Morphometrics as a Tool for the Study of Genetic Variability of Honey Bees

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This study was conducted to determine whether the classical morphometric method is a good tool for investigating biodiversity of honey bee or not. The research material was consisted of the worker bee samples collected from 55 apiaries in different locations in Turkey. They were surveyed for only two morphometric characters. Due to common characters exit in all morphometric studies of honey bee, the wing length and the cubital index were chosen. In our study, phylogenetic tree obtained and the results given in graphics showed that morphometric method was a good tool for studying of morphological genetic variability. But it may be better if it should be replaced by modern geometrik morphometric method.

A review concerning the concept of classical and modern morphometric methods were also been emphesized.

Keywords: Morphometry, honeybee, Türkiye, genetic biodiversity

Bal Arılarının Genetik Değişkenliği Üzerine Çalışmalarda Bir Araç Olarak Morfometri

Bu çalışma yalnızca iki morfometrik karekter aracılığıyla Türkiye bal arısı biyoçeşitliliğini belirlemek, elde edilen sonuçları benzer çalışmaların verileriyle karşılaştırmak ve böylece klasik morfometrik methodun bu tür çalışmalardaki etkinliğini irdelemek amacıyla düzenlenmiştir.

Çalışma için Türkiye'nin farklı yerlerindeki 55 arılıktan işçi arı örnekleri toplanmıştır. Örnekler şimdiye kadar yapılan çalışmaların çoğunda ele alındığı gibi, kubital index ve ön kanat uzunluğu bakımından incelenmiştir. Elde edilen verilerin istatistiki analiz sonuçları klasik morfometrik methodun çeşitli yerel sonuçları karşılaştırmak konusundaki etkinliği bakımından hala geçerli bir araç olduğunu, fakat modern geometrik morfometrik method ile değiştirilirse çok daha etkin bir araç olabileceğini göstermiştir. Eserde klasik ve modern morfometrik metodlara ilişkin genel bir değerlendirme de yapılmıştır.

Anahtar kelimeler: Morfometri, balarısı, Türkiye, genetik çeşitlilik

Introduction

The concept of genetic variability of honeybee ecotypes of Turkiye were studied by several researches. Acording to Ruttner(1988), three subspecies are found in Turkiye: *A. m. anatoliaca*, *A. m. caucasiaca* and *A. m. meda*. *A. m. caucasica* occurs in the extreme northeast of Anatolia and along the eastern Black Sea coast of Turkey. *A. m. meda* is found ın the southeast and *A. m. anatoliaca* occurs all over the rest of Turkiye including Thrace. The Anatolian honey bees in western Turkey, in a region surrounded by İstanbul, Bursa, Eskişehir and Isparta in the east form a distinct subgroup within *A. m. anatoliaca*, ‘‘the western Anatolian bees’’. After that Ruttner, honey bee subspecies were studied extensively by different approaches and there are another *A. mellifera* subspecies were observed. Presence of *A. m. carnica* in European part of Turkiye and *A. m. syriaca* in southeast of Turkiye were also shown (Smith et al., 1997; Palmer et al., 2000; Kandemir et al., 2006).

But still there are enigma and unanswered questions about honeybee biodiversity of Turkey, such as distribution of *A.m.caucasica* as true range of *A. m caucasica?* (Smith et al, 2002). In European Turkiye typical *Anatoliaca* is found with quite different characteristics. In Bulgaria, close to the Turkish border and in Greek Thracia, unhybridized Macedonians are found. In order to answer these questions, and installing honey bees genetic conservatories, it is necessary to collect information on biological diversity of honeybee ecotypes of Turkiye. .Morphometric studies have provided a large amount of information on the structure of *Apis mellifera L.* subspecies. During the last 15 years molecular markers such as mitochondrial and microsatellites have been used to analyse genetic diversity. Most of the time these studies were carried out on restricted areas of the home range using non homologous markers. It is rather difficult to combine these results at the European scale (Garnery et. al., 2004). Morphometrics as a tool for the study of genetic variability of honeybees are more common everywhere. So it is possible to combine these results of morphometrics tools at the European scale.

