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 Kaftanoglu (14) used 21, Güler and Kaftanoglu (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
apararies). 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 lğdır 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

<table>
<thead>
<tr>
<th>Populations</th>
<th># of N</th>
<th>Cubital Wing A</th>
<th>Cubital Wing B</th>
<th>Wing C Value</th>
<th>Wing D Value</th>
<th>Wing Length</th>
<th>Wing Width</th>
<th>Cubital Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>4</td>
<td>29</td>
<td>0.564 ± 0.011</td>
<td>0.203 ± 0.006</td>
<td>0.776 ± 0.007</td>
<td>1.881 ± 0.008</td>
<td>8.807 ± 0.022</td>
<td>2.983 ± 0.017</td>
</tr>
<tr>
<td>Kırklareli</td>
<td>27</td>
<td>233</td>
<td>0.531 ± 0.006</td>
<td>0.197 ± 0.004</td>
<td>0.978 ± 0.015</td>
<td>1.875 ± 0.005</td>
<td>8.859 ± 0.016</td>
<td>3.452 ± 0.058</td>
</tr>
<tr>
<td>Edirne</td>
<td>19</td>
<td>93</td>
<td>0.530 ± 0.008</td>
<td>0.232 ± 0.004</td>
<td>0.828 ± 0.005</td>
<td>1.898 ± 0.012</td>
<td>8.784 ± 0.031</td>
<td>2.925 ± 0.015</td>
</tr>
<tr>
<td>Bolu</td>
<td>20</td>
<td>167</td>
<td>0.522 ± 0.007</td>
<td>0.243 ± 0.006</td>
<td>0.857 ± 0.006</td>
<td>1.883 ± 0.010</td>
<td>8.822 ± 0.030</td>
<td>2.959 ± 0.015</td>
</tr>
<tr>
<td>Ankara</td>
<td>4</td>
<td>28</td>
<td>0.473 ± 0.019</td>
<td>0.202 ± 0.004</td>
<td>0.767 ± 0.012</td>
<td>1.813 ± 0.004</td>
<td>8.467 ± 0.007</td>
<td>2.805 ± 0.010</td>
</tr>
<tr>
<td>Ardahan</td>
<td>20</td>
<td>105</td>
<td>0.530 ± 0.005</td>
<td>0.245 ± 0.004</td>
<td>0.875 ± 0.003</td>
<td>1.887 ± 0.006</td>
<td>8.975 ± 0.015</td>
<td>2.996 ± 0.011</td>
</tr>
<tr>
<td>Artvin</td>
<td>18</td>
<td>151</td>
<td>0.539 ± 0.004</td>
<td>0.252 ± 0.005</td>
<td>0.897 ± 0.004</td>
<td>1.891 ± 0.008</td>
<td>8.971 ± 0.027</td>
<td>2.956 ± 0.010</td>
</tr>
<tr>
<td>Iğdır</td>
<td>5</td>
<td>26</td>
<td>0.529 ± 0.008</td>
<td>0.229 ± 0.015</td>
<td>0.919 ± 0.041</td>
<td>1.879 ± 0.019</td>
<td>8.585 ± 0.051</td>
<td>2.881 ± 0.003</td>
</tr>
<tr>
<td>Kars</td>
<td>9</td>
<td>63</td>
<td>0.530 ± 0.009</td>
<td>0.271 ± 0.002</td>
<td>0.847 ± 0.007</td>
<td>1.868 ± 0.007</td>
<td>8.765 ± 0.019</td>
<td>2.871 ± 0.010</td>
</tr>
<tr>
<td>Naxcivan</td>
<td>15</td>
<td>85</td>
<td>0.559 ± 0.004</td>
<td>0.229 ± 0.006</td>
<td>0.857 ± 0.004</td>
<td>1.829 ± 0.013</td>
<td>8.593 ± 0.029</td>
<td>2.888 ± 0.012</td>
</tr>
</tbody>
</table>

