

POPULATION GENETIC STUDIES OF GREY WOLVES (CANIS LUPUS L. 1758) FROM BOSNIA AND HERZEGOVINA

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Introduction & Aims

The grey wolves of Bosnia and Herzegovina form a subpopulation of the Dinaric-Balkan wolf population and represent one of Europe's least studied wolf populations. Since the Dinaric-Balkan population is a valuable source of genetic diversity for neighboring populations, comprehensive assessments are warranted. First genetic researches encompassed analyses of mtDNA control region, but recently, analyses of autosomal microsatellite loci have been included.

A field monitoring (by using photo trapping) has also been implemented for the population of grey wolf at several localities across Bosnia and Herzegovina with the aim to detect wolves presence at each locality and to make conclusions about their behavior and abundance.



The main aims of wolf population genetic studies in Bosnia and Herzegovina are: i) determination of the genetic variability ii) estimation of population structure of the grey wolf population from Bosnia and Herzegovina iii) estimation of levels of gene flow and inbreeding iv) evaluation of genetic signals of a bottleneck v) detection of mtDNA haplotypes and vi) detection of wolf presence at the specific locality.

Study area



Fig. 1. Map showing the sampling localities of grey wolves from Bosnia and Herzegovina

Materials & methods

Genetic analyses

- N=69 (muscle tissue samples)
- 18 microsatellite autosomal loci – The Canine Genotypes™ Panel 1.1 kit (Finzymes, Thermo Fisher Scientific, Finland)
- Control region of mtDNA – CR1, CR2R primers (Palomares et al., 2002)



Fig. 2. Software which have been used for genetic analyses

Field activities

- Photo trapping – 6 photo traps, 5 localities (feeding places, nature, wolf transects)
- Non-invasive sampling – throughout monitored localities (hair, feces)

Results & Discussion

Analyses of microsatellite loci

Moderately high heterozygosity was detected ($H_o=0.73$, $H_e=0.76$) for the total sample and absence of signs of genetic bottleneck and inbreeding. A weak signal of population structure was detected by using different software (SPCA in R, TESS, DAPC in R) ($K=2$), where some individuals from the south and south-east part of the country formed separate cluster (Figure 3a, b, c) (Šnjegota et al., 2017).

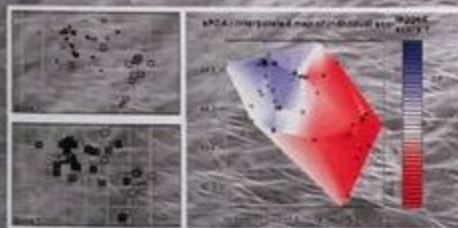


Fig. 3a. Results of grey wolf population structure from Bosnia and Herzegovina by applying SPCA analysis in R. Signal signs of wolf population at the western and eastern part of Bosnia and Herzegovina in comparison to the wolves from the other parts of the country. Left: 100 smallest black dots represent differentiated from large white squares. Left down: levels of grey color signify differences. Right: differences in color signify higher level of differentiation among individuals. Right: interpolated map of individual wolves.

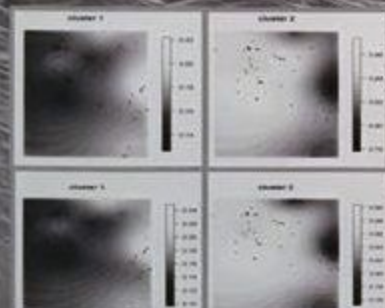


Fig. 3b. Results of grey wolf population structure from Bosnia and Herzegovina by applying TESS analysis in R. Two clusters were detected. Genetic introduction of structure population for individuals belonging to the two detected clusters – TESS analysis. The lighter areas correspond to the two clusters. The darker lines of background represent level of genetic differentiation. It can be clearly observed a genetically separated with very few wolves, within the cluster 2.



Fig. 3c. Results of grey wolf population structure from Bosnia and Herzegovina by applying DAPC analysis in R. Bar plot showing the number of detected clusters (K=2). Each color corresponds to the genetic cluster. Each line represents one individual. The lighter lines of bar plot correspond to individuals from the eastern and central part of Bosnia and Herzegovina.

Analyses of CR mtDNA

Tab. 1. Results of the basic genetic parameters of CR mtDNA analyses for grey wolf from Bosnia and Herzegovina.

n	h	Hd	π	k
69	10	0.752±0.00085	0,0183	k=4,835.

(n = number of individuals; h = number of haplotypes; Hd = nucleotide diversity; π = nucleotide diversity; k = average number of pairwise differences)

Photo trapping

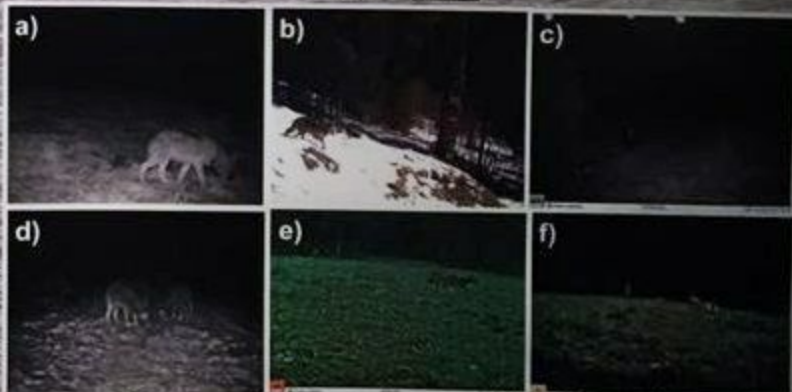


Fig. 4. Monitored grey wolf presence at the monitored localities within Bosnia and Herzegovina. a) Young wolf, b) Young wolf, c) Young wolf, d) Adult wolf, e) Adult wolf, f) Adult wolf.

Rufford Foundation projects

- 2018 Let's make better future for grey wolves in Bosnia & Herzegovina: coordination of grey wolf population
- 2016 The Grey Wolf Conservation in Bosnia & Herzegovina – The next step
- 2015 Monitoring of grey wolf population from Bosnia & Herzegovina with the aim to restore a viable population

Management implication

Continued genetic monitoring based on the results of our study may provide further insights into the genetic diversity and structure of the Dinaric-Balkan wolf population, and in monitoring of individuals, and provide estimates of the contribution of Dinaric-Balkan wolves to newly-formed wolf populations in Western Europe.

References

- Palomares, F., Godoy, J.A., Pita, A., O'Brien, S.J., Johnson, W.E., 2002. Fecal genetic analysis to determine the presence and distribution of elusive canines: design and feasibility for the Iberian lynx. *Mol. Ecol.* 11, 2171–2182.
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