

LANDMARK-BASED MORPHOMETRIC STUDY IN THE FORE AND HIND WINGS OF AN IRANIAN RACE OF EUROPEAN HONEYBEE (*APIS MELLIFERA MEDA*)

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Abstract

Honeybee (*Apis mellifera*) populations are usually distinguished using standard morphometric methods, mainly based on multivariate analysis of distances and angles. Recently, geometric morphometrics, another method of statistical analysis of shape, has been developed. This research was conducted on European honeybees in Iran in 2012. Multivariate analysis on hind wings identified significant differences between honeybee populations from different areas and significant differences in centroid size of fore wings in different geographical areas ($F = 10.6$, $p = 0.000$). Populations from nine areas were classified using discriminate function analysis based on shape variables of fore and hind wings. Cross-validation tests based on discriminant functions of front wings correctly classified 68.2% of the colonies, and cross-validation tests based on discriminant functions of hind wings correctly classified 43% of the colonies.

Keywords: *Apis mellifera meda*, fore wing, geometric morphometrics, honeybee, landmark.

INTRODUCTION

The honeybee *Apis mellifera* L. is widespread in Africa, Europe, and parts of Asia with a wide diversity of subspecies that can be classified with morphometric tools (Ruttner et al., 1978). Honeybees differ in their morphology, behavior, and physiology according to the environmental conditions to which they have adapted (Ruttner, 1992). According to Ruttner (1988), there are at least 24 *Apis mellifera* subspecies grouped into three or four evolutionary branches, based on morphometric data. With the help of unvaried statistics, the discriminations were improved (Alpatov, 1929); additionally, the first attempts to classify bee subspecies were based mainly on color and size (Ruttner et al., 1978). Discriminating among honeybee species is important for beekeeping and preserving honeybee bio-

diversity (Tofilski, 2004). The first comprehensive classification and distribution of Iranian honeybees was done in 1985 by Ruttner et al. (1985). The name *A. m. meda* was first given by Skorikov in 1929 to a honeybee in the Soviet Union close to the Iranian border (Ruttner, 1988). Multivariate analysis of 63 samples from across Iran and 142 samples from neighboring countries (Turkey, Iraq, Syria, Lebanon, Israel, Jordan, and Cyprus) resulted in partly overlapping clusters. Ruttner et al. (1985) discriminated six local populations for *A. m. meda*, as follows: West and Central Iran (Azerbaijan-Iranian highlands), the subtropical coast of the Caspian Sea (Mazandaran), Northeast Iran (Mashad), Southeast Iran (Kerman), Iraq, and Southeast Anatolia, from Van Lake to Hatay. Another modern morphometric method that is also very promising for shape studies is

geometric morphometrics based on the description of shape in Cartesian coordinates (Bookstein, 1991). Instead of distances and angles, it uses the coordinates of points called landmarks. The landmarks are superimposed by translation, scaling, and rotation. After superposition, the landmark configurations differ only in shape and can be analyzed by multivariate statistical methods (Zelditch et al., 2004). In honeybees, geometric morphometric analyses of wing shape have provided many new insights into characters and identification of populations or lineages (Francoy et al., 2008; 2009; Tofilski, 2008; Miguel et al., 2011).

The aim of this study was comparison of venation variations in the front and hind wings of honeybee populations in different areas using a geometric morphometric method. Questions we addressed with this method were whether geographically isolating landforms such as mountains, valleys, or other natural obstacles affect morphological characteristics; whether this geometric morphometric method can discriminate honeybee populations in nine areas based on the front and hind wings; and which wing (front or hind wing) better discriminates area populations.

