

POPULATION-GENETICAL CHARACTERISTICS OF THE BEE POPULATION OF VOJVODINA

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In 1893. Jovan Živanović notes that based on the color of the abdomen of bees, two varieties of bees are present in Vojvodina, one dark and the other with two yellow rings on abdomen. Up to the present time there are mixed opinions about the presence of one or more populations of bees in Vojvodina, especially about the presence of the yellow variety in Banat. The aim of this paper is to establish the existence of one or more populations of bees, using genetic analysis of bees. Evaluation of genetic connections, diversity within the population and structure of the bee population in Vojvodina, were calculated on the basis of allele variation of 25 microsatellite loci. A genetic typification of the following microsatellites was performed: *A8, A14, A24, A29, A43, A79, A88, A113, Ac11, Ac88, Ac139, Ac306, Ap15, Ap68, Ap85, Ap90, Ap223, Ap224, Ap226, Ap249, Ap273, Ap274, Ap288, At168, At188*. 92% or 23 loci proved to be polymorphic in samples of bees from Srem and Bačka, and 88% or 22 loci proved to be polymorphic in samples of bees from Banat. Heterozygosity calculated for the whole population is not significantly different from the expected heterozygosity. It was found that the obtained genetic differences between bees of Srem and Bačka, and Banat region are not sufficient for these two populations to be considered separate.

Key words: *A. m. carnica*, alleles, bee, genetic analysis

INTRODUCTION

In the literature and magazines since the second half of the nineteenth century, opinion can be encountered that the bees in the area of the Pannonian plain are different from the bees

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from the mountain landscape to the west of this area, which belong to the race of *Apis mellifera carnica*.

JOVAN ŽIVANOVIĆ, at the end of the 19th century, states that in Srem two bee varieties are present: one is dark, while the other has two yellow rings on abdomen and is similar to the Italian bee (*Apis mellifera ligustica*). At the beginning of the 20th century, BUTTEL-REEPEN, believe that Banat bee shows hybrid characteristics between *Apis mellifera carnica*, *Apis mellifera mellifera* i *Apis mellifera ligustica*, and therefore cannot be set aside as a separate race. Grozdanić comparing Banat bee with *Apis mellifera carnica*, *Apis mellifera cypria*, *Apis mellifera ligustica* i *Apis mellifera syriaca* races, notes that in the area of Banat and in the surrounding areas there is one "yellow" bee form, which cannot be described as any one of the existing Mediterranean and Anatolia form of bees. Citing the results of their research, the same author suggests that this bee should be set aside as a special race and should be named *Apis mellifera banatica*. However about the mid of 20th century Goetze states that the Banat bee is probably a local form of some "major bee race" (PIHLER, 2012).

NEDIĆ (2007), stating Goetze, that the area of the Alps to the Carpathians, and south to the Vardar is the area of distribution for *Apis mellifera carnica* and that yellow bees of Pannonian plain aren't formed by breeding with *Apis mellifera ligustica*, but represent Kranjska bee conditioned by habitat.

Lacking the studies (most of the conclusions are made on the basis of different color of bees), there are very different opinions about the taxonomic place of bees from the Pannonian plain, especially the yellow Banat bee form. This has led the Krunić (sixties of the 20th century) to carry out extensive morphological examination of bees from this region. Examining the cubital index, hantel index, radial index, precubital index, the number of rear wing hooks of working bees, the length of the tongue, scutellum coloring and hair coloring of the thorax, Krunić comes to the conclusion that the bees from the area of Vojvodina and Slavonia, regardless of color, differ from *Apis mellifera carnica* in most morphological characteristics, and represent a homogenous entirety, and can therefore be set aside as a special race, and he suggests the *Apis mellifera Panonica* name (NEDIĆ, 2009).

RUTTNER (1988) examining the taxonomy of honeybees, lists Banat bee only as a variety of *Apis mellifera carnica banatica*, citing studies from Adam in 1983. year, which found that in this variety the occurrence of yellow rings on abdomen is only more frequent. Morphometric study of bees from the area of the Pannonian plain has not ended with this, so GAJGER *et al.* (2007), examined the cubital index of continental Croatian bees, stating that its value ranged from 1.4 to 3.9, and that only 25.6% of bees belong to Kranjska bee race, while 61.8% of bees are in the zone of overlapping with other races. RADOŠ *et al.* (2008), examining the morphometry of yellow honey bee in Vojvodina, reveals that the values are slightly lower than is the case of morphometry of bees in central Serbia. STANIMIROVIĆ *et al.* (2005), during the study of chromosomes of Banat bee ecotype (from the vicinity of Belgrade), Sjeničko-Pešterski (from Sjenica-Pešter) and Timok ecotype (from Timok), points to a strong inter-ecotypic chromosomal polymorphism of *A. m. carnica* in Serbia.

