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İR FAN KANDEMİR

MERAL KENCE

AYKUT KENCE

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Morphometric and Electrophoretic Variation in Different Honeybee (*Apis mellifera* L.) Populations

İrfan KANDEMİR

Department of Biology, Devrek Faculty of Arts and Sciences, Zonguldak Karaelmas University, 67100 Zonguldak - TURKEY

Meral KENCE

Department of Biology, Faculty of Arts and Sciences, Middle East Technical University, 06531 Ankara - TURKEY

Aykut KENCE

Department of Biology, Faculty of Arts and Sciences, Middle East Technical University, 06531 Ankara - TURKEY

E-mail: aykut@metu.edu.tr

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Abstract: Honeybee colonies from Turkey, Naxcivan (Azerbaijan) and Austria were studied for morphometric and electrophoretic variation and for comparison among different subspecies. Out of six enzyme systems, four were found to be polymorphic with 14 allozymes. Discriminant function analysis of 10 morphometric variables resulted in a total of four groups. Cluster analysis of electrophoretic data showed a similar type of groupings. Among the four polymorphic loci, *Mdh-1* showed the highest and *Est-3* the lowest differentiation among populations.

Key Words: Morphometry, electrophoresis, allozymes, honeybee

Farklı Balarısı (*Apis mellifera* L.) Toplumlarında Morfometrik ve Elektroforetik Varyasyon

Özet: Türkiye, Nahcivan (Azerbaycan) ve Avusturya'dan örneklenen balarısı kolonilerinin morfometrik ve elektroforetik varyasyonları ve alttürler arası ilişkileri çalışıldı. Altı enzim sisteminden dört tanesi toplam 14 allozim ile polimorfik bulundu. On morfometrik karakterin ayrışım fonksiyonu analizi sonucu dört grup ortaya çıktı. Elektroforetik verilerin kümelendirme analizi benzer şekilde gruplar oluşturdu. Polimorfik lokuslar içinde *Mdh-1* en yüksek, *Est-3* ise en düşük toplumlarası farklılaşmayı göstermektedir.

Anahtar Sözcükler: Morfometri, elektroforez, allozim, balarısı

Introduction

Bodenheimer (1) was the first to classify honeybee subspecies in Turkey. He suggested that basically there were four subspecies present which were *Apis mellifera caucasica*, *A. m. remipes*, *A. m. ligustica*, and *A. m. syriaca* in Turkey. However, a formal taxonomic status of the Anatolian honeybee (*A. m. anatoliaca*) based on a few museum specimens has been published by Maa (2). Later Adam (3) visited Turkey three times, in 1954, 1962 and 1972, and he observed the behavioral and physiological performances of Anatolian honeybees.

The honeybee (*Apis mellifera* L.) is endemic to Europe, Africa, and western Asia. They differ in their morphology, behavior, and physiology according to the environmental conditions they have adapted to (4).

Among these subspecies at least five of them (*A. m. anatoliaca*, *A. m. meda*, *A. m. caucasica*, *A. m. syriaca* and *A. m. carnica*) are known to be present in Turkey (5-8). Honeybee subspecies were studied extensively by morphometry, allozymes and mtDNA (9). Honeybee populations from Austria (*A. m. carnica*) are one of the mostly studied subspecies among other honeybees. For the morphometrical discrimination of *Apis mellifera* subspecies, more than 35 characters were determined by Ruttner (10). In morphometric studies of honeybees, Cornuet and Garnery (11) used 6, Darendelioğlu and Kence (12) used 23, Kandemir et al. (13) used 12, Güler and Kaftanoğlu (14) used 21, Güler and Kaftanoğlu (15) used 20, and Güler et al. (16) used 19 characters. However, these data in association with multivariate

statistical and electrophoretic analyses provided a good discrimination among populations of the subspecies. Although *A. m. carnica* was well studied there is no study corresponding to honeybee populations from Naxcivan with respect to morphometry and allozymes. Honeybee populations from Turkey, however, were studied extensively after 1995. Kandemir and Kence (5) and Asal et al. (17) reported the allozyme variation in central Anatolian honeybee populations. Further extensive morphometric and allozymic studies in Turkey (7) showed that Turkey is close to the origin of genetic diversity in honeybee evolution. Smith et al. (6) and Palmer et al. (8) reported the mtDNA variation present in Turkey and with respect to mtDNA, and that Turkish honeybees belonged to the Eastern Mediterranean C lineage.

