

SPATIAL GENETIC ANALYSIS OF ROE DEER FROM THE NORTHERN SERBIAN PROVINCE OF VOJVODINA

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Stefanović M., N. Karaiskou, N. Veličković, M. Kovačević, Z. Ristić, M. Djan (2019): *Spatial genetic analysis of roe deer from the northern Serbian province of Vojvodina.*- Genetika, Vol 51, No.3, 1127-1138.

Ecological and evolutionary processes responsible for genetic variation are affected by geographical distribution of samples, therefore combined analysis of genetic and spatial information can help to a better understanding of these processes. Mitochondrial DNA control region was sequenced in 103 individuals from 11 different localities crossing Northern Serbian Province of Vojvodina. Sixteen haplotypes were detected, with high haplotype diversity values (0.898 ± 0.012), that were clustered in two spatially and genetically differentiated groups named: East and West. The haplotype diversity was higher in East group, while nucleotide diversity and average number of nucleotide differences were higher in West group. Conservation plans and management strategies should always take into consideration genetic data that helps to understand spatial genetic patterns and/or effect of landscape features in dispersal between animal populations.

Keywords: *Capreolus capreolus*, genetic clustering, mtDNA, Serbia

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INTRODUCTION

Population genetic structure is not necessarily explained by geographic locality of individuals, therefore a priori definition of populations could mask real spatial patterns, due to cryptic structure or undetected migrants (MANEL *et al.*, 2003). As many ecological and evolutionary processes that influence genetic variation are mediated by space, the joint analysis of genetic and spatial information can lead to a better understanding of these processes (GUILLOT *et al.*, 2009). Recent development in spatial statistical methods, mainly through Bayesian clustering, has emerged as a powerful tool for understanding how space and landscape features could affect population structuring (FRANTZ *et al.*, 2009; FRANÇOIS and DURAND, 2010). Climatic fluctuations during Pleistocene have had a strong influence on shaping genetic variability of various temperate mammal species (HEWITT, 1999), and several studies have underlined the impact of landscape features to gene flow not only for large mammals (e.g. SCHREGEL *et al.*, 2012) that have restricted dispersal capabilities but also for medium-sized species such as roe deer that are expected to be relatively mobile (COULON *et al.*, 2006; KUEHN *et al.*, 2007). Understanding the spatial distribution of the genetic variability of the individuals, and the influence of landscape and environmental features in shaping it, is important for the development of proper management and conservation plans (MANEL *et al.*, 2003; PALSBOELL *et al.*, 2007).

The European roe deer (*Capreolus capreolus*) is one of the most common and most abundant ungulate species in Europe, widely distributed from the Mediterranean region towards the north up to Scandinavia (ANDERSEN *et al.*, 1998). Between the late 19th and early 20th centuries, the roe deer distribution was reduced due to habitat fragmentation, deforestation, hunting pressure and other kind of human-induced disturbances (BURBAITÉ and CSÁNYI, 2009; RANDI *et al.*, 2004). From the beginning of the 20th century, roe deer populations started to increase again (BURBAITÉ and CSÁNYI, 2009), probably as a consequence of improvement of habitats quality and development of proper game management plans (MERIGGI *et al.*, 2008). Roe deer represents one of the most biologically and economically valuable game species in Northern Serbian Province of Vojvodina, whose habitats are mostly formed by agricultural lands (PRENTOVIĆ *et al.*, 2012). Over the last fifty years density of roe deer population have doubled in lowland habitats in Vojvodina, just in decade from 2001 to 2011, the average density increased from 1.8 animals per km² to 2.4 animals per km² (PRENTOVIĆ *et al.*, 2012). Even though there is a trend in increasing population density of roe deer in Vojvodina, it is still below the natural habitats potential. In order to assess long term potential for survival and reproduction of this species in Vojvodina, genetic monitoring and investigation of genetic structure is important in developing proper sustainable conservation management plans in this area.

