

Comparison of two morphometric methods for discriminating honey bee (*Apis mellifera* L.) populations in Turkey

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Received: 07.04.2011

Accepted: 19.09.2012

Published Online: 25.02.2013

Printed: 25.03.2013

Abstract: In this study, we compared 2 different methodologies (traditional morphometrics, TM, and geometric morphometrics, GM) in order to determine their ability to discriminate the honey bee populations distributed throughout Turkey. In TM, 16 morphometric characters were measured from the forewings of different honey bee populations from Turkey. A total of 20 landmarks were utilized for the GM analysis. Multivariate statistical analysis of data obtained from the 2 methodologies showed that GM was more successful (81.5%) than TM (70.4%). While the GM method is much simpler and easier compared to the standard morphometric measurement of size characters and angles of wing venations, the discrimination ability of GM on the honey bee populations was greater than that of TM. In addition, TM is restricted to distance characters and rotation of distances; GM not only includes these measurements indirectly, but also allows for wing shape analysis using the landmark approach.

Key words: Geometric morphometrics, traditional morphometrics, honey bee subspecies, Turkey

1. Introduction

Anatolia is an important gene center for honey bee (*Apis mellifera* L.) subspecies and ecotypes because of its geographical position, climatic conditions, and ecological diversity (Kandemir and Kence, 1995). Based on multivariate statistical analysis of standard morphometry data, Ruttner (1988) demonstrated that 4 honey bee subspecies were present in Anatolia. *Apis mellifera anatoliaca* is distributed throughout Central Anatolia, the Aegean and Mediterranean regions, and a large part of the Black Sea region. *A. m. caucasica* is distributed in Northeastern Anatolia, *A. m. meda* in Southeastern Anatolia, and *A. m. carnica* in the Thrace region (Kandemir et al., 2000).

In the years following Ruttner's research (1988), honey bee populations from Anatolia were studied extensively by using standard morphometric characters (Güler and Kaftanoğlu, 1999a; 1999b; Kandemir et al., 2000; Adl et al., 2007), allozymes (Asal et al., 1995; Kandemir and Kence, 1995; Kandemir et al., 2000), mitochondrial DNA (Smith et al., 1997; Palmer et al., 2000; Kandemir et al., 2006), and microsatellite analysis (Bodur et al., 2007) in order to reveal the presence of genetic variation with respect to morphometry, biochemistry, and genetics, as well as the distribution of honey bee subspecies in Turkey. According to Kandemir and Kence (1995) and Palmer et al. (2000), *A.*

m. anatoliaca, *A. m. meda*, *A. m. caucasica*, *A. m. syriaca*, and *A. m. carnica* are known to be distributed in Turkey. Özdil et al. (2009) also strengthened knowledge of the presence of the aforementioned subspecies by mtDNA sequencing data.

The use of standard measurements in honey bee characterization and identification, here called traditional morphometrics (TM), has been improved with multivariate analysis of morphometric characters. In standard morphometry on honey bee subspecies, wing shape has been studied by using angles and distances (reviewed in Ruttner, 1988). These angles and distances have generally been combined with other size characters and analyzed through multivariate statistical analysis. In order to discriminate honey bee subspecies, geometric morphometrics (GM), a relatively new field dealing with the study of wing shape by using landmarks, has been used instead of standard morphometry in recent years. In honey bees, GM analyses of wing shape have provided many new insights, into either the characters or the identification of populations or lineages (Francoy et al., 2008, 2009; Tofilski, 2008; Miguel et al., 2011). These studies demonstrated that wing GM appears more appropriate than TM in order to better differentiate honey bee subspecies and indicated that the new methodologies were very effective and fast for the identification and discrimination of honey bees. The

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objective of this paper is to use the forewing to compare the effectiveness of both TM and GM for discriminating the honey bee populations or subspecies in Turkey.

2. Materials and methods

A total of 162 colonies were sampled from 15 locations belonging to different geographic regions in Turkey between the years 2000 and 2007 (Figure 1). Honey bees were preserved in 70% ethanol until morphometric examinations were carried out. The forewings of 10 worker bees per colony were mounted between microscope slides in distilled water, and left forewings were photographed with a Leica MZ16 camera–microscope system.

