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Allozyme genetic polymorphism in Bulgarian honey bee (*Apis mellifera* L.) populations from the south-eastern part of the Rhodopes

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ABSTRACT

Allozyme genetic polymorphism in Bulgarian honey bee populations from four different locations in the south-eastern part of the Rhodopes Mountain was studied on six enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to six genetic loci. Allozyme analysis revealed that all studied loci were polymorphic in almost all investigated populations. The observed heterozygosity was found to range from 0.110 to 0.208 and Nei's genetic distance – between 0.016 and 0.061 among the studied populations. These honey bee populations were clustered in two groups in the UPGMA dendrogram. The Tihomir population was in a separate clade while other three populations (Kardzhali, Krumovgrad and Dolni Yurutci) were grouped together.

Key words: Honey bee, *Apis mellifera*, allozymes, polymorphism, Bulgaria

Introduction

Bulgarian honey bees were investigated for the purposes of the selection ever since the 30s of the last century (Lazarov, 1935; 1936). Different studies onto the morphological features have been performed in the period of 1967-1975 and the obtained results were used as a basis of the selective work with bees in Bulgaria during the period 1971-1990 (Velichkov, 1970). In the past, the local Bulgarian bee was threatened by many activities (eg. queen rearing and importation of foreign queens), which have had an impact on the genetic variability of the honey bees all over the country.

The allozyme variability of honey bee populations from some regions in Bulgaria was first assessed by Ivanova et al. (1996) and Ivanova & Popov (1997). The genetic structure of the local honey bee population from the gene bank of the National Selective Centre, maintained by artificial insemination and populations of *A. mellifera* from different locations of the country were also compared and analyzed (Ivanova et al. 2007, 2010a,b, 2011). The present study was focused on the detection of genetic variability among honey bee populations from different locations in the south-eastern part of the Rhodopes Mountain – a region that was not studied in this aspect previously.

Materials and Methods

Honey bee samples were collected from managed colonies of four different locations (Kardzhali, Krumovgrad, Dolni Yurutci and Tihomir) in the south-eastern part of the Rhodopes Mountain in Bulgaria. Totally about 300 worker bees (five to seven colonies per a population, 7 to 10 individuals per a colony) were tested. Collected worker bees were transported to the laboratory alive and frozen at -20ºC until used. The thorax (for MDH, ME, EST, PGM and HK) or total (for ALP) homogenization and electrophoresis in polyacrylamide gel were done according to Ivanova et al. (2007).

Six enzymic systems were studied: MDH (malate dehydrogenase, EC 1.1.1.37); ME (malic enzyme, EC 1.1.1.40); EST (esterase, EC 3.1.1), ALP (alkaline phosphatase, EC 3.1.3.1); PGM (Phosphoglucomutase, EC 5.4.2.2) and HK (Hexokinase, EC 2.7.1.1). Buffers and electrophoretic conditions for each enzymic system were the same as described in Boyer (1961), Gahne (1967) and Shaw

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& Prasad (1970). Enzyme activities were visualized by histochemical staining (Harris & Hopkinson, 1976) and allozymes were numbered according to their relative anodal mobility.

Results from the isoenzymic analysis were statistically processed using BIOSYS-1 (Swofford & Selander, 1981) and PHYLIP (Felsenstein, 1993) software packages. The UPGMA (Sneath & Sokal, 1973) phylogenetic tree was obtained by genetic distance matrix methods.

Results

The studied enzymic systems correspond to 6 genetic loci, MDH-1, ME, EST-3, ALP, PGM and HK, which were polymorphic in most of the investigated populations (Table 1).

Table 1. *Allele frequencies in the studied populations*.

