ALLOZYME VARIABILITY IN POPULATIONS OF LOCAL BULGARIAN HONEY BEE

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ABSTRACT

Genetic variation of honey bee populations from six different locations corresponding to tree geographic regions: North-western, North-central and North-eastern of Bulgaria was studied on 6 enzymic systems (MDH, ME, EST, ALP, PGM and HK) corresponding to 6 genetic loci. Allozyme analysis revealed that all loci studied were polymorphic in almost all populations studied. The mean number of alleles per locus varied from 1.8 to 2.5. The estimated percentage of polymorphic loci was between 50% and 100%. The observed and expected heterozygosities (H_o and H_e) ranged from 0.17 to 0.221 and 0.250 to 0.315, respectively. There are not significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in most populations ($0.99 > P > 0.1$). The estimated mean F_{ST} value from allozyme data was 0.0443 which shows that 4.43% of the overall genetic diversity observed was among populations, as opposed to 95.57% within populations. The values of genetic distance range from 0.002 to 0.036. UPGMA dendrograms were constructed.

Keywords: Honey bee, *Apis mellifera*, allozymes , Bulgaria

Introduction

The honey bee, *Apis mellifera* L., is a species with great economic importance and exists in different ecological conditions in the world. About twenty six subspecies of *A. mellifera* are recognized mainly on the base of classical morphometry (26, 27 and 33). According to Ruttner's morphometric analysis (26), *A. m. macedonica* subspecies occurs in Bulgaria but according to Petrov (25), a native type, *A. m. rodopica* exists in the country.

Honey bees in Bulgaria have been studied by many authors for the purposes of selection (19, 20 and 36).

The allozyme variability of local type and honey bee populations from some regions in Bulgaria were analyzed in many articles (12, 14 and 15). Genetic variation in honey bee populations from Bulgaria and Turkey was investigated on the base of isoenzyme and Randomly Amplified Polymorphic DNA (RAPD) analyses (13).

Although there are many different studies concerning race status and the degree of genetic diversity of local Bulgarian honey bee, they are mainly based on classical morphometry and partially on biochemical-genetic analysis. Hence, the aim of this study is to investigate variation in honey bee populations from different bases of National Bee Breeding Association where the local type *A. m. rodopica* has been reared.

Materials and Methods

Honey bee samples were collected from six different locations corresponding to tree geographic regions: North-western (Chereshovitca), North-central (Morava, Batin and Pordim) and North-eastern (General Kiselovo and Elenovo).

The thorax homogenization and electrophoresis in polyacrylamide gel were done according to Ivanova (12). Five colonies (30 to 45 individuals) per populations were tested.

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,

electrophoretic conditions and histochemical staining for each enzymic system used were as in Boyer (4); Gahne (9); Shaw and Prasad (28), Harris and Hopkinson (11) and Ivanova (12). Alleles were designed with respect to their relative mobility, as the mobility of the most common allozyme used as standard (mobility 100).

Allele frequencies, 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, Nei's genetic distance (D) (23), and Wright's fixation index, F_{ST} (37) were calculated using BIOSYS-1 (35), values of F_{ST} range from 0 (no population subdivision) to 1 (complete population subdivision). Phylogenetic trees were constructed using values of genetic distance (23), by UPGMA (34) method using the PHYLIP (8) software package.

Results and Discussion

Data about allele frequencies, percentage of polymorphism and heterozygosity (Ho and He) are presented in **Table 1** and **Table 2.**

TABLE 1

Allele Frequencies in local honey bee populations studied

	Batin	**G.Kiselovo**	**Elenovo**	**Morava**	**Pordim**	**Chereshovitca**
Locus						
MDH-1						
65	0.458	0.304	0.462	0.417	0.674	0.304
100	0.542	0.696	0.538	0.583	0.326	0.482
80	0	0	0	0	0	0.214
ME						
100	0.9	0.913	0.737	0.9	0.978	0.773
110	0.1	0.087	0.105	0.1	0.022	0.227
90	0	0	0.158	0	0	0
EST-3						
80	0	0.045	0	0	0	0
100	0.958	0.932	1	1	0.978	0.9
88	0	0.023	0	0	0	0.06
118	0.042	0	0	0	0.022	0.04
ALP						
80	0.5	0.545	0.6	0.542	0.511	0.552
100	0.5	0.455	0.3	0.458	0.489	0.448
90	0	0	0.1	0	0	0
PGM						
100	0.923	0.857	0.957	0.909	0.891	0.926
114	0.077	0.143	0.043	0.091	0.109	0.074
HK						
87	0.036	0.022	0.021	0	0	0.015
100	0.964	0.922	0.979	0.933	0.895	0.971
110	0	0.056	0	0.067	0.105	0.015

Data about genetic distance calculated according to Nei (23) are given in **Table 3**. Based on genetic distance, UPGMA (34) dendrograms (**Fig. 1 and 2**) are constructed also.