2.1. Morphometric analysis

For standard morphometric analysis, a total of 16 morphometric characters were biometrically measured with the camera combined with a morphometric measurement program (Bee2 ©, Meixner and Meixner, 2004). Morphometric characters included forewing length (FWL), forewing width (FWW), cubital A (CuA), cubital B (CuB), cubital index, and forewing angles (A4, B4, D7, E9, G18, J10, J16, K19, L13, N23, O26) (Ruttner, 1988). The mean values of measurements of 10 bees in each colony were calculated. To discriminate the honey bee populations based on morphometric characters,

multivariate statistical analyses were performed on mean values of measurements.

2.2. Geometric morphometric analysis

For GM, each left wing image was photographed and stored in the computer. A total of 20 landmarks on the forewings were identified according to Bookstein's (1990) classification (Figure 2). tps files were prepared using tpsUtil 1.40 (Rohlf, 2008a), and landmarks were digitized on the images using tpsDig 2.11 (Rohlf, 2008b). Later, landmarks were superimposed using a generalized least-square algorithm (GPA; Rohlf, 1999) in Morpheus (Slice, 2002). This landmark-based morphometric method removes all nonshape variation that can be attributed to differences in the location, orientation (or rotation), or scale of the specimens. A multivariate analysis of variance (MANOVA) and pairwise tests were carried out on the landmark data by using Morpheus (Slice, 2002) in order to compare honey bee populations. Superimposed x , y coordinate data were then used as the data set for multivariate statistical analyses of honey bee populations.

Honey bee populations were assigned to 5 groups according to Ruttner's (1988) classification. To assess the variation between the 5 study populations, multivariate statistical analyses (canonical variate analysis, principal component analysis, and discriminant function analysis)



Figure 1. Sampling locations in Turkey (Thrace: 1–2; Aegean: 3; Central Anatolia/Mediterranean: 4–11; Southeastern Anatolia: 12–13; Northeastern Anatolia: 14–15).

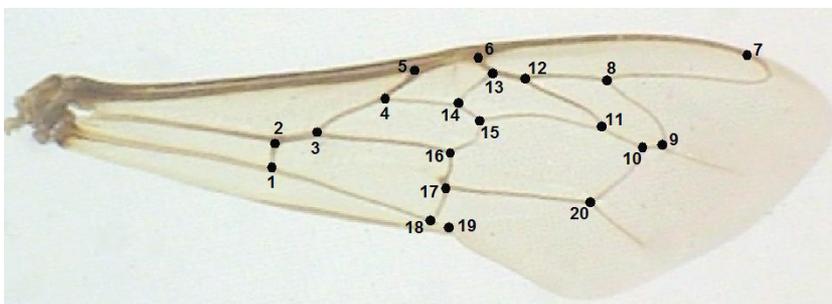


Figure 2. Location of landmarks on *Apis mellifera* worker forewing.

were carried out on standard and geometric morphometrics data collected from the same set of samples taken from different geographic regions of Turkey. All classification rates were estimated using leave-one-out (cross-evaluation) classification. Statistical analyses were carried out with the computer software programs SPSS 13.0 (SPSS, 2004) and PAST 1.47 (Paleontological Statistics; Hammer et al., 2001). A UPGMA cluster analysis (Rohlf, 2004) was performed on Mahalanobis distances of both sets of morphometric data to show the clustering among honey bee populations.

3. Results

3.1. Standard morphometric analysis

Analysis of variance (ANOVA) of morphological characters showed that 15 out of 16 characters (i.e. all except G18) displayed statistically significant differences among honey bee populations ($P < 0.05$). Canonical variate analysis of wing measurements plotting CV 2 against CV 1 could not clearly separate the 5 honey bee populations. Total shape variation was explained by 4 axes as 68.1%, 26.3%, 4.1%, and 1.5%, respectively. The first 2 axes explained 94.4% of the total variation. Although population-specific groupings were obvious, there were large overlaps between Aegean, Central Anatolia/Mediterranean, and Southeastern Anatolia (PCA plot shown in Figure 3A). The scatter plot of CV 3 against CV 2 did not separate the groups at all. Table 1 summarizes the colony assignments with respect to regions based on traditional morphometrics. It is seen that 72.2% of colonies were identified correctly in their original groups with standard morphometric characters of forewing as the discriminant criteria. Cross-validation tests based on discriminant functions correctly classified 70.4% of the colonies.

