Genetic characterization of *Apis mellifera macedonica* (type "*rodopica"*) populations selectively controlled in Bulgaria

Genetichen analiz na populatsii *Apis mellifera macedonica* (tip "*rodopica"*) podlozheni na selekcionen kontrol v Bulgariya

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Abstract

The genetic variability in selectively controlled in Bulgaria local honey bee populations, representing *Apis mellifera macedonica* subspecies (type "*rodopica*"), has been studied by usage of alloenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci. Totally 324 worker bee individuals from 9 different local populations belonging to breeding stock of National Bee Breeding Association were included in this investigation. All of the studied loci were found to be polymorphic in most of the populations with the exception of EST-3 locus which was established to be fixed in two of investigated populations. Polymorphism with three alleles was ascertained for MDH, ME, ALP and PGM loci and with four alleles – for EST-3 and HK loci. The most common alleles in all of the investigated populations were ME 100, EST-3 100, PGM 100 and HK 100. Two private alleles (frequency < 0.05) were found for two of the studied populations. The calculated level of polymorphism was between 88.33% and 100%. The observed and expected heterozygosities were found to range from 0.186 to 0.301, and from 0.205 to 0.305, respectively. The calculated mean Fst level was 0.028. Allele frequencies of all studied loci were used to estimate Nei's (1972) genetic distance, which was established to range between 0.001 and 0.028 among the selectively controlled populations studied. The assignment test showed a high level of consolidation for the all studied populations.

Keywords: allozymes, *Apis mellifera*, bee breeding and selection, genetic variability

Abstrakt

V nastoyashtoto izsledvane, na genetichen aloenzimen analiz sa podlozheni selektsionno kontrolirani populatsii medonosni pcheli ot devet (9) bazi na Natsionalnata Razvadna Asotsiatsiya po Pchelarstvo v Balgariya, prinadlezhashti kam podvid Apis mellifera macedonica (mesten tip “rodopica”). Ustanoveno e, che vsichkite izsledvani 6 aloenzimni lokusi sa polimorfni v pochti vsichki ot prouchvanite populatsii, s izklyuchenie na EST-3 lokusat, fiksiran s ediniya si alel (EST-3 100) v dve ot populatsiite. Trialelen polimorfizam e ustanoven za MDH, ME, ALP i PGM lokusite, a chetirialelen – za EST-3 i HK lokusi. Izsledvaneto sochi, che s nay-golyama chestota na sreshtane v izsledvanite populatsii sa alelite ME 100, EST-3 100, PGM 100 i HK 100. Konstatirano e nalichieto na dva chastni alela (chestota < 0.05) v dve ot prouchvanite populatsii. Izchisleni sa niva na polimorfizam v diapazon ot 88.33% do 100%. Nablyudavanata i ochakvana heterozigotnost varirat mezhdu 0.186 i 0.301, i mezhdu 0.205 i 0.305, saotvetno. Izchislenoto sredno nivo na fiksatsionniya indeks (Fst) e 0.028. Ustanovenite genetichni distantsii po Nei (1972) sa v granitsata ot 0.001 do 0.028. Provedeniyat asignatsionen test (test za prinadlezhnost) pokazva visoko nivo na konsolidatsiya sred podlozhenite na selektsionen kontrol i vklyucheni v nastoyashtoto izsledvane populatsii.

Klyuchovi dumi: aloenzimi, *Apis mellifera*, genetichna izmenchivost, pchelarstvo i selektsiya

Introduction

Honeybee selection in Bulgaria has long traditions and history. The local Bulgarian honeybees have been studied morphologically since the 30s of the last century (Lazarov, 1935, 1936; Velichkov, 1970). During the period of 1967-1975 mainly morphological studies of the local bee have been carried out and the results obtained have been used as a basis for organization of bee selection in Bulgaria over the period of 1971-1990.