In this study, allozymic and morphometric variations in honeybee populations from Turkey were studied and compared with honeybee populations extending from west to east (Austria to Naxcivan-Azerbaijan) in order to see the changes in morphometric characters and allozyme frequencies and to assess subspecific relationships of populations distributed in this region.

Materials and Methods

Honeybee colonies were collected from eight populations in Turkey, one location from Naxcivan (Azerbaijan) and one location from Austria. A total of 135 colonies were sampled. Approximately 1000 honeybee workers were used in the analysis (all from stationary apiaries). For sampling details of morphometric and electrophoretic analysis see Kandemir et al. (7). The statistical analysis (discriminant function analysis) was performed with SPSS/PC, SYN-TAX and NTSYS program packages on only morphometric data. For allozyme study 6 enzyme systems known to be polymorphic in honeybees were selected: *Pgm-1*, *Mdh-1*, *Hk*, *Est-3*, *Me*, and *Pgi*. The electrophoresis was carried out on thorax homogenates on 12% horizontal starch gel. Enzyme activity was visualized using standard histochemical staining methods (5). For each locus allozyme frequency, average heterozygosities, population differentiation values (F_{ST}) and a phylogenetic relationships were computed by the BIOSYS-1 program.

Results

In the present study, 10 morphometric variables were measured for morphometric analysis. Their original measurements and standard errors are given in Table 1.

Morphometric analysis:

Except for metatarsus length, all of the 9 morphometric variables were significantly different among the populations ($P < 0.001$). Figure 1 shows the results of discriminant function analysis. Four groups can be visualized: (i) Ankara colonies (Central Anatolia) formed a cluster, (ii) honeybee populations from the European part of Turkey (Kırklareli and Edirne) including the neighboring locality Bolu and Austrian colonies clustered together. (iii) The third group, however, consisted of honeybee populations from northeastern Turkey, and the fourth group (iv) consists of Naxcivan (Azerbaijan) populations.

The 1st axis explained 43%, the 2nd axis 24.1% and the 3rd axis 15.1% of the total variation. Thus 82.2% of the total variation could be explained by the first three canonical variates.

Allozymes

Of the six enzyme systems assayed with horizontal starch gel electrophoresis, four (*Pgm-1*, *Mdh-1*, *Hk*, and *Est-3*) were found to be polymorphic and two (*Pgi* and *Me*) exhibited invariant banding pattern. All isozymes were designated using relative mobilities with respect to the most common isozyme used as a standard (Mobility 100) (Table 2).

The highest number of alleles was observed in Edirne. The maximum polymorphism was observed (33.3) in Edirne, Kars, Kırklareli, and Austria colonies. Kırklareli has the highest mean heterozygosity (0.140) among the 10 populations studied. Ankara, Austria, Naxcivan, Bolu, and Iğdır were in Hardy-Weinberg equilibrium for all enzyme systems. Edirne populations deviated from H-W equilibrium only in *Mdh-1* enzyme system. Kırklareli, Artvin, and Kars populations, however, showed deviations in favor of *Pgm-1* heterozygotes. Ardahan populations deviated for *Pgm-1* and *Hk* enzyme systems.

The highest differentiation among the populations was caused by the *Mdh-1* enzyme system. The lowest differentiation, however, was due to *Est-3*. The homogeneity X^2 test among populations showed highly significant ($P < 0.001$) heterogeneity due to the differences in allele frequencies.

Table 1. Original values (mm) of 10 morphometrical characters (A, wing characters; B, leg characters) in honeybee populations from Turkey, Austria, and Naxcivan.