Discriminant function analysis on size characters of forewing demonstrated that 55.6% of the colonies were

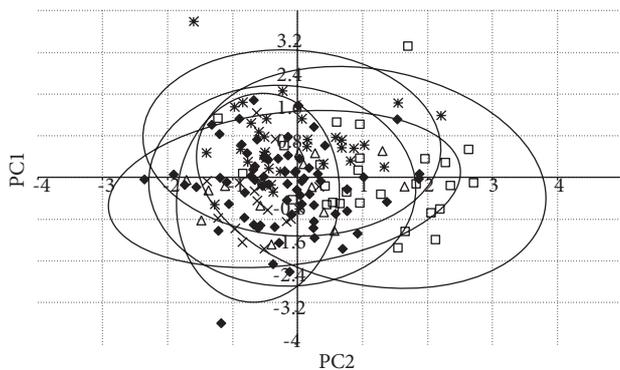


Figure 3A. Scatter plot of principle component analysis of honey bee populations from different geographic regions based on TM (Thrace = *; Aegean = x; Central Anatolia/Mediterranean = ◆; Southeastern Anatolia = △; Northeastern Anatolia = □).

correctly classified into their original groups. Discriminant function analysis based on angles of wing venations displayed a higher percentage of correct classification (60.5%).

3.2. Geometric morphometric analysis

We found significant differences among honey bee populations ($P < 0.001$) based on MANOVA. Pairwise comparisons were followed and significant differences between the groups were found, except in those of Central Anatolia and Southeastern Anatolia ($P < 0.001$). ANOVA of Cartesian coordinates of the landmarks on the forewing showed that 37 out of 40 Cartesian coordinates of landmarks displayed statistically significant differences among colonies of honey bee groups ($P < 0.05$). Canonical variate analysis scatter plot based upon 20 landmarks showed 4 groups. Only the Central Anatolia/Mediterranean and Southeastern Anatolia groups overlapped on scatter plot (PCA plot shown in Figure 3B). Total shape variation was explained by 4 axes as 62.6%, 27.2%, 8.8%, and 1.4%, respectively. The first 2 axes described 89.8% of the total variation among the 5 honey bee groups. Table 2 summarizes the colony assignments with respect to regions based on geometric morphometrics. All colonies were assigned to their original group with a high probability (88.3%). Cross-validation tests based on discriminant functions correctly classified 81.5% of the colonies.

When data from both morphometric approaches were allocated to 5 groups according to Ruttner's (1988) discriminations, a phenogram of honey bees based on wing morphometric characters (standard morphometry) demonstrated that the Thrace, Aegean, and Central Anatolia/Mediterranean groups were clustered very closely and this group, together with Southeastern Anatolia, made up a larger cluster. Northeastern Anatolia remained as a distinct unit within the constructed phenogram (Figure 4A). A phenogram of honey bees based on landmarks demonstrated

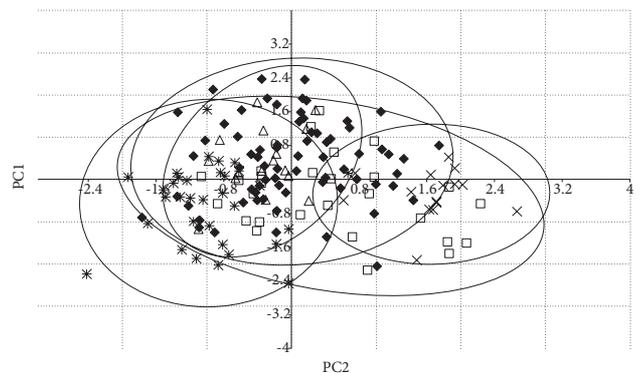


Figure 3B. Scatter plot of principle component analysis of honey bee populations from geographic regions based on GM (Thrace = *; Aegean = x; Central Anatolia/Mediterranean = ◆; Southeastern Anatolia = △; Northeastern Anatolia = □).