Since 1999 a new National Program for beebreeding has been announced and its main purpose was to ensure the gene pool conservation of the local Bulgarian honey bee which has proven biological and productive advantages and good adaptation to the specific local conditions (Petrov, 2010). In this aspect, morpho-ethological investigations by specific characteristics have been carried out in the country in order to determine the race standard (Petrov, 1990, 1993, 1995, 1997, Petrov and Ivanova, 2009) of the local honey bee. In addition, some biochemical and molecular-genetic researches of the polymorphism in some protein, isoenzyme and DNA systems have been performed since 1996 (Ivanova, 1996; Ivanova and Bouga, 2009; Ivanova et al., 2007, 2008).

The aim of the present study was to investigate, characterize and compare genetic variability in different selectively controlled and purposefully used in National Bee breeding Program *Apis mellifera macedonica* (type “*rodopica*”) populations and to provide information on population genetic parameters appropriate for diagnostic

markers. Data obtained in the research could be useful for the selection activities with honey bees in Bulgaria and also, in complex with morpho-ethological characteristics - as clear scientific bases for national honeybee breeding.

Materials and methods

Honey bee samples

Honey bee samples were collected from managed colonies of *Apis mellifera macedonica* subspecies (type *rodopica*) reared in Bulgaria. Totally 324 worker bee individuals from 9 different local populations belonging to breeding stock of National Bee Breeding Association were tested in this investigation. Ten colonies per populations were included in the study. Three to five individuals were taken from each colony. Collected worker bees were transported to the laboratory alive and stored at -20°C until being used. The thorax homogenization and electrophoresis in polyacrylamide gel were performed according to Ivanova (1996).

Allozyme analysis

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), 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 used for each enzymic system were as it was described in Meixner et al. (2013).

Statistical Analyses

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, mean value of gene flow (Nm), F statistics concerning heterozygosity in total population (F_{IT}), within subpopulation (F_{IS}) and for subpopulations within the total population (F_{ST}), and Nei’s genetic distances (Nei, 1972), were calculated using GENALEX software package (Peakall and Smouse, 2006).

Results

In this research the enzyme systems studied (MDH-1, ME, EST-3, ALP, PGM and HK) were polymorphic in all of the populations, at the 95% level, having three or four different alleles in the studied populations (Figure 1.). Three alleles were detected at MDH-1 ($MDH^{\mathbf{65}}$, $MDH^{\mathbf{80}}$ and $MDH^{\mathbf{100}}$), ME ($ME^{\mathbf{90}}$, $ME^{\mathbf{100}}$ and $ME^{\mathbf{106}}$), ALP (ALP^{80}, $ALP^{\mathbf{90}}$ and $ALP^{\mathbf{100}}$) and PGM (PGM^{80}, PGM^{100} and PGM^{114}) loci. Four alleles were detected at EST-3 ($EST^{\mathbf{80}}$, $EST^{\mathbf{88}}$, $EST^{\mathbf{100}}$ and $EST^{\mathbf{118}}$) and HK (HK^{87}, HK^{100}, HK^{110} and HK^{121}) loci.