For morphometrical discrimination of *A. mellifera* subspecies more than 35 characters, with hair, size, fore wing length and color, were determined by Ruttner (1988). The most common preferable characters to study honeybee biodiversity have been cubital index, fore wing lenght and some wing venation angles (Cornuet et. al., 1991; Kandemir et. al., 1995; Güler and Kaftanoğlu, 1999a; Güler and Kaftanoğlu 1999b; Güler et. al., 2002.)

The shape of organisms and biological structures have been of scientific interest for centuries. This is understandable because biological shape of the most conspicuous aspects of an organism’s phenotype provides a link between the genotype and the environment (Ricklefs and Miles, 1994).

Historically, the study of shape variation has been concentrated on using distances between landmarks in biological structures, as well as angles and distance ratios. These variables were generally combined and analyzed through an array of canonical and cluster analyses called “traditional morphometrics” which is based on multivariate analysis of distances, angles and ratios (Marcus, 1990). This approach has been gradually replaced by the modern geometric morphometric methods (Rohlf and Marcus, 1993).

Traditional morphometric approaches are based on the application of standard multivariate analyses of arbitrary collections of distance measures, ratios, and angles. These variables typically represent only part of the information that may be obtained from the relative positions of the landmarks on which these measurements are based.

The relatively new field of geometric morphometrics represents an important new paradigm for the statistical study of shape variation and its covariation with other variables.

The fundamental advances of geometric morphometrics over traditional approaches include the way one measures the amount of difference between shapes (using Procrustes distance), the elucidation of the properties of the multidimensional shape space defined by this distance coefficient, the development of specialized statistical methods for the study of shape, and the development of new techniques for the graphical representations of the results (Rohlf, 2000).

To illustrate the method mentioned above an example is the honey bee (*Apis mellifera L.*). Twenty-seven subspecies of A. mellifera are recognized on the basis of morphometric characters according to Ruttner (1988,1992) and Sheppard et al. (1997,2003).

In traditional geometric morphometric, the methods for measuring angles practised as follow. Fore wings are spread in wet condition, slightly dried and covered for measurement. For measuring angles and indices a clear definition of the measuring points is needed. In analysis crossing points of the imaginary midline of the veins were consistently taken. These points are marked on a transparent paper and the angles measured (Ruttner, 1988).

The modern geometric morpometrics analysis of honey bees uses the coordinates (x,y) of 19 landmarks (figure 1) located at vein intersections of the fore wings, as seems to be informative about wing’s size and shape.

Images of the forewing are obtained using a video camera mounted on a microscope with a 1x objective. The coordinates of 19 landmarks are recorded, and the measurements are taken using the TPSdig program (Rohlf, 2003).

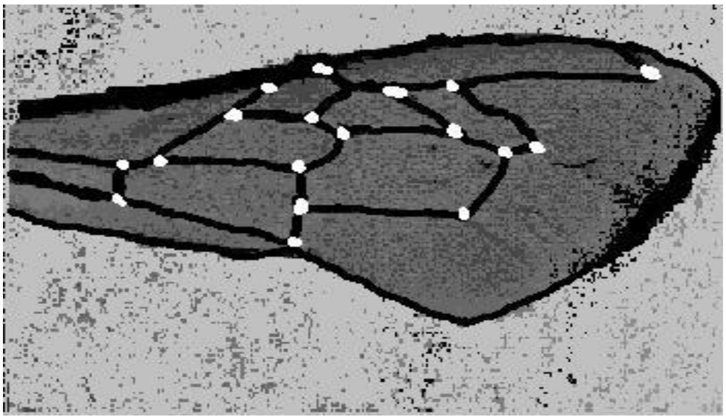


Figure 1. The nineteen landmarks used in geometrics morphometrics in honey bees

Landmark coordinates are superimposed using a Generalized least Squares (GLS) Procreates Superimposition (Rohlf and slice, 1990): Specimens are centred, normalized to unit centred-size (Bookstein, 1991) and interactively rotated to minimize the sum-of squared distances between each location and its sample mean, centroid size is analyzed separately by ANOVA or together with shape parameters (i.e. Procrustes residuals) Shape differences are analyzed by multivariate analysis of variance (MANOVA), Canonical Variate Analyses (CVA) and Multiple Discriminate Analyses, and shape patterns along the canonical axes are estimated by multivariate regression (Monteiro , 1999).

