Iranian Int. J. Sci. **5***(2), 2004, p.151-166*

Morphometric and Allozyme Variability in Persian Bee Population from the Alburz Mountains, Iran

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(received: 4/9/2003 ; accepted: 9/8/2004)

Abstract

Apis mellifera meda is one among those two dozen subspecies which are the most widely distributed in the world. While in our first description, its distribution is restricted to North Persia, Lencoron (on the Caspian Sea coast), in later studies it extends westward, to the north of Iraq and Syria, and to the southeastern parts of Turkey. To investigate the geographic variation and population structure of the honey bees of northern Iran that are among the least studied populations of *Apis mellifera*, samples were collected in the Alburz mountains, in the south of the Caspian Sea.

A total of 46 colonies were sampled from six different locations (three in the highlands: Khanchay, Sefar, Zanjan; and three in the lowlands, Gholikandy, Gultepe and Duzteran Fig.1). Out of six enzymes assayed, four were found to be polymorphic (*Pgm*, *Hk*, *Mdh*, and *Est*) and two (*Pgi* and *Me*) displayed invariant banding patterns. Gene frequencies, enzyme heterozygosities and usefulness of gene frequencies to Hardy-Weinberg expectations were analyzed with the BIOSYS-1 package. The overall grand mean heterozygosity for all populations was calculated as 0.052±0.036. A Distance Wagner analysis based on the Prevosti distance divided the honey bee samples into two groups according to their origin from lowlands and highlands. Morphmetric variations of the colonies were assessed using parameters measured from hind and fore-wing and from legs. Different multivariate analyses were applied for the determination of different honey bee populations in the Alburz Mountains in Iran.

Keywords: *Apis, mellifera, Iran, Allozyme, Turkey.*

Introduction

The Persian bee, *Apis mellifera meda*, was first described by Skorikov (1929) based on the length of the tongue and the shape of the abdominal sternites which are no longer used in taxonomy (Ruttner *et al*., 1985). In our first description, the distribution is given as North Persia, Lencoron (Located on the Caspian Sea); however , the distribution of *A.m.meda* later extends to the north of Iraq and Syria and also to the southeastern parts of Turkey (Ruttner, 1988).

Iran is the most populated Asian country in terms of honey bee colonies. Current statistics (FAO, 2002) reported that there are more than 3 million colonies present in Iran. However, the presence of this much honey bee colonies are not projected well into honey yield in Iran. This is also well seen from the current statistics of honey export from Iran. There are several reasons for this low productivity. Firstly, honey bee keeping experience is still inappropriate and does not meet the requirements of modern bee keeping technology. Secondly, migratory bee keeping practices are not performed effectively. Lastly and may be the most importantly the population of honey bee decreases due to improper medical treatments and queen replacements (Honey bee keeping observations in Iran in 2002).

Compared to the other population of *Apis mellifera*, Persian honey bee population has been less extensively studied. Morphometric analysis has been used to study *A. m. meda* and its variation in relation to surrounding subspecies. The morphometric differences between low and high lands were also shown (Ruttner *et al*., 2000), but genetic variability in terms of allozymes and DNA is unknown.

From the morphometric analysis, Ruttner (1988) reported that *A. m. meda* is placed in the centre of the Middle Eastern group (*A. m. syria, A. m. anatoliaca, A. m. caucasica*). Persian honey bee subspecies, *Apis mellifera meda*, is distributed in a large geographic area. Ruttner (1988) mentioned that within this large geography there are six major population of the subspecies and some of these population formed distinct groups; however, some overlap each other. He also added that this subspecies is at the beginning of its taxonomic radiation. That is why it is important to carry out an extensive survey in Iran and try to find out the extend of morphometric and genetic variation in Persian honey bee population. The preliminary results of this study was

presented at in APIMONDIA congress in Slovenia in August 2003 (Kandemir *et al*., 2003). Enzyme polymorphisms in honey bee subspecies have been extensively studied since 1980 (Badino *et al*., 1983; 1985; 1988; Del Lama *et al*., 1985; 1988; Sheppard and McPheron, 1986; Sheppard and Berlocher, 1984; 1985; Sheppard, 1988; Kandemir and Kence, 1995). However there is no study on the allozyme variability in honey bee population in Iran.