MATERIAL AND METHODS

The colonies were sampled from nine areas (three colonies in each area) in different geographic regions of Iran (Abhar: 36°8' N, 49°13' E;

Tarom (Emamkandi): 42°95' N, 48°9' E; Zanjan (Hajarash): 36°40' N, 48°30' E; Khoramdareh: 35°31' N, 49°17' E; Ijrood: 36°5' N, 32°49' E; Khodabandeh: 38°14' N, 39°16' E; Mahneshan: 37°10' N, 48°10' E; Zanjan (Nikpey): 36°89' N, 46°22' E; Tarom (Dastjerdeh): 42°52' N, 49°11' E) (Tab. 1). Sampling was conducted from hives that had not migrated for many years. Samples of honeybees were preserved in preservative solution (30 parts distilled water, 15 parts 95% ethanol, 7 parts 38% to 40% formaldehyde and 2 parts acetic acid) to prevent sample deformation. Forty samples of honeybees were collected from each colony. Collected samples from each area were mixed and 40 specimens were randomly selected. Then, 40 right front and 20 hind wings were separated and mounted in Canada balsam. Digital photos were taken from mounted wings using a DP12 camera and a SZX12 OLYMPUS stereomicroscope. Eighteen landmarks on fore wings and six on hind wings were digitized on photos by tpsDig software (Fig. 1). Next, digitized landmark outputs were analyzed by tpsRelw, tpsReg (Rohlf, 2010), and NtSys Pc. 2. Finally, samples of nine areas were classified with discriminant function analysis (DFA) by SPSS ver. 18. All non-shape variations of these landmarks such as orientation (or rotation), scale, and size were removed. A multivariate analysis of variance (MANOVA) was carried out on the landmark data to compare honeybee populations.

Table 1.

List and code of collection sites of <i>Apis mellifera</i> in Iran				
Population	Fore wing		Hind wing	
	Code	Sample number	Code	Sample number
Abhar	A	40	a	20
Tarom (Emamkandi)	B	40	b	20
Zanjan (Hajarash)	C	40	c	20
Khoramdareh	D	40	d	20
Ijrood	E	40	e	20
Khodabandeh	K	40	k	20
Mahneshan	M	40	m	20
Zanjan (Nikpey)	N	40	n	20
Tarom (Dastjerdeh)	T	40	t	20

RESULTS

Variance of coordinates of 18 landmarks on fore wings and 6 landmarks on hind wings as well as coordinates of landmarks of average shape were evaluated. At the front wing, the fourteenth landmark, the junction of the Rs and 2r-m veins, had the maximum variation ($S^2 = 0.00005$) in collected populations from nine areas of Iran; landmarks 12, 18, and 13 had the next greatest variations. Furthermore, the eighth landmark,

the junction of the 1m-cu and cu veins, had the minimum variation ($S^2 = 0.00001$) because the eighth landmark is near the centroid of the wing and landmarks 12, 18, and 13 are much further from the centroid. At the hind wing, the third landmark, the junction of the M and cu veins ($S^2 = 0.00019$), and the fourth landmark, the junction of the M and r-m veins ($S^2 = 0.00003$), had the maximum and minimum variations, respectively (Fig. 1 and 2).