In the last two decades, extensive genetic analyses have been done in European roe deer populations, employing different molecular markers, and aiming to estimate the levels of genetic variability and population differentiation, the effects of habitat fragmentation for conservation and management purposes, as well as to shed light on the phylogeography of the species (ZACHOS *et al.*, 2006, and references cited within). Available data on Serbian roe deer populations based on allozyme analyses indicated lower genetic variability and polymorphism values in comparison to similar analysis done in Central, Southern and Eastern Europe roe deer populations (MILOŠEVIĆ-ZLATANOVIĆ *et al.*, 2005). The results of this study also suggested that nonrandom mating and game management might have had an influence on the genetic structure of roe deer populations in Serbia (MILOŠEVIĆ-ZLATANOVIĆ *et al.*, 2005). However, RANDI *et al.* (2004) using mitochondrial DNA (mtDNA) and microsatellites markers detected high

haplotype diversity values and moderate to high heterozygosity values for Serbian population unraveling the discrepancy between different molecular markers.

Since genetic variability supports a species evolutionary potential and since knowledge of spatial structuring of genetic diversity is of fundamental importance for the development of a sustainable management program, the aim of this study was to analyze spatial distribution and clustering of genetic diversity in roe deer from Northern Serbian Province of Vojvodina, based on the variability of mtDNA sequences.

MATERIAL AND METHODS

Muscle tissue samples from 103 roe deer individuals were collected from 11 localities across the Northern Serbian Province of Vojvodina during the winter hunting season of 2014-2015 (Fig. 1A). The name of localities, their abbreviations (in parenthesis) and the number of sampled individuals per locality are: Bački Petrovac (BP) – 29; Novo Miloševo (NM) – 22; Novi Kneževac (NK) – 13; Šid (SI) – 9; Torda (TO) – 8; Kanjiža (KA) – 6; Nova Crnja (NC) – 5; Novi Bečej (NB) – 5; Žitište (ZI) – 3; Itebej (IT) – 2; and one individual from Hetin (H).

Total genomic DNA was extracted using a slightly modified standard proteinase K digestion, phenol/chloroform extraction procedure (SAMBROOK and RUSSELL, 2001). A partial fragment of the first hypervariable domain of the mtDNA control region (CR-1) was amplified following the procedure published by ROYO *et al.* (2007) using the primer pair L-Pro and H-16493 (5' CGT CAG TCT CAC CAT CAA CCC CCA AAG C 3'; 5' TGA GAT GGC CCT GAA GAA AGA ACC 3'; DOUZERY AND RANDI 1997). The PCR products were purified following the ExoI-SAP protocol (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and capillary sequencing was conducted with an ABI 3730xl DNA Analyzer (Applied Biosystems Inc., Foster City, CA, USA).

Sequences were aligned using Geneious Pro v.5.3.4 (DRUMMOND *et al.* 2011), while final adjustments were done by eye. The dataset consisted of 103 sequences with the full alignment length of 475 bp (474 bp excluding one site with indel position).

Molecular DNA polymorphism indices for all sequences (h - haplotype diversity, π - nucleotide diversity, k - mean number of pairwise differences) and their corresponding variances were calculated in DnaSP 5.10.01 (LIBRADO and ROZAS, 2009) and ARLEQUIN 3.5.1.3 (EXCOFFIER and LISCHER, 2010), while the latter was further used to calculate the neutrality tests (Fu's F_s and Tajima's D test), as well as the significance of the fit of observed mismatch distribution to the expected values under the demographic expansion model.

Median-Joining network (BANDELT *et al.*, 1999) was calculated using Network v5.0.0.0 (available at <http://www.fluxus-engineering.com>) applying the default settings ($\epsilon = 0$ and the variable sites weighted equally = 10), in order to assess relationships among roe deer haplotypes detected in this study. In order to obtain a wider perspective on phylogenetic position of roe deer haplotypes revealed in this study, a new dataset was formed which included 161 haplotype sequences published by RANDI *et al.* (2004) with haplotypes generated in this study. Because different primer pairs were used in different studies, some of the haplotypes collided together, resulting in a final data set comprising of 94 haplotypes with total length 430 bp.

