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RESEARCH ARTICLE

STUDYING THE VARIATION OF WING SHAPE AND SIZE FOR IRAQI HONEY BEE WORKER *APIS MELLIFERA* (HYMENOPTERA : APIDAE) COLLECTED FROM BAGHDAD AND DIYALA PROVINCES BY USING GEOMETRIC MORPHOMETRIC OF WING

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ABSTRACT

There are several different methods of classifying Insect. One of these methods that were used in this research is Geometric Morphometric of wing to study the variation shape and size of the right front wing of Iraqi honeybee worker collected from the provinces of Baghdad and Diyala in Iraq. Geometric Morphometric is an essential tool for honey bee races discrimination and characterization. Such vital tool has been applied widely in honey bee researches. The results showed that the mean centriod size of the right front wing is 764.34 and 768.92 for specimens of Baghdad and Diyala provinces respectively and did not show any significant differences in the mean of the centroid size for the right front between the colonies of the two provinces. When reclassification percent using discriminate analysis the results showed that the all specimens of Baghdad and Diyala provinces are completely identical. Furthermore results showed that no significant differences between specimens of Baghdad and Diyala provinces in the size and shape of the right front wing by using Analysis of variance.

INTRODUCTION

The Honeybee, *Apis mellifera,* is social insects noted for providing their nests with large amounts of honey . A colony of honeybees is a highly complex cluster of individuals that functions virtually as a single organism. Honeybee (Hymoneptera: Apidae) is also a social insect known as the most economically valuable insect because of its honey production and pollinating activities (Lawal and Banjo, 2010). *A. mellifera* otherwise known as western honeybee is naturally spread in Europe, Africa and Western Asia (Howpage, 1991; Miguel *et al*., 2011). *A. mellifera* is about 1.2 cm (about 0.5 inch) long, the head and thorax, or midsection, are somewhat bristly and vary in colour according to the strain. Two large compound eyes and three simple eyes, or ocelli, are located on top of the head. Keen eyesight is complemented by two sensitive, odour-detecting antennae (Encyclopedia Britannica, 2012). as regards the environment shows that the species possesses such plasticity and genetic variability that this could give rise to the selection of development cycles suited to different environmental conditions (Mazeed, 2004; Rattanawannee *et al.,* 2010). The shape of organisms and their 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). There is a strong relationship between morphometric structures and climatic data, suggesting that adaptation and natural selection are occurring. Several works with *A. mellifera* involving morphological characteristics showed that there is a strong influence of the environment in the morphology of the same ones (Milne *et al.,* 1986). Various honey bee colonies, races and species were discriminated by employing morphometric analysis(Raina and Kimbu, 2005; Farhoud and Kence, 2005; Shaibi *et al*., 2009; Nedić *et al*., 2011). Many studies have shown that wings of many insect species including that of intraspecific groups or populations and subspecies can be identified based on wing morphology alone(Gaston and O'Neill, 2004; Steinhage *et al*., 2007). For example, the development of geometric morphometric analysis defined as a statistical analysis of shape (Marcus *et al*., 1996) has been employed to clarify the relationships of closely related taxa such as bees (Aytekin *et al*., 2007; Drauschke *et al*., 2007; Mendes *et al*., 2007; Francoy *et al*., 2006, 2008), Diptera (de la Riva *et al.,* 2001; Hoffmann and Shirriffs 2002; Moraes *et al*., 2004) and Hemiptera (Gumiel *et al.,* 2003; Jaramillo *et al*., 2002). The tool has been successful in the taxonomy of honeybees subspecies and temporal variations in populations of Africanized honeybees (Francoy *et al*., 2009). The common method for the characterization and classification of honey bee subspecies is based mainly on measuring honey bee wing characters, which