The objectives of this study were to determine the genetic variability with respect to six enzyme systems and 26 morphometric characters, and to compare the results with the published data from surrounding honey bee populations.

Materials and Methods

Honey bee samples were collected in July 2002 from south of the Caspian Sea, Iran. The area is mountainous and the high lands reach 2500 meters. Bees were collected from the south of the Alburz Mountains. Lowland bees are on the plain areas not more than 800 meters in altitude. Six locations, three from highland (Hancayi, Sefar, Zanjan) and three from lowland (Gultepe, Duzteran, Gholikandy) and 12 apiaries were visited. A total of 46 honey bee colonies were sampled. Honey bee thoraces were grinded in distilled water and run on 12% starch gels. Allozymes were detected after gels were stained for Esterase (*Est*, E.C. 3.1.1), Hexkinase (*Hk*, E.C. 2.7.1.1), Phosphoglucomutase (*Pgm*, E.C. 5.4.2.2 formerly E.C. 2.5.7.11), Malate dehydrogenase (*Mdh*, E.C. 1.1.1.37), Malic enzyme (*Me*, E.C. 1.1.1.40) and Phosphoglucose isomerase (*Pgi*, E.C. 5.3.1.9). Detailed experimental procedures were described in Kandemir and Kence (1995). A total of 26 morphometric variables from wings and legs were measured on both sides (left and right) (Table 1). For morphometric study, samples collected from 77 locations were dissected into legs and wings. Forewings and hind legs were mounted onto microscope slide with a plastic tape. Prepared specimens were observed under the Olympus dissection microscope and images were projected onto JVC TV screen by a JVC video camera. Morphometric characters were measured on both sides and the data was calibrated with a factor of 11.5 and 8.5 to convert the measurements into millimeters (Table 1).

Table 1. Morphometric characters measured on both sides of the body and their abbreviations.

	Characters	Abbreviation
	On the right side	
1	Forewing Length	RFWL
2	Forewing Width	RFWW
3	Forewing Cubital A	RcuA
4	Forewing Cubital B	RcuB
5	Forewing D Length	RWDL
6	Forewing C Length	RWCL
7	Hindwing Length	RHWL
8	Hindwing Width	RHWW
9	Hindwing Hamuli number	RHAM
10	Femur Length	RFL
11	Tibia Length	RTL
12	Metatarsus Length	RMTL
13	Metatarsus Width	RMTW
	On the left side	
14	Forewing Length	LFWL
15	Forewing Width	LFWW
16	Forewing Cubital A	LCuA
17	Forewing Cubital B	LCuB
18	Forewing D Length	LWDL
19	Forewing C Length	LWCL
20	Hindwing Length	LHWL
21	Hindwing Width	LHWW
22	Hindwing Hamuli number	LHAM
23	Femur Length	LFL
24	Tibia Length	LTL
25	Metatarsus Length	LMTL
26	Metatarsus Width	LMTW

Statistical Analysis

A total of more than 500 honey bee samples were used in electrophoresis. Genotypes were determined for six enzyme systems for 46 honey bee colonies. Gene frequencies, enzyme heterozygosities, usefulness of gene frequencies to Hardy-Weinberg expectations were analyzed with the BIOSYS-1 package (Swofford and Selander, 1981). Morphometric data from 26 morphological characters were used in univariate and multivariate statistical analysis. Analysis of Variance (ANOVA) (SPSS package), Principical Component Analysis (NTSYS-pc, Rohlf, 1992), Discriminant Function Analysis (SYNTAX-pc, Padoni, 1993) and also Fluctuating Asymmetry values (Palmer and Strobeck, 1986) were calculated using the formula (1) for the honey bee populations.

$$FA = \frac{\sum (R_i - L_i)^2}{N} \qquad \text{Formula (1)}$$

Results

In the present study, 13 morphometric variables were measured for morphometric analysis. Their original measurements and standard errors were given in Table 1 (A and B). In addition to morphometric analysis 6 enzyme systems were utilized and gene frequencies were calculated and tabulated in Table 3 (Tables 2 and 3).