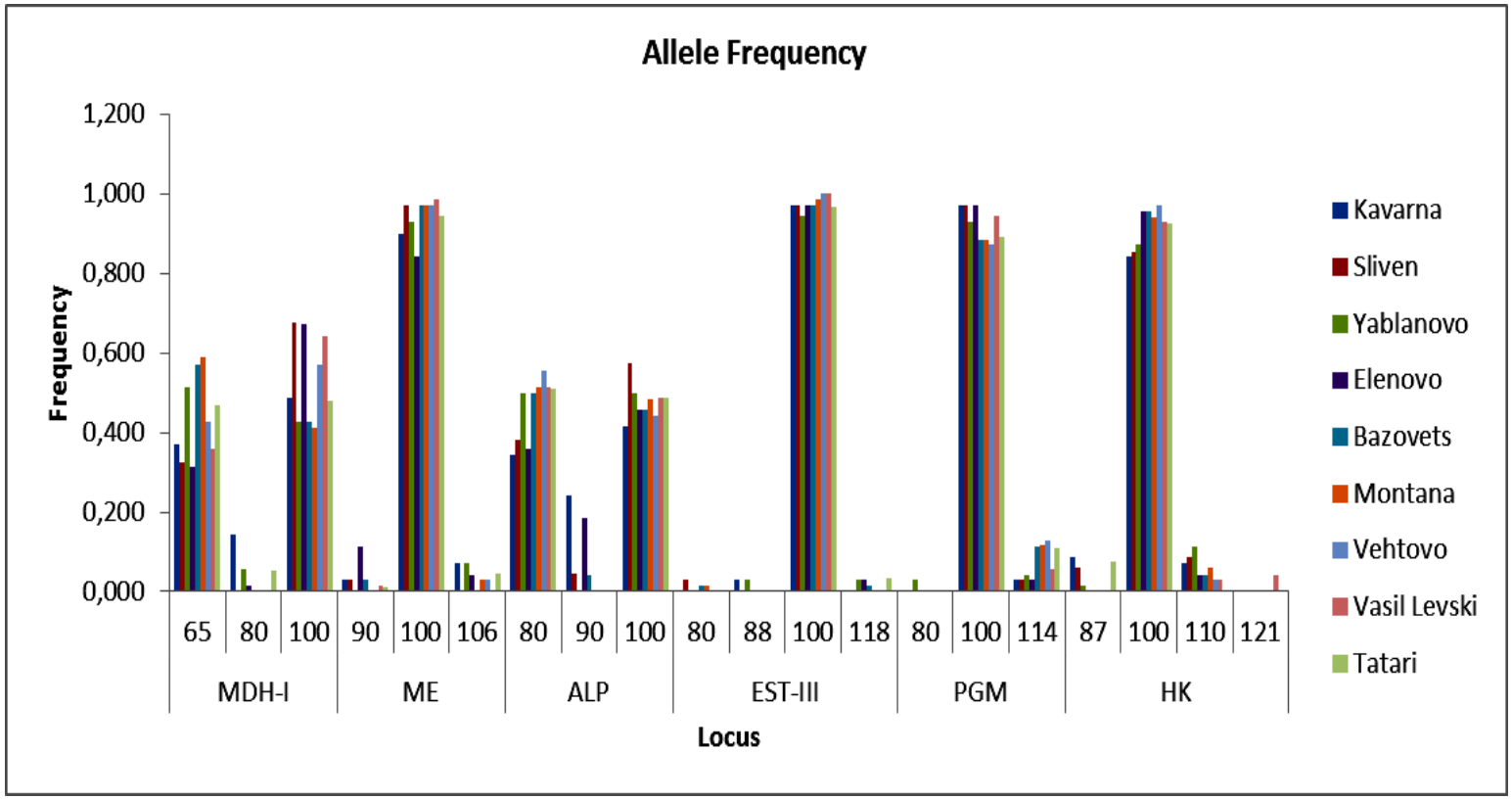

Figure 1. Allele frequencies for the loci studied

Figura 1. Alelni chestoti za izsledvanite lokusi

Table 1. Number of alleles (Na), number of effective alleles (Ne), percentage of polymorphic loci (%P), observed (Ho) and expected (He) heterozygosity (Standard errors – SE are included) per a population studied. The number of worker bees per a population is presented in brackets.

Tablitsa 1. Broy aleli (Na), efektiven broy aleli (Ne), protsent polimorfni lokusi (%P), poluchena (Ho) i ochakvana heterozigotnost (He) (Standartnata greshka e vklyuchena) za izsledvanite populatsii. Broyat izsledvani pchelni individi za populatsiya e predstaven v skobi.

Pop		Na	Ne	%P	Ho	He
Kavarna (35)	Mean	2.667	1.689	100	0.219	0.305
	SE	0.211	0.328		0.099	0.108
Sliven (34)	Mean	2.333	1.402	100	0.211	0.232
	SE	0.211	0.180		0.081	0.086
Yablanovo (35)	Mean	2.667	1.490	100	0.248	0.276
	SE	0.211	0.199		0.108	0.081
Elenovo (35)	Mean	2.500	1.517	100	0.229	0.258
	SE	0.224	0.265		0.094	0.098
Bazovets (35)	Mean	2.333	1.432	100	0.195	0.237
	SE	0.211	0.204		0.085	0.091
Montana (34)	Mean	2.000	1.402	100	0.186	0.231
	SE	0.000	0.182		0.077	0.086
Vehtovo (35)	Mean	1.833	1.390	83.33	0.190	0.220
	SE	0.167	0.187		0.080	0.091
Vasil Levski (35)	Mean	2.000	1.359	83.33	0.214	0.205
	SE	0.258	0.181		0.110	0.089
Tatari (46)	Mean	2.333	1.468	100	0.301	0.258
	SE	0.211	0.206		0.120	0.086
Total (324)	Grand mean	2.296	1.461	96.30	0.221	0.247
	SE	0.073	0.069	2.45	0.030	0.028

