Applied Zoology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 67 (4), 2015: 567-572

Allozyme Analysis of Selectively Reared Populations of Honey Bee, *Apis mellifera* Linnaeus, 1758, in Kosovo

Valentina Peševa[1], Ivan Y. Stoyanov[2], Bojan Andjelkovic[3], Mića Mladenović[4], Vida H. Georgieva[2], Evgeniya N. Ivanova[2*]

[1] Faculty of Agriculture, University of Priština, 38219 Lešak, Serbia
[2] Section of Genetics, Department of Developmental Biology, Plovdiv University „Paisii Hilendarski", 24 Tzar Assen Street, 4000 Plovdiv, Bulgaria.
[3] Institute for Forage Crops, Kruševac, 37215 Globoder, Serbia
[4] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Belgrade, Serbia

Abstract: The genetic variability of selectively reared honey bees *Apis mellifera* from Kosovo was studied within and among populations. We used six alloenzymic systems (MDH-1, ME, EST-3, ACP, PGM and HK), which corresponded to six loci. Five of the studied enzyme systems. *i.e.* MDH 1, ACP, EST, PGM and HK, manifested intra- and inter-population polymorphism with two or three alleles. ME locus was monomorphic in all of the tested honey bee populations. The observed and expected heterozygosities ranged from 0.104 to 0.164 and from 0.093 to 0.130, respectively. The percentage of polymorphic loci among the studied honey bee populations from Kosovo was between 16.7% and 50%. The calculated NEI's genetic distance ranged between 0.001 and 0.019. The estimated mean F_{ST} value from allozyme data was 0.028.

Key words: *Apis mellifera*, allozymes, genetic variability, Kosovo

Introduction

The biodiversity and conservation status of the European subspecies of honey bee have been recently reviewed by De la Rúa *et al*. (2009), Meixner *et al*. (2009) and Francis *et al.* (2014). Due to commercially attractive traits as high honey production and gentleness, the subspecies *A. m. carnica* Pollman, 1879 is now mainly spread across the Central and Eastern Europe on the territory of Austria, Slovenia, Croatia, Bosnia and Herzegovina, Albania, Serbia (including Kosovo), Hungary and Romania (Ruttner 1988). According to Mladenović, Simeonova (2010), honey bee breeding in Kosovo is based on rearing *Apis mellifera carnica*. At the same time, "*carnica – macedonica*" hybrid zones in the southern part of Serbia (Stevanovic *et al*. 2010, Muñoz *et al*. 2012) and the presence of *A. m. macedonica* Ruttner, 1988 populations in Macedonia and probably in some parts of Albania (Uzunov et al., 2014) are discussed.

Although different genetic tools (classical and geometric morphometry, allozyme electrophoresis, mitochondrial and microsatellite DNA analyses) are applied to study the genetic diversity of honey bees in Europe (Meixner *et al*. 2013), until now mainly classical morphometry has been used for characterisation of the populations of *A. mellifera* from Kosovo (Grozdanić 1926, Mladenović, Simeonova 2010). Recently, some *A. m. carnica* populations from Serbia, Montenegro and Poland were studied using alloenzyme analysis (Ivanova 2010, Ivanova *et al*. 2012) but there is not any information about alloenzymic genetic variability in populations of honey bee from Kosovo. In this study, honey bee populations identified as *A. m. carnica* by

*Corresponding author: e.ivanova@gmail.com, geneiv@uni-plovdiv.bg

applying classical morphometry were studied using polyacrylamide gel electrophoresis on six gene-enzyme systems. These populations are under selective control in Kosovo. The purpose of the study was to examine genetic variability in these populations using allozyme analysis. Data obtained in the study could be useful for honey bee selection and conservation on the territory of the Balkan Peninsula.