Table 2. Morphometric measurements of 26 characters (A) from Right and (B) from Left

A

Loc	N	RFWL	□SE	RFWW	□SE	RCubA	□SE	RCubB	□SE	RDL	□SE	RCL	□SE	RHWL	□SE
1	90	9.367	0.016	3.107	0.008	0.558	0.004	0.229	0.003	2.080	0.006	0.920	0.003	6.492	0.074
2	30	9.406	0.023	3.118	0.013	0.561	0.008	0.230	0.005	2.070	0.010	0.924	0.006	6.638	0.017
3	117	9.201	0.081	3.090	0.007	0.555	0.004	0.237	0.003	2.053	0.005	0.912	0.003	6.475	0.057
4	120	9.265	0.014	3.067	0.006	0.539	0.004	0.254	0.015	2.053	0.005	0.907	0.003	6.524	0.012
5	38	9.379	0.030	3.074	0.012	0.555	0.008	0.237	0.005	2.072	0.010	0.915	0.006	6.565	0.022
6	60	9.317	0.018	3.133	0.008	0.546	0.006	0.247	0.003	2.062	0.007	0.932	0.003	6.557	0.016

Loc	N	RHWW	□SE	RHAM	□SE	RFL	□SE	RTL	□SE	RML	□SE	RWW	□SE	CI
1	90	1.875	0.005	21.811	0.153	2.757	0.007	3.318	0.010	2.042	0.007	1.184	0.006	2.437
2	30	1.884	0.007	22.233	0.335	2.733	0.009	3.301	0.012	2.065	0.011	1.149	0.012	2.434
3	117	1.833	0.019	21.888	0.161	2.714	0.007	3.259	0.008	2.012	0.006	1.145	0.004	2.336
4	120	1.849	0.005	21.683	0.136	2.700	0.006	3.257	0.007	2.013	0.006	1.145	0.005	2.124
5	38	1.844	0.010	22.421	0.271	2.706	0.010	3.279	0.013	2.042	0.010	1.136	0.007	2.343
6	60	1.857	0.006	22.267	0.228	2.720	0.007	3.281	0.010	2.055	0.009	1.156	0.006	2.211

B

Loc	N	LFWL	□SE	LFWW	□SE	LCubA	□SE	LCubB	□SE	LDL	□SE	LCL	□SE	LHWL	□SE
1	90	9.306	0.017	3.046	0.008	0.558	0.004	0.228	0.003	2.000	0.005	0.901	0.003	6.765	0.013
2	30	9.342	0.022	3.057	0.012	0.545	0.007	0.235	0.006	1.984	0.007	0.896	0.006	6.860	0.022
3	117	9.143	0.081	3.004	0.027	0.550	0.004	0.238	0.005	1.974	0.005	0.898	0.003	6.637	0.059
4	120	9.226	0.014	3.022	0.006	0.525	0.004	0.237	0.003	1.973	0.004	0.893	0.003	6.700	0.011
5	38	9.317	0.030	3.033	0.012	0.543	0.008	0.237	0.004	1.981	0.008	0.896	0.005	6.767	0.021
6	60	9.286	0.018	3.081	0.009	0.518	0.006	0.244	0.004	1.975	0.008	0.916	0.004	6.637	0.113

Loc	N	LHWW	□SE	LHAM	□SE	LFL	□SE	LTL	□SE	LML	□SE	LMW	□SE	CI
1	90	1.844	0.005	21.456	0.160	2.764	0.007	3.303	0.009	2.046	0.008	1.179	0.005	2.447
2	30	1.847	0.007	22.433	0.310	2.726	0.011	3.277	0.010	2.055	0.008	1.159	0.007	2.321
3	117	1.824	0.005	21.889	0.158	2.721	0.007	3.250	0.007	2.012	0.006	1.142	0.004	2.311
4	120	1.817	0.005	21.600	0.159	2.723	0.005	3.250	0.006	2.018	0.006	1.145	0.004	2.214
5	38	1.808	0.009	22.500	0.274	2.730	0.011	3.280	0.013	2.041	0.009	1.146	0.005	2.295
6	60	1.759	0.043	22.117	0.192	2.764	0.007	3.283	0.009	2.062	0.007	1.160	0.005	2.122

Table 3. Allele frequencies of four polymorphic enzymes from six *A.m.meda* populations in Iran.

