Research Article

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Genetic Comparison between Local *Apis mellifera macedonica* Ruttner, 1988 Selectively Reared for Production of Bee Queens and Swarms in Bulgaria and Honey Bee Colonies with Indicative Hygienic Behaviour

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Abstract: The genetic polymorphism of malate dehydrogenase (MDH-1) and esterase (EST-3) loci in selectively reared for production of bee queens and bee swarms in Bulgaria local honey bee *Apis mellifera macedonica* was studied in comparison with a group of honey bee colonies with indicative hygienic behaviour. Totally 851 worker bees collected from the selection bee rearing bases and 414 worker bee individuals from colonies with manifested hygienic behaviour were used for this comparative study. Both of the studied loci were found to be polymorphic all of the studied honey bee groups. Polymorphism with two and three alleles was found for MDH-1 locus and with four and five alleles – for EST-3 locus for compared hygienic colonies and those under selective control. Levels of polymorphism between 50% and 100% were calculated for the studied honey bee groups. The observed and expected heterozygosities (H_o and H_e), mean F_{ST} and Nm values were calculated and compared. The observed similarities and slight differences among honey bee groups under selective control and this with indicative hygienic behaviour were discussed.

Key words: *Apis mellifera*, polymorphism, hygienic behaviour, genetic markers

Introduction

Morphological investigations have been done in the past in order to be used for honeybee selection in Bulgaria (Lazarov 1935, 1936, Velichkov 1970). In 1999, a new national program for beebreeding has been announced and accepted by the Ministry of Agriculture and Foods of Bulgaria. This program was actualized and enriched in 2013 (Petrov & Ganev 2013) as a breeding program for preservation of the local for the country Bulgarian honey bee. The main purpose of the program was to preserve the gene pool of the local Bulgarian honey bee, which has proven biological and productive advantages, good adaptation to the specific local conditions and valuable behaviour traits (Petrov 2010). In accordance with this aim and in order to characterize the subspecies standard of Bulgarian honey bees, mainly morpho-ethological investigations by specific characteristics have been carried out in the country (Petrov 1990, 1993, 1995, 1997, Petrov & Ivanova 2009). During the period after 1996, some isoenzyme and molecular genetic studies on the polymorphism in honey bee populations from Bulgaria have been done (Ivanova 1996, Ivanova & Bouga 2009; Ivanova *et al.* 2007, 2008). Recently, the relationships between genotype and behavioural characteristics of honey bees are much interesting

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for selection and the scientists are in search of new approaches for their investigating. Hygienic behaviour and grooming are much important among selection programmes because of their relation to colony health and disease control (Büchler *et al.* 2010). In this aspect, the artificial selection seems to support natural selection, as increasing hygienic or grooming behaviour should help the bees to remove several pathogens and parasites otherwise causing diseases (Uzunov *et al.* 2014).

The aim of the present study was to characterize and compare genetic variability in groups of local *Apis mellifera macedonica* Ruttner, 1988 colonies, selectively reared for production of bee queens and bee swarms in Bulgaria and honey bee colonies with indicative hygienic behaviour and to define the levels of genetic similarity and distinction between them. Data obtained in this study could be useful for future selection with honey bees in Bulgaria and also as a scientific basis for purposeful bee breeding activities.

Materials and Methods

Honey bee samples were collected from managed colonies of *Apis mellifera macedonica* subspecies reared in Bulgaria. Totally, 851 worker bees from honey bee colonies, forming two groups – for queen production and for swarm productions and belonging to breeding stock of National Bee Breeding Association were tested in this investigation. Additionally, 414 worker bees from a group of colonies with indicative hygienic behaviour were included in the same study.

Testing of colonies for hygienic behaviour was carried out by modified method (Gurgulova *et al.* 2003), different from that of Taber & Gilliam (1988) and similar to the one applied by Petrov (1997). As colonies with indicative hygienic behaviour have been used those that have cleaned over 95% of the cells in the outlined area on the 24th hour after jabbing.

The thorax homogenization and electrophoresis in polyacrylamide gel were performed according to Ivanova (1996).

Two enzyme systems corresponding to two loci were studied: MDH (malate dehydrogenase, EC 1.1.1.37) and EST (esterase, EC 3.1.1). Buffers, electrophoretic conditions and histochemical staining used for each enzymic system were as it was described in Meixner *et al.*(2013).

Allele frequencies, mean number of alleles per locus, proportion of polymorphic loci, observed (H_o) and expected (H_e) heterozygosity, deviation from the Hardy-Weinberg equilibrium, mean value of gene flow (Nm) and F statistics were calculated using GENALEX software package(Peakall & Smouse 2006).

