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Genetıc Diversity of Bee Ecotypes in Turkey and Evidence for Geographical Differences

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Abstract

Honeybees collected from 56 different areas of Turkey were analysed, using 12 morphometric characters. The multivariate statistical analysis of data and discriminant function analysis established seven different ecotypes spreading according to different coordinates of regions. UPGMA dendrogram based on the Mahalonobis distance showed that the studied colonies were clustered in four main regional groups like A. m. anatoliaca in central Anatolia, A.m. caucasica in the northern Anatolia, A. m. meda in southern and south-eastern Anatolia and A. m. carnica in the European part of Turkey.

Keywords: *Apis mellifera* /morphometry/geographical variability/Turkey**.**

Introduction

Bodenheimer (1941) [4] classified for the first time honeybee subspecies in Turkey. He discriminated seven zones with different types: *A. m. caucasica* in North - eastern Anatolia, *A. m. remipes* in Elazığ, *A. m. anatoliaca* in central Anatolia and the bees of Western Turkey (west of line İstanbul-Bursa-Eskişehir-Isparta) as different from the other types. The remaining three types were described respectively as intermediate between the Anatolian and Caucasian bee, yellow transcaucasians and Syrians. Later on, Adam (1983) [1] observed the behavioral and physiological performances of Anatolian honeybees and he reached the same conclusions as Bodenheimer (1941) [5], there were four subspecies and many intermediates types present in Turkey.

Ruttner (1988) [24] claimed that Asia Minor, including Anatolia, appears to be the genetic centre for honeybee subspecies. According to the multivariate statistical analysis of morphometric data, *A. m. anatoliaca* is placed in the centre of the Middle Eastern group (*A. m. armeniaca, A. m. adami*, *A. m. syriaca*, *A. m. caucasica*, *A. m. meda* and *A. m. cypria*). Ruttner's (1988) [24] presentation of morphometric, behavioral and ecological data showed that three of these honeybee subspecies, *A. m. anatoliaca*, *A. m. caucasica* and. *A. m. meda* naturally have been in Turkey.

Recently, honeybee subspecies from Anatolia were studied extensively using molecular markers, mtDNA analysis [25, 20, 16, 17, 7, 19], izoenzymic analysis (13, 14, 15) and microsatellite and RAPD analysis (12, 11, 16). *A. m. carnica* has been recorded in Thrace [25, 14, 15, 5, 6]. Later studies of mtDNA provided evidence of a fourth lineage of *Apis mellifera* in the extreme South near the Syrian border of Turkey, *A. m. syriaca* has been described based on restriction site and sequence data [8, 20, 16].

Turkey (part of Asia Minor) forms the intersection of three continents, Asia, Europe and Africa and incorporates diverse climatic and ecological conditions. Thus, it is not surprising that numerous honeybee subspecies and ecotypes have been described from this

region. But within the last 15-20 years, migratory beekeeping has become widespread in Turkey. The extensive practice of migratory beekeeping and commercial breeding might promote the gene flow between different races, and result in homogenization of the honeybee population of Turkey.

The aim of this study is to determine the different ecotypes of Turkish honeybees and to find out their phylogenetic relationships, in order to contribute to the conservation of local honeybees populations.

Materials and methods

Honeybee samples were collected from 56 different locations of nine different geographic regions of Turkey (Fig. 1, Tab. I).

Table 1. Localities, altitudes and coordinates of study areas in Anatolia

	Bee yards	**localities**	La	Lo	Al
1	1	Malkara	41	27	210
	2	Hayrabolu	41	27	18
	3	Muratlı	41	28	79
	4	Çerkezköy	41	28	150
	5	Çorlu	41	28	128
	6	Saray	41	28	151
	7	Lüleburga	41	27	76
	8	Kırklareli	42	27	199
	9	Keşan	41	27	97
	10	Meriç	41	26	22
	11	Kocahıdır	41	26	28
	12	İpsala	41	26	36
	13	Enez	41	26	3
	14	İstanbul	41	29	24
2	15	Bursa	40	29	188
	16	Yalova	41	30	0
	17	Çanakkale	40	26	0
	18	Bozcaada	40	26	1
	19	Gökçeada	40	26	135
	20	Balıkesir	40	28	136
	21	İzmit	41	30	0
3	22	Aksaray	38	34	1044
	23	Eskişehir	40	30	870
	24	Çankırı	41	34	723
4	25	İzmir	38	27	37
	26	Muğla	37	28	632
	27	Uşak	39	29	956
	28	Kütahya	39	30	935