Locations	# of hives	# of bees	Pgm 100	Pgm 128	Hk 87	Hk 100	Mdh 65	Mdh 87	Mdh 100	Est 70	Est 100
Hancayi	9	90	1.000	0.000	0.256	0.744	0.111	0.039	0.850	0.000	1.000
Sefar	3	30	1.000	0.000	0.050	0.950	0.033	0.000	0.967	0.000	1.000
Zanjan	12	117	1.000	0.000	0.034	0.966	0.132	0.034	0.833	0.017	0.983
Gholikandy	12	120	0.992	0.008	0.033	0.967	0.096	0.000	0.904	0.000	1.000
Gultee	4	38	0.961	0.039	0.000	1.000	0.039	0.000	0.961	0.000	1.000
Duzteran	6	60	0.875	0.125	0.000	1.000	0.025	0.000	0.975	0.000	1.000

Morphometric analysis

Out of the 13 morphometric variables, four were found to be significantly different among populations ($P<0.001$) (Table 4).Other variables are not significant among populations. Figure 1 shows the result of discriminant function analysis. Two main groups were

visualized, in one group, low land populations clustered and in the other group high land populations, from Alburz mountains in Iran (Table 4 , Fig 2).

Table 4. Univariate (ANOVA) result of 13 morphometric variables of Persian honey bee populations.

Characters	Among Group SSQ	Within Group SSQ	F Ratio
Forewing Length	.02	.00	2.560*
Forewing Width	.00	.00	2.056ns
Forewing Cubital A	.00	.00	6.694**
Forewing Cubital B	.00	.00	0.904ns
Forewing D Length	.00	.00	1.394ns
Forewing C Length	.00	.00	2.139ns
Hindwing Length	.02	.00	4.150**
Hindwing Width	.00	.00	1.782ns
Hindwing Hamuli number	1.04	.63	1.664ns
Femur Length	.00	.00	1.872ns
Tibia Length	.00	.00	2.184ns
Metatarsus Length	.00	.00	2.173ns
Metatarsus Width	.00	.00	4.175**

The 1st axis explains 45.12%, the 2nd axis 23.54% and the 3rd axis 19.75 of the total variation respectively (Table 5). 88.41 % of the total variation could be explained by the first three canonical variates. Contributions of the morphometric variables to discrimination on the first three vectors can be seen in Table 6. In Table 6, Forewing Cubital A value contributes the most in separation of groups along the first axis. Forewing D lenght and Metatarsus With contribute the most to discrimination of groups along the second and third axis respectively(Table 5 and 6).

Table 5. Eigenvalues, % of variance and cumulative variance (only eigenvalues over 1 shown in table) of canonical variates.

Canonical Variates	Eigenvalues	Eigenvalues %
1	1.69	45.12
2	0.88	23.54
3	0.74	19.75