MATERIALS AND METHODS

Samples of bees were collected from nine locations in Vojvodina (northern part of Serbia) and with three locations in Bačka, Banat and Srem (Table 1). On each of the nine sites, samples were taken from two colonies with frames with sealed brood.

During the sampling of bees in all nine sites, GPS coordinates were recorded.

Genetic analyzes of bees were conducted on Faculty of Agricultural Sciences, Aarhus University in Denmark, Research Centre Flakkebjerg.

DNA isolation was carried out using standard procedures (WALSH *et al.*, 1991). Individual PCR microsatellite amplification was performed using Thermal cycler.

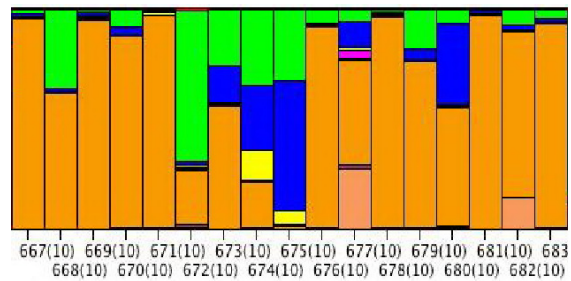
Table 1. Locations from which the bee samples were collected, with the names of the owners, the places where the samples were taken and GPS coordinates of hives.

Locality	Number of samples	GPS coordinates
Kikinda (Banat)	(3 i 4)	45 ⁰ 47,650'N; 20 ⁰ 26,350'E
Janošik (Banat)	(5 i 6)	45 ⁰ 10,309'N; 21 ⁰ 00,364'E
Mužljanski rit (Banat)	(7 i 8)	45 ⁰ 15,484'N; 20 ⁰ 19,661'E
Savino selo (Bačka)	(1 i 2)	45 ⁰ 30,303'N; 19 ⁰ 31,614'E
Bogojevo (Bačka)	(9 i 10)	45 ⁰ 31,062'N; 19 ⁰ 06,636'E
Bajmok (Bačka)	(11 i 12)	45 ⁰ 59,815'N; 19 ⁰ 24,543'E
Susek (Srem)	(13 i 14)	45 ⁰ 10,794'N; 19 ⁰ 31,475'E
Stari Slankamen (Srem)	(15 i 16)	45 ⁰ 08,221'N; 20 ⁰ 15,640'E
Adaševci (Srem)	(17 i 18)	45 ⁰ 02,576'N; 19 ⁰ 15,859'E

Review of genetic parameters bee samples from Vojvodina was calculated on the basis of variations of 25 microsatellite loci (MEIXNER *et al.*, 2013). Genetic typification was performed on the following primers: *A8, A14, A24, A29, A43, A79, A88, A113, Ac11, Ac88, Ac139, Ac306, Ap15, Ap68, Ap85, Ap90, Ap223, Ap224, Ap226, Ap249, Ap273, Ap274, Ap288, At168, At188*.

Genetic analyzes were visualized by ABI310 DNA sequencioner. Determination of allele frequencies was performed by direct measurement of the allelic frequencies for each locus; Inter-population genetic variations of microsatellites were determined based on the average heterozygosity and average expected heterozygosity (NEI, 1987), the average number of alleles per locus and percentage of locus polymorphism. Loci are considered polymorphic when the frequency of the predominant allele is less than 0.95.

The existence of subpopulations within populations were examined using Wright's index by using WEIR and COCKERHAM (1984) method, based on the variances. Means of F-static parameters were obtained for each locus using the jackknife procedure (WEIR, 1996). The significance of the estimated F-statistic parameters was determined by permutations of alleles within the whole population, genotypes within the whole population and alleles within the sample.



Picture 1. Genetic analysis of Vojvodina bee samples, visualized by ABI310 DNA sequencer (*Samples in sequencer in the following order : Savino Selo 1-(667), Savino Selo 2-(668), Kikinda 3-(669), Kikinda 4-(670), Janošik 5-(671), Janošik 6-(672), Mužljanski rit 7-(673), Mužljanski rit 8-(674), Bogojevo 9-(675), Bogojevo 10-(676), Bajmok 11-(nije uspela analiza) Bajmok 12-(677), Susek 13-(678), Susek 14-(679), Stari Slankamen 15-(680), Stari Slankamen 16-(681), Adaševci 17-(682), Adaševci 18-(683).*)

RESULTS AND DISCUSSION

Determination of the genetic parameters of samples of bees from Vojvodina was calculated on the basis of variations in 25 microsatellite loci. Genetic typification was performed with the following primers: *A8, A14, A24, A29, A43, A79, A88, A113, Ac11, Ac88, Ac139, Ac306, Ap15, Ap68, Ap85, Ap90, Ap223, Ap224, Ap226, Ap249, Ap273, Ap274, Ap288, At168, At188.*

92% or 23 loci proved to be polymorphic in samples of bees from Srem and Bačka, and 88% or 22 loci proved to be polymorphic in samples of bees from Banat. Loci are considered to be polymorphic when the predominant allele frequency is less than 0.95. Total of 121 alleles was detected. The highest number of alleles - 17, was found on the locus *A29*, and the lowest - 2, at loci *Ap249, Ap273, Ap288, Ap274*

Table 2. Average calculated (H_{obs}) and expected (H_{exp}) heterozygosity.