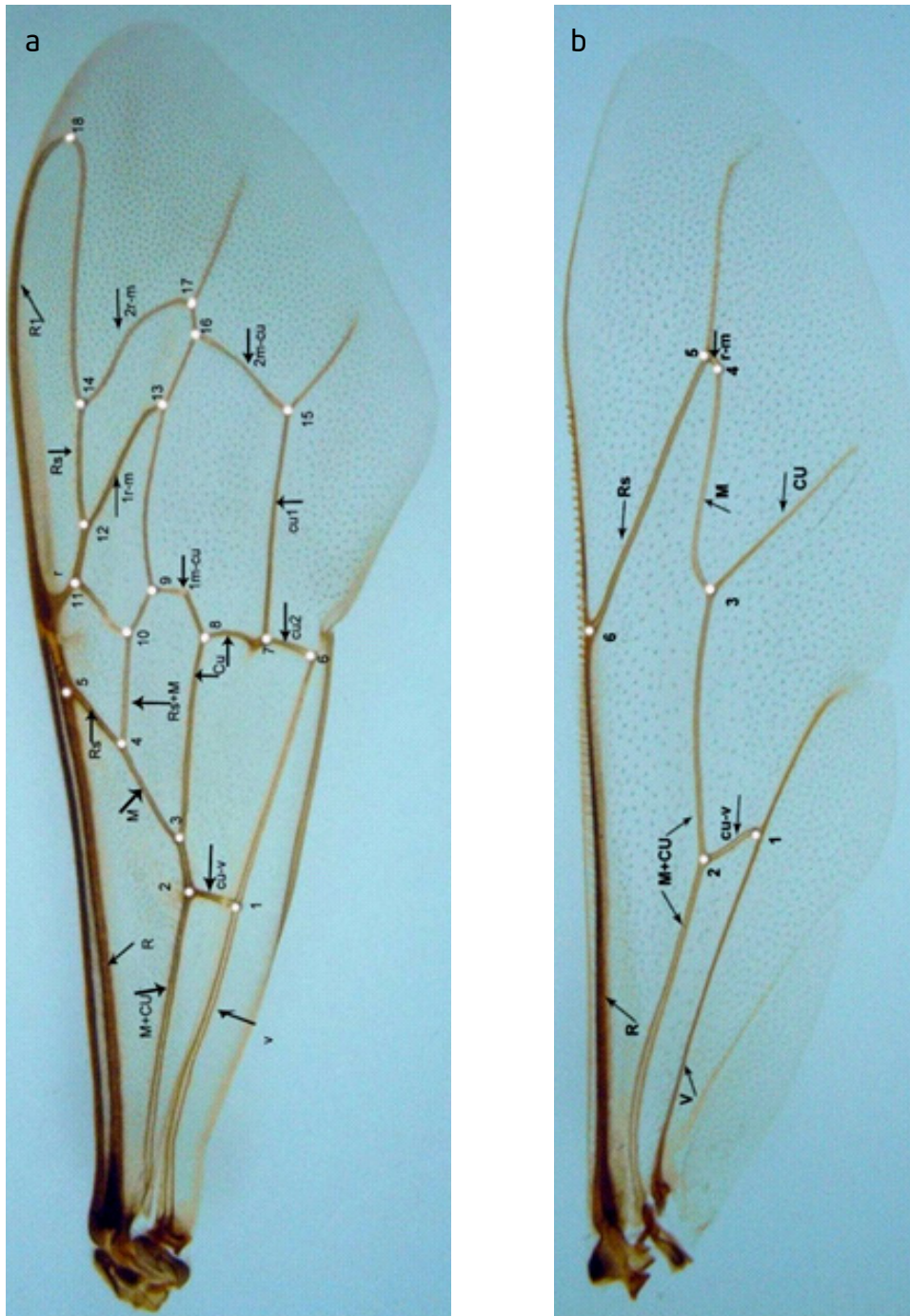


Fig. 1. Eighteen landmarks and six landmarks on vein junctions of fore wing (a) and hind wing (b) of *Apis mellifera*.

Allometry testing on fore wings and hind wings was performed. The results identified a significant difference between the size (centroid size) of the front wings and 32 shape variables (Wilks' lambda = 0.705, $p = 0.0001$); therefore, the shape variations of the fore wings were not uniform. Then, with the increasing of size of the front wings, wing shape changed. In addition,

there was allometry in hind wings between centroid sizes and six shape variables (Wilks' lambda = 5.89, $p = 0.0001$).

Results of MANOVA of fore wings (with 32 shape variables) showed significant differences between populations of the nine areas ($F = 4.97$, $p = 0.000$). Moreover, multivariate analysis on hind wings (with eight shape