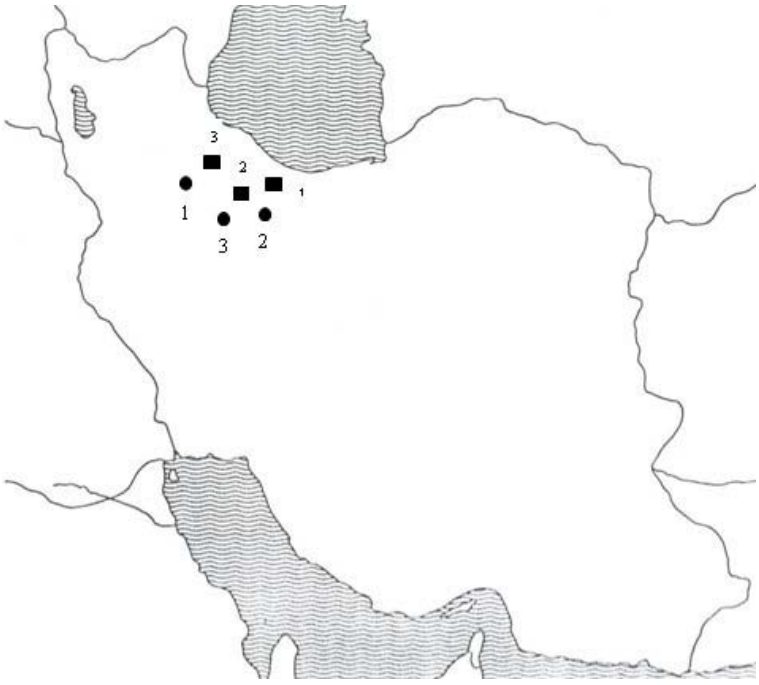

Figure 1:Samples from six different locations (three in the highlands: (•) Hancayi , Sefar , Zanjan ; and three in the lowlands: (■) Gholikandy , Gultepe and Duzteran).

Table 6. Contributions of 13 morphometric variables in Discriminant Function Analysis on the first three vectors.

Characters	C.V. 1	C.V. 2	C.V. 3
Forewing Length	6.407	-4.756	-7.560
Forewing Width	-5.879	10.468	10.299
Forewing Cubital A	50.314	-17.209	-6.227
Forewing Cubital B	-12.868	-6.426	-13.667
Forewing D Length	2.342	28.169	11.433
Forewing C Length	-19.141	-24.094	4.383
Hindwing Length	2.377	-15.665	9.486
Hindwing Width	10.089	7.869	-11.455
Hindwing Hamuli number	-0.960	-0.556	0.167
Femur Length	-6.551	6.852	3.788
Tibia Length	7.685	4.758	-0.761
Metatarsus Length	-9.594	-4.123	0.846
Metatarsus Width	-1.789	25.573	34.672

Fluctuating Asymmetry values were also calculated and tabulated in Table 7. The highest heterogeneity were obtained in Zanjan populations which has the lowest asymmetry values of all morphometric variables (Table 7).

Allozymes

Of the six enzyme systems assayed with horizontal starch gel electrophoresis, four were found to be polymorphic (*Pgm-1, Mdh-1, Hk*, and *Est-3*) and two (*Pgi* and *Me*) exhibited invariant banding pattern. All isozymes designated follow relative mobilities with respect to the most common isozyme used as a standard (Mobility 100) (Table 3).

Pgm-1: Two alleles were observed for *Pgm* locus in this study: *Pgm-100* and *Pgm-128*. The most common allele (*Pgm-100*) ranges between 0.875 and 0.992 in three polymorphic populations.

Hk: *Hk* locus showed four alleles: *Hk-77* and *Hk-100*. The most common allele (*Hk-100*) ranges between 0.966 and 0.950 in four polymorphic populations.

Mdh-1: The most variant enzyme system was polymorphic in all six populations. The polymorphism of *Mdh-1* in this study consisted of three alleles: *Mdh-65, Mdh-87* and *Mdh-100*. The most common allele (*Mdh-100*) frequency ranges between 0.875 and 0.992 in polymorphic populations.

Est-3: Three alleles were found for this locus: *Est-70, Est-100* and *Est-130*. The most common allele (*Est-100*) was found to be 0.983 in one polymorphic population.

Pgi & Me: One invariant banding pattern was observed for both of the enzyme systems.

