THE 3rd INTERNATIONAL SYMPOSIUM ON HUNTING

"Modern aspects of sustainable management of game populations"

Proceedings of 3rd International Symposium on Hunting with Abstract book

Organizers

Balkan Wildlife Scientific Society

Zemun-Belgrade, 26-28. September, 2014.

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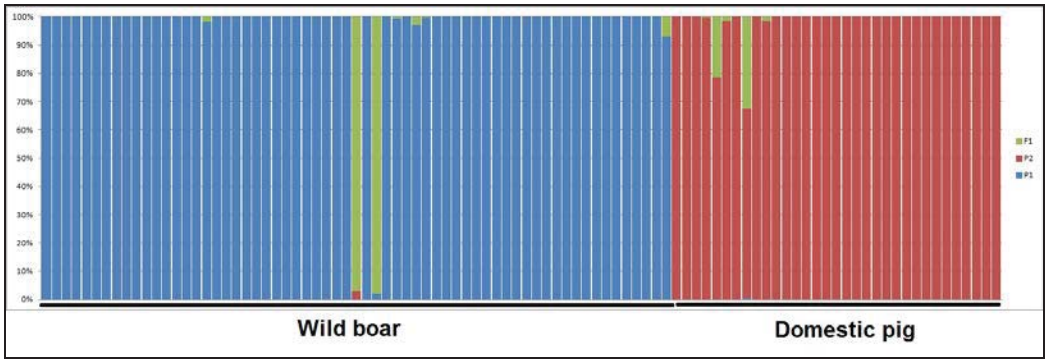

Figure 2. Results from the simulation considering two parental (P1 & P2) and one hybrid classes (F1), for pairwise analysis of wild boars and domestic pigs, using NEWHYBRIDS. Each individual is represented by a vertical bar. Likelihoods of assignment to parental and F1 classes are plotted in different colours. Horizontal bars represent a priori geographic origin.

Since the wild boar is the game species that provides great economic income to hunters and hunting associations and hunting grounds in Vojvodina Province are one of the most important in the region, it is very important to consider the implications of obtained results for the sustainable management of this species. Obtained results point out that management strategies of wild boar populations in Vojvodina should be narrowed to preserve observed level of genetic variability and on prevention of uncontrolled translocations and crossbreeding with domestic pigs.

Conclusion

The recent expansion of wild boar populations in Serbia raised concern about the investigation of genetic diversity and structure of wild boar populations in order to develop adequate management strategies. Since wild boars can crossbred with domestic pigs producing fertile hybrids, it is very important to examine percentage of hybridization. In this study high genetic variability was observed in both wild boars and domestic pigs. In the analysis of 63 wild boars, only two was shown to be hybrids between wild boars and domestic pigs. This result point out that for sustainable management of wild boars in hunting grounds in Vojvodina Province contacts of wild boars and domestic pigs should be strictly controlled in order to prevent introgression of domestic pig genes to wild boars and to maintain registered high genetic diversity.

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GENETIC VARIABILITY OF GREY WOLF (*Canis lupus*) POPULATION IN BOSNIA AND HERZEGOVINA

Šnjegota D.[1], Djan M.[2], Veličković N.[2], Popović D.[2],
Trbojević I.[1], Obreht D.[2], Ćirović D.[3]

Brief Introduction: Previous analyses of genetic diversity in grey wolf populations from Europe showed that grey wolves from the Balkans retained high portion of variability comparing to other European grey wolf populations. The main goal of this research was to determine genetic variability of grey wolf population from Bosnia and Herzegovina based on mtDNA control region sequence variability.
Material and Methods: Muscle tissue samples of 17 grey wolf individuals were collected. Total DNA was extracted and partial fragment of mitochondrial control region was amplified and sequenced.
Results: The final length of sequences in the dataset was 283bp, among which 10 were variable positions (9 parsimony informative sites and 1 singleton variable site). In total, four haplotypes were detected in grey wolf population from Bosnia and Herzegovina. Haplotype diversity was h=0.625±0.083, nucleotide diversity, π=0.012, while average number of nucleotide differences was k=3.515. One haplotype showed high frequency (52.9%), one intermediate frequency (35.3%) and two haplotypes were rare and detected only per one individual (5.9% each). Mismatch distribution analysis showed statistically significant deviation from the null hypothesis that the observed data fit the sudden expansion model (Ssd=0.136; p=0.03). Fu's *Fs* and Tajima's *D* tests of neutrality showed positive, although not statistically significant values. Multimodal mismatch distribution and positive values of neutrality tests may indicate past population size decline.
Conclusion: We have found high genetic variability within analysed grey wolf population, as expected for the Dinaric-Balkan grey wolf populations, as compared with other European wolf populations. Detection of present genetic diversity and demographic history is important for determination of population structure and sustainable management of the population.