An application of this method on Greek honey bees (Hatjina *et al*., 2004): A geometric morphometrics analysis using the coordinates of 19 landmarks located at vein intersections of the left wings was contacted on a sample of 450 honey bees collected from 11 populations of the Greek mainland, 17 populations from Ionian islands and 47 populations from Crete island. Landmark coordinates were processed, using MS Excel, TPS (Rohlf, 2003) and NTSYS (Rholf, 1990) program packages. The analysis on wing shapes revealed significant information on population differentiation. The results showed that the populations studied are mixed, may be due to beekeeping manipulations (hives moving and commercial breeding).

The geometric morphometric analysis is very informative concerning biogeographical variability, morphometric discrimination of populations

The methods of geometric morphometrics were used to analyse differences between three honeybee subspecies, *A. m. mellifera, A. m.carnica, A. m. caucasica*. From every subspecies a hundred workers were collected. As landmarks, 18 vein junctions were chosen. After obtaining the wings images, the vein junction were detected automatically using Drawings software Generalized Procreates analysis followed by Canonical Variants Analysis was used to compare the shape of venation. The discrimination based on the geometric morphometrics proved to be succesful. The analysis revealed many differences between the subspecies (Tofilski, 2004).

This method is not only used for vein junctions but also used for cubital index- a ratio of lengths of two wing veins. Traditionally to obtain the veins lengths, the wing is usually scanned and a computer mouse used to point the vein junctions on a computer screen. Pointing the landmarks is time consuming and often associated with errors because the exact position of a landmark is ambiguous, particularly when veins are wide.

In order to automate the process of cubital index determination, computer program was used (Tofilski, 2004). The program accepts greyscale images of honey bee wings. The wing venation outline is extracted by converting the image into black and white. A thinning algorithmic is applied to the outline. As a result the veins became single lines and vein junctions become points. Coordinates of the points are used to calculate the cubital index. Automatic determination of the cubital index is not only faster but also more repeatable than traditional method (Tofilski, 2004).

Table 1. Average cubital index and length of fore wings for populations from Turkey

Sample place	Cubital İndex	wing lenght	Sample place	Cubital İndex	wing lenght
Istanbul	2,467	9,144	Sinop	2,569	9,154
Gokceada	2,300	9,035	Hakkari	2,407	8,919
Malkara	2,539	9,029	Zonguldak	2,087	9,109
Yalova	2,268	9,090	Trabzon	2,212	9,240
Corlu	2,440	9,042	Osmaniye	2,382	8,827
Saray	2,278	8,995	Bartin	1,885	9,269
Aksaray	2,314	9,002	Mersin	2,119	9,073
Kesan	2,261	9,000	Adana	2,560	8,867
Balikesir	2,418	8,788	K. Maras	2,109	8,935
Canakkale	2,220	8,985	Eskisehir	2,100	9,013
Izmir	2,145	8,763	Adiyaman	2,382	8,935
Mugla	2,312	8,969	Diyarbakir	2,264	8,856
Bozcaada	2,159	8,941	Malatya	2,245	9,044
Usak	2,166	8,898	Bayburt	2,505	9,219
Kutahya	2,581	9,077	Rize	2,153	9,156
Cerkezkoy	2,335	9,056	Isparta	2,363	9,100
Bursa	1,932	9,014	Giresun	1,956	9,115
Duzce	2,282	9,263	Corum	2,347	9,002
Muratli	2,531	9,044	Gumushane	2,140	9,156
Izmit	2,449	9,100	Elazig	2,187	9,254
Kirklareli	2,240	8,949	Erzincan	2,070	9,177
Denizli	2,412	9,046	Van	2,007	9,048
Luleburgaz	1,987	8,938	Agri	2,317	9,181
Hayrabolu	2,390	8,821	Meriç	2,268	8,815
Aydin	2,496	8,930	Kocahidir	2,248	8,949
Cankiri	2,203	9,141	Ipsala	2,312	8,857
Artvin	2,146	8,958	Enez	2,358	8,911
Bingol	2,628	8,783			
Min *	**1,885**	**8,763**			
Max *	**2,628**	**9,269**			
Aver *	**2,281**	**9,018**			
C.V *	**%5.78**	**%39.7**			