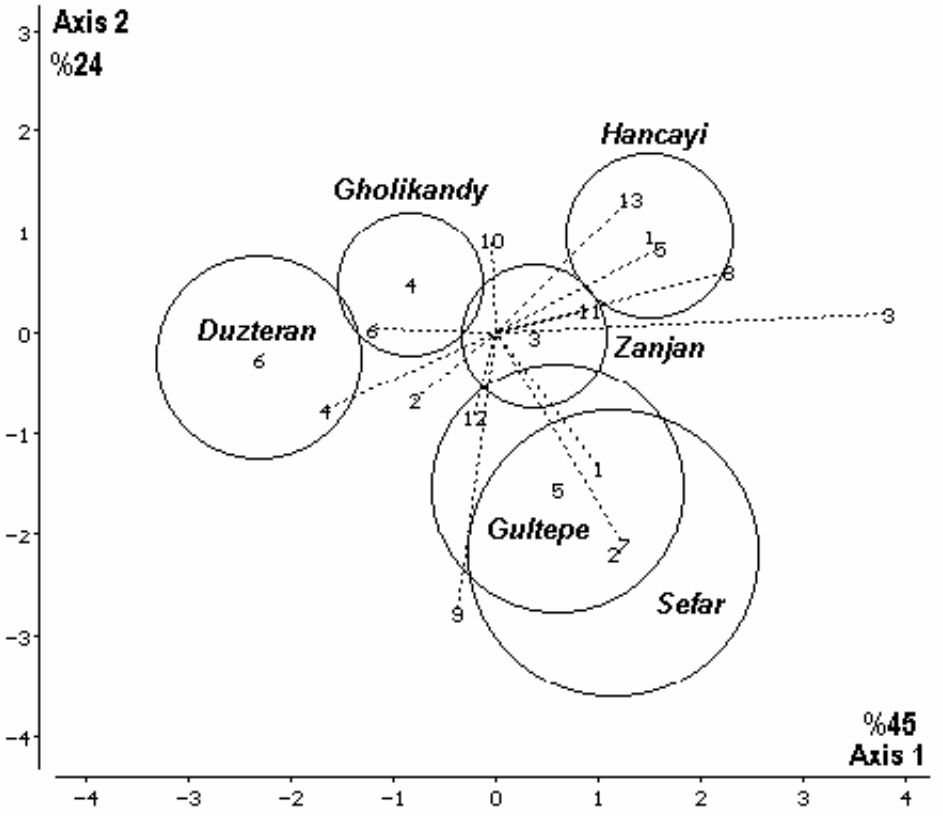

Figure 2: Discriminant Function Analysis of 13 morphometric variables showing clustering among 6 Persian honey bee populations.

Table 7. Fluctuating Asymmetry values of morphometric characters and the populations.

Loc	N	FWL	FWW	Cu A	Cu B	FWDL	FWCL
1	90	0.009	0.006	0.002	0.001	0.008	0.001
2	30	0.010	0.006	0.002	0.001	0.009	0.002
3	117	0.009	0.005	0.002	0.002	0.008	0.001
4	120	0.006	0.003	0.003	0.001	0.007	0.001
5	38	0.010	0.004	0.002	0.001	0.010	0.001
6	60	0.006	0.005	0.003	0.001	0.011	0.001

Loc	HWL	HWW	HAM	FL	TL	MTL	MTW	FA values
1	0.574	0.003	2.844	0.003	0.003	0.002	0.002	3.457
2	0.060	0.003	3.267	0.003	0.004	0.002	0.003	3.372
3	0.403	0.014	1.922	0.004	0.003	0.002	0.002	2.376
4	0.039	0.003	2.783	0.003	0.003	0.002	0.002	2.856
5	0.046	0.003	2.605	0.002	0.003	0.001	0.002	2.690
6	0.043	0.063	2.517	0.004	0.003	0.001	0.002	2.659

Table 8 shows the summary of genetic variability at six loci in all populations. The highest number of alleles was observed in Zanjan. The maximum polymorphism was observed (33.3) in Hancayi colonies. At the same time, Hancayi has the highest mean heterozygosity (0.108) among 6 populations studied. Overall heterozygosity for all enzyme systems was calculated as 0.052 ± 0.036 in the present study for all honeybee populations. All lowland populations (Gholikandy, Gultepe and Duzteran) were in Hardy Weinberg equilibrium for all enzyme loci, whereas there were deviations from Hardy Weinberg equilibrium for *Hk* (Hancayi, Sefar, Zanjan) and *Mdh* (Zanjan) in favor of heterozygotes in high land populations (χ^2 test, $P<0.05$)(Table 8).

Table 8. Summary of genetic variation based on six allozyme loci in six populations of A.m.meda in Iran.