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were considered as strong tool (Rattanawannee *et al.,* 2010). Standard morphometric was used in honey bee studies by measuring different wing angles, indices and distances (Ruttner, 1988) while geometric morphometric was used in honey bee studies by measuring the coordinates of fore wing points to calculate the centroid size (Tofilski, 2008). Not many studies were done on geometric morphometric of honey bees. Concerning hind wings, only hind wing distances and number of hooks were used widely during morphometric studies (Abou- Shaara *et al*., 2012) but no hind wing angles were incorporated in the morphometric analysis previously Wing characters were found to be affected by different factors e.g. temperature (Tan *et al*., 2005), season (Mattu and Verma, 1984) and bee age (Herbert *et al*., 1988). The right fore and hind wings were used by some authors during their morphometric analysis (Andere *et al.,* 2008; Uzunov *et al.,* 2009; Mladenović *et al.,* 2011 and Abou-Shaara *et al.,* 2012) while left wings were used by others (Bouga and Hatjina, 2005 and Tofilski, 2008). Fluctuating asymmetry and directional asymmetry were studied by some authors (Smith *et al.,* 1997 and Schneider *et al.,* 2003). Smith *et al.* (1997) found that honey bee drones were with higher directional asymmetry than females while Schneider *et al.* (2003) found higher fluctuation asymmetry of shape for European and hybrid workers than for African workers. This research aims to determine the effect of environmental conditions on the variation of the shape and size of the wing of a worker honeybee Iraqi collected from two different regions in Iraq by using Geometric Morphometric of Wing.

MATERIALS AND METHODS

Research Site and Collection of Specimens

This research was performed in the Zoology laboratory, Department of Biology, Faculty of Education for pure Science, University of Diyala, Diyala. Iraq. Random Specimens of 30 honeybee workers were collected from 2 colonies situated in the Baghdad and Diyala provinces in Iraq 15 Specimen from each colony for each province, Where the coordinates of the Baghdad province, is 33˚ 15 N, 44˚ 29 E and the annual average temperature is 32.99c◦ and the rate of annual rainfall is 160.5Mm , While the coordinates of Diyala province, is 33˚ 35 N , 44˚ 35 E and the annual average temperature is 33.63c◦ and the rate of annual rainfall is 190.8Mm . Specimens of the honeybee collected were stored separately in 70% ethanol in a small labeled container according to their province of collection.

Specimens processing and data collecting

All right front wings were processed and mounted on glass slides with Hoyer mounting solution. The right front wing images were captured using a digital camera on a stereomicroscope (40X). The study used 16 landmarks (Fig. 1) following type I (venation intersections) design Bookstein (1991). The coordinates of landmarks were digitized by the COO module of software (see "Software"). The connections between 16 landmarks provided polygons used for further analysis, including comparison of wing size and shape. The coordinates of landmarks were superimposed (translation, scaling, rotation) using the MOG module (see "Software") which computed procrustes superimposition, centroid sizes, and partial warps (as shape variables).

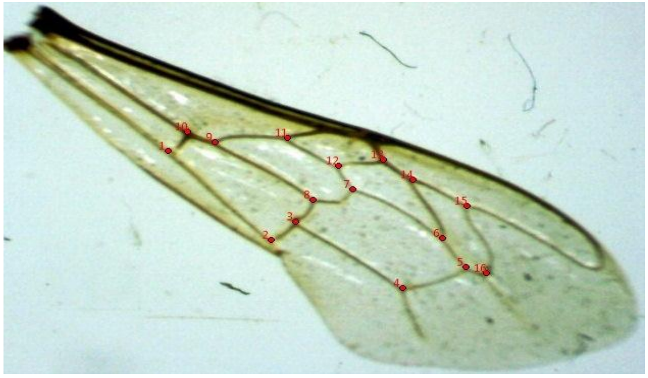


Fig. 1. Sixteen landmarks on the *A. mellifera* right front wing used in wing geometric analysis

Size

Centroid size, an isometric estimator of wing size, was calculated from the square root of the sum of the squared distances between the centre of the polygon and each landmark. Then all centroid size values and their variances were compared by non-parametric analysis based on permutations (1000 runs).