** The figures given last four raws showed the general statistics for all laocations sampled*

Classical morphometrics is still an informative approach herewith molecular and geometric morphometrics analysis for detecting study intraspecific variation at the population level.

Materials and Methods

In order to present an application of classical morphometric approach worker honey bee samples were collected in June- September

2006 from the collection sites in Turkiye. Data obtained were sourced from worker honey bee samples collected from 55 different area of Turkiye. Three colonies were sampled from each locations. Fifteen honey bees worker were taken from entrance of each hive. They were put in small label boxes and kept in %99 ethanol until final processing.

In this study we used classical morphometric method to investigate the genetic diversity. Two characters were choosen for application of classical morphometric approach. Previous studied had shown that these characters were both commonly used and the best characters among others.

The characteristics studied were forewing length and cubital index. As concerning the classical morphometrics analysis, for wings the body kept in % 70 lactic acid for 24 hours in order to tissues to be soften for better resolution. Fore wings were put between transparent tapes fastened on a 5x5 cm slide frame and were projected them on a screen by slide projector. By this method the length and width of fore and hind wings were measured as well as distances ‘’a’’ and ‘’b’’ values of cubital vein.

The results from classical morphometrics analysis were statistically processed using MS Excel and NTSYS program package (STAND, SMINT, SAHN, TREE program) as well as, CORRESP program for correspondence analysis phlogenetic tree was constructed based on UPGMA method using the same package.

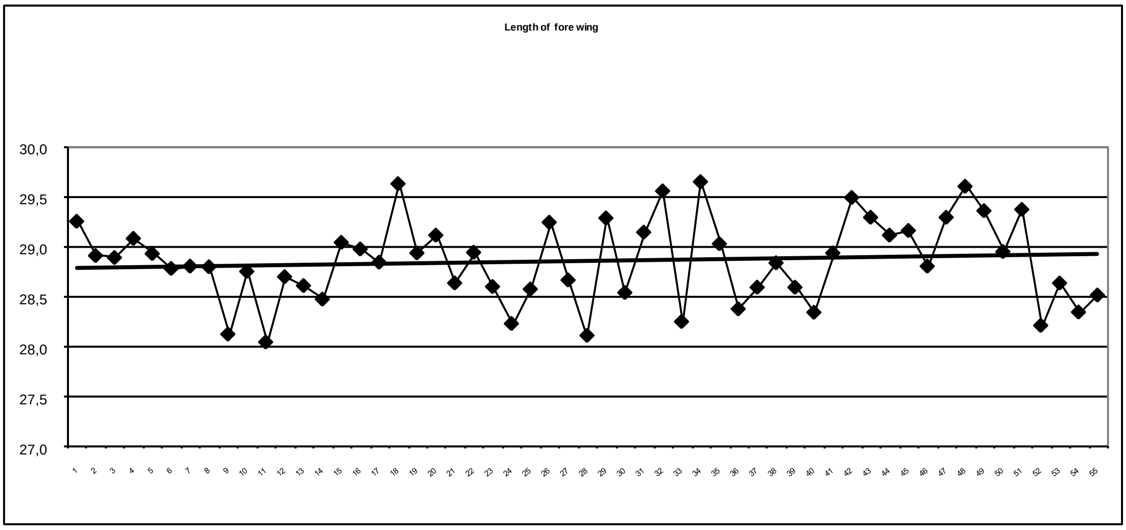
Results

In the present study, 2 morphometric variables were measured for morphometric analysis. Their average measurements were given in table 1. In Turkiye honeybee populations, cubital index ranged between 1.885 and 2.628. Bingöl population from the East of Türkiye exhibited quite a high cubital index (2.628) and Bartın population from the Northwest, exhibited the lowest cubital index(1.885).