	Kardzhali	Krumovgrad	D.Yurutci	Tihomir
MDH-1				
65	0.578	0.429	0.455	0.136
100	0.422	0.357	0.545	0.864
80	0	0.214	0	0
ME				
100	1	0.95	0.9	0.889
106	0	0.05	0	0.111
90	0	0	0.1	0
EST-3				
100	0.957	0.9	0.8	0.925
118	0	0.05	0	0.025
94	0.043	0.05	0.2	0.05
ALP				
80	0.524	0.357	0.313	0.25
100	0.476	0.643	0.688	0.75
PGM				
100	0.917	0.955	1	1
114	0.083	0.045	0	0
HK				
87	0.044	0.013	0.03	0.019
100	0.956	0.988	0.97	0.981

Alleles were designed with respect to their relative mobility, as the mobility of the most common allozyme were used as a standard (mobility 100). Two alleles at the MDH-1 locus (MDH^{65} and MDH^{100}) were detected in three of the populations (Kardzhali, Dolni Yurutci and Tihomir). A third allele (MDH^{80}) was observed in the Krumovgrad population. MDH^{100} allele frequency was higher in Dolni Yurutci and Tihomir, whereas MDH^{65} was with a higher frequency in the Kardzhali population and with the highest one in the Krumovgrad population. Three alleles were found at the ME locus (ME^{90}, ME^{100} and ME^{106}) and the ME^{100} was fixed in the Kardzhali population and at higher frequency in the other studied populations. EST-3 was polymorphic with three alleles (EST^{94}, EST^{100}, and EST^{118}). The EST^{100} allele was at higher frequencies in all populations. The ALP locus was polymorphic with two alleles (ALP^{80} and ALP^{100}) in all studied populations. The allele ALP^{100} was with higher frequency in all populations except Kardzhali. Two alleles were detected at PGM locus (PGM^{100} and PGM^{114}). PGM^{100} was fixed in Dolni Yurutci and Tihomir population and PGM^{114} was present in the Kardzhali and Krumovgrad populations. The HK locus was polymorphic with two alleles – HK^{87} and HK^{100} as the last one was with higher frequency in all populations.

The mean number of alleles per locus varied from 1.8 (Kardzhali and Dolni Yurutci) to 2.3 (Krumovgrad). The estimated percentage of polymorphic loci was 50% in Kardzhali and 66.7% in the other three populations using the 0.95 criterion (Table 2).

The observed and expected heterozygosities (H_o and H_e) ranged from 0.110 (Tihomir) to 0.208 (Kardzhali) and from 0.168 (Tihomir) to 0.255 (Krumovgrad), respectively (Table 2). For most of the loci there are not significant deviations of the genotype frequencies from the Hardy-Weinberg expectations in most populations ($0.970 \geq P \geq 0.05$).

Table 2. *Observed and expected heterozygosity in the tested populations.*

Population	Mean number of alleles per locus	Percentage polymorphic loci (P=0.95)	H_o	H_e
Kardzhali	1.8±0.2	50	0.208±0.105	0.222±0.091
Krumovgrad	2.3±0.2	66.7	0.196±0.064	0.255±0.103
D.Yurutci	1.8±0.2	66.7	0.187±0.095	0.254±0.084
Tihomir	2±0.3	66.7	0.110±0.056	0.168±0.058

Chi-Square tests showed that only for some loci the deviations were generally in favor of the homozygotes.

The estimated mean FST value from allozyme data was 0.072, which shows that only 7.2% of the observed overall genetic diversity was among the populations and 92.8%

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within the populations.

The values of genetic distance (Nei, 1972) were calculated using the allele frequencies (Table 1) and range from 0.016 (between Kardzhali-Krumovgrad and Krumovgrad-Dolni Yurutci) to 0.061 (between Tihomir and Kardzhali) (Table 3).

Table 3. *Genetic distances (Nei, 1972).*

Population	Kardzhali	Krumovgrad	D.Yurutci	Tihomir
Kardzhali	***	0.016	0.022	0.061
Krumovgrad		***	0.016	0.044
D.Yurutci			***	0.028
Tihomir				***

In the UPGMA (Sneath & Sokal, 1973) dendrogram, the Tihomir population is in a separate cluster (Figure 1). The rest of the studied populations were grouped together in another cluster with two branches – first one with the Kardzhali population and the second one – with the Krumovgrad and Dolni Yurutci populations.

Discussion

Generally, five alleles (MDH^{65}**,** MDH^{80}, MDH^{87}, MDH^{100} and MDH^{125}**)** were detected on MDH-1 locus (Meixner et al., 1994; Kandemir & Kence, 1995; Kandemir et al., 2000; Bouga et al., 2005; Ivanova, 2010) in different populations from Europe, Turkey, Brazil and USA.