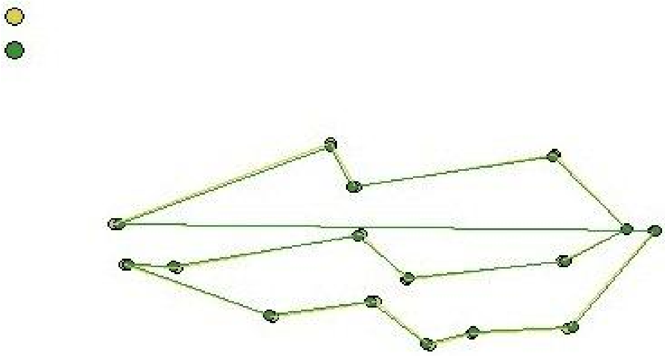
Shape

The shape variables of the wings were computed with discriminate analysis (Caro-Ria˜no, *et al*., 2009).

Software

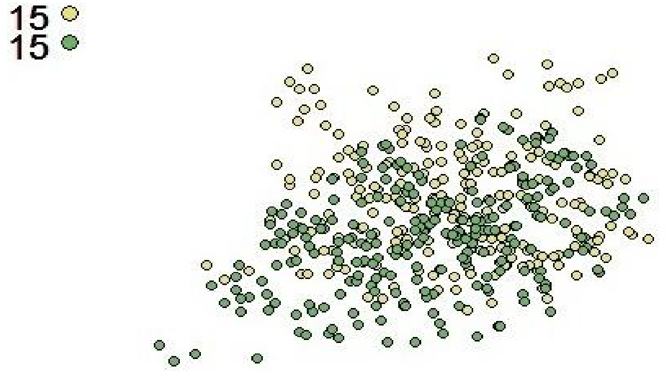
Collecting landmarks made use of the COO module. Centroid size and partial warp scores were obtained from the MOG module. All discriminate analyses were PAD module . Analysis of asymmetry in the shape and size of the wing were performed by the ASI module (software components are available at http://www. mpl.ird.fr/morphometrics) .

RESULTS



X = of – 0.405 to 0.364, Y = of – 0.134 to 0.171

Fig. 2. Mean coordinates of landmark in the right front wing of Iraqi honeybee worker, Yellow color represents Baghdad province specimens and Green color represents Diyala province specimens



X = of – 553.094 to 410.906 , Y = of - 522.094 to 146.6

Fig. 3. Output of consensus configuration by the GPA Procrustes superimposition method locating the 16 landmarks for each individual wing of the two colonies. Yellow color represents Baghdad province specimens and Green color represents Diyala province specimens

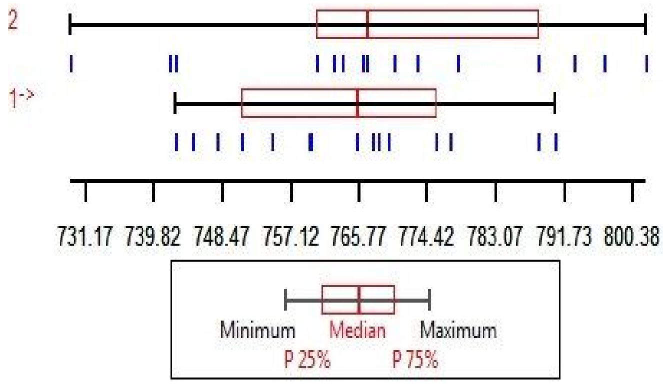


Fig. 4. Variation of the centroid size of wings for Iraqi honeybee worker *A.mellifera* according to localities Baghdad and Diyala, Each box shows the group median separating the 25 th and 75 th the quartiles. Vertical bars under the boxes represent the wings numbers 1 and 2 in the Fig. represent Baghdad and Diyala provinces respectively. Units are pixels. P, percentile