It was calculated that, the mean number of alleles per locus varied from 1.833 (Vehtovo) to 2.667 (Kavarna and Yablanovo) and the effective number of alleles varied from 1.359 (Vasil Levski) and 1.689 (Kavarna) (Table 1.). The estimated percentage of polymorphic loci, using the 0.95 criterion, was 100% in almost all of studied populations with exceptions of Vasil Levski and Vehtovo where the level of polymorphism was calculated as 83.3% (Table 1.).

In the present study, the observed and expected heterozygosities (Ho and He) ranged from 0.186 (Montana) to 0.301 (Tatari) and from 0.205 (Vasil Levski) to 0.305 (Kavarna), respectively (Table 1.). The calculated mean expected heterozygosity was 0.247.

Two private alleles were observed in two of the studied populations – Yablanovo (PGM80) and Vasil Levski (HK121).

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 showed that the deviations were generally in favor of homozygotes.

The calculated F statistic giving information about the heterozygosity in all populations is shown in Table 2. The mean heterozygosity in total populations FIT averaged to 0.188 (-0.017 – 0.495). Mean heterozygosity within subpopulation FIS was 0.164 (-0.032 – 0.480). The fixation coefficients of subpopulations for the loci studied within the total populations, measured as FST value, varied from 0.015 (EST-3) to 0.042 (MDH-1), with a mean of 0.028 (Table 2.).

Table 2. Mean expected (He) and observed (Ho) heterozygosity, F statistics and Nm values per locus for all populations

Tablitsa 2. Sredna ochakvana i poluchena heterozigotnost za lokus, F-statistika i potok na geni (Nm) za vsichki populatcii

Locus	Mean He	Mean Ho	Fis	Fit	Fst	Nm
MDH-1	0.502	0.516	-0.028	0.016	0.042	5.634
ME	0.105	0.071	0.325	0.348	0.034	7,034
ALP	0.537	0.500	0.070	0.095	0.027	8.951
EST-3	0.047	0.049	-0.032	-0.017	0.015	16.685
PGM	0.138	0.114	0.172	0.191	0.024	10.368
HK	0.152	0.079	0.480	0.495	0.029	8.467
		Mean	0.164	0.188	0.028	9.523
		SE	0.084	0.082	0.004	1.579

The calculated in this study mean value of gene flow (Nm) was 9.523.

The genetic distances (Nei, 1972) ranged from 0.001 (between Buzovetc and Montana) to 0.028 (between Elenovo and Montana).

Summary of population assignment outcomes to 'self' or 'other' population is presented in Figure 2.