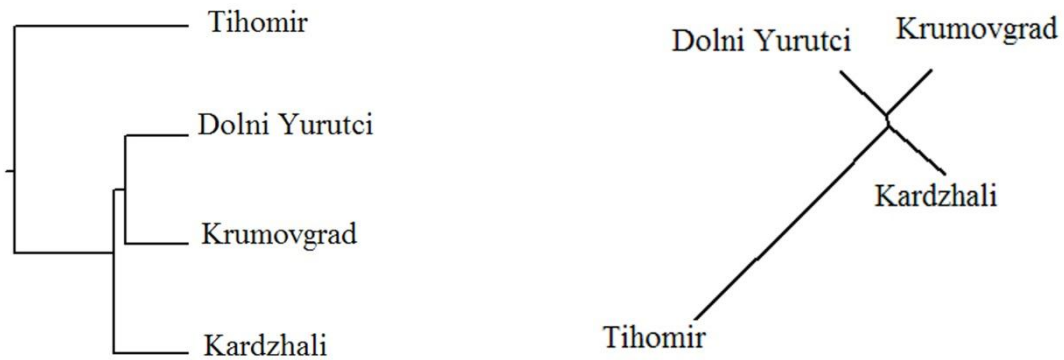

Figure 1. *Relationships of studied populations as shown in UPGMA (Sneath and Sokal, 1973) dendrograms*.

Three alleles at this locus (MDH^{65}**,** MDH^{80} and MDH^{100}) were detected in *A. mellifera carnica* and *A. mellifera caucasica* populations from Poland and two alleles in *A. mellifera macedonica* populations from Bulgaria, where MDH^{80} was absent (Ivanova et al., 2011). Dedej et al. (1996) reported two MDH-1 alleles (MDH^{100} and MDH^{65}) for *A. mellifera macedonica* in Greece, but according to a recent research of Bouga et al. (2005) this locus has three alleles (MDH^{100}, MDH^{80} and MDH^{65}) in Greece and the most frequent of them is MDH^{80}. The frequency of MDH^{100}, according to Badino et al. (1988), is decreasing from South to North of Greece, but remains high in the eastern region near the border with Turkey. One more allele (MDH^{125}) was found in *A. mellifera carnica* from Serbia (Ivanova, 2010). Comparing the results of our study with those of Bouga et al. (2005) we should note that in the studied Bulgarian honey bees the MDH^{80} allele is present only in the Krumovgrad population.

The presence of three alleles was found at ME locus (ME^{70}, ME^{100} and ME^{106}**)** in different European *A. mellifera* populations (Sheppard & Berlocher, 1984; Sheppard & Berlocher, 1985; Sheppard & Mcpheron, 1986). The ME locus was found to be invariant in honey bee populations from Turkey (Kandemir et al. 2000, 2005). Dedej et al. (1996) reported no polymorphism in the ME locus too, but according to Bouga et al. (2005) this locus is polymorphic with two alleles – ME^{100} and ME^{79} in *A. mellifera macedonica* populations from Greece.

ME^{100} was found to be fixed in the investigated *A. mellifera carnica* populations from Serbia (Ivanova, 2010). Ivanova et al. (2011) reported three alleles at this locus (ME^{90}, ME^{100} and ME^{106}) and the highest frequency for ME^{100} in all studied populations from Poland and Bulgaria. It was detected that ME^{106} allele was with the lowest frequency in "*macedonica*" bees and with the highest frequency in "*caucasica*" bees. One more allele (ME^{90}) was

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observed in an *A. mellifera macedonica* population from Thrace in Bulgaria. In the present study three alleles of this locus were detected in the studied populations from Kardzhali region where ME^{100} was the most frequent allele and the ME^{90} was found only in the population of Dolno Yurutci.

