Sus scrofa attila* and *S.s. scrofa* is shifted much more to the west and corresponds with the border between the Carpathians and the Hercynians. The southern European populations also show significant genetic structuring of populations [3].

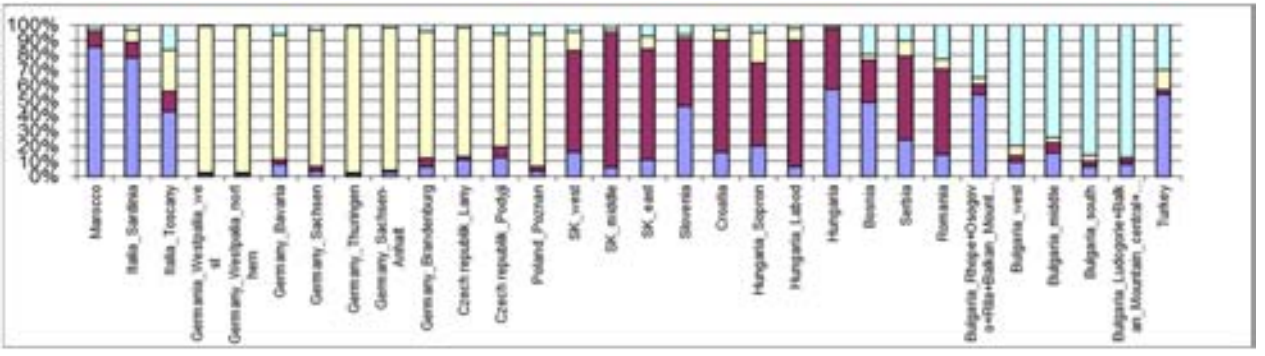

Fig. 2 Results of the structure analysis of wild boar populations in Europe-wide study.

The third case study was aimed at the study of brown bear populations along the Carpathians. We have used in total about 300 tissue samples from legally culled brown bears in Slovakia and some additional samples of hair and faeces, which were later compared with about 120 samples from Greece. For the analyses, 13 microsatellites were used in three multiplexes. Overall differentiation was shown between the Romanian and Slovak brown bear populations and within in Slovakia there was a separation of northern and central Slovakian populations with some migrants. This was due to the landscape fragmentation as the consequence of building the water reservoir, highway and industrial infrastructure in the valley between the High and the Low Tatras [12], [13].

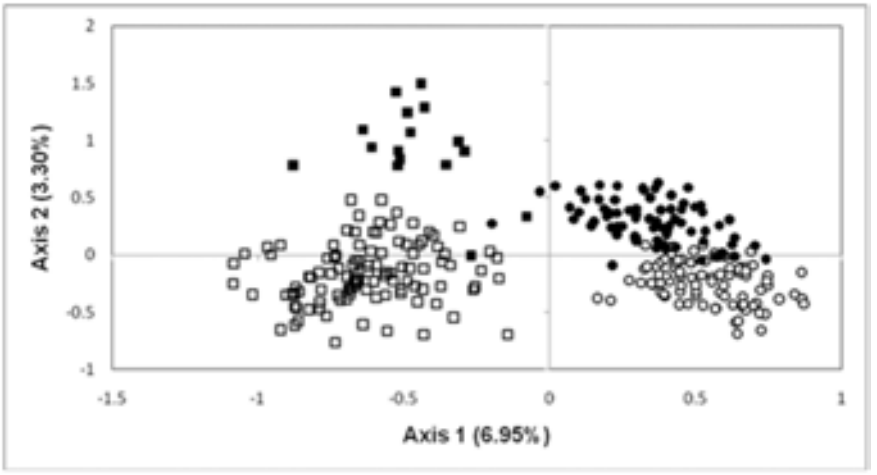

Fig. 3 Fragmentation of brown bear populations from Carpathians (empty squares – Romania; full squares – Eastern Slovakia; full circles – northern Slovakia and empty circles –central Slovakia) (Straka *et al*., 2012)

Finally, we have made comparison of chamois populations belonging to four subspecies – *Rupicapra rupicapra rupicapra, R. r. balcanica, R. r. tatrica* and *R. r. carpatica*. We have analyzed in total 395 samples (tissues, bones) and used 24 microsatellites optimized for three multiplex reactions and two singleplexes. A very good differentiation has been shown between three subspecies *Rupicapra rupicapra rupicapra* (populations 1–7)*, R. r. balcanica* (populations 10–11)*, R. r. tatrica* (population 13), except *R.r. carpatica* (populations 12) for which we did not have enough samples yet. Some populations of *R.r. balcanica* (populations 8–9) were characterized by admixture of individuals possessing genes of two subspecies due to the translocations of animals to support the populations size and trophy quality. Since these sites are at present in the national park Velebit, it seems to be difficult to control the genetic purity of populations and upgrade their conservation status.

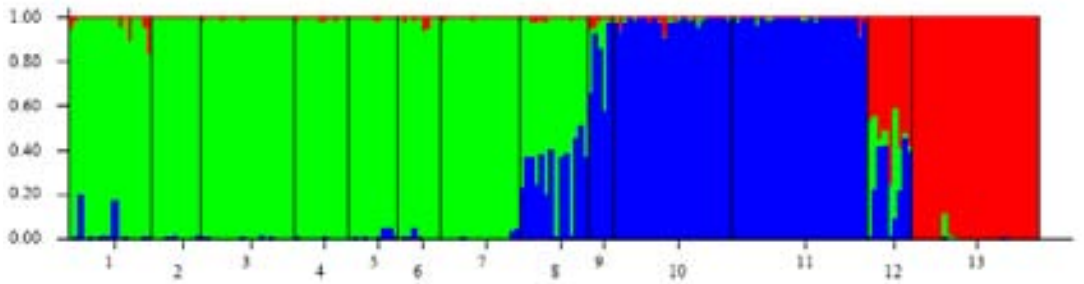

Fig. 4 Results of the STRUCTURE analysis of the chamois populations from Alps, Tatras and Balkan.

Conclusion

Four case studies, which shows the large-scale studies of genetic structure and differentiation of wildlife (red deer, wild boar, brown bear and chamois) populations, showed that it is possible to use microsatellites of the nuclear DNA for studying population differentiation on large geographical scales and/or phylogeographic pattern that could contribute to the understanding the intra specific structure and position of specific lower taxonomical units usually described in the past on the basis of morphological variation.

Besides the analyses using nuclear microsatellites, it would be helpful to compare the given results also with pattern of mitochondrial DNA variation. This could enlighten the historical background of present populations with regard to the migration processes in postglacial period and/or the recent translocation activities.

Acknowledgement

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