A) Wing characters

Populations	# of Hive	N	Cubital A	Cubital B	Wing C Value	Wing D Value	Wing Length	Wing Width	Cubital Index
Austria	4	29	0.564 ± 0.011	0.203 ± 0.006	0.776 ± 0.007	1.881 ± 0.008	8.807 ± 0.022	2.983 ± 0.017	2.783 ± 0.085
Kırklareli	27	233	0.531 ± 0.006	0.197 ± 0.004	0.978 ± 0.015	1.875 ± 0.005	8.859 ± 0.016	3.452 ± 0.058	2.718 ± 0.057
Edirne	19	93	0.530 ± 0.008	0.232 ± 0.004	0.828 ± 0.005	1.898 ± 0.012	8.784 ± 0.031	2.925 ± 0.015	2.293 ± 0.060
Bolu	20	167	0.522 ± 0.007	0.243 ± 0.006	0.857 ± 0.006	1.883 ± 0.010	8.822 ± 0.030	2.959 ± 0.015	2.171 ± 0.061
Ankara	4	28	0.473 ± 0.019	0.202 ± 0.004	0.767 ± 0.012	1.813 ± 0.004	8.467 ± 0.007	2.805 ± 0.010	2.348 ± 0.108
Ardahan	20	105	0.530 ± 0.005	0.245 ± 0.004	0.875 ± 0.003	1.887 ± 0.006	8.897 ± 0.016	2.966 ± 0.011	2.173 ± 0.048
Artvin	18	151	0.539 ± 0.004	0.252 ± 0.005	0.879 ± 0.004	1.891 ± 0.008	8.971 ± 0.027	2.956 ± 0.010	2.157 ± 0.050
İğdir	5	26	0.529 ± 0.008	0.229 ± 0.015	0.919 ± 0.041	1.879 ± 0.019	8.858 ± 0.051	2.881 ± 0.003	2.352 ± 0.199
Kars	9	63	0.530 ± 0.009	0.271 ± 0.002	0.847 ± 0.007	1.868 ± 0.007	8.765 ± 0.019	2.871 ± 0.010	1.953 ± 0.044
Naxcivan	15	85	0.559 ± 0.004	0.229 ± 0.006	0.857 ± 0.004	1.829 ± 0.013	8.593 ± 0.029	2.888 ± 0.012	2.469 ± 0.071

B) Leg characters

Populations	# of Hive	N	Metatarsus Length	Metatarsus Width	Femur Length	Tibia Length
Austria	4	29	1.954 ± 0.014	1.114 ± 0.001	2.940 ± 0.017	2.435 ± 0.013
Kırklareli	27	233	2.018 ± 0.014	1.282 ± 0.014	3.011 ± 0.024	2.811 ± 0.038
Edirne	19	93	1.965 ± 0.016	1.165 ± 0.007	2.803 ± 0.023	2.395 ± 0.020
Bolu	20	167	1.991 ± 0.028	1.157 ± 0.007	2.843 ± 0.026	2.447 ± 0.036
Ankara	4	28	1.957 ± 0.021	1.150 ± 0.006	2.971 ± 0.025	2.442 ± 0.011
Ardahan	20	105	1.986 ± 0.012	1.192 ± 0.005	3.110 ± 0.018	2.456 ± 0.010
Artvin	18	151	2.023 ± 0.065	1.184 ± 0.005	3.049 ± 0.022	2.456 ± 0.013
İğdir	5	26	2.004 ± 0.047	1.157 ± 0.044	3.075 ± 0.019	1.963 ± 0.034
Kars	9	63	1.936 ± 0.015	1.138 ± 0.013	3.054 ± 0.026	2.443 ± 0.010
Naxcivan	15	85	1.907 ± 0.013	1.128 ± 0.009	2.987 ± 0.011	2.317 ± 0.017

Table 2. Gene frequencies of four polymorphic enzymes in honeybee populations from Turkey, Naxcivan (Azerbaijan), and Austria.

Populations	# of Hive	N	PGM 45	PGM 75	PGM 100	HK 77	HK 87	HK 100	HK 110	MDH 65	MDH 87	MDH 100	MDH 116	EST 70	EST 100	EST 130
Austria	4	29	-	1.000	-	-	-	1.000	-	0.879	-	0.121	-	-	0.931	0.069
Kırklareli	27	233	-	0.758	0.242	-	-	1.000	-	0.367	-	0.633	-	0.004	0.996	-
Edirne	19	93	0.027	0.925	0.048	-	0.016	0.984	-	0.231	-	0.758	0.011	0.005	0.984	0.011
Bolu	20	167	-	0.760	0.240	0.006	-	0.994	-	0.018	-	0.982	-	0.012	0.988	-
Ankara	4	28	-	1.000	-	-	-	1.000	-	-	-	1.000	-	-	1.000	-
Ardahan	20	105	-	0.800	0.200	0.033	0.005	0.952	-	0.005	-	0.995	-	-	1.000	-
Artvin	18	151	-	0.752	0.248	0.007	-	0.993	-	0.003	-	0.997	-	-	1.000	-
İğdir	5	26	-	0.750	0.250	-	-	1.000	-	-	-	1.000	-	-	1.000	-
Kars	9	63	-	0.730	0.270	-	-	0.984	0.016	-	-	0.889	0.111	-	1.000	-
Naxcivan	15	85	-	0.888	0.112	-	-	0.982	0.018	-	0.012	0.988	-	-	1.000	-