Materials and Methods

Honey bee samples were collected during the summer of 2013 from managed colonies in Kosovo: Kosovsko Pomoravlje with locations Budriga (Bd) and Pasjane (Ps); Central Kosovo and Metohija with locations Laplje Selo (Lps) and Livadje (Lv), and Northern Kosovo and Metohija with locations Lešak (Ls) and Slatina (Sl) (Fig. 1). They were identified as *A. m. carnica* using classical morphometry.

Worker bees from each location were transported to the laboratory alive and stored at -20^{0}C. Five colonies per population, 10–12 honey bees per colony, in total 322 worker bees per allozyme systems were tested in the study. The thorax and the whole body homogenisation and electrophoresis in polyacrylamide gel were performed according to standard methods for characterising subspecies and ecotypes of *Apis mellifera* (see Meixner *et al*. 2013).

Six enzymic systems corresponding to six loci were studied: MDH (Malate dehydrogenase, EC 1.1.1.37); ME (Malic enzyme, EC 1.1.1.40); EST (Esterase, EC 3.1.1); ACP (Acid phosphatase, EC 3.1.3.2); PGM (Phosphoglucomutase, EC 5.4.2.2) and HK (Hexokinase, EC 2.7.1.1). Buffers, electrophoretic conditions for each of the used enzymic systems and visualisation of enzyme activities were done following Staykova *et al.* (2010) and Meixner *et al*. (2013).

GenAlEx 6.5 (Peakall, Smouse 2012) add-on was used in Excel (Microsoft) to calculate frequency-based statistics and population assignment, which gives information on genetic determination of populations based on heterogeneity within and among them. Polymorphism in different allozyme systems was considered at the 99% level in order rare alleles to be characterised and compared. Mean number of alleles per locus, proportion of polymorphic loci at the 95% level, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium and Nei's genetic distance (D) (NEI 1972), were calculated using BIOSYS-1 (Swofford & Selander 1981).

Allele frequencies, level of polymorphism and heterozigosity calculated previously (Ivanova *et al*. 2012) concerning *Apis mellifera carnica* and *Apis mellifera macedonica* populations from Montenegro (Bijelo Polje and Sutomore), Serbia (Vršac and Vranje) and Bulgaria (Plovdiv and Smolyan) were used as referent data for comparison with populations of honey bee from Kosovo. Referent data were marked with * in the text and in the tables.

Results

We found that five of the studied enzyme systems (MDH-1, EST-3, ACP, PGM and HK) were polymorphic in almost all the studied enzyme systems were polymorphic in most of the populations, having three to six different alleles (Table 1). ME locus was monomorphic in all of the tested honey bee populations. In total, two alleles were detected at MDH-1 (MDH^{65} and MDH^{100}), ACP (ACP^{80}, ACP^{100}) and PGM (PGM^{100} and PGM^{114}) loci and three alleles at EST-3 (EST^{80}, EST^{100} and EST^{118}) and HK (HK^{87}, HK^{100} and HK^{121}) loci.

In the present study, mean number of alleles per locus in Bulgarian populations varied between 1.8 (Razgrad) and 3.5 (Burgas). (Ls) to 2.0 (Lv). The estimated percentage of polymorphic loci was 16.7% in Bd, Ls and Sl, 33.3% in LpS and Ps and 50% in Lv. The observed and expected heterozygosities were found to range from 0.104 to 0.164 and from 0.093 to 0.130, respectively (Table 2).

There were significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in most populations ($P \geq 0.001$). Chi-Square tests (df: 1–3) showed that the deviations were generally in favour of homozygotes.

The estimated mean F_{ST} value was 0.029 meaning that only 2.9% of the overall genetic diversity observed was among the studied populations, as opposed to 97.1% within them. Summary of population assignment outcomes to 'Self' or 'Other' population show low assignment values (28% – to "self" population and 72% – to "other" populations: Fig. 2).

The values of genetic distance (Nei 1972) were calculated using the allele frequencies and ranged between 0.001 and 0.019 (Table 3).