A wide range of morphometric variation occurs throughout in Turkey (Figure 2). Cubital index showed significantly differences (39.7 %) among the populations studied.

Table 2. Comparative measurement of cubital index (A) and length of fore wing (B) of *A.mellifera* ecotypes respectively according to different researcher

Apis m. subspecies	(A) cubital index			(B)length wing	
	Sheppard at al., 2003	Ruttner, 1988	Bouga at al., 2001	Ruttner, 1988	Bouga at al., 2001
Mellifera	1.82±0.2	1.84		9.33	
Carnica	2.69±0.18	2.59±0.42		9.40	
Ligustica	2.52±0.12	2.55		9.21	
Caucasica	2.14±0.12	2.16±0.31		9.32	
Anatolica	2.25±0.22	2.24±0.18		9.19	
Meda	2.5±0.23	2.56±0.72		8.97	
Armenica	2.61±0.16	2.61±0.42		9.07	
Macedonica		2.59	2.55	9.18	9.14
Adami		1.89±0.18	2.30	9.09	9.07
Cypria		2.72±0.36		8.87	
Pomonella	2.24±0.2				
Syriaca		2.28±0.37		8.48	



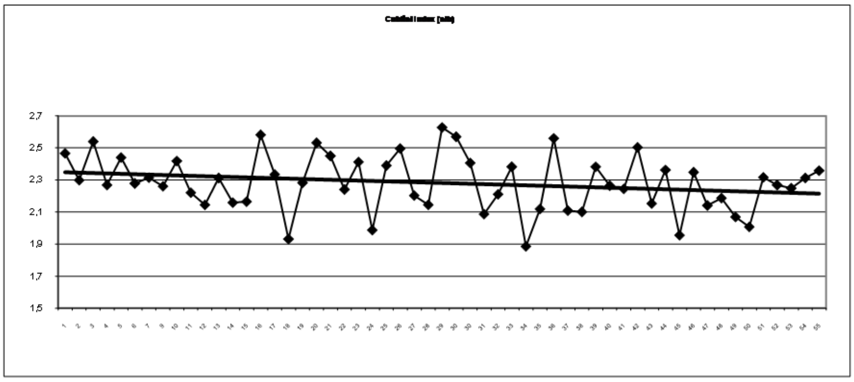


Figure 2. The distribution of measurement of length of fore wing and cubital index of Turkey

1.İstanbul 2.Gökçeada 3.Malkara 4.Yalova 5.Çorlu 6.Saray 7.Aksaray 8.Keşan 9.Balıkesir 10.Çanakkale 11.İzmir 12.Muğla 13.Bozcaada 14.Uşak 15.Kütahya 16.Çerkezköy 17.Bursa 18.Düzce 19.Muratlı 20.İzmit 21.Kırklareli 22.Denizli 23.Lüleburgaz 24.Hayrabolu 25.Aydın 26.Çankırı 27.Artvin 28.Bingöl 29.Sinop 30.Hakkari 31.Zonguldak 32.Trabzon 33.Osmaniye 34.Bartın 35.Mersin 36.Adana 37.Maraş 38.Eskişehir 39.Adıyaman 40.Diyarbakır 41.Malatya 42.Bayburt 43.Rize 44.Isparta 45.Giresun 46.Çorum 47.Gümüşhane 48.Elazığ 49.Erzincan 50.Van 51.Ağrı 52.Meriç 53.Kocahıdır 54.İpsala 55.Enez

UPGMA (unweighed pair group method analysis) dendogram (figure 3) based on the results from morphometric approach show no reginal compact cluster. There is inter population variability in the population studied. UPGMA dendrogram based on the results from morphometric approach shows that Aydın, Diyarbakır, İzmit populations were discriminated from the rest ones. Artvin, Trabzon, Düzce were formed first cluster. In the second cluster İzmir and Lüleburgaz population formed differet cluster and the others formed two different coherent goups.