Results and Discussion

In this study, the enzyme systems analyzed (MDH and EST) were polymorphic in all the studied honey bee groups (Table 1). Totally, three alleles were detected at MDH-1 locus – MDH^{65}, MDH^{80} and MDH^{100} and five alleles – at EST-3 locus –EST^{80}, EST^{88}, EST^{94}, EST^{100}and EST^{118}. The most frequent alleles for the compared honey bee groups were MDH-1^{100} and EST-3^{100} – Figure 1. The MDH-1^{80} allele was found in the gene pool of the groups which were under selective control for queen and swarm production. Its frequency was less than 5%. EST-3^{94} allele was not detected in honey bee colonies with indicative hygienic behaviour but one of the rarer alleles – EST-3^{80} was found with higher frequency (0.071) in their gene pool – Table 1.

The number of alleles per locus for honey bee groups under selective control (bee queen and swarm production) was a little bit larger than for the group with indicative hygienic behaviour (Tables 2 and 3). The number of the effective alleles was rather similar for the compared groups (Tables2 and 3). The estimated percentage of polymorphic loci, using the 0.95 criterion, was 50% in both bee groups which were under selective control and 100% in the group of colonies with indicative hygienic behaviour. Without a criterion, percentage of polymorphic loci for all the studied groups was 100% (Table 1). In the present study, the mean levels of the observed and expected heterozygosity (Ho and He) for the different compared groups ranged from 0.261 to 0.354 and from 0.298 to 0.332, respectively (Table 3).

Table 1. Allele frequencies for the studied loci in compared honey bee groups

Locus	Allele	Queen production	Swarm production	Indicative hygienic behaviour
MDH-1	65	0.393	0.438	0.402
	80	0.023	0.031	0.000
	100	0.584	0.531	0.598
EST-3	80	0.013	0.006	0.071
	88	0.002	0.006	0.017
	94	0.004	0.002	0.000
	100	0.959	0.974	0.901
	118	0.022	0.012	0.011
P=0.95		50%	50%	100%
P=1.0		100%	100%	100%

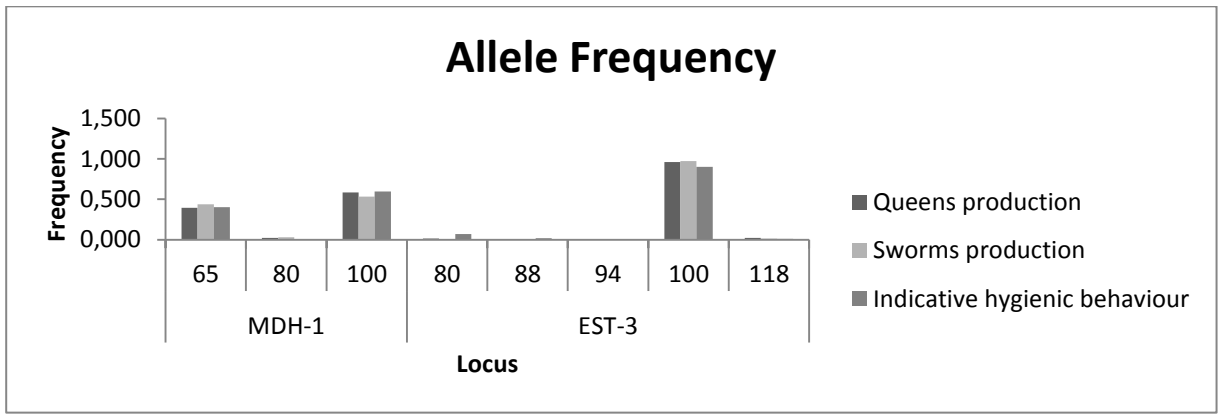

Fig. 1. Allele distributions for the loci studied with information about allele frequencies

Table 2. Number of samples per group (N), number of alleles (Na), number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity and F index

Groups	Locus	N	Na	Ne	Ho	He	F
Queen production	MDH-1	458	3.000	2.016	0.452	0.504	0.103
	EST-3	458	5.000	1.088	0.070	0.081	0.133
Swarm production	MDH-1	324	3.000	2.106	0.522	0.525	0.007
	EST-3	324	5.000	1.054	0.052	0.052	-0.018
Indicative hygienic behaviour	MDH-1	414	2.000	1.926	0.510	0.481	-0.060
	EST-3	414	4.000	1.224	0.198	0.183	-0.084

Table 3. Mean number of alleles (Na), mean number of effective alleles (Ne), mean levels of observed (Ho) and expected (He) heterozygosity and F index (Standard errors are included)

Groups		Na	Ne	Ho	He	F
Queen production	Mean	4.000	1.552	0.261	0.292	0.118
	SE	1.000	0.464	0.191	0.212	0.015
Swarm production	Mean	4.000	1.580	0.287	0.288	-0.006
	SE	1.000	0.526	0.235	0.237	0.012
Indicative hygienic behaviour	Mean	3.000	1.575	0.354	0.332	-0.072
	SE	1.000	0.351	0.156	0.149	0.012
Total	Mean	3.667	1.569	0.301	0.304	0.014
	SE	0.494	0.203	0.090	0.091	0.036

The higher levels of heterozygositisy in the group with indicative hygienic behaviour (Tables 2 and 3) which is in accordance with the higher level of polymorphism at 0.95 criterion calculated for the same group should be mentioned.