Discussion

In the present study, five out of the six examined loci were found to be polymorphic (including rare alleles with frequencies less than 5%). In similar studies, in total five alleles at MDH-1 locus were detected (Kandemir *et al*. 2000, Bouga *et al*. 2005, Ivanova *et al*. 2012, Meixner *et al*. 2013, Francis *et al*. 2014) in populations from Europe. In the present

study, we observed two alleles at this locus, MDH^{65} and MDH^{100}; this was in agreement with Dedej *et al.* (1996) who found the same alleles in other studied *A. m. carnica* populations from the Balkans. One more allele (MDH-1^{125}**)** was reported by Ivanova *et al*. (2012) for the Vranje population (Serbia). Similar with referent data for Bulgarian* *A. m. macedonica* populations, for one population of *A. m. carnica* from Montenegro* and one from Serbia*, MDH^{100} was the more common allele in Bd, LpS and Sl populations from Kosovo. MDH^{65} was with higher frequency in Ls, Lv and Ps as it was detected also in Sutomore (Montenegro*) and Vršac (Serbia*; Table 1).

Similar with referent data for Serbian* *carnica* populations (Table 1), in the present study ME locus was found to be monomorphic, while in other European populations three alleles of this locus were detected (ME^{90}, ME^{100} and ME^{106}; see Ivanova *et al*. 2012, Meixner *et al*. 2013). ME locus was found to be invariant for studied bee populations from Turkey (Kandemir *et al*. 2000, 2005), but according to Bouga *et al*. (2005) this locus is polymorphic with two alleles (ME^{100} and ME^{79}) in some honey bee populations from Greece. Ivanova *et al*. (2011) reported two alleles (ME^{100} and ME^{106}) in selectively reared populations of *A. m. carnica* from Poland.

EST-3 locus was polymorphic and exhibited three or more alleles in other European honey bee populations (Bouga *et al*. 2005, Ivanova *et al*. 2010a,b, Ivanova *et al*. 2011, Bouga *et al*. 2011). In the present study, three alleles (EST^{80}, EST^{100} and EST^{118}) were detected in the Pasjane population and two alleles (EST^{100} and EST^{118}) in the other studied honey bee populations from Kosovo. It must be noted that for referent data from Montenegro*, Serbia* and Bulgaria* two additional alleles were reported – EST^{94} and EST^{105} (Table 1).

The ACP locus was found to be polymorphic with two alleles, ACP^{100} and ACP^{80} in Cyprus (Bouga *et al*. 2005) and with three alleles (ACP^{100}**,** ACP^{90} and ACP^{80}) in Bulgaria (Staykova, Ivanova 2011). In the present research ACP locus had also two alleles (ACP^{100} and ACP^{80}) in all of the studied honey bee populations, except Sl where ACP^{100} allele was fixed (Table 1).

According to the reviewed information by Meixner *et al*. (2013), the PGM locus was found as invariant or polymorphic in different studies. In accordance with similar studies on honey bee populations from Serbia, Montenegro, Bulgaria and Greece (Ivanova 2010, Ivanova *et al*. 2010b, 2012), Kosovo PGM locus was found to be also polymorphic with two alleles – PGM^{100} and PGM^{114} (Table 1). Referent data show that PGM^{114} allele is with higher frequencies (0.105 – 0.125) in Sutomore*, Vršac* and Vranje* than in other studied *A. m. carnica* and *A. m. macedonica* populations (Table 1).