		localities	La	Lo	Al
	29	Denizli	38	29	418
	30	Aydın	38	28	57
5	31	Düzce	41	31	168
	32	Zonguldak	41	32	96
	33	Bartın	42	32	12
	34	Sinop	42	35	0
	35	Çorum	41	35	864
6	36	Gümüşhan	40	39	1228
	37	Bayburt	40	40	1924
	38	Rize	41	41	1
	39	Giresun	41	38	0
	40	Trabzon	41	40	191
	41	Artvin	41	42	386
7	42	Hakkari	38	44	1504
	43	Van	39	43	1727
	44	Bingöl	39	40	1139
	45	Elazığ	39	39	1121
	46	Erzincan	40	39	1186
	47	Ağrı	40	43	1641
	48	Malatya	38	38	975
8	49	Isparta	38	31	1085
	50	Osmaniye	37	36	110
	51	Mersin	37	35	0
	52	Adana	37	35	13
	53	Maraş	38	37	654
9	54	Adıyaman	38	38	685
	55	Diyarbakır	38	40	663
	56	Urfa	37	38	940

La: **latitude,** Lo: **longitude,** Al: **altitude**

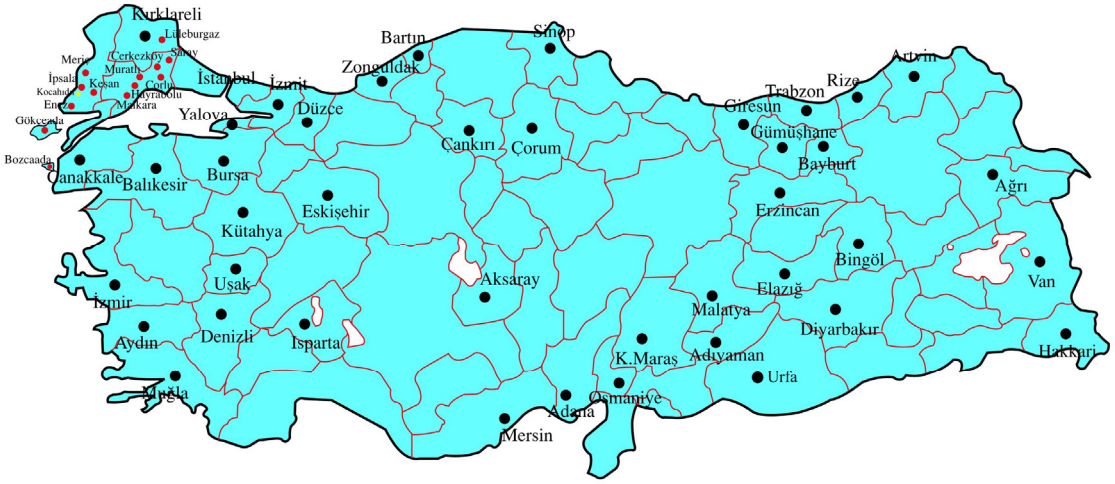

Figure 1. Sampling sites

Sampling was carried out mostly from small managed apiaries, in which requeening of colonies was mostly natural and there were no migratory beekeeping and commercial breeding. Approximately 2700 worker bees were collected from 56 localities, and were placed into small plastic vials with 96 % alcohol, which were labelled and brought to laboratory.