### B) Leg characters

<table>
<thead>
<tr>
<th>Populations</th>
<th># of N</th>
<th>Metatarsus A Length</th>
<th>Metatarsus A Width</th>
<th>Femur Length</th>
<th>Tibia Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>4</td>
<td>29</td>
<td>1.954 ± 0.014</td>
<td>1.114 ± 0.001</td>
<td>2.940 ± 0.017</td>
</tr>
<tr>
<td>Kırklareli</td>
<td>27</td>
<td>233</td>
<td>2.018 ± 0.014</td>
<td>1.282 ± 0.014</td>
<td>3.011 ± 0.024</td>
</tr>
<tr>
<td>Edirne</td>
<td>19</td>
<td>93</td>
<td>1.965 ± 0.016</td>
<td>1.165 ± 0.007</td>
<td>2.803 ± 0.023</td>
</tr>
<tr>
<td>Bolu</td>
<td>20</td>
<td>167</td>
<td>1.991 ± 0.028</td>
<td>1.157 ± 0.007</td>
<td>2.843 ± 0.026</td>
</tr>
<tr>
<td>Ankara</td>
<td>4</td>
<td>28</td>
<td>1.957 ± 0.021</td>
<td>1.150 ± 0.006</td>
<td>2.971 ± 0.025</td>
</tr>
<tr>
<td>Ardahan</td>
<td>20</td>
<td>105</td>
<td>1.986 ± 0.012</td>
<td>1.192 ± 0.005</td>
<td>3.110 ± 0.018</td>
</tr>
<tr>
<td>Artvin</td>
<td>18</td>
<td>151</td>
<td>2.032 ± 0.005</td>
<td>1.184 ± 0.005</td>
<td>3.049 ± 0.022</td>
</tr>
<tr>
<td>Iğdır</td>
<td>5</td>
<td>26</td>
<td>2.004 ± 0.047</td>
<td>1.157 ± 0.044</td>
<td>3.075 ± 0.019</td>
</tr>
<tr>
<td>Kars</td>
<td>9</td>
<td>63</td>
<td>1.936 ± 0.015</td>
<td>1.138 ± 0.013</td>
<td>3.054 ± 0.026</td>
</tr>
<tr>
<td>Naxcivan</td>
<td>15</td>
<td>85</td>
<td>1.907 ± 0.013</td>
<td>1.128 ± 0.009</td>
<td>2.987 ± 0.011</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Populations</th>
<th># of N</th>
<th>PGM A</th>
<th>PGM B</th>
<th>PGM C</th>
<th>PGM D</th>
<th>HK A</th>
<th>HK B</th>
<th>HK C</th>
<th>HK D</th>
<th>MDH A</th>
<th>MDH B</th>
<th>MDH C</th>
<th>MDH D</th>
<th>EST A</th>
<th>EST B</th>
<th>EST C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>4</td>
<td>29</td>
<td>-</td>
<td>1.000</td>
<td>0.879</td>
<td>-</td>
<td>0.121</td>
<td>-</td>
<td>-</td>
<td>0.931</td>
<td>0.069</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kırklareli</td>
<td>27</td>
<td>233</td>
<td>-</td>
<td>0.758</td>
<td>0.367</td>
<td>-</td>
<td>0.633</td>
<td>0.004</td>
<td>0.996</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edirne</td>
<td>19</td>
<td>93</td>
<td>0.027</td>
<td>0.925</td>
<td>0.037</td>
<td>-</td>
<td>0.984</td>
<td>0.018</td>
<td>0.982</td>
<td>0.012</td>
<td>0.988</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolu</td>
<td>20</td>
<td>167</td>
<td>-</td>
<td>0.760</td>
<td>0.994</td>
<td>-</td>
<td>0.982</td>
<td>0.012</td>
<td>0.988</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankara</td>
<td>4</td>
<td>28</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ardahan</td>
<td>20</td>
<td>105</td>
<td>-</td>
<td>0.800</td>
<td>0.952</td>
<td>-</td>
<td>0.995</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artvin</td>
<td>18</td>
<td>151</td>
<td>-</td>
<td>0.752</td>
<td>0.993</td>
<td>-</td>
<td>0.997</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Iğdır</td>
<td>5</td>
<td>26</td>
<td>-</td>
<td>0.750</td>
<td>0.984</td>
<td>-</td>
<td>0.989</td>
<td>0.111</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kars</td>
<td>9</td>
<td>63</td>
<td>-</td>
<td>0.730</td>
<td>0.889</td>
<td>-</td>
<td>0.988</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naxcivan</td>
<td>15</td>
<td>85</td>
<td>-</td>
<td>0.888</td>
<td>0.988</td>
<td>-</td>
<td>0.988</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 Kırklareli (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 Kırklareli are isolated from other parts of Turkey in that few migratory beekeepers visited this region. This may explain the differences between Kırklareli 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 Kırklareli 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 Kırklareli 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 $P_{0}Q$ 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 electrophoretical 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).

References

13. Kandemir, I., Kandemir, G., Kence, M., Ind, A., Kence, A.: Morphometrical and electrophoretical discrimination of honeybees from different regions of Turkey. XXXIVth International Apicultural Congress in Apimondia, 14-19 August 1995, Lausanne, Switzerland.