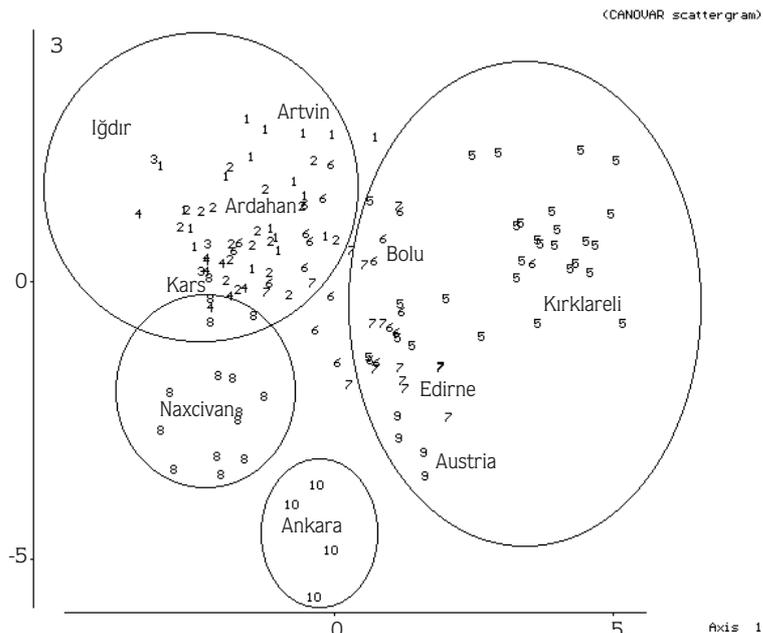


Figure 1. Two dimensional clustering in Discriminant Function Analysis of 10 populations from Turkey, Naxcivan (Azerbaijan), and Austria based on morphometric data.

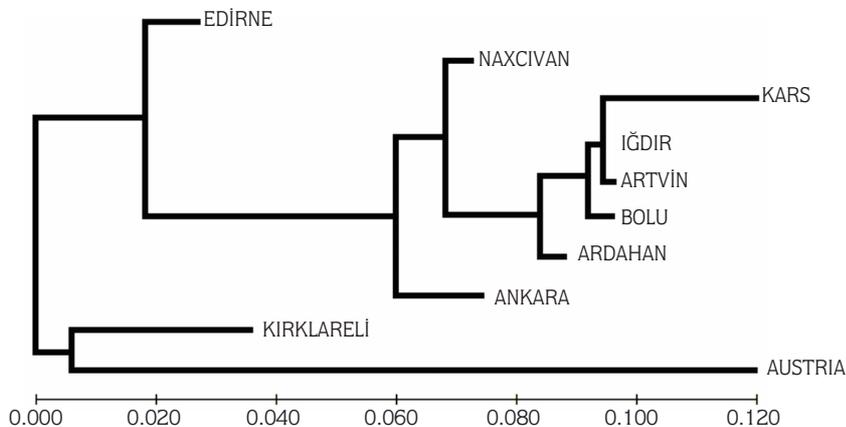


Figure 2. Distance-Wagner clustering of 10 honeybee populations based on Prevosti Distance calculated from allozyme data.

Figure 2 shows the phylogenetic relationships among populations as revealed by the distance-Wagner analysis based on the Prevosti genetic distance calculated from allozyme data. More or less similar types of groupings were observed (see Figure 1). Austrian colonies and Kırklareli colonies formed the first cluster. In the second cluster, Anatolian honeybees (Ankara, Bolu, Artvin, Kars, Ardahan, and Iğdır) and Naxcivan formed a coherent group, whereas Edirne was on the second branch of this cluster.

Discussion

A wide range of genetic and electrophoretic variation occurs in a geography extending from Austria to Naxcivan (Azerbaijan). *A. m. carnica*, *A. m. anatoliaca*, and *A. m. caucasica* subspecies could be differentiated morphometrically in this study as would be expected according to Ruttner (4). Honeybee populations from Naxcivan (Azerbaijan) were close to the *A. m. caucasica*

cluster (Artvin, Ardahan, Kars, and Iğdır) but did not coincide with it exactly. The colonies from Naxcivan are probably *A. m. meda*, which were classified as *remipes* by Bodenheimer (1). Honeybees from Anatolia (Ankara, Beypazarı) were found to be distinct from other two clusters and they represent *A. m. anatoliaca*. Thrace populations were thought to be *A. m. carnica* and are clustered with Austrian colonies, which were known to be *A. m. carnica* (Figure 1). On the one hand, cubital index, a diagnostic morphometric ratio for honeybee subspecies, also showed similarity between honeybee populations from Kirklareli (2.718) and Austria colonies (2.783). On the other hand, Edirne populations exhibited quite a low cubital index (2.293). Migratory beekeepers from Anatolia frequented Edirne quite often, whereas honeybee populations of Kirklareli are isolated from other parts of Turkey in that few migratory beekeepers visited this region. This may explain the differences between Kirklareli and Edirne populations.