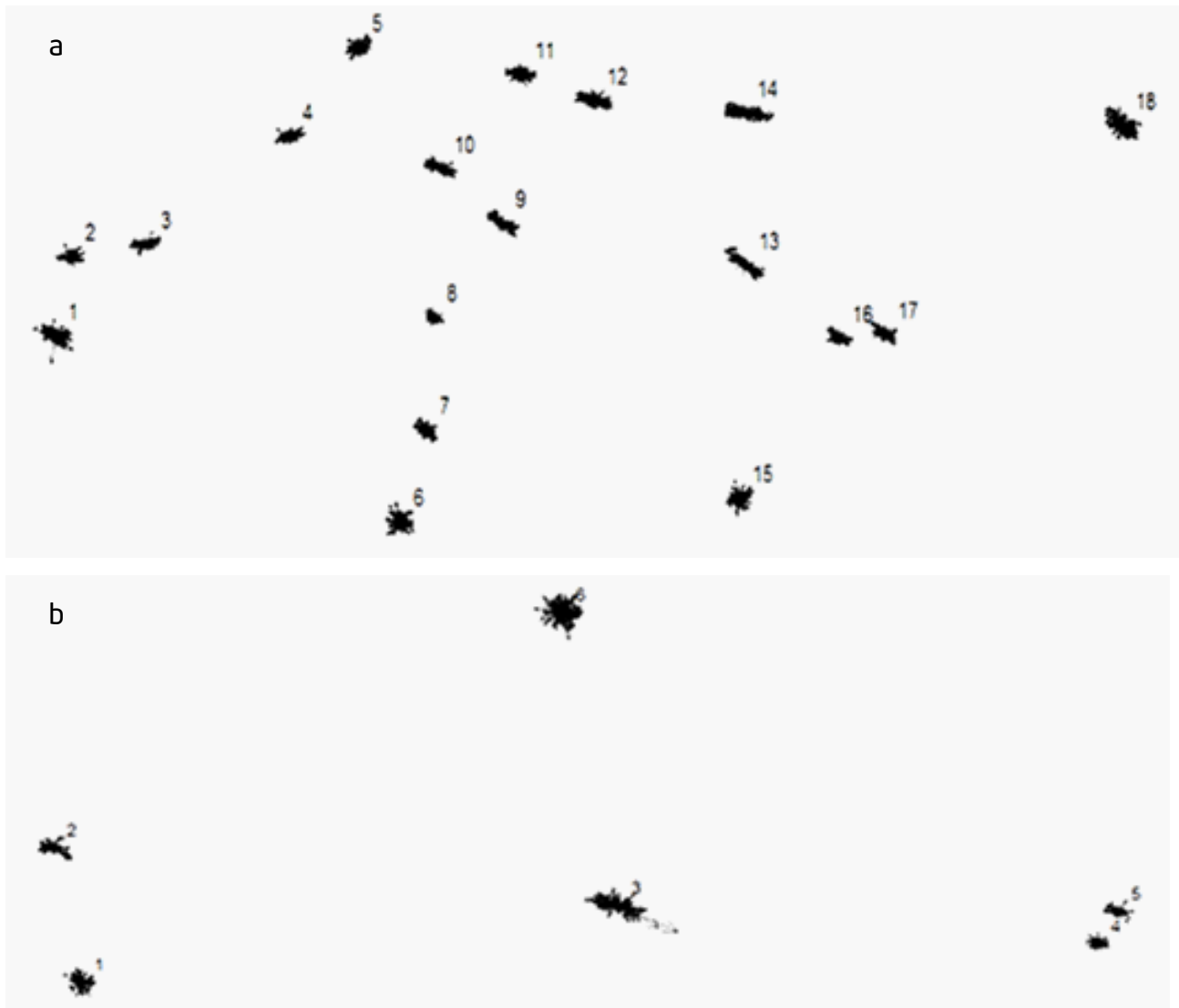


Fig. 2. Average of schematic variations of 18 landmarks on the fore wing (a) and 6 landmarks on hind wings (b) of *Apis mellifera*.

Table 2.

MANOVA for shape variables of fore and hind wings of *Apis mellifera* in different geographical populations of Iran

Worker wing	Wilks' lambda	F	df1	df2	p
Fore wing	0.041	4.97	256	2506.1	0.000
Hind wing	0.281	3.64	64	946.7	0.000

variables) identified significant differences among honeybee populations of the nine areas ($F = 3.64$, $p = 0.000$) (Tab. 2).