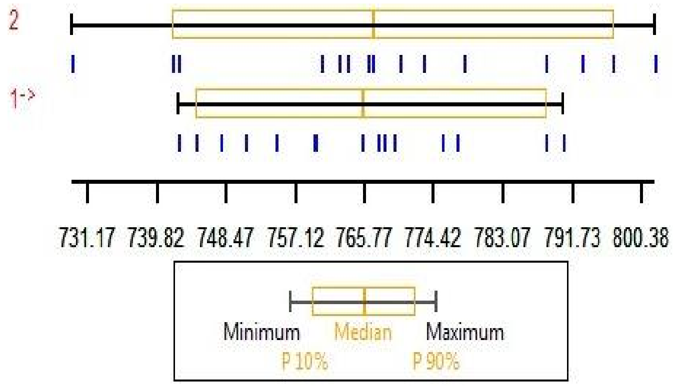
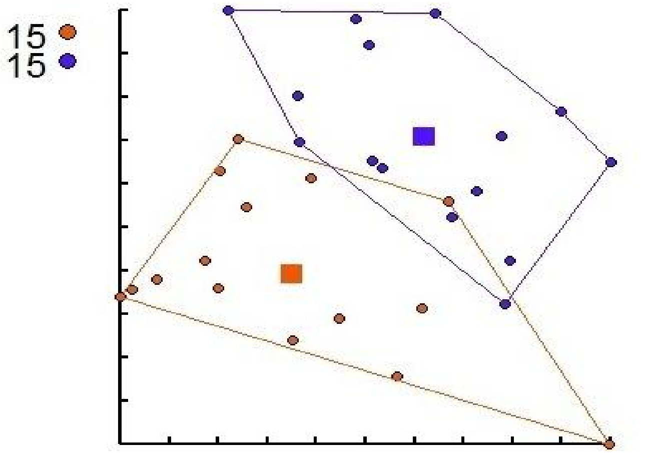


Fig. 5. Variation of the centroid size of wings for Iraqi honeybee worker *A. mellifera* according to localities Baghdad and Diyala , Each box shows the group median separating the 10 th and 90 th the quartiles. Vertical bars under the boxes represent the wings numbers 1 and 2 in the Fig. represent Baghdad and Diyala provinces respectively. Units are pixels. P, percentile



X = of – 0.022 to 0.023 , Y = of – 0.025 to 0.020

Fig. 6. Scatter plot of the principle component analysis of Iraqi honey bee worker *A.mellifera* colonies according to localities Baghdad and Diyala provinces based on Geometric Morphometric, Brown spots represent Baghdad province specimens and Blue spots represents Diyala province specimens, Brown square represent mean centroid size of the right front wing for the Baghdad province specimens = 764.34 and Blue square represent mean centroid size of the right front wing for the Diyala province specimens = 768.92

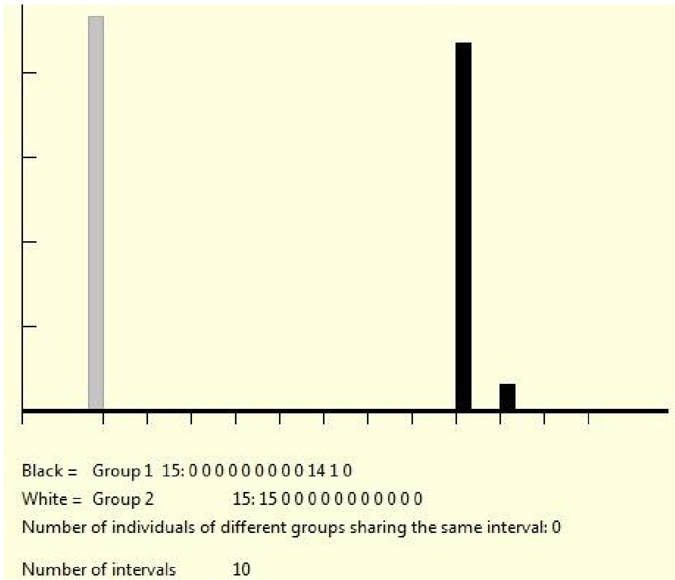


Fig. 7. Discriminate analysis of two colony Iraqi honeybee worker *A. mellifera*. The Mahalanobis distances between centroids size were as follows: Baghdad province specimens to Diyala province specimens = 37.66, Black color in this figure represented to Baghdad province specimens and White color represented to Diyala province specimens.