Key words: grey wolf, genetic variability, mtDNA, Bosnia and Herzegovina

Introduction

The grey wolf (*Canis lupus*) is a species that was very abundant and widely distributed in Europe until the end of the 19th and beginning of the 20th century, when the decline of its populations was observed (Boitani, 2000). The main reasons for grey wolf populations decline in Europe are widespread destruction of its habitats, human prosecution and decreases in natural prey (Delibes, 1990; Randi et al., 2000; Randi, 2011). Delibes (1990) stated that during the population decline period in Europe, two isolated populations survived, one in Italy and one in Iberia, while larger populations remained in the Balkans and Eastern Europe (Boitani, 2000; Lucchini et al., 2004; Gomerčić et al., 2010). Some of the Balkans countries have protected the grey wolf species, but Bosnia and Herzegovina is not one of them. The available data estimate approximately 400 individuals in Bosnia&Herzegovina (Boitani, 2000; Milenković et al., 2007), and according to the Association of hunting organization in Bosnia and Herzegovina, there are around 350 wolves (www.slobih.ba). Distribution of the grey wolf populations

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in Bosnia and Herzegovina is very wide and includes karstic fields and mountains (Sofradžija and Muzaferović, 2007) at the border with Croatia, Montenegro and the Dinaric region. Continuous distribution and documented gene flow between wolves from Bosnia, Croatia and Slovenia was reported and they were grouped in large Dinaric-Balkan population (Štrbenac et al., 2005, 2008) together with populations from Serbia, Montenegro, Albania and FYR Macedonia (Gomerčić et al., 2010).
The Balkan grey wolf population represents the border between populations from Eastern and Western Europe (Djan et al., 2014) and genetic substructuring on north-south axis has been observed. The first data on genetic diversity of Dinaric-Balkan grey wolf population showed high genetic diversity and differentiation to "western" and "eastern" subpopulations with different demographic histories (Djan et al., 2014).
The aim of this study was to detect level of genetic diversity in grey wolf population in Bosnia and Herzegovina and to determine genetic structure, as well as to find evidence of possible population expansion and/or bottleneck, using analysis of sequence variability of mtDNA control region.

Material and Methods

Material

Genetic variability analysis of the grey wolf population in Bosnia and Herzegovina included 17 muscle tissue samples, collected during legal hunts at several localitites (Fig. 1). Tissue samples were stored at -20ºC prior to analysis.

Figure 1. Geographic positions of sampling localities.

Molecular analysis

Extraction of total mtDNA from each muscle tissue sample was done using phenol chloroform extraction (Sambrook and Russel, 2001). Partial fragment of mtDNA control region was amplified using CR1 and CR2R primers (Palomares et al., 2002) in a total reaction volume of 25µl. Reaction mixture for PCR contained 0.2mM dNTPs, 0.1 µM of each primer, 2.5mM $MgCl_2$, 1U Taq polymerase and 1xreaction buffer. PCR amplification was performed by following conditions: denaturation at 95ºC for 5 min; 35 cycles of amplification (each cycle begins at 94ºC for 40s; 55ºC for 50s and 72ºC

for 1 min) and final extension at 72ºC for 10min. The PCR products were purified using Exo-Sap protocol. Sequencing was conducted with an ABI3730xl genetic analyzer (Applied Biosystems).

Data analyses

Sequence alignment was performed in BioEdit software (Hall 1999), and final adjustments were done by eye. Our dataset consisted of 17 CR-1 mtDNA sequences with final length 283bp. DNASP (Librado and Rozas 2009) was used for calculations of basic parameters of genetic diversity (haplotype diversity, nucleotide diversity, average number of pairwise differences) and mismatch distribution analysis and neutrality tests (Fu's Fs and Tajima's) were performed in same program.
In order to test hypothesis of population structure, sample was divided to „west" and „east" subpopulations and AMOVA analysis was done in Arlequin (Excoffier and Lischer 2010), together with mismatch distribution analyses for both subpopulations and total population. Median Joining network was constructed in order to visualize mutational pathways among found haplotypes using Network 4.6.0.0 software (http://www.fluxus-engineering.com/sharenet.htm).

Results

Amplification of the grey wolf mtDNA control region from Bosnia and Herzegovina was successful for all 17 samples. Analysis of 17 mtDNA sequences, with a total length of 283bp, revealed presence of 10 variable positions (9 parsimony informative sites and 1singleton variable site) and 4 haplotypes (Table 1). Haplotype diversity was 0.625±0.083, nucleotide diversity was 0.012, and average number of nucleotide differences was 3.515. One haplotype showed high frequency (52.9%), one intermediate (35.3%) and two haplotypes were rare and detected only per one individual (5.9% each).

Table 1. Control region mtDNA haplotypes of grey wolf from the territory of Bosnia and Herzegovina with associated variable positions.

Haplotypes	Variable positions										N	%
	47	66	93	135	151	161	165	176	183	192		
H1	T	T	G	C	T	C	T	T	G	A	9	0.529
H2	.	.	A	T	.	T	C	.	A	.	6	0.352
H3	C	C	A	C	C	C	T	C	.	G	1	0.058
H4	T	C	A	C	C	.	.	C	.	G	1	0.058

N- number of individuals sharing the same haplotype. %- percentage of total number of haplotypes.

Calculated parameters of genetic diversity show high genetic variability of analysed grey wolf population, which was expected, since high genetic diversity in grey wolf populations from the Balkans was reported before (Gomerčić et al., 2010; Fabri et al., 2013; Djan et al., 2014). The grey wolf populations from the Balkans, generally, have higher genetic variability in comparison to other European populations, most probably due to moderate size fluctuations in the past as reported for Bulgaria (Randi et al., 2000) and Croatia (Gomerčić et al., 2010; Fabbri et al., 2013). The high genetic diversity was also revealed using microsatellites (Lucchini et al., 2004; Moura et al., 2013).

The mismatch distribution analysis showed statistically significant deviation from the null hypothesis corresponding to the model of the rapid expansion (Ssd=0.136; p=0.03). Fu's *Fs* and Tajima's *D* tests of neutrality showed positive, but statistically insignificant values. Observed multimodal mismatch distribution and positive values of neutrality tests showed that no population expansion is present, even though it might be that recent population decline has happened. The reflection of recent population size decline to population genetic variability and structure was previously reported by Fabbri et al. (2013) and Djan et al. (2014).