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# GENETIC DIVERSITY OF BELA KRAJINA PRAMENKA COMPARED TO THREE CROATIAN SHEEP BREEDS – A PRELIMINARY STUDY

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Original scientific paper

***SUMMARY***

***In the Balkan Peninsula, different populations of Pramenka were the most common sheep breed. Due to different ecosystems and climate environment, these sub-populations have evolved genetic differences. The largest influence on the local genotype of Bela Krajina Pramenka in Slovenia based on the literature data could have Lika Pramenka from the neighbouring Croatia and some other Pramenka breeds from Bosnia and Herzegovina. Herein, genetic diversity within Slovenian Bela Krajina Pramenka sheep (BP) compared to three Croatian breeds, Cres island sheep (CRE), Lika Pramenka sheep (LIK), and Istrian Pramenka (IST) was studied using ten microsatellite markers. Bela Krajina Pramenka had relatively high genetic diversity shown by the mean number of alleles per microsatellite locus (7.20±2.04) and expected heterozygosity (0.72±0.03). The delta K method reviled four clusters as the most appropriate fit. The STRUCTURE software formed four unique distinct clusters equal to the actual number of analysed populations. Therefore, Bela Krajina Pramenka was found to be an authentic breed based on ten microsatellite markers and compared to three geographically closest sheep breeds. Some admixture among the included populations was found as well.***

***Key-words: Bela Krajina Pramenka, microsatellite markers, genetic diversity***

## INTRODUCTION

In the countries of the former Yugoslavia, different populations of Pramenka were once the most common sheep breeds. In the central Europe it was known also as »Zackel« (Drăgănescu & Grosu, 2010). These sub-populations have evolved genetic differences due to different ecosystems and climate environment and they were usually named the region or village (Mitić, 1984). In the sixties of the previous century Muck (1956) described three major sheep breeds in Slovenia; Pramenka (Bovška, Istrian, Bela Krajina), Jezersko-Solčava and Bergamasca. Zagožen (1984) stated that the Pramenka was a descendant of the Balkan mouflon crossed with the wild Asian steppe sheep.

The largest influence on the local genotype of Bela Krajina Pramenka in Slovenia could have Lika Pramenka from the neighbouring Croatia and some other Pramenka populations from Bosnia and Herzegovina (Grabrijan, 1997). Today, Bela Krajina Pramenka is one of the four Slovenian autochthonous sheep breeds, besides Jezersko-Solčava sheep, Bovška sheep and Istrian Pramenka. The Bela Krajina Pramenka is widespread in the Southeast part of Slovenia near the river Kolpa, mainly for lamb production. Total population was estimated at 900 purebred animals in the year 2014. Therefore, the breed is endangered by the national rules. Lika Pramenka, the most similar to Bela Krajina Pramenka by type traits and purpose, is reared across the border, in Croatia, on the pastures of Lika and Gorski Kotar. Istrian Pramenka is an indigenous breed of the Northern Adriatic area. Today, the initially transhumant Istrian Pramenka population is fragmented in separate populations in Slovenia, Croatia and Italy. Cres island sheep is a dual-purpose breed used mostly for meat production (Salamon et al., 2015).

The knowledge on the genetic diversity of breeds such as Bela Krajina Pramenka is of high importance for conservation of endangered populations. However, other

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three Slovenian autochthonous sheep breeds (Jezersko-Solčavska sheep, Bovška sheep, Istrian Pramenka) were included in genetic analyses before (Kavar et al., 2002; Dalvit et al., 2008). Istrian Pramenka from Slovenia was precisely compared with Croatian population of Istrian Pramenka (Salamon et al., 2015) as well. Numerous studies describe different sheep breeds using microsatellite markers to assess genetic diversity. Peter et al. (2007) explained genetic diversity of 57 European and Middle-Eastern sheep breeds, while Ćinkulov et al. (2008) studied genetic diversity and structure of seven West Balkan Pramenka population. Bela Krajina Pramenka has never been included in such genetic analyses. Therefore, the aim of the present study was to provide a preliminary research in understanding genetic diversity of Bela Krajina Pramenka compared to the three Croatian sheep breeds.