Concerning the classical morphometrics analysis, heads of worker bees were kept in 30 % alcohol for the measurements of proboscis, while the rest of the body in 70 % of lactic acid for 24 hours, in order to soften the tissues for better resolution. We put fore and hind wings between transparent tapes fastened on a 5x5 cm slide frame and we projected them on a screen by a slide projector. The frames were magnified 32 times larger than normal. By this method we measured length and width of fore and hind wings, as well as distances a and b of cubital veins. The hind leg was fixed on microscope slide by Entellan fixative and covered by a cover slip. We measured the length of femur, tibia and basitarsus as well as the width of basitarsus by microscopic stereoscope in 20x magnification. The length of proboscis was measured by microscopic stereoscope in 10x magnification. The measurements were subsequently converted into millimetres.

The statistical analysis was performed using SPSS 14.0.1 package [27]. Univariate (ANOVA) and multivariate (MANOVA) statistical analysis, as well as discriminant function analysis, were performed on the data from 12 morphological characters. The results from classical morphometrics analysis were also statistically processed using NTSYS 1.70 package [22] and the phylogenetic tree was constructed, based on UPGMA method, using the same package.

Finally, multiplex regression analysis was applied to the morphometric variables using latitude, longitude and altitude as independent variables.

Results

In the present study, 12 morphometric characters were measured for morphometric analysis. The mean values of these measured characters and standard error were recorded.

1. ANOVA and DFA

Analysis of variance (ANOVA) of the data showed out of 12 morphometric variables four, FWL ($P<0.001$), HWL ($P<0.000$), HWW ($P<0.006$) and PL ($P<0.000$)) were found to be significantly different among populations. Discriminatory power of all morphometric characters are given ın Table II.

Table 2. Morphometrical characters entered into the discriminant functions ranked according to their discriminatory power.

No	Characters	Wilks' Lambda	F-statistic	P(sig.)
1	FWL	.854	3.693	**.001***
2	FWW	.929	1.642	.116
3	A	.935	1.492	.163
4	B	.989	.235	.984
5	CI	.977	.646	.738
6	HWL	.786	5.891	**.000***
7	HWW	.884	2.835	**.006***
8	FL	.926	1.737	.093
9	TL	.957	.963	.467
10	BL	.943	1.319	.237
11	BW	.924	1.772	.086
12	PL	.844	4.012	**.000***

*P<0.05

The discriminant functions were used to classify the colonies and to determine the percentages of correctly classified colonies and highest posterior probability of each colony being in any cluster was computed. Wilks' lambda statistic was used to test for significant differences in the cluster vector by means of each characters used in the discriminant functions. The homogeneity of intercolonial and intracolonial variances at each region was tested using F-statistic.

When colony means were subjected to discriminant function analysis on the basis of nine geographical regions, Thracen, Marmara, Aegean, Eastern Black, South Eastern Anatolia, Mediterranean and Western Black and Central Anatolian, seven distinct cluster of sampling colonies were formed on the plot. Samples from South-eastern Anatolia could not be distinguished from those in the Mediterranean region, and samples from central Anatolian slightly overlapped with those from Western Black Sea. Discriminant function analysis (DFA) of colony means from 9 geographic regions of Turkey revealed the existence of seven statistically separable morphometric groups clustered four main branches.

Fig. 2 shows the results of discriminant function analysis presented in a two dimensional plot; the 1st axis explains 41.4 %, the 2nd axis 25 % of the total variation respectively 66.4 % of total variation could be explained by the first two canonical variants (P<0.05).