Locations	Mean # of alleles per locus	Percentage of polymorphic loci*	Mean heterozygosity observed	Mean Heterozygosity Expected
Hancayi	1.5±0.3	33.3	0.120±0.081	0.108±0.070
Sefar	1.3±0.2	16.7	0.017±0.011	0.027±0.018
Zanjan	1.7±0.3	16.7	0.053±0.037	0.065±0.046
Gholikandy	1.5±0.2	16.7	0.046±0.031	0.043±0.028
Gultepe	1.3±0.2	0.0	0.026±0.017	0.026±0.016
Duzteran	1.3±0.2	16.7	0.050±0.041	0.045±0.036

**A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.*

Table 9 shows the population differentiations (F_{ST}) based on the studied enzyme systems. The highest differentiation among populations was caused by the *Hk* enzyme system. The lowest differentiation, however, was due to *Est-3*. The homogeneity $\div^2$ test (9) among populations showed highly significant ($P<0.001$) heterogeneity due to the differences in allele frequencies (Table 10).
Figure 3 shows the phylogenetic relationship among populations as revealed by the Distance-Wagner analysis based on the prevosti genetic distance. Two main clusters were formed separating the low and high land populations in Persian honey bees.

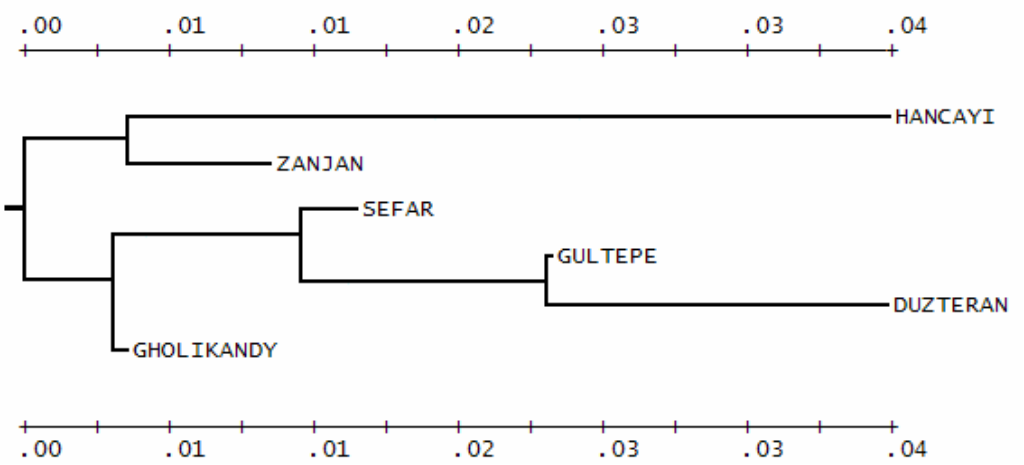

Figure 3: Distance Wagner tree based on Prevosti distance of allozyme data of six honey bee populations in Iran.

Table 9. Summary of F-statistics at all loci.

Locus	F(IS)	F(ST)	F(IT)
PGM-1	-0.1117	0.0803	-0.0225
HK	-0.0846	0.1296	0.0556
MDH-1	0.0562	0.0249	0.0797
EST-3	-0.0174	0.0128	-0.0044
Mean	-0.0106	0.0676	0.0577

Table 10. Contingency chi-square analysis at all loci.

Locus	# of Alleles	Chi Square	DF	P
PGM-1	2	73.065	5	0.00000
HK	2	117.643	5	0.00000
MDH-1	3	36.686	10	0.00006
EST-3	2	11.607	5	0.04060
Totals		239.000	25	0.00000

Discussion

With its wide range of geography, Iran harbors different *Apis mellifera meda* population adapted to various geographical regions extending from east to west and from south to north. Within this huge geography Rutther (1988) reported the presence of sevaral Persian honey bee populations, indicating that there was not just one *Apis mellifera meda* population. In the present work a small area on the south of Caspian Sea was studied in a number of populations sampled

from the south-west end of the Alburz Mountains. This work is the most recent and complete one on the honey bee population in Iran.