HK locus was described as monomorphic for honey bee populations from Europe and as polymorphic (with two to five alleles) in Brazil (Meixner *et al*. 2013) and in Turkey (Kandemir *et al.* 2000, 2005). Ivanova (2010) described a different allele (HK^{121}) in one of the studied Serbian *A. m. carnica* populations (Ivanova 2010). In total, three alleles were found at the HK locus (HK^{87}, HK^{100} and HK^{121}) in the present investigation. HK^{100} was more com-

Fig. 1. Study area: 1-6 – Kosovo (1 – Lesak; 2 – Slatina; 3 – Livadje; 4 – Lapje selo; 5 – Budriga; 6 – Pasjane); 7-8 – Montenegro (7 – Bijelo Polje; 8 – Sutomore); 9-10 – Serbia (9 – Vršac; 10 – Vranje); 11-12 – Bulgaria (11 – Plovdiv; 12 – Smolyan)

Table 1. Allele frequencies in the studied populations. N – number of the samples. Referent data (IVANOVA *et al*. 2012) are marked with *

Locus	Allele	Budriga (Kosovo)	Pasjane (Kosovo)	Laplje Selo (Kosovo)	Livadje (Kosovo)	Lesak (Kosovo)	Slatina (Kosovo)	Bijelo Polje (Montenegro*)	Sutomore (Montenegro*)	Vršac (Serbia*)	Vranje (Serbia*)	Plovdiv (Bulgaria*)	Smolyan (Bulgaria*)
MDH	**N**	54	54	54	53	53	54	54	54	54	53	53	50
	65	0.389	0.509	0.472	0.698	0.519	0.481	0.425	0.595	0.600	0.432	0.36	0.405
	100	0.611	0.491	0.528	0.302	0.481	0.519	0.575	0.405	0.400	0.500	0.64	0.595
	125	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0	0
ME	**N**	54	54	54	53	53	54	54	54	54	53	53	45
	90	0.000	0.000	0.000	0.000	0.000	0.000	0.104	0.043	0.000	0.000	0.034	0
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.833	0.935	1.000	1.000	0.879	0.935
	106	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.022	0.000	0.000	0.086	0.065
EST	**N**	54	54	54	53	53	54	54	54	54	53	53	44
	80	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.025	0.027	0.000	0.00	0.00
	94	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.00
	100	0.991	0.972	0.944	0.953	0.981	0.972	0.923	0.950	0.947	0.925	0.976	0.946
	105	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.00	0.00
	118	0.009	0.019	0.056	0.047	0.019	0.028	0.058	0.025	0.026	0.075	0.008	0.054
ACP	**N**	54	53	54	53	54	54						
	80	0.037	0.009	0.028	0.057	0.009	0.00						
	90	0.963	0.991	0.972	0.943	0.991	1.00						
PGM	**N**	54	54	54	53	53	54	54	54	54	53	53	31
	100	0.972	0.952	1.000	0.962	1.000	0.963	0.921	0.882	0.895	0.875	0.935	0.957
	114	0.028	0.075	0.000	0.038	0.000	0.037	0.079	0.118	0.105	0.125	0.065	0.043
HK	**N**	54	54	54	53	53	54	54	54	54	53	53	52
	87	0.000	0,000	0.000	0.019	0.000	0.028	0.000	0.000	0.000	0.000	0.063	0
	100	0.981	1.000	0.991	0.972	1.000	0.972	0.924	0.919	0.890	0.879	0.896	0.978
	110	0.000	0.000	0.000	0.000	0.000	0.000	0.076	0.081	0.094	0.087	0.042	0.022
	121	0.019	0.009	0.009	0.009	0.000	0.000	0.000	0.000	0.016	0.034	0.00	0.00

Table 2. Mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected heterozygosity (H_e). Referent data (Ivanova *et al*. 2012) are marked with *