Centroid sizes of fore wings and hind wings were compared in different geographical areas of Iran. Results showed a significant difference in centroid size of fore wings ($F = 10.6$, $p = 0.000$). Front wings of honeybee populations in Zanjan (Nikpey area) had maximum centroid size ($C = 600.6$), but the populations of the Khodabandeh area did not differ. Additionally, the front wings of the Mahneshan population had the minimum centroid size ($C = 582.9$) (Tab. 3). Analysis of hind wing centroid sizes revealed significant differences among the nine areas ($F = 3.70$, $p = 0.001$). The honeybee population of Tarom (Emam kandi) had the maximum centroid size ($C = 231.02$) but did not differ significantly from the populations of Abhar, Zanjan (Hajarash), Khoramdareh, Ilrood,

Khodabandeh, and Zanjan (Nikpey). The hind wings of the honeybee population of Tarom (Dastjerdeh) had the minimum centroid size ($C = 223.8$).

Populations of nine zones based on shape variables of fore and hind wings were classified by DFA. Cross-validation tests based on discriminant functions of front wings correctly classified 68.2% of the colonies (Tab. 4). Statistical analysis results for 32 shape variables of the front wings by DFA showed that the honeybee population of Khodabandeh was almost separated (90%) from other areas. Cross-validation tests based on discriminant functions of hind wings correctly classified 43% of the colonies (Tab. 5). Statistical analysis results for eight shape variables of the hind wings by DFA showed that the honeybee population of Tarom was almost separated (65%) from other areas (Fig. 3).

Table 3.

Comparison of centroid size means in fore wings of *Apis mellifera*
in different geographical areas of Iran

Population	N*	K	T	E	A	D	B	C	M
Fore wing	600.6 ^a	598.4 ^{ab}	594 ^{bc}	592.8 ^c	592 ^c	591.2 ^c	590.8 ^c	585.8 ^d	582.9 ^d

*Different letters indicate collection sites; see Table 1.

Table 4.

Summary of the colony assignments of fore wing
with respect to regions based on geometric morphometrics

Area	A**	B	C	D	E	K	M	N	T
A	26* (65%)	-	-	-	-	-	-	-	-
B	-	28 (70%)	-	-	-	-	-	-	-
C	-	-	24 (60%)	-	-	-	-	-	-
D	-	-	-	28 (70%)	-	-	-	-	-
E	-	-	-	-	29 (72%)	-	-	-	-
K	-	-	-	-	-	36 (90%)	-	-	-
M	-	-	-	-	-	-	29 (72%)	-	-
N	-	-	-	-	-	-	-	20 (50%)	-
T	-	-	-	-	-	-	-	-	26 (65%)

Percent classifications are in parentheses; * - number of specimens;

**different letters indicate collection sites; see Table 1.

Table 5.

Summary of the colony assignments of hind wings with respect to regions based on geometric morphometrics									
Area	a**	b	c	d	e	k	m	n	t
a	8* (40%)	-	-	-	-	-	-	-	-
b	-	3 (15%)	-	-	-	-	-	-	-
c	-	-	9 (45%)	-	-	-	-	-	-
d	-	-	-	8 (40%)	-	-	-	-	-
e	-	-	-	-	11 (55%)	-	-	-	-
k	-	-	-	-	-	8 (40%)	-	-	-
m	-	-	-	-	-	-	9 (45%)	-	-
n	-	-	-	-	-	-	-	8 (42%)	-
t	-	-	-	-	-	-	-	-	13 (65%)

Percent classifications are in parentheses; * - number of specimens;

**different letters indicate collection sites; see Table 1.

Relations of geographical populations were evaluated using the UPGMA (unweighted pair group method with arithmetic means) method. The cladogram resulting from the UPGMA cluster analysis of the front wing showed that the two populations of Zanjan (Hajarash) and Zanjan (Nikpey) were similar. Cluster analyses divided populations into three main groups. The first group included the populations of Abhar, Zanjan (Hajarash), Zanjan (Nikpey), and Ijrood. The second group included two populations of Tarom (Dastjerdeh and Emamkandi), and a third group contained Khodabandeh (Fig. 4). Cluster analyses of the hind wing revealed relatively different results, with the Tarom populations (Dastjerdeh and Emamkandi) separated from other populations. Additionally, population of Khodabandeh was classified with Abhar and Ijrood populations (Fig. 5).