Due to unequal number of individuals sampled per each locality and in order to avoid any possible bias in a priori definition of populations, spatially-explicit Bayesian clustering of individuals was applied using GENELAND ver 4.0.5 (GUILLOT *et al.*, 2005). The model based on multinomial distribution of genotypes, population memberships and linkage equilibrium was

used with a total of 2.000.000 iterations in 3 independent runs and number of clusters K ranging from 1 to 10. The analyses were repeated five times in order to assess the consistency of the results. Three additional runs were performed using the same parameters with selected fixed K value. In addition, spatially explicit Bayesian clustering method implemented in BAPS v.6.0 (CORANDER *et al.*, 2008) was used, based on individual's coordinates and without a priori population membership information. BAPS was run with the maximal number of groups (K) set to vary from 1 to 10, with each K replicated 10 times, while the results were averaged based on the probability likelihood scores.

Molecular DNA polymorphism indices, neutrality tests and mismatch distribution analysis were also calculated for each detected spatial group using DnaSP and ARLEQUIN, as described above. Genetic differentiation within and between detected clusters was further assessed by calculating Φ_{ST} pairwise differences and through the analysis of molecular variance (AMOVA) using ARLEQUIN. Furthermore, the effect of isolation by distance was tested by a Mantel's test in the IBD program (JENSEN *et al.*, 2005).

RESULTS

In the dataset of 103 roe deer control region mtDNA sequences from Vojvodina 16 different haplotypes were detected (Fig. 1B), designated as C1-C16 (GenBank accession numbers: KY114497-KY114515). The total number of polymorphic sites was 23, with twenty two parsimoniously informative transitions and one indel position. Three of the haplotypes were the most abundant (C1, C2, C13), accounting for 50% of the total sample size, while the majority of haplotypes were represented with low frequencies, ranging from 1.94% to 7.76% and three haplotypes (C7, C10, C11) were unique (Fig. 1B). Haplotype diversity value was 0.898 ± 0.012 , while nucleotide diversity (π) was 0.012 and the average number of nucleotide differences (k) was 5.876 (Table 1).

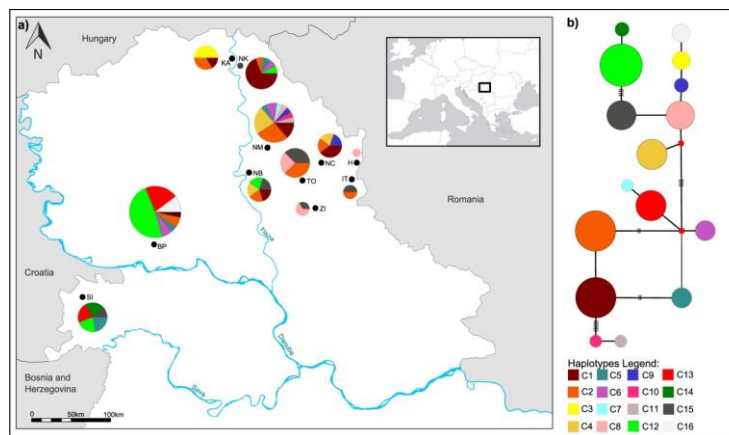


Fig. 1. A) Map of sampling localities represented with black dots and haplotype distribution in Northern Serbian Province of Vojvodina represented by pie charts which size correspond to number of haplotypes. B) Median-joining network of sixteen roe deer haplotypes revealed in this study. Circle sizes correspond to number of individuals having each haplotype. Locality abbreviations correspond to those described in text.

Table 1. Molecular diversity indices and neutrality tests in roe deer population from Vojvodina Province

	WEST	EAST	Total sample
Number of individuals (n)	38	65	103
Number of haplotypes (h)	9	13	16
Haplotype diversity (Hd)	0.777 ± 0.054	0.863 ± 0.023	0.898 ± 0.012
Nucleotide diversity (π)	0.012	0.010	0.012
k	6.134	4.984	5.876
Number of polymorphic sites (S)	17	21	23
Number of transitions (Ti)	16	20	22
Number of transversions (Tv)	0	0	0
Number of indels	1	1	1
Mismatch distribution – SSD (P)	0.054 (0.13)	0.026 (0.15)	0.006 (0.71)
Tajima's D neutrality test (P)	1.549 (0.95)	0.434 (0.601)	0.872 (0.79)
Fu's F_s neutrality test (P)	2.850 (0.91)	0.593 (0.67)	0.997 (0.75)

k – average number of pairwise differences, SSD- sum of squared deviations.