All of the six genetic loci (MDH-1, ME, EST-3, ALP, PGM and HK) were found to be polymorphic in most of the populations studied, at the 95% level. (**Table 1**).

Two alleles were detected at MDH-1 locus (MDH^{100} and MDH^{65}) in five of the populations, however a third allele, MDH^{80} was observed in Chereshovitca. MDH^{100} allele frequency was high in G. Kiselovo, whereas MDH^{65} was at a higher frequency in Pordim population.

Totally, three alleles were found at the ME locus (ME^{90}, ME^{100} and ME^{106}) and the allele ME^{100} was at high frequency in all populations studied but ME^{90} was present only in Elenovo population. EST-3 was polymorphic with four alleles (EST^{80}, EST^{88}, EST^{100} and EST^{118}). EST^{100} allele was at high frequencies in all populations and was fixed in two (Elenovo and Morava). ALP locus was polymorphic with two alleles in five of the populations studied (ALP^{100} and ALP^{80}), but a third allele, ALP^{90} was observed in Elenovo population. ALP^{80} allele was with a higher frequency in almost all populations except Batin, where frequencies of ALP^{100} and ALP^{80} alleles were equal. PGM locus was polymorphic with two alleles (PGM^{100} and PGM^{114}). PGM^{100} was most common allele in all populations studied. The HK locus was found to be polymorphic with three alleles (HK^{87}, HK^{100} and HK^{110}) in G. Kiselovo and Chereshovitca populations. $HK^{87}$and HK^{100} alleles were established in Batin and Elenovo, and HK^{100} and HK^{110} - in Morava and Pordim. HK^{100} was at highest frequency in all populations.

The mean number of alleles per locus varied from 1.8 (Morava) to 2.5 (Chereshovitca). The estimated percentage of polymorphic loci was between 50% (in Elenovo) and 100% (in Chereshovitca) (**Table 2**).

TABLE 2

Observed and Expected Heterozygosity in the populations tested

Population	Mean sample size per locus	Mean no. of alleles per locus	Percent Polymorphic loci(P=0.95)	H_o	H_e
Batin	36±3.3	2±0	66.7	0.221±0.062	0.25±0.084
G.Kiselovo	37±0.2	2.3±4.6	100	0.266±0.079	0.271±0.065
Elenovo	32.5±1.1	2.2±0.3	50	0.224±0.122	0.27±0.104
Morava	30.3±3.3	1.8±0.2	83.3	0.211±0.109	0.249±0.084
Pordim	41.7±3.8	2±0	66.7	0.182±0.069	0.237±0.08
Chereshovitca	29.5±1.6	2.5±0.2	83.3	0.17±0.049	0.315±0.092

The observed and expected heterozygosities (H_o and H_e) ranged from 0.17 (Chereshovitca) to 0.221 (Batin) and 0.250 (Batin) to 0.315 (Chereshovitca), respectively (**Table 2**). There were not significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in most populations ($0.99 > P > 0.1$). The estimated mean F_{ST} value was 0.0443 which showed that 4.43% of the overall genetic diversity observed was among populations, as opposed to 95.57% within populations. The values of genetic distance (23) were calculated using the allele frequencies (**Table 1**) and ranged from 0.002 (between Morava and Batin) to 0.036 (between Chereshovitca and Pordim populations) (**Table 3**). In UPGMA dendrograms (**Fig. 1 and 2**) Morava, Batin and G. Kiselovo were clustered together, and Chereshovitca, Elenovo and Pordim formed the other tree branches of the tree.

In similar studies, five alleles on MDH-1 locus were detected (1, 2, 3, 10, 16, 17, 21, 22, 24, 29; 30, 31 and 32) in different populations from Europe, Brazil, Asia and USA. Comparing our results with that of a recent research of Bouga et al. (5) we noted that in *A. m. macedonica* in Greece there were three alleles (MDH^{100}, MDH^{80} and MDH^{65}) in MDH-1 locus and the most frequent of them was MDH^{80}. However, in Bulgarian honey bees MDH^{80} allele was present only in

Chereshovitca population with low frequency.

Three alleles at ME locus, ME^{70}, ME^{100} and ME^{106} were found in *A. mellifera* populations in Norway (30), Italy (31) and western Czechoslovakia (32). The ME locus was found nearly fixed in Kenya where, in one colony, a previously unknown allele, ME^{117} was found (22).