Region	H_{obs}	Standard error	H_{exp}	Standard error
Srem and Bačka	0.419	0.055	0.432	0.052
Banat	0.432	0.067	0.411	0.055
Whole population	0.426	0.043	0.421	0.037

Calculated heterozygosity (Table 2) in the whole population is not significantly different from the expected heterozygosity. The average calculated heterozygosity of the Banat bee population was slightly higher than the average expected heterozygosity, while the average calculated heterozygosity for the bee population in Srem and Bačka was slightly smaller than the average expected heterozygosity.

In order to present more clearly the population-genetic structure of analyzed bee population in Vojvodina and determine the possible existence of subpopulations within a population, F statistic parameters were calculated (Table 3). These parameters can be presented as a measure of correlation of alleles of an individual and are related to the coefficient of consanguinity, which shows the degree of non-random connection of alleles of an individual. As such, the F-statistic parameters show the effect of consanguinity within subpopulations (F_{IS}), among subpopulations (F_{ST}), and within the whole population (F_{IT}) (MURRAY, 1996).

Table 3. The results of F-statistic parameters for each locus individually and an average

Locus	F_{IS}	F_{IT}	F_{ST}
A8	0.204	0.258	0.067
A14	-0.094	-0.067	0.025
A24	-0.059	-0.022	0.035
A29	-0.087	-0.042	0.042
A43	-0.060	-0.029	0.029
A79	-0.086	-0.029	0.053
A88	0.208	0.212	0.006
A113	-0.084	-0.065	0.018
Ac11	-0.060	-0.035	0.023
Ac88	-0.073	-0.035	0.035
Ac139	0.098	0.212	0.127
Ac306	-0.108	-0.081	0.025
Ap15	-0.097	-0.085	0.010
Ap68	-0.119	-0.068	0.045
Ap85	-0.042	-0.008	0.032
Ap90	-0.162	-0.119	0.037
Ap223	0.129	0.245	0.133
Ap224	0.237	0.258	0.027
Ap226	-0.274	-0.201	0.058
Ap249	0.248	0.257	0.013
Ap273	-0.091	-0.043	0.043
Ap274	-0.048	-0.023	0.023
Ap288	1.000	1.000	0.091
At168	-0.252	-0.178	0.060
At188	0.351	0.355	0.006
Mean	0.027	0.067	0.043
Standard error	0.052	0.050	0.007

The average values of fixation indexes, derived by microsatellite allele frequencies of 25 bees from the Vojvodina territory, were as follows: $F_{IS} = 0.027$; $F_{IT} = 0.067$ i $F_{ST} = 0.043$. Based on the calculated F_{ST} value, we see that 4.3% of the total genetic variation can be attributed to differences among populations, while individual differences make 89.5%.

According to HARTL and CLARK (1989), the calculated value of F_{ST} shows us that the genetic differences between populations were very small, and that it is most likely to be one bee population.

Based on the results obtained in this study, it can be generally concluded that in the sample of the bees from the Vojvodina, there are two types of bees or two separate populations. Genetic differences that were identified between analysed bees in Srem and Bačka and region of Banat were not enough for these two populations to be considered separate.

CONCLUSION

Based on the results obtained by genetic analyses of bees sampled from 9 locations in Vojvodina, based on 25 microsatellite allele frequencies, the following conclusions can be made:

- The calculated heterozygosity (0.426) in the whole population is not significantly different from the expected heterozygosity (0.421). Expected heterozygosity values in Srem and Bačka bees (0.432) and bees from the Banat region (0.411) were not significantly different from the calculated (0.419 and 0.432).

- Based on the average values of fixation indexes of Vojvodina bees, $F_{IS} = 0,027$; $F_{IT} = 0.067$, we can conclude that a high degree of consanguinity is present in both populations.

- Based on the calculated F_{ST} – value of 0.043 for the bees of Vojvodina, we conclude that the genetic differences between populations were very small, and that it is most likely to be one bee population.

It may be concluded that in the sample of the Vojvodina bees, there are no two types of bees or two separate populations. Identified genetic differences between analysed bees in Srem and Bačka and region of Banat are not sufficient for these two populations to be considered separate.