Median-joining network of larger data set consisting of 94 haplotypes revealed the existence of three haplogroups-clades: West, East and Central as previously described by RANDI *et al.* (2004). Eight haplotypes revealed in this study (C3, C4, C8, C9, C12, C14, C15, C16) belonged to East clade, six haplotypes (C1, C2, C5, C6, C7, C13) belonged to Central clade and two haplotypes (C10, C11) fall into West clade (data not shown). Additionally, three new haplotypes were detected (C10, C11, C14) in comparison to those published by RANDI *et al.* (2004).

Geneland analysis of the dataset of 103 roe deer mtDNA sequences from Vojvodina revealed the existence of 3 clusters: the one cluster named WEST was comprised of individuals from two localities situated in the western part of studied region (SI and BP) with assigned probabilities higher than 80%, and the other two clusters named EAST-N and EAST-S were comprised of individuals from localities situated in the eastern part of Vojvodina Province that were further divided in northern and southern subgroup (Fig. 2A, B). These two eastern clusters were supported with lower assigning probabilities and few individuals from localities NB and NC showed inconsistency in cluster assignment during multiple runs. When the analysis was run with k fixed to 2, Geneland results supported the existence of two clusters with very high probability (>98%) for each individual: the WEST one (as previously assigned), and the EAST one, consisting of both EAST-N and EAST-S subgroups (Fig. 2A). Having in mind the higher posterior probabilities and the consistency in multiple runs, all further analyses were done on two genetically differentiated groups named: WEST and EAST. It's worth mentioning here that East and Central European clades/ lineages mentioned above were abundant in both of the two differentiated groups with relatively proportional contribution to each group.

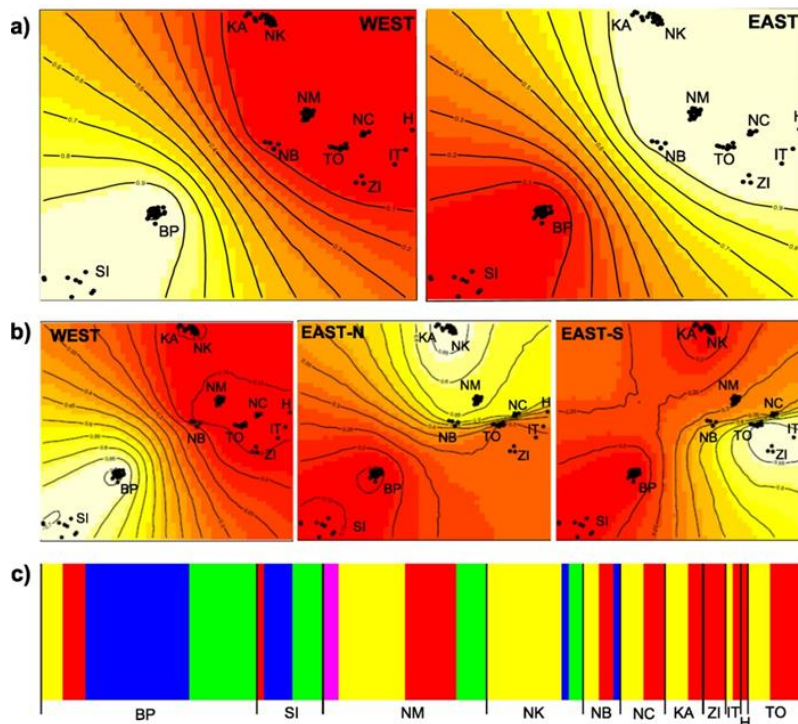


Fig. 2 A) Geneland results of spatial structuring of roe deer individuals from Vojvodina for $k = 2$; B) Geneland results for $k = 3$; C) BAPS spatial results of clustering individuals into five groups represented per sampling locality.