Lines	Mean no. of alleles per locus (± s.e)	Percent Polymorphic loci (P=0.95)	H_o	H_e
Budriga (Kosovo)	1.8±0.2	16.7	0.118±0.077	0.113±0.073
Lapje selo (Kosovo)	1.7±0.2	33.3	0.164±0.131	0.111±0.080
Lesak (Kosovo)	1.5±0.2	16.7	0.139±0.128	0.093±0.082
Livadje (Kosovo)	2.0±0.3	50.0	0.119±0.074	0.130±0.063
Pasjane (Kosovo)	1.8±0.3	33.3	0.104±0.082	0.120±0.080
Slatina (Kosovo)	1.7±0.2	16.7	0.141±0.124	0.123±0.078
Bijelo Polje (Montenegro*)	2.5±0.2	100	0.213±0.075	0.306±0.084
Sutomore (Montenegro*)	2.3±0.2	100	0.222±0.074	0.26±0.074
Vrsac (Serbia*)	2.2±0.3	83.3	0.254±0.098	0.245±0.082
Vranje (Serbia*)	2.3±0.3	83.3	0.272±0.107	0.282±0.093
Plovdiv (Bulgaria*)	2.5±0.2	83.3	0.23±0.115	0.258±0.075
Smolyan (Bulgaria*)	2.0±0.0	66.7	0.196±0.083	0.222±0.085

Table 3. Genetic distances (Nei 1972)

Bd	LpS	Ls	Lv	Ps	Sl	
0.000						Bd
0.002	0.000					LpS
0.003	0.001	0.000				Ls
0.019	0.010	0.007	0.000			Lv
0.003	0.002	0.001	0.008	0.000		Ps
0.002	0.001	0.001	0.010	0.001	0.000	Sl

mon in most of the studied populations and fixed in Ls and Ps. As in Plovdiv* population from Bulgaria (0.063), HK^{87} allelic variant was detected in Lv and Sl honey bees but with lower frequencies (0.019–0.028, respectively). HK^{121} was detected in Bd, LpS and Lv, as well as in other Serbian* *carnica* populations with similar frequencies (Table 1).

Looking at the allele frequencies discussed we should point out that in some aspects Kosovo honey bee populations are similar to other Serbian populations of *A. m. carnica* (fixation of ME^{100} allele, presence of HK^{121} and EST^{80} alleles). On the other hand, some similarities between Kosovo and Bulgarian *A. m. macedonica* populations could be found also (frequencies of PGM alleles, presence of HK^{87} allele).

Low percentage of polymorphic loci (16.7–50%), together with low values of H_o, H_e (0.104–0.164 and 0.093–0.123, respectively) and F_{ST} (0.029) are in relation with low levels of genetic differentiation among the studied honey bee populations from Kosovo. In comparison with referent data (Table 2), it could be seen that all these levels are lower than detected in the studied by Ivanova *et al*. (2012) populations of *A. m. carnica* and *A. m. macedonica*, which shows a higher level of consolidation for the investigated in this study populations

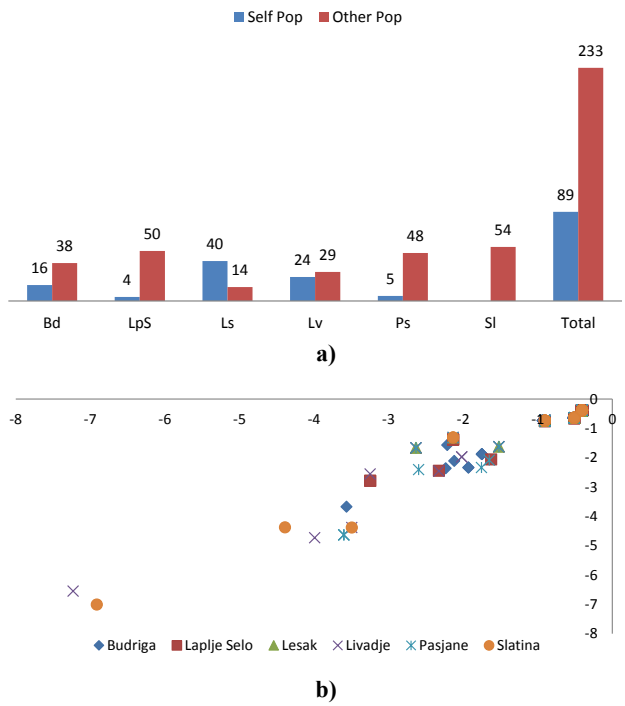

Fig. 2. a). Summary of population assignment outcomes to 'Self' or 'Other' population; b) Population assignment graph

from Kosovo. These low levels of polymorphism and F_{ST}, as well as low genetic distances obtained in this study are also in connection with the selective control on the studied populations from Kosovo.