The pattern we observed in the discriminant function analysis based on morphometric data is also supported by the clustering obtained from distance-Wagner analysis (Figure 2) based on allozyme data. High frequency of *Mdh-65* allele has high loadings on the separation of Kirklareli and Austria colonies from the rest of the groups. Although Edirne populations joined with the other group, they are separated enough from those groups as was the case for Ankara populations. Bolu populations joined closely within populations from northeast Turkey (Artvin, Ardahan, Kars); this may be because the Bolu ecotype is under the similar climatic influences (Black Sea) as the Artvin, Ardahan, and Kars honeybee populations. The grouping from discriminant function analysis of morphometric data and the clustering obtained from the distance-Wagner analysis based on Prevosti distance from allozyme data agreed well with one another.

In European honeybee populations, *Mdh-65* frequencies ranged between 0.879 and 0.231, whereas populations from the Asian side have the highest frequency in Bolu (0.018). Here again the Edirne population has the lowest *Mdh-65* frequency (0.231) on the European side, which indicates the effect of gene flow from Anatolia due to migratory beekeepers. Nevertheless, the European and Asian populations can easily be distinguished on the basis of *Mdh* frequencies. The highest F_{ST} values (0.3157) were observed for this

enzyme system. This high F_{ST} is largely due to differences between the European and Asian populations used in this study. Similarly *Hk* locus is fixed for *Hk-100* in Kirklareli and Austria honeybees, whereas *Hk* locus in Edirne exhibits small amount of genetic variation evidencing introgression from Anatolia. *Hk* locus in all of the honeybee populations on the Asian side showed polymorphism. As one goes southward this polymorphism increases (7). However, the F_{ST} value observed for *Hk* is the lowest (0.0144) among the four polymorphic loci in this study, indicating the lowest differentiation between the populations.

Est-3 enzyme systems are variable in Anatolia, Thrace, and Austria but fixed for *Est-100* in northeast Turkey and Naxcivan where *A. m. caucasica* is distributed. No electrophoretic variability was detected in Ankara samples probably because of the small sample size. However, in a previous detailed study in Central Anatolia (Ankara) by Kandemir and Kence (5), electrophoretic variability in these four enzyme systems was reported.

European and Anatolian honeybee populations were well separated in the discriminant function analysis using morphometric variables and distance-Wagner clustering based on allozymes. According to mtDNA data (18) *Apis mellifera* populations in the European and Asian parts of Anatolia could not be well distinguished. Instead the data showed that all European and Anatolian populations belonged to C lineage, as Smith et al. (6) reported earlier. mtDNA results add the new finding that there is another lineage in southern Anatolia (Hatay) that can be classified into A (19) or O lineage (8,20) based on restriction analysis and sequencing of mtDNA, but also showed that there are not unique patterns related to the European part of Turkey. On the other hand, Palmer et al. (8) stated that Tekirdağ populations have Eastern Type 2 restriction pattern, which has P₀Q sequence on their *COI-COII* intergenic region, but this unique pattern is only found in Hatay colonies in southern Turkey. Based on this restriction pattern, Palmer et al. (8) concluded that Thrace populations were not different from Anatolian honeybee populations. It is clearly shown in the present work that both electrophoretic and morphometrical data separated both populations well but not mtDNA. There seems to be a conflict with our results and those in Palmer et al. (8) or this could be attributed to migratory beekeeping or gene flow between two populations (Tekirdağ and Hatay).

Each character and combinations of characters show different distributions. On this basis it is difficult to place boundaries between the populations of honeybees separating subspecies. Bodenheimer (1), studying honeybee races in Turkey, reached the conclusion that honeybees show local variations within subspecies.

Louveaux (21) also suggested the presence of ecotypes in honeybees. The presence of great genetic diversity in Anatolian honeybee populations as we have observed suggests that Anatolia may be close to the center of origin of honeybees (4).

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