Bayesian clustering model implemented in BAPS revealed the existence of five different groups in roe deer dataset from Vojvodina. One group comprised of just two individuals sampled at the same locality (NM, marked as pink in Fig. 2C) while the rest of the individuals were spread in the remaining four groups (Fig. 2C). The majority of individuals from BP and SI were assigned into two main groups (marked as blue and green – Fig. 2C) that were mainly found in Western Vojvodina, while the individuals from the other sampling localities were assigned in the rest two groups (marked as yellow and red) mainly spread in Eastern Vojvodina. This structuring pattern in general is in accordance with the GENELAND results differentiating the Western from the Eastern populations of Vojvodina Province. Analysis of molecular variance also supported a genetic differentiation, since even though most of the genetic variability was due to differences among individuals within groups (80.49%), there were three times as much variation among groups than among populations within groups indicating a certain genetic differentiation (Table 2). Even though natural populations can show some degree of structuring due to geographic location alone, Mantel's test did not reveal any significant correlation between genetic and geographic distance ($r = 0.187$; $p > 0.05$).

Table 2. Analysis of molecular variance (AMOVA) in roe deer population from Vojvodina Province.

	% of variation	Fixation index	P value
Among groups	14.78	$\Phi_{CT} = 0.14778$	0.00000
Among populations within groups	4.73	$\Phi_{SC} = 0.05552$	0.03396
Within populations	80.49	$\Phi_{ST} = 0.19510$	0.00000

Genetic differentiation between WEST and EAST was further supported by significant pairwise Φ_{ST} value ($\Phi_{ST} = 0.15654$, $P < 0.0001$). Even though some previously published roe deer sequences from Serbia by RANDI *et al.* (2004) can be downloaded from GenBank, exact locality for each haplotype cannot be retrieved from the original reference, and therefore these sequences were not included in the clustering analyses.

The haplotype diversity was higher in EAST group, while nucleotide diversity and average number of nucleotide differences were higher in WEST group (Table 1). All the neutrality tests yielded positive values, even though not significant, while the mismatch distribution failed to reject the hypothesis of demographic expansion (Table 1) probably due to past fluctuations in population size.

DISCUSSION

The majority of ungulate species are threatened globally by human induced habitat changes like fragmentation, urbanization and over-hunting. Understanding of the spatial distribution of genetic variation and how it is related to changes in landscape features is enhanced through recent technological development of statistical analysis through integration of genetic and spatial data (HALL and BEISSIGNER, 2014). Bayesian clustering methods are pronounced computational approaches for inferring genetic structuring and assigning individuals in groups taking into account spatial individual information which increases the power in detection of underlying population structure (FRANZ *et al.*, 2009). GENELAND clustering based on the variability of mtDNA control region sequences in roe deer from Northern Serbian Province of Vojvodina indicated the presence of two main spatially and genetically differentiated groups: EAST and WEST. Additionally, BAPS, although it can overestimate the number of clusters (LATCH *et al.*, 2006), and its sensitivity in detecting population structure could be also affected by a small overall genetic differentiation in roe deer populations from Vojvodina, depicted the general spatial grouping of individuals in accordance with GENELAND results supporting the WEST -EAST structuring pattern.

The observed structuring pattern could be explain as a consequence of a several possible reasons, like the asymmetric contribution of different European clades/lineages to local population. If this region acted as a contact zone between European clades, each cluster (WEST and EAST) might have received disproportionate contribution from different clades and thus be structured. On the other hand, this hypothesis may be ruled out by the fact that a relatively proportional contribution of the most abundant clades (Central and East) was observed in the two differentiated groups described in Vojvodina area. Another process that can affect population structuring is isolation by distance, since gene flow and migration can be affected by geographic distance. However, this was not the case since we did not detect significant correlation between