The present results provide new data on allozyme variability in *A. mellifera* honey bee populations from Kosovo and could be successfully used in further comparative population-genetic studies. The fixed alleles, the alleles with the highest frequencies, the private alleles (including alleles rarer than 5%) and the specific genetic differences found between

the compared populations of *A. mellifera* could be successfully used as genetic markers in further population-genetic studies in Kosovo, the Balkan Peninsula and Europe.

Acknowledgments: This study was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, Projects TR 46009 (2011-2014) and TR 31057 (2011-2014).

References

Bouga M., G. Kilias, P.C. Harizanis, V. Papasotiropoulos, S. Alahiotis 2005. Allozyme variability and phylogenetic relationships in honey bee (Hymenoptera: Apidae: *A. mellifera*) populations from Greece and Cyprus. – *Biochemical Genetics*, **43**: 471-484.

Bouga M., C. Alaux, M. Bienkowska, R. Büchler, N. Carreck, E. Cauia, R. Chlebo, B. Dahle, R. Dall'olio, P. De La Rúa, A. Gregorc, E. Ivanova, A. Kence, M. Kence, N. Kezic, H. Kiprijanovska, P. Kozmus, P. Kryger, Y. Le Conte, M. Lodesani, A. Murilhas, A. Siceanu, G. Soland, A. Uzunov, J. Wilde 2011. A review of methods for discrimination of honey bee populations as applied to European beekeeping. – *Journal of Apicultural Research*, **50** (1): 51-84. DOI: 10.3896/IBRA.1.50.1.06.

De la Rua P., R. Jaffe, R. Dall'Olio, I. Munoz, J. Serrano 2009. Biodiversity, conservation and current threats to European honeybees. – *Apidologie,* **40**: 263–284. DOI: 10.1051/apido/2009027

Dedej S., A. Biasiolo, R. Piva 1996. Morphometric and alloenzymatic characterisation in the Albanian honeybee population *Apis mellifera* L. – *Apidologie,* 27: 121-131.

Francis R.M., P. Kryger, M. Meixner, M. Bouga, E. Ivanova, S. Andonov, S. Berg, M. Bienkowska, R. Buchler, L. Charistos, C. Costa, W. Dyrba, F. Hadjina, F. Panasiuk, H. Pechhacker, N. Kezic, S. Korpela, Y. Le Conte, A. Uzunov, J. Wilde 2014. The genetic origin of honey bee colonies in the COLOSS Genotype-Environment Interactions Experiment. – *Journal of Apicultural Research*, **53** (2). DOI: 10.3896/IBRA.1.53.2.02.

Ivanova E. 2010. Investigation on genetic variability in honeybee populations from Bulgaria, Greece and Serbia. – *Biotechnology & Biotechnological Equipment*, **24** (2): 385-389.

Ivanova E., T. Staykova, P. Petrov 2010a. Allozyme variability in populations of local Bulgarian honey bee. – *Biotechnology & Biotechnological Equipment*, **24** (2): 371-374.

Ivanova E., P. Petrov, M. Bouga, N. Emmanouel, R. Ivgin-Tunka, M. Kence 2010b. Genetic Variation In Honey Bee (*Apis mellifera* L.) Populations From Bulgaria. – *Journal of Apicultural Science*, **54** (2): 49-60.

Ivanova E., M. Bienkowska, P. Petrov 2011. Allozyme Polymorphism and Phylogenetic Relationships in *Apis mellifera* Subspecies Selectively Reared in Poland and Bulgaria. – *Folia biologica (Kraków)*, **59** (3-4): 121-126. DOI:10.3409/fb59_3-4.09-13.