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REFERENCES

- BUCHLER, R., S. ANDONOV, K. BIENEFELD, C. COSTA, F. HATJINA, N. KEZIĆ, P. KRYGER, M. SPIVAK, A. UZUNOV, J. WILDE (2013): Standard methods for rearing and selection of *Apis mellifera* queens. [Journal of Apicultural Research](#); 52(1):1-29.
- GAJGER, T.I., Ž. MATAŠIN, Z. PETRINEC (2007): Bee wing index in continental Croatia Conference on Native Breeds and Varieties as part of Natural and Cultural Heritage 13. – 16. Novembr 2007, Šibenik
- HARTL, D.L., A.G. CLARK (1989): Principles of population genetics. Sinauer Associates, Inc., Sunderland, USA.
- MEIXNER, D. M., A.M. PINTO, M. BOUGA, P. KRYGER, E. IVANOVA, S. FUCHS (2013): Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. [Journal of Apicultural Research](#); 52 (4), pp.27.
- MURRAY, B.W. (1996): The estimation of genetic distance and population substructure from microsatellite allele frequency data. McMaster University, Ontario, Canada.
- NEDIĆ, N., M. MLADENović, LJ. STANISAVLJEVIĆ (2007): Biological and production characteristics of certain lines of honey bee in Serbia. *Biotechnology in Animal Husbandry* vol. 23 (5-6), pp. 389 – 398.
- NEDIĆ, N., LJ. STANISAVLJEVIĆ, M. MLADENović, J. STANISAVLJEVIĆ (2009): Molecular characterization of the honeybee *Apis mellifera carnica* in Serbia. *Archives of Biological Sciences, Belgrade*, vol.61 (4):587-598.
- NEI, M. (1987): Molecular evolutionary genetics. Columbia University Press, New York, USA.
- PIHLER, I. (2012): Genetic and morphometric characteristics of two types of cornual bees. PhD thesis, University of Novi Sad, Faculty of Agriculture.

- RADOŠ, RENATA., M. MLADENOVIĆ (2008): Morphometric characteristics of yellow honey bee in Vojvodina. XVI scientific conference "Quality and selection in beekeeping to Europe", 9 - 10. February 2008., Faculty of agriculture, Belgrade, Book of plenary lecture, pp. 21 - 25.
- RUTTNER, F. (1988): Biogeography and Taxonomy of Honeybees. Springer – Verlag, Berlin.
- STANIMIROVIC, Z., J. STEVANOVIC, M. ANDJELKOVIC (2005): Chromosomal Diversity in *Apis mellifera carnica* from Serbia, *Apidologie*, 2005, vol. 36, no. 1, pp. 31–42.
- WALSH, PS, DA METZGER, R. HIGUCHI (1991): Chelex 100_ as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10:506–513.
- WEIR, B.C., C.C. COCKERHAM (1984): Estimating F-statistics for the analysis of population structure. *Evolution*, 38, 1358-1370.
- WEIR, B.C. (1996): Genetic data analysis. Sunderland, MA: Sinauer.

GENETIČKO-POPULACIONE KARAKTERISTIKE PČELA VOJVODINE

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Izvod

Još 1893. Jovan Živanović na osnovu boje abdomena pčela, konstatuje da su na području Vojvodine prisutna dva varieteta pčela, jedan tamni i drugi sa dva žuta prstena na abdomen. Sve do današnjeg vremena postoje podeljena mišljenja oko prisutnosti jedne ili više populacija pčela u Vojvodini naročito oko prisutnosti žutog varieteta u Banatu.

Cilj ovog rada je da se pomoću genetskih analiza pčela utvrdi postojanje jedne ili više populacija pčela.

Ocena genetičke povezanosti, unutar populacijska raznolikost i struktura populacije pčela u Vojvodini, izračunata je na bazi varijacije alela 25 lokusa mikrosatelita. Izvršena je genetska tipizacija sledećih mikrosatelita: A8, A14, A24, A29, A43, A79, A88, A113, Ac11, Ac88, Ac139, Ac306, Ap15, Ap68, Ap85, Ap90, Ap223, Ap224, Ap226, Ap249, Ap273, Ap274, Ap288, At168, At188. 92% ili 23 lokusa su se pokazali kao polimorfni u uzorcima pčela iz Srema i Bačke, a 88% ili 22 lokusa su se pokazali kao polimorfni u uzorcima pčela iz Banata. Izračunata heterozigotnost na nivou cele populacije se nije statistički značajno razlikovala od očekivane heterozigotnosti. Utvrđeno je da dobijene genetičke razlike između analiziranih pčela iz regiona Srema i Bačke i regiona Banata nisu dovoljne da se ove dve populacije mogu smatrati razdvojenim.

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