The calculated mean observed and expected heterozygosities for all groups were 0.301 and 0.304, respectively. There were found significant deviations of genotype frequencies from Hardy-Weinberg expectations at both the loci in the groups for bee queen and bee swarm production ($P \geq 0.001$) which is in connection with their selective control (Table 4). Data for the calculated F statistic which gives information concerning levels of heterozygosity in all compared honey bee groups are presented in Tables 2 and 3. The calculated F_{ST} and Nm are given in Table 5. The low levels of F_{ST} (0.002 – 0.012) correlated with the high levels of Nm which demonstrated low levels of genetic differentiation between the studied honey bee groups.

The calculated genetic distances (Nei 1972) ranged from 0.002 to 0.005 between different groups which demonstrated high similarity and was in accordance with data obtained from the applied assignment test – Figure 2. Our explanation concerning the observed in the present study low levels of genetic differentiation and high genetic similarity between the compared honey bee groups is related to the applied bee breeding activities. It is known that the local Bulgarian honey bee *A. m. macedonica* is a base for realization of National bee breeding programme. It is well adapted to the local conditions and characterized by valuable biological and productive quali-

Table 4. Chi-square test for deviation from Hardy-Weinberg equilibrium

Groups	Locus	DF	ChiSq	Prob	Signif
Queen production	MDH-1	3	34.572	0.000	***
	EST-3	10	129.047	0.000	***
Swarm production	MDH-1	3	54.007	0.000	***
	EST-3	10	0.235	1.000	ns
Indicative hygienic behaviour	MDH-1	1	1.485	0.223	ns
	EST-3	6	5.002	0.544	ns

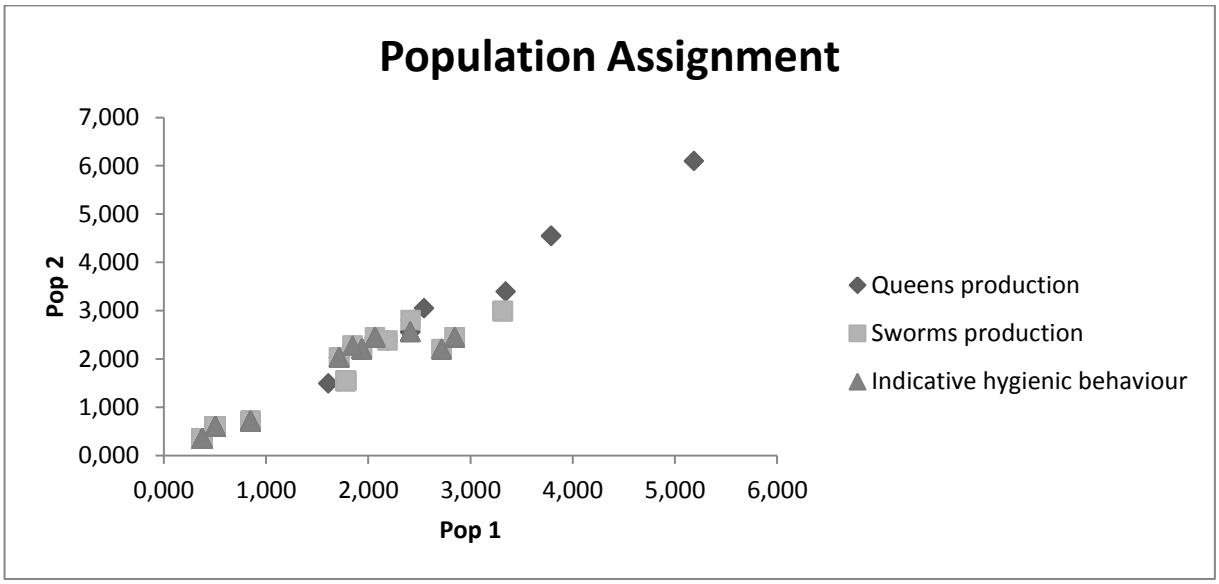

Fig. 2. Level of assignment between the individuals from the studied honey bee groups

Table 5. F_{ST} and Nm over the compared all honey bee groups

Pop1	Pop2	Fst	Nm
Queen production	Swarm production	0.002	128.425
Queen production	Indicative hygienic behaviour	0.007	36.477
Swarm production	Indicative hygienic behaviour	0.012	21.004

ties. The good hygienic behaviour is one of them. On the other hand, the included in the study honey bee colonies with indicative hygienic behaviour are selected only on this behaviour character and do not belong to the bee breeding bases performing National breeding program, which explains the slight differences found between the compared two groups.

Conclusions

Polymorphism with two and three alleles was found for MDH-1 locus and with four and five alleles – for EST-3 locus for the compared honey bee groups under selective control and for the group with indicative hygienic behaviour. Levels of polymorphism between 50% and 100% were calculated for the studied honey bee groups. The calculated mean F_{ST}, Nm and genetic distances (Nei 1972) showed low level of genetic differentiation. High genetic similarity among the studied honey bee groups under selective control and those with indicative hygienic behaviour was observed.

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