Morphometric variability

Although there is some mixing among populations, it is obvious that there are two populations detected earlier by morphometrics (Ruttner *et al*., 2000). Ruttner *et al*. (2000) reported the presence of the two populations on the 36th parallel. As we collected the samples from the similar field, we also obtained similar results. Cubital index values of the Iranian honey bee populations were similar to honey bee populations from Cyprus and Anatolia (Kandemir *et al*., 2000).

In terms of body size all Middle Eastern honey bees were almost of equal size. The same similarities were also observed in several of their behavioral and physiological properties. This is well reported in several of previous works by Adam (1983), and Ruttner (1988). They frequently visited Turkey and Middle East to collect and take queen bees to their country for breeding purposes (Adam, 1983). Even in the beginning of 19th century F. Benton from US, visited Middle East and established an apiary in Palestine and Cyprus for breeding purposes, shipping several queens to US (Adam, 1983; Ruttner, 1988; Strange, 2001).

Although not all the morphometric variables were significantly different among populations, their discriminatory powers were well observed in Figure 1. Low land populations except Gultepe were smaller in size than Highland populations, as expected. The dashed lines in the Figure were well scattered all around the graph indicating that the variables had discriminatory loadings in all directions and one can find the value of each character in the Figure 2.

Fluctuating asymmetry is a measure of environmental stress, and we noted that the highland populations experienced more developmental stress than that of lowland population. This could be the result of several reasons: homozygosity, inbreeding mutations, and hybridization. However comparing the allozyme results ,except Hancayi populations, this could be reasonable. We expect low levels of fluctuating asymmetry values for Hancayi, however we found the highest heterozygosity in this population.

Allozyme variability

The first allozyme variability study showed that Persian bees have considerable amount of biochemical variation (0.056). This value was the second highest heterozygosity estimated after Kandemir *et al*. (2000). The pattern observed in the discriminant function analysis based on morphometric data is also supported by the clustering obtained from Distance -Wagner analysis and UPGMA (Figure 1-2) based on allozyme data.

The deviations obtained in some of the populations could be resulted from migratory beekeeping in mountainous areas (observations by two of the authors). In lowland areas, however, we did not observe any migratory beekeeping operations. Instead, colonies were still maintained in a traditional way and there was no sign of modern beekeeping (treatment of disease, queen replacement, etc.).

We did not observe any introgression between Iranian and Turkish honey bees, although beekeepers told that there was queen importation from Turkey to Iran. Especially, on the way to Iran we re-sampled colonies close to Iranian border and analyzed those samples as well. As reported previously (Kandemir, 1999; Kandemir et al., 2000) Mdh^{116} allele observed in Eastern locations in Turkey close to Iranian border, was present in most of the re-sampled locations, but this allele was not present in Iran. Similarly there was no variation in *Est* locus in Eastern Turkey; however this locus was variable in one of the populations in Iran. *Pgm* locus was highly variable in Turkey but not much variable in Iran.

Another interesting result is the observation of high variability in the *Mdh* locus. Allele frequencies in this locus show a clinal and gradual north-south variation in Turkey (Kandemir *et al*., 2000) and this phenomenon has been known in other regions as well (Cornuet, 1986; Nielson *et al*., 1994). The frequency of the Mdh^{65} allele is high in European honey bees and gradually declines towards the east and finally disappears in the south of Turkey (fixed for Mdh^{100}) (Kandemir, 1999). However, this allele reappears in Iran with very high frequency, especially on highland populations. One possible interpretation of this phenomenon is the different stability of *Mdh*

alleles at different environmental temperatures as reported by Cornuet *et al*. (1995).

The present study was the first allozyme study in the litrature; however, it is not complete. This work only covered a small portion of a huge country. So, further and more detailed studies with extensive sampling (covering all Iran and including molecular works such as mtDNA RFLP), sequencing or microsatellites and also morphometrics should help to resolve Persian honeybee population structure and sub-specific status.

Acknowledgments

We wish to thank Prof. Dr Inci Togan and Zeki Kaya, Department of Biology, METU, Ankara, Turkey for laboratory support. This work was collaboratively supported from grants of Zonguldak Karaelmas University, Turkey and Zanjan University, Iran.

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