## MATERIAL AND METHODS

### Animals and DNA extraction

Blood samples of Bela Krajina Pramenka sheep (BP; n = 29) from Slovenia as well as blood the ones of the three Croatian breeds, Cres island sheep (CRE; n = 25), Lika Pramenka sheep (LIK; n = 25), and Istrian Pramenka (IST; n = 35) were collected. Animals of BP and IST breed were unrelated within the sampled group and originated from 12 and 18 flocks, respectively while animals of CRE and LIK originated from one flock each. A total of 114 animals were included in the analysis. The DNA was isolated from blood samples using Blood Genomic DNA Kit (GenEltueTM; Sigma-Aldrich®, St. Louis, MO, USA). For the initial selection of microsatellite markers, multiplex reactions were optimized using fluorescent-labelled primers and hot-start polymerase (JumpStart™ REDTaq® ReadyMix™; Sigma-Aldrich®, St. Louis, MO, USA). Ten markers had been selected from the sheep diversity list recommended by the Food and Agriculture Organization of the United Nations (FAO, 2011). Diluted PCR products were processed in a 16-capillary electrophoresis ABI3130XL Genetic Analyser, with two of the PCR-multiplex reactions. Genetic diversity parameters were estimated for ten microsatellite loci.

### Data analysis

Allele frequency, the mean number of alleles (MNA), polymorphic information content (PIC), observed heterozygosity (Ho) and heterozygosity expected (He) under the Hardy-Weinberg (HWE) equilibrium assumption across the markers and the populations were calculated using the Excel Microsatellite Toolkit v. 3.1 (Park 2001). Genetic variation and the distribution of genetic diversity among and within the groups were determined by the analysis of molecular variance (AMOVA) using the GenAlEx 6.501 Software (Peakall and Smouse, 2012). Individual assignment in the populations was investigated using the STRUCTURE software 2.3.1 (Pritchard et al., 2000). We performed 10 runs to choose the appropriate number of inferred clusters (K), fitting K from 1 to 5. The burn-in period for all runs was 35 000 iterations, and data were collected during the period of 15 000 iterations. To choose the optimal K, the delta K method was used (Evanno et al., 2005).

## RESULTS AND DISCUSSION

A total of 106 different alleles were found in 114 genotyped individuals. The average number of alleles per locus was 10.60. The highest number of detected alleles recorded was 18 for marker HUJ616. The PIC values per marker varied from 0.543 for MAF214 to 0.821 for HH47 (Table 1).

**Table 1. Genetic diversity parameters estimated for ten microsatellite loci**

| Marker | A | Ho | He | HWE | Fst | Fis | PIC |
|---|---|---|---|---|---|---|---|
| CP34 | 6 | 0.735 | 0.763 | n.s | 0.002 | 0.050 | 0.724 |
| JMP58 | 10 | 0.770 | 0.840 | p=0.0008 | 0.140* | -0.016 | 0.818 |
| JMP29 | 15 | 0.763 | 0.787 | n.s | 0.106* | -0.056 | 0.753 |
| BM8125 | 8 | 0.696 | 0.682 | n.s | 0.109* | -0.078 | 0.647 |
| DYMS1 | 11 | 0.726 | 0.723 | n.s | 0.059* | -0.035 | 0.698 |
| VH72 | 8 | 0.737 | 0.793 | n.s | 0.125* | -0.028 | 0.763 |
| MAF214 | 7 | 0.477 | 0.598 | p=0.0053 | 0.133* | 0.194* | 0.543 |
| MCM140 | 10 | 0.781 | 0.789 | n.s | 0.055* | -0.033 | 0.757 |
| HH47 | 13 | 0.761 | 0.843 | p=0.0017 | 0.109* | 0.027 | 0.821 |
| HUJ616 | 18 | 0.639 | 0.737 | n.s | 0.023* | 0.191* | 0.704 |
| Overall | 106 | 0.708 | 0.756 | | 0.087 | 0.020 | |

A - number of alleles per locus, Ho - average observed heterozygosity, He - average expected heterozygosity, HWE – significant deviation from the Hardy-Weinberg equilibrium (n.s. - not significant), Fst - genetic difference among populations, PIC - polymorphic information content, Fis = coefficient of inbreeding (estimates and significance of the deviation of HW equilibrium per population across the 10 loci),* $P<0.01$

In the global population, and accounting for the multiple tests performed (ten loci, four populations), three loci were found to be in Hardy-Weinberg disequilibrium. The AMOVA analysis showed a significant and higher source of variation within (89.52%) than among (8.67%) the populations. Estimated inbreeding coefficients (Fis) were estimated for each locus in the global population. The Fis values for the markers ranged from -0.078 (BM8125) to 0.194 (MAF214). Moreover, two markers (MAF214 and HUJ616) showed positive and significant ($P<0.01$) Fis values.

Genetic diversity of the Bela Krajina Pramenka was relatively high (Table 2), as shown by the mean number of alleles per microsatellite locus (7.20 ± 2.04) and mean expected heterozygosity (0.72 ± 0.03). The observed heterozygosity was very similar between Bela Krajina Pramenka (0.72 ± 0.03) and Lika Pramenka (0.72 ± 0.02), followed by Istrian Pramenka (0.71 ± 0.028), while Cres Pramenka (0.66 ± 0.06) had the lowest Ho. The largest difference between Ho and He was found for Cres island sheep.