Ivanova E., M. Bouga, T. Staykova, M. Mladenovic, S. Rasic, L. Charistos, F. Hatjina, P. Petrov 2012. The genetic variability of honey bees from the Southern Balkan Peninsula, based on alloenzymic data. – *Journal of Apicultural Research*, **51** (4): 329-335. DOI: 10.3896/IBRA.1.51.4.06.

Kandemir I., M. Kence, A. Kence 2000. Genetic and morphometric variation in honeybee (*Apis mellifera* L.) populations of Turkey. – *Apidologie*, **31**: 343-356.

Kandemir I., M. Kence, A. Kence 2005. Morphometric and electrophoretic variation in different honeybee (*Apis mellifera* L.) populations. – *Turkish Journal of Veterinary and Animal Science*, **29**: 885-890.

Meixner M. D., C. Costa, P. Kryger, F. Hatjina, M. Bouga, E. Ivanova, R. Büchler 2009. Conserving diversity and vitality for honey bee breeding. – *Journal of Apicultural Research,* **49** (1): 85–92. DOI: 10.3896/IBRA.1.49.1.12.

Meixner M. D., M. A. Pinto, M. Buoga, P. Kryger, E. Ivanova, S. Fuchs 2013 Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. – *Journal of Apicultural Research*, **52** (4). DOI 10.3896/IBRA.1.52.4.05.

Mladenović M., V.D. Simeonova 2010. The variability of wing nervature angles of honey bee from the north kosovo area. – *Biotechnogy & Biotechnological Equipment*, **24**: 427-432.

Muñoz I., J. Stevanovic, Z. Stanimirovic, P. De la Rúa 2012. Genetic variation of *Apis mellifera* from Serbia inferred from mitochondrial analysis. – *Journal of Apicultural Science,* **56** (1): 59-69.

Nei M. 1972. Genetic distance between populations. – *American Naturalist*, **106**: 283-292.

Peakall R., P.E. Smouse 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. – *Bioinformatics*, **28** (19): 2537-9. http://dx.doi.org/10.1093/bioinformatics/bts460

Ruttner F. 1988. Biogeography and Taxonomy of Honeybees, Springer Verlag, Berlin.

Staykova T., E. Ivanova, P. Tzenov, Y. Vasileva, D. Arkova-Pantaleeva, Z. Petkov 2010. Acid phosphatase as a marker for differentiation of silkworm (*Bombyx mori*) strains. – *Biotechnology & Biotechnological Equipment*, **24** (2): 379-384.

Staykova T., E. Ivanova 2011. Concerning genetic variability and usable isozyme markers for characterization of *A. mellifera* L. populations and *B. mori* L. breeds in Bulgaria. Advances in Bulgarian Science, 20-28.

Stevanovic J., Z. Stanimirovic, M. Radakovic, R.S. Kovacevic 2010. Biogeographic study of the honey bee (*Apis mellifera* L.) from Serbia, Bosnia and Herzegovina and Republic of Macedonia based on mitochondrial DNA analyses. – *Russian Journal of Genetics,* **46** (5): 603-609.

Swofford D. L., R. B. Selander 1981. BIOSYS-1: A computer program for the analysis of allelic variation in genetics Rel. 1.0. Department of Genetics and Development University of Illinois at Urbana-Champaign, Urbana, Illinois 60801, USA, 65 p.

Uzunov A., M. D. Meixner, H. Kiprijanovska, S. Andonov, A. Gregorc, E. Ivanova, M. Bouga, P. Dobi, R. Büchler, R. Francis, P. Kryger 2014. Genetic structure of *Apis mellifera macedonica* in the Balkan Peninsula based on microsatellite DNA polymorphism. – *Journal of Apicultural Research*, **53** (2): 288-295.

Received: 18.12.2014
Accepted: 21.06.2015