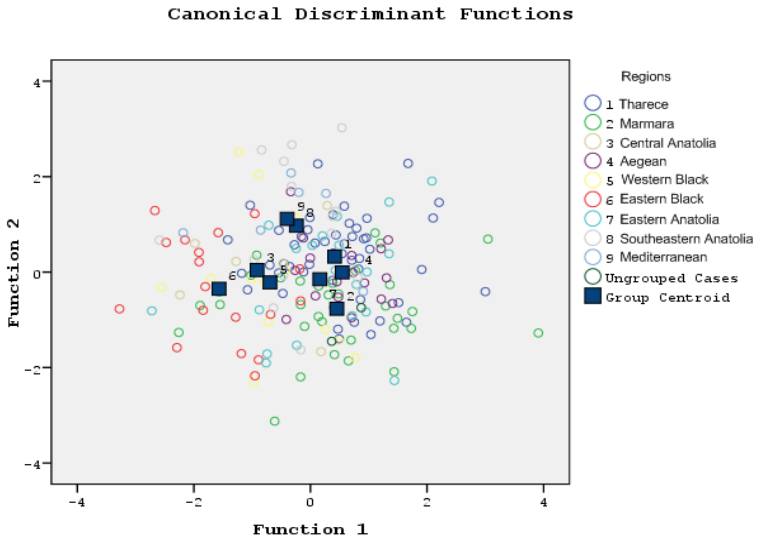

Figure 2. Two dimensional clustering in Discriminanat Function Analysis of colony means from 9 geographic regions in Turkey

Hind wing and proboscis length values contribute the most in separation of groups along the first axis. Fore wing and hind wing length contribute the most to discrimination of groups along the second axis respectively morphometrical characters entered into the discriminant functions ranked according to their discriminatory power are shown in Tab.III.

Table 3. Structure Matrix (Contributions of 12 morphometric variables in Discriminant Function Analysis on the vectors).

	Functions							
variable	1	2	3	4	5	6	7	8
AKU	.647(*)	.494	-.089	-.168	-.185	.344	-.077	-.163
DU	.501(*)	-.466	-.040	-.233	.033	-.257	.216	.065
AKG	.049	.565(*)	-.442	-.231	-.298	-.057	.117	.036
ÖKU	.386	.500(*)	-.440	.159	.160	-.035	.104	.410
ÖKG	.282	.170	-.463(*)	.063	.069	-.162	.127	.114
FU	.152	.303	.405(*)	.360	-.226	-.254	-.308	.277
BU	-.169	.303	.132	-.123	.435(*)	.041	.235	-.222
a	-.202	-.295	-.253	-.051	-.312	.570(*)	.034	.208
TU	.111	.181	-.026	-.372	.323	.035	-.517(*)	.480
BG	.127	.308	.508	-.301	-.006	-.093	.353	.534(*)
b	.105	.034	-.161	-.016	-.049	.247	.037	.364(*)
Kİ	-.174	-.181	.034	.052	-.004	.133	.020	-.201(*)

*Largest absolute correlation between each variable and any discriminant function

Mahalanobis distances between each groups were determined. The shortest Mahalanobis distances were found to be between Thracen and Eastern populations and the longest one between the Aegean and Eastern Black populations.

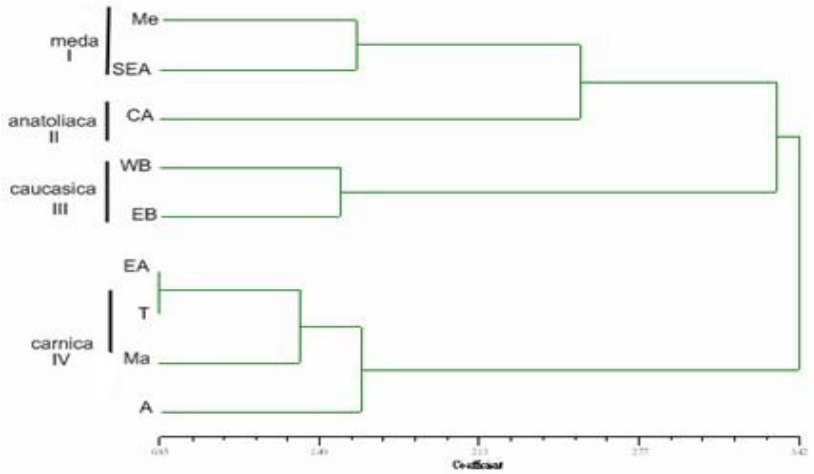

Figure 3. UPGMA Phenogram of populations from nine geographic regions based on Mahalonobis distances among centroids of groups in discriminant function analysis.
(Me: Mediterranean, SEA: Southeast Anatolian, CA: Central Anatolia, WB: Western Black, EB: Eastern Black, EA: East Anatolia, T: Thracen, Ma: Marmara, A: Aegean)

Fig. 3 shows the phylogenetic relationships among populations as revealed in UPGMA (Unweighted Pair Group Analysis) dendrogram, based on the Mahalonobis distance calculated from morphometric data. Honeybee populations of Turkey exactly divided into four main groups. In the first clade Mediterranean and South-eastern Anatolia are clustered together (group I), and this group together with Central Anatolian population (II) make up a larger cluster. Colonies from Western Black Sea and Eastern Black Sea (III) are very close. The Western population of Anatolia (Thrace, Marmara and Aegean region) (IV) remained as distinct units within this phenogram. Thrace (IV) and Marmara formed together with the Aegean population forming a coherent group.