genetic and geographic distances. This structuring could be also explained by the existence of possible barriers to gene flow. Even though mtDNA is a uniparentally inherited marker which reflects rather past events, it is noteworthy to mention that the observed structuring coincides with the existence of the only high traffic highway-road through Vojvodina Province – European route E75, fenced approximately during the past 15 years. Based on microsatellites data, the influence of the transportation infrastructure and fenced highways on genetic divergence of roe deer was also observed in a population from Switzerland (HEPENSTRICK *et al.*, 2012; KUEHN *et al.*, 2007), while COULON *et al.* (2006) have shown that roe deer at different sides of highways and waterways in southern France may not be considered as a part of a single panmictic population. It seems that fenced highway roads may act as barrier to gene flow, and can increase genetic differentiation among individuals, even though only few generations have passed since their construction (HOLDEREGGER and DI GIULIO, 2010). We should keep in mind that mitochondrial DNA is not the best molecular marker for assessing gene flow and structure caused by recent barriers (due to its uniparental inheritance and high mutation rates). For this reason additional molecular markers like nuclear markers are needed to support the previous hypothesis.

Spatial distribution of haplotypes revealed in this study was also slightly indicative of the observed WEST - EAST structuring (Fig. 1B), since seven of 16 detected haplotypes were presented only in the eastern group, three only in the western group, while six were shared between those two groups (Fig. 1A). Overall haplotype diversity in 103 roe deer samples from Vojvodina was 0.898 and in accordance with previous findings for Vojvodina, but lower in comparison to roe deer population from Eastern and Central Serbia (RANDI *et al.*, 2004). Haplotype diversity was higher in the Eastern group, while nucleotide diversity and average number of pairwise differences were lower than in the Western group, which might be attributed to different sample size. The highest haplotype diversity was found in NM, with three haplotypes being unique for this location.

Possible sub-structuring of Eastern group in EAST-N and EAST-S (as indicated from Geneland analysis for $k = 3$) could be supported by the presence of roe deer migrants foraging from Western Romania. Even though roe deer are territorial animals, with low dispersal activities restricted to a few kilometers (LINNELL *et al.*, 1998), local hunters observed that Romanian roe deer are attracted by winterfeeding places in Vojvodina. Supplementary feeding of ungulates has been widely used as a conservation and management tool in most of European roe deer populations, and it is even obligatory by law in some countries (APOLLONIO *et al.*, 2010). However, the lack of available Romanian roe deer sequences in Genbank does not allow us to test the validity of this hypothesis.

Mismatch analysis revealed the presence of an expansion signal in roe deer from Serbia, while non-significant positive Fu's F_{ST} and Tajima's D values could be indicative of a past decline detected in roe deer populations throughout Europe.

Even though roe deer populations in Europe are starting to increase again, it is still important not only to understand just the effect of geographic distances on genetic differentiation, but also the spatial distribution of genetic variability and to detect possible obstacles to gene flow in order to develop proper conservation and management plans. It is even more important in the case of roe deer, since there is no breeding dispersal in adult roe deer in comparison to most other ungulates and they generally remain faithful to their home range from one year to the next (DANILKIN and HEWISON, 1996). Our results suggest that there is a spatial

distribution of genetic variability, but also indicate a possible effect of fenced highways as a barrier to gene flow in roe deer individuals from Vojvodina. It is clear that urbanization and ongoing road construction can further provoke habitat fragmentation. Maintaining of continual gene flow between fragmented clusters is a necessary step in order to avoid negative consequences of further genetic differentiation within each cluster. In further spatial genetics analyses of roe deer from Vojvodina, a more comprehensive sampling is suggested, as well as continuous monitoring with use of additional more informative nuclear markers, especially since structuring based solely on mtDNA sequences may underestimate current level of genetic differentiation.

ACKNOWLEDGMENTS

The study was supported by the Provincial Secretariat for Science and Technological Development of Autonomous Province of Vojvodina, Grant No. 104-401-4099/2013-07-2b-1