DISCUSSION

Morphometric identification techniques, which have improved considerably thanks to new com-

putational techniques, have also improved in practicality because they require little technical knowledge or specialized equipment (Francoy et al., 2008). Standard morphometrics has long been applied to discriminate honeybee subspecies, but such studies take considerable time to complete (Francoy et al., 2008). Geometric morphometric methods are more practical and easier and accomplished in a much shorter time because all procedures are based on computer-assisted technology (Zelditch et al., 2004).

Three factors likely drive wing shape: (1) environmental pressures such as latitude (Alpatov, 1929), altitude (Verma et al., 1994; Hepburn et al., 2000), and climate (Hepburn et al., 2001; Radloff et al., 2005a,b; Tan et al., 2008); (2) sexual selection (Radloff et al., 2003); and (3) abiotic factors such as temperature (Soose, 1954) and season (Mattu and Verma, 1984). The current work showed that populations with small geographical distances have more morphological similarities. Oyerinde et al. (2012) confirmed that a number of factors can

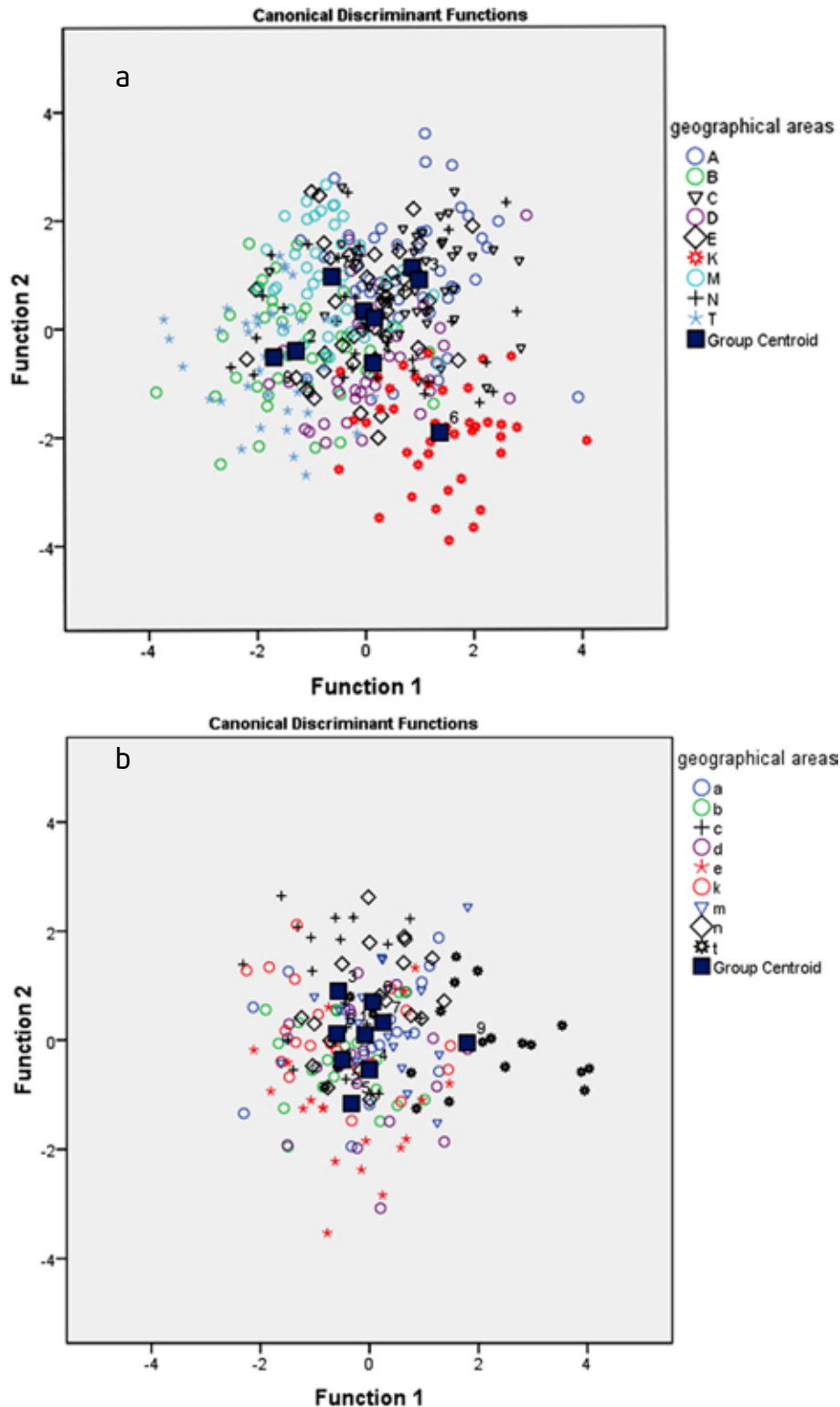


Fig. 3. Scatter plot of discriminant analysis in fore wings (a) and hind wings (b) of *Apis mellifera* in different geographical areas.

affect honeybee taxonomy, especially agro-ecological zone factors. Amssalu et al. (2004) evaluated Ethiopian honeybees at five locations (northeast, west, east, southeast, and central Ethiopia). Results showed that *A. mellifera woyigambell* and *A. m. monticola* were sited in the

southeast and the north mountains in dry and semi-humid climates, respectively. Farshineh et al. (2007) compared *A. m. meda* populations of Iran (Orumieh, Tbriz, and Tehran) with populations in different zones of Turkey (Kiseher and Beypazari) and *A. m. carnica* of

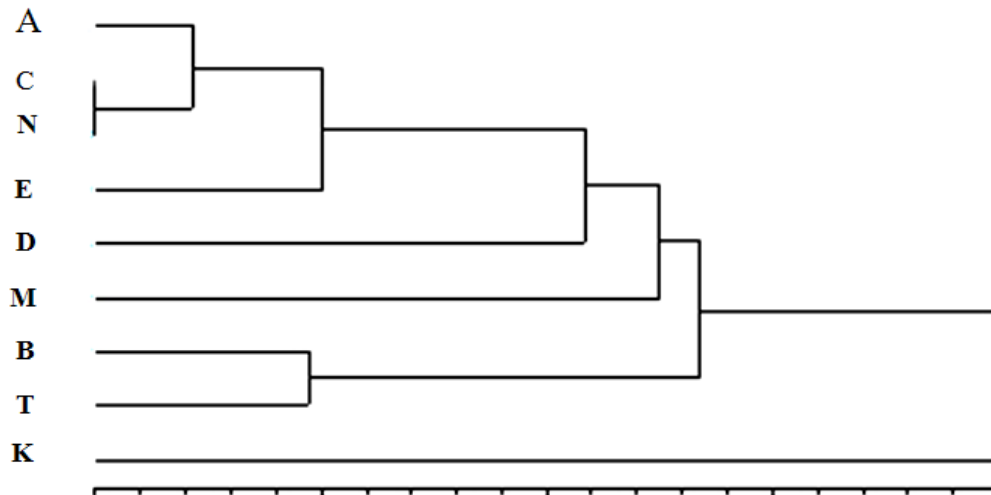


Fig. 4. Dendrogram resulting from a UPGMA cluster analysis of samples from geographical populations using data from the fore wing.