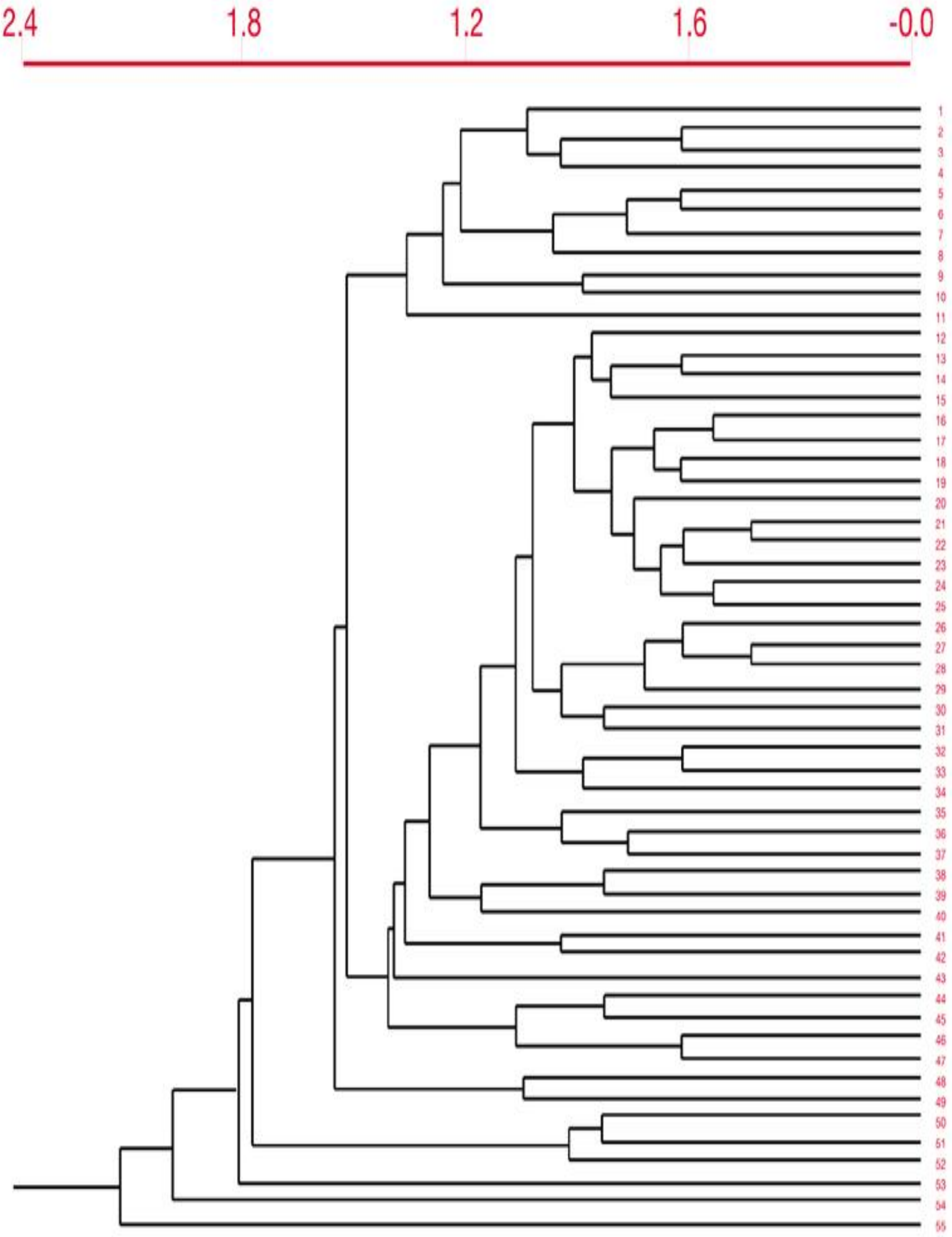


Figure 3. UPGMA Dendogram drawn on the base of (UPGMA) method.