The EST-3 locus was polymorphic and exhibited three alleles (EST^{70}, EST^{100} and EST^{130}) in Czechoslovakian (Sheppard & McPheron, 1986) and in Central Anatolian honey bees (Kandemir & Kence, 1995). Three alleles were detected also in *A. mellifera macedonica* from Greece (Bouga et al., 2005). Ivanova et al. (2010a) reported that EST^{100} was fixed in the regions of Rhodopes Mountain of Bulgaria and its frequency is rather high in Thrace regions of Bulgaria and Turkey. It was found that EST-3 locus had two or three alleles (EST^{94}, EST^{100} and EST^{118}) in *A. mellifera carnica* and *A. mellifera caucasica* population from Poland and in *A. mellifera macedonica* populations from Bulgaria (Ivanova et al., 2011). The results about the polymorphism found in our study are quite similar to the reported above. EST^{100} was the most common allele in all of these populations.

In previous studies of Ivanova (2010) three other alleles of EST-3 locus (EST^{80}, EST^{88} and EST^{105}) were described in populations of *A. mellifera carnica* (from Serbia and Montenegro) and *A. mellifera macedonica* (from Bulgaria and Greece). In the current investigation these three alleles were not detected.

The ALP locus was found as polymorphic with two alleles (ALP^{100} and ALP^{80}**)**. ALP^{80} was more frequent allele in Greece (Bouga et al., 2005; Ivanova, 2010) and in Bulgaria (Ivanova et al., 2010a). It was detected that the ALP locus had three alleles in *A. mellifera carnica* honey bees (from Poland and Serbia) and in *A. mellifera caucasica* (from Poland) – ALP^{80}**,** ALP^{90} and ALP^{100}**,** and two – ALP^{100} and ALP^{80} – in "*macedonica*" honey bees from Bulgaria (Ivanova, 2010; Ivanova et al., 2011). In the present study, the ALP locus has the same two alleles.

The PGM locus was studied by many researchers (Mestriner & Contel, 1972; Brueckner, 1974; Nunamaker & Wilson, 1980; Badino et al., 1983; Sheppard & Berlocher, 1985) but Del Lama et al. (1985) first reported the presence of three alleles at this locus in Africanized bee populations and two alleles in *A. mellifera carnica* originating from Germany. Meixner et al. (1994) found three alleles of which PGM^{120} was previously unreported. Similar to the results of this study, the PGM locus was found to be polymorphic with two alleles (PGM^{100} and PGM^{114}) in populations from Serbia, Montenegro, Bulgaria and Greece (Ivanova, 2010; Ivanova et al., 2010a, b), where PGM^{100} was the more common or fixed allele. A third allele (PGM^{80}) was found in *A. mellifera caucasica* population from Poland (Ivanova et al., 2011).

The HK locus was monomorphic in many European honey bee populations (Sheppard & Berlocher, 1985, Sheppard & McPheron, 1986, Badino et al., 1988, Del Lama et al., 1990). It was found to be polymorphic with two alleles (HK^{87} and HK^{100}) in Africanized bee populations from Brazil and Central America (Del Lama et al. 1988, 1990). Later studies determined four alleles at this locus (Kandemir & Kence, 1995). Kandemir et al. (2000) detected one more allele (HK^{77}) in honey bee populations from Turkey. For Polish and Bulgarian populations of *A. mellifera carnica*, *A. mellifera caucasica* and *A. mellifera macedonica* totally three alleles were found (HK^{87}, HK^{100} and HK^{110}) and HK^{100} was the most common allele (Ivanova et al., 2011). In a previous research, one more allele (HK^{121}) was detected in Serbian *A. mellifera carnica* populations (Ivanova, 2010).

In the studied populations from Kardzhali region only HK^{87} and HK^{100} alleles were detected.

As concerning the topology of the UPGMA dendrogram, it is very interesting that Tihomir population is in a separate clade and seems to be different from the other populations, which according to the topology of the dendrogram, seems to be much more similar.

It must be also noted that in all studied populations a moderate percentage of polymorphic loci is found. F_{ST} value of 0.072 indicates a low level of genetic differentiation among the studied populations.

The results of this research provide new information concerning the genetic variability of honey bee populations from south-eastern part of the Rhodopes Montain in Bulgaria and could be used in further investigations for different population-genetic comparisons, in order to be able to determine conservation areas of local interest.

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