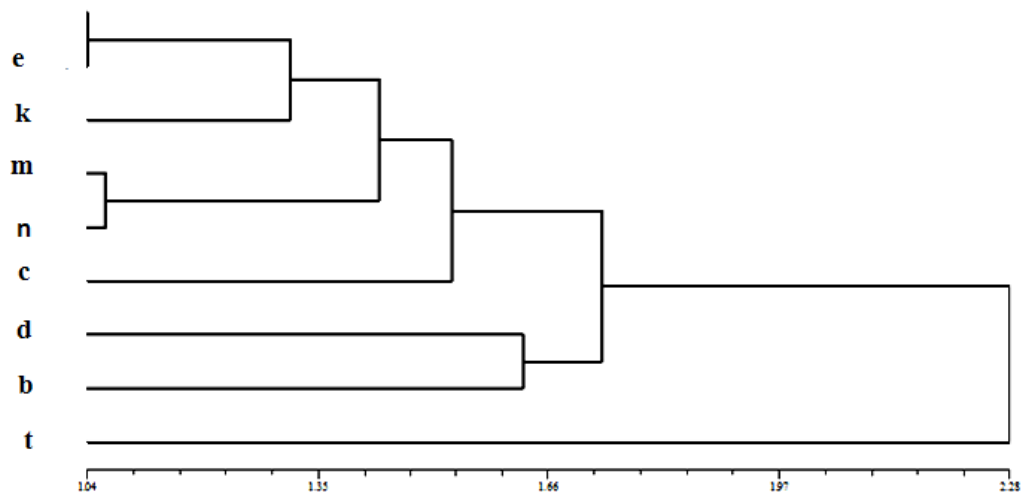


Fig. 5. Dendrogram resulting from a UPGMA cluster analysis of samples from geographical populations using data from the hind wing.

northern Turkey. They found that honeybee populations in Iran were smaller than honeybee populations in Turkey. Moradi and Kandemir (2004) evaluated the morphometric and allozyme variability of *A. m. meda* in the Alburz Mountains in Iran. Also, samples from seven honeybee populations – 5 *Apis m. meda* from Iran and 2 from Turkey, one belonging to *A. m. meda* and one to *A. m. caucasica* – were investigated using morphometric, mtDNA, and microsatellite analyses and no variation was observed in the Dral restriction of COI-COII intergenic region in mitochondrial DNA, yielding a single C lineage mitotype (Kence et al., 2009).

Özkan and Kandemir (2010) evaluated honeybee populations using a geometric morphometric method in western Turkey. They concluded that

these honeybees were different from populations of other zones in Turkey. Other work was conducted on *A. mellifera* in 17 zones of Greece using a geometric morphometric method: 19 landmarks were marked on fore wings of 450 collected samples, and the authors found that geographical distances cause differential characteristics (Hatjina et al., 2004). Tofilski (2008) assessed three species – *A. m. mellifera*, *A. m. carnica*, and *A. m. caucasica* – by traditional morphometric and geometric morphometric methods. Four distances and eleven angles of wings were assessed, and 18 landmarks were marked on vein junctions. This author concluded that geometric morphometrics was 84.9% successful while standard morphometry was 83.8% successful. The results showed that

geometric morphometrics was marginally more reliable than standard morphometry for discrimination of honeybee subspecies. Kandemir et al. (2009) studied the shape diversity of hind and front wings of *Apis florae* by geometric morphometrics in Iran. Statistical analysis showed significant differences between different areas. Discriminant analysis in the current work revealed that the front wing offered better discrimination (68.2%) than the hind wing (43%). Tofilski (2008) has used front wing characteristics for discriminating races and populations of *A. mellifera* by standard morphometrics. In the case of front wings discrimination, geometric morphometrics was 84.9% successful and standard morphometry was 83.8% successful. Özkan and Kandemir (2013) used front wings of *A. mellifera* for discrimination comparison of populations by geometric morphometrics and traditional morphometrics. Front wings discriminated (81.5%) different areas by geometric morphometrics.

CONCLUSIONS

Use of wing morphometric features and landmark variations can serve as an effective tool for grouping *Apis mellifera*, and this technique can be adopted for discerning honeybees. This application is in line with the report of successful use of morphometrics of *A. mellifera* by Andere et al. (2008) and the use of wing landmarks in bumble bees by Aytekin et al. (2007). Finally, front wing measures discriminated area populations better than hind wing measures using a geometric morphometric method.

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