Table 1. Summary of the colony assignments with respect to regions based on TM. Percent classifications are in parentheses; N denotes the number of colonies.

Groups	Thrace	Aegean	Central Anatolia/ Mediterranean	Southeastern Anatolia	Northeastern Anatolia	N
Thrace	31 (93.9)	-	2 (6.1)	-	-	33
Aegean	-	-	13 (86.7)	1 (6.7)	1 (6.7)	15
Central Anatolia/Mediterranean	2 (2.9)	1 (1.4)	63 (90.0)	3 (4.3)	1 (1.4)	70
Southeastern Anatolia	1 (6.3)	-	11 (68.8)	3 (18.8)	1 (6.3)	16
Northeastern Anatolia	1 (3.6)	-	7 (25.0)	-	20 (71.4)	28

Table 2. Summary of the colony assignments with respect to regions based on GM. Percent classifications are in parentheses; N denotes the number of colonies.

Groups	Thrace	Aegean	Central Anatolia/ Mediterranean	Southeastern Anatolia	Northeastern Anatolia	N
Thrace	33 (100)	-	-	-	-	33
Aegean	-	15 (100)	-	-	-	15
Central Anatolia/Mediterranean	2 (2.9)	1 (1.4)	64 (91.4)	2 (2.9)	1 (1.4)	70
Southeastern Anatolia	-	-	9 (56.3)	6 (37.5)	1 (6.3)	16
Northeastern Anatolia	2 (7.1)	-	1 (3.6)	-	25 (89.3)	28

that the Central Anatolia/Mediterranean group and the Southeastern Anatolia group were clustered very closely, and this, together with the Northeastern Anatolia group, made up a larger cluster. Thrace and Aegean populations remained as a distinct unit within the constructed phenogram (Figure 4B). Except for the Southeastern Anatolia group, this phenogram reflected the similar results of Kandemir et al. (2000) concerning honey bee populations from different geographic regions in Turkey.

4. Discussion

Although standard morphometry has long been applied to discriminate honey bee subspecies, it takes much time to complete these studies (Francoy et al., 2008). GM methods are more practical, easier, and accomplished in a short time, since all procedures are based on computer-assisted technology (Zelditch et al., 2004). This new approach is cost-effective, as well, and all the software and programs related to GM can easily be downloaded.

In this study we compared both TM and GM methods and we found that GM data yielded much better discrimination of honey bee populations than TM. In multivariate statistical analysis of data obtained from the 2 methodologies, we found that GM was more successful (81.5%) than TM (70.4%). Earlier studies also emphasized

that GM was marginally more reliable than standard morphometrics for discrimination of honey bee subspecies (Francoy et al., 2008; Tofilski, 2008; Kandemir et al., 2011). Moreover, Miguel et al. (2011) pointed out that geometric morphometrics appear more suitable than mitochondrial DNA analysis or traditional morphometrics.

In TM studies of honey bees, the size characters of wings in general and angles of wings in particular were widely used to investigate phylogeny and phylogeography of honey bees. According to earlier studies of honey bees, measurement of size characters and color were found to be highly interrelated with geographic parameters (Diniz-Filho et al., 1999; Ruttner et al., 2000). Otherwise, wing angles were interrelated with phylogeny rather than phylogeography (Diniz-Filho et al., 1999). Venation was described either by 11 angles in TM or by the coordinates of 20 landmarks (40 x, y coordinates) in GM. According to Bookstein (1991), the use of size-free coordinates instead of distances, rotations, or angles leads to more exhaustive descriptions of geometric forms in biology. While TM is restricted to distance and ratios of distances, GM not only includes these measurements indirectly but also allows for wing shape analysis by using the landmark approach. The number of characters (40 x, y coordinates) used is thought to be more in GM and all the characters were size-free;