**Table 2. Within-population genetic diversity parameters derived from ten microsatellites**

| Population | n | Ho | He | MNA |
|---|---|---|---|---|
| Bela Krajina Pramenka | 29 | 0.72 ± 0.03 | 0.72 ± 0.03 | 7.20 ± 2.04 |
| Cres island sheep | 25 | 0.66 ± 0.06 | 0.71 ± 0.03 | 5.90 ± 2.02 |
| Lika Pramenka | 25 | 0.72 ± 0.02 | 0.71 ± 0.03 | 7.60 ± 1.71 |
| Istrian Pramenka | 35 | 0.71 ± 0.03 | 0.69 ± 0.03 | 6.00 ± 1.15 |

n - sample size, Ho – average observed heterozygosity (± SD), He - average expected heterozygosity (± SD), MNA - mean number of alleles

Expected heterozygosities obtained in this study were lower than reported by Ćinkulov et al. (2008) based on 15 microsatellites in Pramenka populations, where the lowest He was 0.74 in Karakacanska Pramenka from Macedonia and the highest 0.81 for Recka Pramenka from Albania. The aforementioned study reported higher Ho in Istrian Pramenka as well (0.76). We found lower expected heterozygosity value for Bela Krajina Pramenka than it was reported for Bovška (0.74) and Jezersko-Solčava sheep (0.76) in the study of Alpine sheep breeds (Dalvit et al., 2008). Much lower population size of Bela Krajina Pramenka compared to Jezersko-Solčava sheep could explain this result.

This result concur with geographical diversity pattern reported for 57 European and Middle-Eastern sheep breeds (Peter et al., 2007). As expected, Swiss high production breeds (Glowatzki-Mullis et al., 2009) showed lower diversity compared to Pramenka sheep populations (Ćinkulov et al., 2008). Expected heterozygosity values of Bela Krajina and Lika Pramenka were obtained between the two aforementioned groups.

To choose the most appropriate number of clusters the method of Evanno et al. (2005) was used. Based on the highest value of ΔK (0.544), the most likely number of clusters (K) was ascertained to be four, being equal to the actual number of the analysed populations. Using $K = 4$ in the STRUCTURE software, four unique, distinct clusters were formed (Figure 1). Each colour represents one cluster and the length of the bar represents the individual's estimated proportion of membership in the cluster. Therefore, Bela Krajina Pramenka was found to be an authentic breed based on ten microsatellite markers and compared to three geographically closest sheep breeds (Lika, Cres and Istrian Pramenka). However, we found some of the animals in all included populations to be admixed, which could be a consequence of geographical proximity.

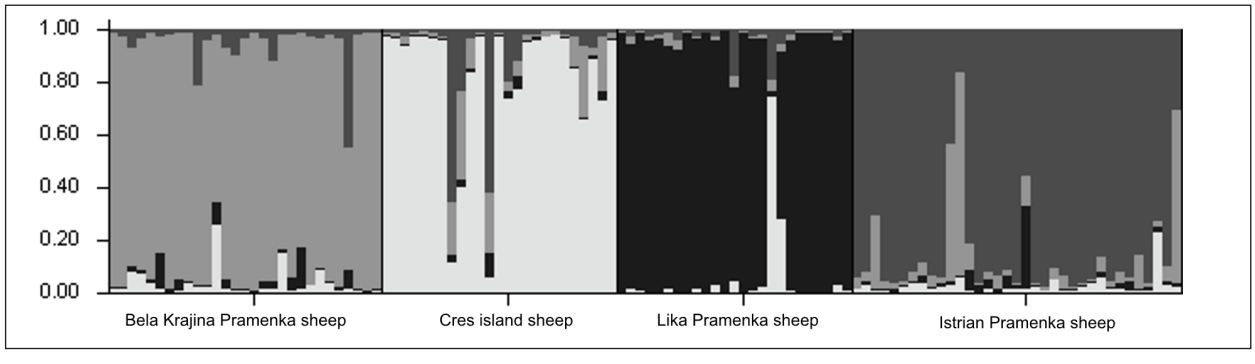


**Figure 1. The population structure of four sheep breeds**

## CONCLUSION

Due to large influence of neighbouring Pramenka populations, genetic diversity of Bela Krajina Pramenka was studied. This study found considerable genetic diversity in the population of Bela Krajina Pramenka, very similar to Lika Pramenka. Despite the geographical proximity and similar type traits, Bela Krajina Pramenka and Lika Pramenka in this study, were considered as two separated breeds. To confirm results from this preliminary study, further analysis with larger number of markers and sheep populations are necessary and recommended.

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