Received, September 03th, 2018

Accepted May 18th, 2019

REFERENCES

- ANDERSEN, R., P., DUNCAN, J.D.C., LINNELL (1998): The European roe deer: the biology of success. Scandinavian University Press, Oslo.
- APOLLONIO, M., R., ANDERSEN, R., PUTMAN (2010): European ungulates and their management in the 21st century. Cambridge University Press, Cambridge.
- BANDEL, H.J., P., FORSTER, A., RÖHL (1999): Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.*, *16*: 37-48.
- BURBAITĚ, L., S., CSÁNYI (2009): Roe deer population and harvest changes in Europe. *Estonian Journal of Ecology*, *58*(3): 169-180.
- CORANDER, J., P., MARTTINEN, J., SIREN, J., TANG (2008): Enhanced Bayesian modeling in BAPS software for learning genetic structure of populations. *BMC Bioinformatics*, *9*: 539-552.
- COULON, A., G., GUILLOT, J.F., COSSON, J.M., ANGIBAULT, S., AULAGNIER, B., CARGNELUTTI, M., GALAN, A.J., HEWISON (2006): Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Mol. Ecol.*, *15*(6): 1669-1679.
- DANILKIN, A.A., A.M., HEWISON (1996): Behavioural ecology of Siberian and European roe deer. Chapman & Hall, London.
- DOUZERY, E., E., RANDI (1997): The mitochondrial control region of Cervidae: evolutionary patterns and phylogenetic content. *Mol. Biol. Evol.*, *14*(11): 1154-1166.
- DRUMMOND, A.J., M., KEARSE, J., HELED, R., MOIR, T., THIERER, B., ASHTON *et al.* (2007): GENEIOUS, ver. 6.0. 5. Biomatters Ltd., Auckland, New Zealand. Available from <http://www.geneious.com/>.
- EXCOFFIER, L., H., LISCHER (2010): Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.*, *10*: 564-567.
- FRANTZ, A.C., S., CELLINA, A., KRIER, L., SCHLEY, T., BURKE (2009): Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *J. Appl. Ecol.*, *46*(2): 493-505.
- FRANÇOIS, O., E., DURAND (2010): THE STATE OF THE ART - Spatially explicit Bayesian clustering models in population genetics. *Mol. Ecol. Resour.*, *10*: 773-784.
- GUILLOT, G., F., MORTIER, A., ESTOUP (2005): GENELAND: a computer package for landscape genetics. *Mol. Ecol. Notes.*, *5*: 712-715.