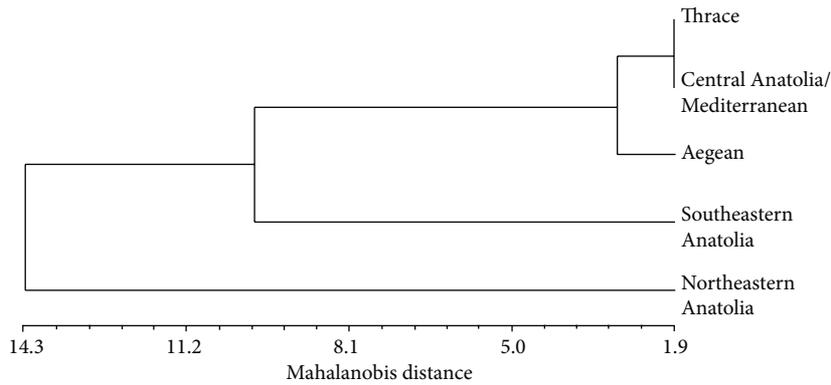


Figure 4A. UPGMA phenogram of honey bee populations from different geographic regions based on traditional morphometrics.

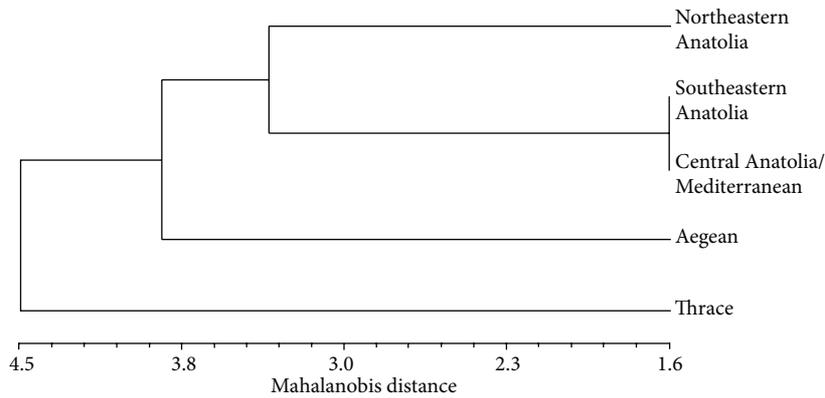


Figure 4B. UPGMA phenogram of honey bee populations from different geographic regions based on geometric morphometrics.

thus, much better discrimination based on wing shape was not surprising. Our findings also show that discriminant analysis of the angles of the forewing performed on the 162 colony means yielded better classification than discriminant analysis of the size characters, and so these results also show the strength of the size-free characters and landmark approach.

This study compared results of TM and GM methods with respect to their potential power in discriminating 5 honey bee populations in Turkey. The multivariate statistical analyses of TM data did not provide any useful separation among honey bee populations. On the other hand, our findings based on multivariate analysis of GM data showed close affinity with Ruttner's (1988) findings. The Central Anatolia/Mediterranean and Southeastern Anatolia groups overlapped on canonical analysis scatter plot; these 2 groups did not demonstrate any statistically significant differences ($P < 0.001$) (Figures 3A and 3B).

When Mahalanobis distances among the 5 groups were allocated according to Ruttner's (1988) discrimination

used in constructing UPGMA dendrograms, this resulted in 2 different topologies (Figures 4A and 4B). The tree obtained from GM data, except for the Southeastern Anatolia group, was more similar to Kandemir et al.'s (2000) findings concerning honey bee populations from different geographic regions in Turkey.

The results of this study clearly showed that measurements of size and angle characters can be sufficient to identify or discriminate honey bee populations, but the new approach, GM, is simple and much more effective than TM in discrimination or identification of honey bee populations in Turkey as well as in the world (Tofilski, 2008; Francoy et al., 2008, 2009; Kandemir et al., 2011; Miguel et al., 2011).

Acknowledgments

This work was supported by the Scientific and Technological Research Council of Turkey - TÜBİTAK-TBAG 107T154 grant to IK.

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