As a morphometric and ecological similarity, group I classified as *A. m. meda* and group II in Central Anatolian classified as *A. m. anatoliaca.* In morphocluster III, honeybee from North and north-eastern Anatolia closely matched *A. m. caucasica.* Fourth group (IV),

west of the line, İstanbul-Bursa-Eskişehir-Isparta, were represented as a distinct cluster (IV) may be *A. m. carnica.*

In addition, DFA was applied on individual data of 56 localities in order to detect different local honeybees. On the two dimensional plot, honeybee samples from some localities, Düzce (Yığılca), Bingöl, Hayrabolu and Balıkesir were formed exceptional group from each other and the rest ones.

2. Regression Analysis

Multiple regression analysis was applied to the morphometric variables using latitude longitude and altitude as independent variables. Five out of 12 morphometric variables positively and significantly correlated to physiographical factors (latitude and longitude). Fore wing length (P<0.001), fore wing width (P<0.018) and hind wing width (0.002) were dependent on latitude. Hind wing length (P<0.002) correlated to both latitude and longitude and proboscis length (P<0.031) dependent on longitude. Morphometric characters showed no significant relationship with altitude (Tab. IV).

Table 4. Results of Multiple regression analysis of morphometric variables on latitude, longitude and altitude

Dependent variables	Regression values (&)	**latitude**	P	**longitude**	P	**altitude**	P
FWL	7.118	0.461	**0.001***	0.294	0.068	0.175	0.286
FWW	2.479	0.342	**0.018***	0.245	0.152	0.143	0.413
A	0.504	0.096	0.523	-0.201	0.268	0.305	0.105
B	0.138	0.206	0.175	0.134	0.460	0.030	0.872
CI	1.593	0.095	0.534	-0.52	0.776	0.198	0.296
HWL	4.786	0.399	**0.002***	0.585	**0.000***	-0.177	0.257
HWW	1.416	0.455	**0.002***	0.158	0.346	0.046	0.789
FL	2.749	-0.045	0.770	0.058	0.755	-0.044	0.818
TL	2.734	0.162	0.273	0.126	0.478	0.221	0.228
BL	2.074	-0.001	0.996	0.103	0.565	-0.305	0.104
BW	1.101	0.089	0.563	-0.075	0.684	0.052	0.785
PL	3.261	0.161	0.271	0.387	**0.031***	-0.120	0.507

(* P< 0.05)

Regression values were calculated using the Formula (Y= &+ b.X) Y: dependent variable; X: independent variable (latitude, longitude and altitude; & regression value; b: grade parameter)

Discussion

In the current study honeybees from Turkey were studied using 12 morphometric characters. Of the twelve characters studied, four (FWL, HWL, HWW, PL) were found to be significantly different between the populations (P<0.05) (Figure 2.).

Although not all morphometric variables were significantly different among the populations, some of their discriminatory powers were well observed in the two dimensional plot. The pattern observed in the dicriminant function analysis with the morphometric data is also supported by the cluster analysis obtained from the UPGMA analysis, in which four main groups were detected as expected despite extensive migratory beekeeping and commercial breeding. Before the period of general modernization of apiculture, the same conclusion was reported (1, 4, 24).

Considering the two dimensional plot in the present study, it is difficult to identify regional boundaries between honey bee population. There is more overlapping among colonies in different regions. This may be not only because of the beekeeping manipulations (migratory beekeeping and commercial breeding) but also due to sampling locations being in different geographic regions but to extent on the same longitude or latitude. In the current study, regression analysis explained well the effect of the geographic coordinates of sampling locations on the morphometric variables.