- GUILLOT, G., R., LEBLOIS, A., COULON, A.C., FRANTZ (2009): Statistical methods in spatial genetics. *Mol. Ecol.*, 18(23): 4734-4756.
- HALL, L.A., S.R., BEISSINGER (2014): A practical toolbox for design and analysis of landscape genetics studies. *Landscape. Ecol.*, 29(9): 1487-1504.
- HEPENSTRICK, D., D., THIEL, R., HOLDEREGGER, F., GUGERLI (2012): Genetic discontinuities in roe deer (*Capreolus capreolus*) coincide with fenced transportation infrastructure. *Basic. Appl. Ecol.*, 13(7): 631-638.
- HEWITT, G.M. (1999): Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.*, 68(1-2): 87-112.
- HOLDEREGGER, R., M., DI GIULIO (2010): The genetic effects of roads: a review of empirical evidence. *Basic. Appl. Ecol.*, 6: 522-531.
- JENSEN, J.L., A.J., BOHONAK, S.T., KELLEY (2005): Isolation by distance, web service. *BMC Genetics*, 6: 13. v.3.23 <http://ibdws.sdsu.edu/>
- KIRSCHNING, J., F.E., ZACHOS, D., CIROVIC, I.T., RADOVIC, S., SAN HMWE, G.B., HARTL (2007): Population genetic analysis of Serbian red foxes (*Vulpes vulpes*) by means of mitochondrial control region sequences. *Biochem. Genet.*, 45(5-6): 409-420.
- KUEHN R., K.E., HINDENLANG, O., HOLZGANG, J., SENN, B., STOECKLE, C., SPERISEN (2007): Genetic effect of transportation infrastructure on roe deer populations (*Capreolus capreolus*). *J. Hered.*, 98(1): 13-22.
- LATCH, E.K., G., DHARMARAJAN, J.C., GLAUBITZ, O.E., RHODES (2006): Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv. Genet.*, 7(2): 295-302.
- LIBRADO, P., J., ROZAS (2009): DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25: 1451-1452.
- LINNELL, J.D.C., P., DUNCAN, R., ANDERSEN (1998): The European roe deer: a portrait of a successful species. In "The European roe deer: the biology of success", Scandinavian University Press, Oslo, pp. 11-22.
- MANEL, S., M.K., SCHWARTZ, G., LUIKART, P., TABERLET (2003): Landscape genetics: combining landscape ecology and population genetics. *Trends. Ecol. Evol.*, 18(4): 189-197.
- MERIGGI, A., F., SOTTI, P., LAMBERTI, N., GILIO (2008): A review of the methods for monitoring roe deer European populations with particular reference to Italy. *Hystrix.*, 19(2): 103-120.
- MILOŠEVIĆ-ZLATANOVIĆ, S., J., CRNOBRNJA-ISAILOVIĆ, S., STAMENKOVIĆ (2005): Allozyme variability and differentiation in Serbian roe deer populations *Capreolus capreolus*. *Acta. Theriol.*, 50(4): 429-444.
- PALSBØLL, P.J., M., BERUBE, F.W., ALLENDORF (2007): Identification of management units using population genetic data. *Trends. Ecol. Evol.*, 22(1): 11-16.
- PRENTOVIĆ, R., D., GAČIĆ, D., CVIJANOVIĆ (2012): Agricultural land in Vojvodina as roe deer habitat – hunting – tourism aspect. *Economics of Agriculture*, 4: 603-615.
- RANDI, E., P.C., ALVES, J., CARRANZA, S., MILOŠEVIĆ-ZLATANOVIĆ, A., SFOUGARIS, N., MUCCI (2004): Phylogeography of roe deer (*Capreolus capreolus*) populations: the effects of historical genetic subdivisions and recent nonequilibrium dynamics. *Mol. Ecol.*, 13(10): 3071-3083.
- ROYO, L.J., G., PAJARES, I., ALVAREZ, I., FERNÁNDEZ, F., GOYACHE (2007): Genetic variability and differentiation in Spanish roe deer (*Capreolus capreolus*): A phylogeographic reassessment within the European framework. *Mol. Phylogenet. Evol.*, 42(1): 47-61.
- SAMBROOK, J., D.W., RUSSEL (2001): Molecular cloning, a laboratory manual. Third edition. Cold Spring Harbor Laboratory, New York
- SCHREGEL, J., A., KOPATZ, S.B., HAGEN, H., BRØSETH, M.E., SMITH, S., WIKAN, I., WARTIAINEN, P.E., ASPHOLM, J., ASPI, J.E., SWENSON, O., MAKAROVA (2012): Limited gene flow among brown bear populations in far Northern Europe? Genetic analysis of the east–west border population in the Pasvik Valley. *Mol. Ecol.*, 21(14): 3474-88.

ZACHOS, F.E., S.S., HMWE, G.B., HARTL (2006): Biochemical and DNA markers yield strikingly different results regarding variability and differentiation of roe deer (*Capreolus capreolus*, Artiodactyla: Cervidae) populations from northern Germany. *J. Zool. Syst. Evol. Res.*, 44(2): 167-174.

**PROSTORNI OBRAZAC GENETIČKE VARIJABILNOSTI SRNDAĆA
NA TERITORIJI AUTONOMNE POKRAJINE VOJVODINA**

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Izvod

Genetička varijabilnost predstavlja evolucionni potencijal vrsta da odgovore na stalno promenljive uslove životne sredine. U ovom radu sagledana je prostorna distribucija genetičke varijabilnosti vrste *Capreolus capreolus* sa teritorije Autonomne Pokrajine Vojvodine, analizom varijabilnosti sekvenci fragmenta kontrolnog regiona mitohondrijalne DNK. Analizom je obuhvaćeno 103 jedinke sa 11 različitih lokaliteta. Otkriveno je 16 haplotipova, sa vrednošću diverziteta od 0.898, dok su vrednosti diverziteta nukleotida i prosečnog broja nukleotidnih razlika iznosile: $\pi = 0.012$ i $k = 5.876$. Uočeno je prisustvo dve genetički i geografski diferencirane grupe, označene kao istočna i zapadna. Diverzitet haplotipova bio je veći u istočnoj grupi, dok je diverzitet nukleotida i prosečan broj nukleotidnih razlika bio veći u zapadnoj grupi.

Primljeno 10.IV 2019.

Odobreno 18. X 2019.