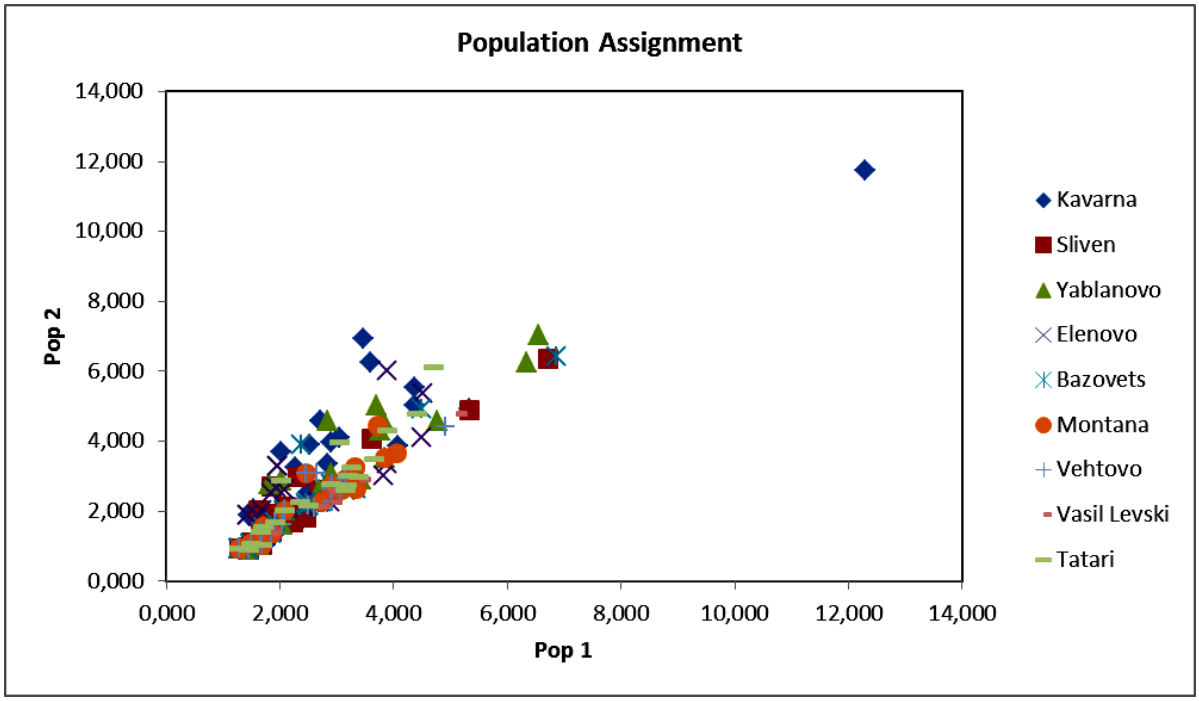

Figure 2. Population assignment between individuals from the populations studied

Figura 2. Populatsionno saotnasyane mezhdu individite ot izsledvanite populatsii

Discussion

Gene heterozygosity is a suitable parameter for investigating the genetic variability. According to the Ott (2001) opinion, a polymorphic locus must have a heterozygosity of at least 0.10. In this aspect and on the base of calculated level of polymorphism it was found in the present investigation that all of the studied six alloenzymic loci had relatively high polymorphisms (83.3% - 100%) with level of expected heterozygosity which ranged between 0.205 and 0.305.

There was not found considerable differences between total mean number of alleles per locus (1.833 - 2.667) and the effective allele number (1.359 - 1.689). The effective number of alleles is the number of alleles with equal frequencies (Ne = 1/(1-H_{exp})). By way of information, alleles with low frequencies contribute very little to the effective number of alleles, so a presence of discrepancy between “number of alleles per locus” and “effective number of alleles per locus” gives information about the presence of alleles with lower (not equal) frequency in the population.

According to Hartl and Clark (2007), F_{ST} levels between 0 and 0.05 indicate low genetic differentiation, between 0.05 and 0.15 – moderate, between 0.15 and 0.25 – high genetic differentiation and levels larger than 0.25 designate highly significant genetic differentiation. According to this information, F_{ST} values for all of the studied in the present investigation loci (0.015 – 0.042), demonstrated low levels of genetic differentiation. As this was mentioned before, the estimated mean F_{ST} value was 0.028 which shows that 2.8% of the overall observed genetic diversity was among populations, as opposed to 97.2% within populations.

The gene flow (Nm) values give the information about genetic divergence or genetic similarity of subpopulations due to the gene flow. If Nm value is smaller than 2, there is still considerable genetic differentiation among subpopulations. Data received in this investigation showed that Nm was greater than 2 for all of the studied loci (5.634 for the MDH-1locus – 16.685 for the EST-3 locus), which indicated very low genetic differentiations among the studied populations.

In accordance with this, the assignment test showed a high level of consolidation for the all studied populations (Figure 2).

Conclusions

The results about allozyme polymorphism characterized in this research give additional detailed genetic information concerning selectively controlled honey bee populations in Bulgaria which belong to local subspecies *Apis mellifera macedonica* (type “*rodopica*”). These results would be useful for more precise description of the local honey bee by giving possible appropriate genetic markers for future selection and conservation.

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