According to mtDNA data, *Apis mellifera* population in the European and Asian parts of Anatolian could be well discriminated. The data showed that all European and Anatolian

populations belonged to "C" linage [25]. In another study based on PCR-RFLP's analysis of mtDNA, Palmer et al. (2000) [20] came to the conclusion that Thrace populations were not different from Anatolian honeybee populations. On the other hand, Kandemir et al. (2005) [15] infer that Thracian populations from the western part of Turkey were supposed to be *A. m. carnica* and were different from Anatolian honeybee populations. Our results show slightly differences. It is clearly shown in the present research, that based on morphometrical data, all western populations (from Marmara, Aegean and Thrace regions) are clustered together, not only the Thrace populations.

Cubital index, a diagnostic morphometric ratio for true range of *A. m. carnica* is 2.56-2.78 mm. [21]. Kandemir et al. (2005) [15] reported high value of cubital index (2.74 mm) for Kırklareli population in Thrace. There seems to be a conflict with our result and those of Kandemir et al., [14,15]. In the present study Thrace population exhibited quite a low cubital index ratio: 2.3 (range 1.99-2.54).

Honeybee population in north-eastern of Turkey, represented by *A. m .caucasica*, exhibited shorter proboscis length (5.95-6.72) than Ruttner's (1988) [24]. Proboscis length, a diagnostic morphometric character for true range of *A. m. caucasica* is 6.9-7.2 Our morphometrical findings for *A. m. caucasica* in consistent with these: Karacaoglu and Fıratlı (1999) [18], reported 6.7 proboscis length; Güler (2001) [9] and Güler et al. (2002) [10] , reported 6.5-6.7 proboscis length with honeybees in North-eastern of Turkey.

Investigating only tongue length (range 6.65-7.10), Skorikov (1929) [26] established four local populations from different geographic region. He stated that there have been different ecotypes of *A. m. caucasica* spread from the Caucasus mountain to the Black Sea coast of Anatolia [26, 2, 3]. On this basis we propose these honeybees may be different ecotypes of *A. m. caucasica.* Beekeepers in Artvin near to Georgia reported that migratory beekeepers from Anatolia frequented Artvin quite often, whereas honeybees of Artvin are isolated legally from other parts of Turkey.

We observed another interesting result, honeybees from some local areas, Düzce (Yığılca) and Bingöl provinces seem to have maintained characteristics of pure populations. Beekeepers from these locations reported that no imported honeybees were introduced into the areas for more than 30 years and colonies are still managed in a traditional way, there is no sign of modern beekeeping manipulations (treatment of diseases, queen replacement, etc). So finding an endemic honeybee population in these areas is perhaps not surprising. Morphometric characteristics of honeybees in Yığılca (Düzce) provinces represent local ecotype of *anatoliaca.* Honeybees of Bingöl provinces may belong to pure *A. m. meda* with shorter forewing length and high cubital index. As Bodenheimer's (1941) [4] classification, *A. m. remipes* was found in Elazığ adjacent to Bingöl. It was reported that *A. m. remipes,* corresponding *A. m. meda* in Ruttner's (1988) [24] classification represents the true Irano-Turanian honeybees.

The significant regression of morphometric variables on latitude and longitude display a structured pattern in the distribution of populations. The spatial nature of this pattern is most likely the result of evolutionary forces acting on the honeybee population. Morphometric variables that showed significant regression on the latitude and longitude also had high loading on the first two axes in the principal component analysis. These results allow us to conclude that there is a connection between the size of honeybees and increasing latitude and longitude.

Conclusion

Our results shows that, with the exception of some regions, beekeeping practices have not significantly influenced the common genetic structure of honeybees in Turkey. So the conclusion about honey bee biodiversity in present and the past are the same. But, On the

other hand, in this study finding the great connection between genetic structure and the geographical location may eventually prove that these four main groups represents the geographically different ecotypes of *A. m. anatoliaca* not the excat races.

The first formal taxonomic classification had been done based on the less honey bees material (Ruttner 1988, page 182). So the result of the past may be not reliable.

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