Genetic Variability in Honey Bee Populations from Northern Bulgaria

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Introduction

Local Bulgarian honey bee is of type "*carnica*" in a broad sense (Engel, 1999). According to Ruttner's morphometric analysis (1988), *A. m. macedonica* subspecies occurs in Bulgaria. Another point of view is the existence in Bulgaria of a native local honey bee *A. m. rodopica* (Petrov, 1995).

The local Bulgarian honey bee is now a basis for selective work in the country. With this regard, a morpho-ethological analysis by specific characteristics has been carried out in order to determine the race belonging of local bees (Petrov, 1993; 1995; 1997). On the other hand, biochemical-genetic researches of polymorphism in some protein and isoenzymic systems have been carried out ever since 1991 (Ivanova *et al*. 1991; 1994; 1998; 2006; 2007; Ivanova, 1996; 1998; Popov *et al*., 2000).

There is the necessity for further research concerning the honey bee diversity in Bulgaria. The related investigation is mainly based on morphometrics analysis and partially on the study of biochemical polymorphisms of some proteins and enzymic systems. The reliable and diverse DNA analyses have been fragmentarily applied in this direction (Ivanova *et al*., 2004; Ivanova *et al.*, 2008). Therefore, the aim of this research is to investigate the genetic variability and phylogenetic relationships of honey bee populations from various regions of North part of Bulgaria using molecular markers such as isoenzymes and mtDNA.

Materials and methods

Honey bee samples were collected from six different locations of the North part of Bulgaria: Drenovets, Ralevo, Pechkovets, Pordim, Buzovets and Vetovo.

The sampling consisted of 1069 worker bees that were used for isienzymic analysis. The thorax homogenization and electrophoresis in 7.5% polyacrylamide gel were according to Ivanova (1996). Four 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); and ALP (alkaline phosphatase, EC 3.1.3.1).

The variation of mtDNA was studied using PCR-RFLP's analysis. The polymerase chain reaction (PCR) was performed as in Bouga *et al*. (2005b). The restriction enzymes used for the 16s rDNA gene segment were Sau3A I, Ssp I, Dra I, Hinc II , EcoR I, for the COI were Nco I, Sau3A I, Fok I, , Bcl I, Ssp I, Sty I, BstU I, and Xho I and for ND5 Dra I, Taq I, Nla III, Hinc II, Fok I and Ssp I.

The results of isoenzymic analysis were statistically performed using BIOSYS-1 (Swofford and Selander, 1981) and PHYLIP (Felsenstein, 1993) software packages.

Results and Conclusions

1. Isoenzymic analysis

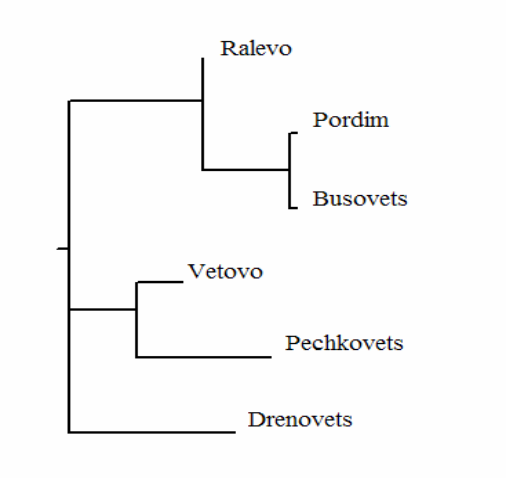
All enzymic systems were polymorphic in most of the populations studied, at the 95% level. Two alleles were detected at MDH-1 locus (MDH^{100} and MDH^{65}) in all populations studied. MDH^{100} allele frequency was at a higher frequency in five of the sixth population studied, whereas MDH^{65} was at a higher frequency only in one (Pordim).

Two alleles were found at the ME locus (ME^{100} and ME^{106}) and the ME^{100} was at higher frequency in all populations studied. EST-3 was polymorphic with three alleles (EST^{80}, EST^{100}, and EST^{118}). EST^{100} allele was at high frequencies in all populations and was fixed in Drenovets and Busovets. ALP locus was polymorphic with two alleles in all populations studied (ALP^{100} and ALP^{80}). ALP^{80} allele was with a higher frequency in all populations.

The mean number of alleles per locus varied from 1.8 to 2.0 and was estimated as follows: 1.8 – in Drenovets and Vetovo, and 2.0 – in Ralevo, Pechkovets, Pordim and Buzovets. The estimated percentage of polymorphic loci was 50% in Pechkovets, Pordim and Buzovets, and 75% in others using the 0.95 criterion. The observed and expected heterozygosities (H_o and H_e) ranged from 0.153 (Pechkovets) to 0.228 (Buzovets) and 0.259 (Pordim) to 0.293 (Drenovets), respectively.

Nei's genetic distances (Nei, 1972) was calculated using the allele frequencies and range from 0.00 (Pechkovets and Pordim) to 0.058 (Drenovets and Pechkovets) in the populations studied. In UPGMA dendrogram two branches are formed. Buzovets, Pordim and Ralevo are grouped together and formed a branch with Drenovets. Vetovo and Pechkovets are grouped in the second one (Fig. 1.).

Figure 1. UPGMA dendrogram based on Nei's genetic distance from allozyme data



Comparing allozyme data of this study with information given by Dedej *et al*. (1996); Bouga *et al*. (2005a); Kandemir *et al*. (2005), we could notice that concerning MDH-1, EST-3, ME and ALP polymorphism, there are differences between Bulgarian honey bees, *A. m. macedonica* and *A. m. carnica*.

2. Mitochondrial DNA analysis

The sizes of PCR-amplified mtDNA segments for all populations examined were found to be about 964bp, 1028bp, and 822bp for 16s rDNA, COI, and ND5 gene segments respectively. No variability was detected among the honey bee populations studied. Comparing the results from these of analogous studies (Bouga *et al*., 2005b; Harizanis *et al.*, 2006), Bulgarian honey bees seem to be discriminated from Greek honey bees. There are differences between the Macedonian honey bee population in Greece and Bulgarian honey bees after the digestion of COI gene segment with the restriction enzymes Sty I and Nco I (Bouga *et al*., 2005b). There are also differences between Greek and Bulgarian honey bees in the digestion pattern of COI with Ssp I, as well as, of ND5 with Hinc II and Fok I..

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