1.İstanbul 2.Bingöl 3.Hakkari 4.Adana 5.Adıyaman 6.İpsala 7.Enez 8.Meriç 9.Hayrabolu 10.Osmaniye 11.Balıkesir 12.Gökçeada 13.Aksaray 14.Isparta 15.Çankırı 16.Yalova 17.Malatya 18.Çorum 19.Kocahıdır 20.Çorlu 21.Saray 22.Kırklareli 23.Keşan 24.Muğla 25.Denizli 26.Bozcaada 27.Mersin 28.Eskişehir 29.Van 30.Elazığ 31.Ağrı 32.Zonguldak 33.Rize 34.Giresun 35.Çanakkale 36.uşak 37.Maraş 38.Malkara 39.Kütahya 40.Çerkezköy 41.Muratlı 42.Sinop 43.Bayburt 44.Bursa 45.Bartın 46.Gümüşhane 47.Erzincan 48.İzmir 49.Lüleburgaz 50.Düzce 51.Trabzon 52.Artvin 53.İzmit 54.Diyarbakır 55.Aydın

Discussion

Different honeybee (*Apis mellifera*) ecotypes, or races allocated to the seven geographic region of Turkey,''*A.m.carnica'', ''A.m.anatoliaca'', ''A.m.caucasca'', ''A.m.syriaca''*were studied. They couldn't be morphometricaly differentiated in this study as would be expected according to Ruttner (1988).

Güler et al. (2001) based on the morphometric variability identified the genotype grown in the ''Camili'' area of the nort eastern Anatolian region is *A. m. caucasica G.* He found that average length of forewing was 9.380±0.0154 and cubital index was 2.0935± 0.11, similar to our result for Artvin (2.146).

Kandemir et al. (2005) claimed that cubital index for honeybee subspecies also showed similarity between honeybee population from Kırklareli (2.718) and Austria colonies (2.783), on contrary with our results. In the present study cubital index for honeybee population from Kırklareli 2.240 was similar of *Anotoliaca* (2.24, 2.25) (Ruttner, 1988; Sheppard 2003).

Average cubital index and wing length in central Anatolia were found as 2.12± 0.028 and 9.15 ±0.014 respectively (Karacaoğlu and Fıratlı, 1998).

According to Gençer and Fıratlı (1999), different population from central Anatolia showed different cubital index values as: 2.203(Kışehir), 2.140(Beypazarı), 2.207 (Çankırı), 2.327 (Eskişehir). Also our results indicated some differences between population studied from central Anatolia, Eskişehir(2.100) and Aksaray(2.314).

Çınar et al (2004) were found, similarity between Muğla and Hatay population to respect of cubital index. Present study showed that Muğla and kırklareli population were included in the same cluster.

Bodenheimer (1942), studying honeybee races in Turkey reached the conclusion that honeybees show local variation within subspecies.

Population from Bingöl (2.628) similar to A. *armenica* (Shapperd et al., 2003) and population from Bartın (1.885) similar to ''*adami''* Ruttner (1988), with Respect to cubital index.

Louveaux (1969) also suggested the presence of ecotypes in honeybees. Sheppard and Meixner (2003) based on the basis of morphometric analysis described a new subspecies, *Apis mellifera pomenella*, in central Asia, it is in general, very similar to *A m. anatoliaca*. Average cubital index for each ecotype showed great similarity (table 2A). Because of the great similarity in Anatolian honeybee population it is concluded that Anatolia may be close to the center of origin of honeybees. Kandemir et al. (2000) also suggested the same opinion from both morphometric and enzyme polymorphism.

Differentiation among the honey bee (*Apis mellifera*) subspecies is usually performed by methods of traditional morphometrics which based on multivariate analysis of distances, angles and ratios. It has been provided that those methods were graphical presentation of results and personal errors. In addition classical methods were required more labour and tedious work for measurments. Many of the problems of tradional morphometrics were solved by geometric morphometrics and it would be better using modern morphometric method.

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