TABLE 3

Genetic distances calculated according to Nei (1972)

Population	Batin R	G.Kiselovo	Elenovo Sh	Morava	Pordim	Chereshovitca
Batin	***	0.008	0.013	0.002	0.014	0.013
G.Kiselovo		***	0.02	0.004	0.033	0.017
Elenovo			***	0.012	0.029	0.019
Morava				***	0.017	0.013
Pordim					***	0.036
Chereshovitca						***

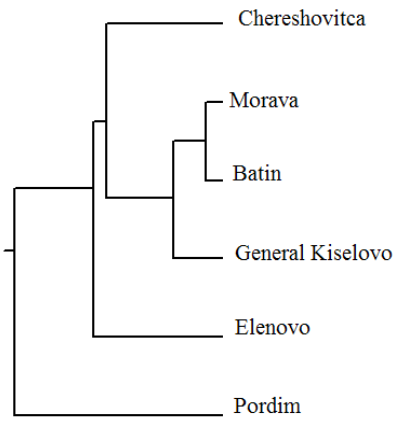

Fig. 1. Relationships of populations studied as shown in UPGMA (Sneath and Sokal, 1973) dendrogram

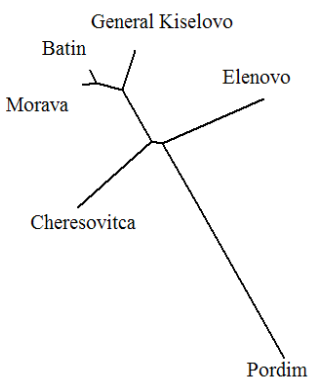

Fig. 2. Relationships of populations studied as shown in UPGMA (Sneath and Sokal, 1973) drowgram

ME locus was found invariant in honey bee populations of Turkey (17, 18) but two alleles (ME^{100} and ME^{79}**)** were detected in Greece (5) where ME^{100} allele frequency was high in all Greek populations studied and the same is in Bulgarian populations, except Elenovo where ME^{90} was found.

EST-3 locus was polymorphic with three alleles, EST^{70}, EST^{100} and EST^{130} in Czechoslovakian (32) and in central Anatolian honey bees (16). Ivanova et al. (13) reported that EST^{100} was fixed in Rhodopes mountainous regions of Bulgaria and its frequency is rather high in Thrace regions of Bulgaria and Turkey. The rare alleles detected in Thrace regions of Bulgaria and Turkey, were EST^{70} and EST^{130}, respectively (13). EST-3 locus had three alleles in *A.m.macedonica* in Greece (5) but in this investigation, EST-3 locus showed four alleles, two of them, EST^{80} and EST^{118} were detected with PAGE, probably correspond to the EST^{70} and EST^{130} detected with starch gel electrophoresis (13, 14, 15).

The ALP locus was polymorphic with two alleles - ALP^{100} and ALP^{80}. ALP^{80} was the most frequent allele in Greece (5). In our research, in one of the populations studied, ALP locus has one more allele – ALP^{90}.

The PGM locus was studied by many researchers but Del Lama *et al*. (6) first reported the presence of three alleles on this locus in Africanized bee populations and two alleles in *A. m. carnica* originating from Germany. Meixner *et al.* (22) found three alleles - PGM^{120} was previously unreported. In our research we found that PGM^{100} was more common allele for Bulgarian honey bee populations. This allele was previously reported as PGM^{75} according to data of starch gel

electrophoresis (13, 15).

HK locus was found to be monomorphic in Norwegian (30), Italian (31), Czechoslovakian (32), Greek (3) and German (7) honey bee populations. However, it was found to be polymorphic with two alleles (HK^{87} and HK^{100}) in Africanized bee populations from Brazil and Central America (7). Later studies determined four alleles on the above mentioned locus (16). Kandemir et al. (17) detected one more allele - HK^{77} in honey bee populations from Turkey. In our previous study (13) three alleles were found in studied regions from Bulgaria and Turkey. HK^{100} was reported as the common allele for all of studied regions. In the present study HK locus was found to be polymorphic with the same three alleles.

Conclusions

The results of this research provide new information concerning the genetic variability of local honey bee populations from Bulgaria. The presence of the EST^{88}, EST^{118}, ME^{90}, ME^{106} and ALP^{90} alleles are reported here for the first time for Bulgarian honey bees. Further investigation, based on complex approach including different methods, is necessary to be done in order to analyze in details genetic structure of honey bee populations from Bulgaria.

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