Table 1. Compared the centroid size of the right front for Iraqi honeybee worker *A.mellifera* between specimens of Baghdad and Diyala provinces

Provinces	M.CS	St.D	Va.	F	P	T	P	A.D
Baghdad	764.34	14.06	197.77	1.99	0.21	0.73	0.47	4.59
Diyala	768.92	19.84	393.57					

M.CS: Mean centroid size, St.D: Standard Deviation, Va.: Variance, P :Probability, A.D : Absolute differences

Table 2. Analysis of variance for asymmetry right front wing size of Iraqi honeybee worker *A.mellifera* specimens of Baghdad and Diyala provinces

Source	SS	df	MS	F	Signification
Modal	422.29	3	140.77	0.42	0.74
Individual	403.92	1	403.92	1.21	0.28
Side	5.65	1	5.65	0.02	0.89
Side•i	12.72	1	12.72	0.04	0.85
Residu	7993.38	24	333.06		

Table 3. Analysis of variance for asymmetry right front wing shape of Iraqi honeybee worker *A. mellifera* specimens of Baghdad and Diyala provinces

Source	SS	df	MS	F	Signification
Modal	0.0013	84	0.000015	0.76	0.95
Individual	0.0005	28	0.000017	0.84	0.70
Side	0.0004	28	0.000013	0.62	0.94
Side•i	0.0005	28	0.000016	0.80	0.76
Residu	0.0137	672	0.000020		

DISCUSSION

Insect wings are the most appropriate structures for geometric morphometric studies (Dvorak *et al.*, 2006). The result of this study showed that the worker honeybees from Baghdad and Diyala provinces were more similar, As is evident in Fig. 2, which shows the coordinates of each match landmarks placed on the right front wing for all specimens of Baghdad and Diyala provinces. In addition, the results also showed that overlapping specimens between Baghdad and Diyala provinces, as is evident in the Fig.3 when using principle component analysis PCA of the 16 wing landmarks. The mean centriod size of the right front wing is 764.34, 768.92 for specimens Baghdad and Diyala provinces, respectively. The results of the statistical analysis using test F and T tests no significant difference in the centroid size of wing right front between specimens Baghdad and Diyala provinces as shown in Table 1. And also there is no variation in the centriod size of the right front wings for Iraqi honeybee worker *A. mellifera* according to localities Baghdad and Diyala when the the median group separating the 25 th and 75 th the quartiles and 10 th and 90 th the quartiles, As shown in the Fig. 4, 5 respectively.

When using the Principal Component Analysis showing clustering between specimens Baghdad and Diyala provinces as shown in the Fig. 6. Furthermore, the results of discriminate analysis showed that no significant differences between specimens Baghdad and Diyala provinces and the Mahalanobis distances between centroids size of the right front wings for specimens Baghdad and Diyala provinces is 37.66 when reclassification percent using discriminate analysis the results showed that the all specimens of Baghdad and Diyala provinces Are completely identical as shown in the Fig. 7. Also used in this study, analysis of variance to determine the asymmetry wings size and shape, results showed that no significant differences between specimens Baghdad and Diyala provinces as shown in the Table 2, 3 respectively. The lack of variation in the shape and size of the right front wing of the Iraqi honeybee worker between two colonies insects from Baghdad and Diyala province, may be due to several reasons, Including the convergence of the environmental conditions of symmetry in terms of annual temperature and rainfall annually in both Baghdad and Diyala province, In addition, near the Baghdad province of Diyala province, where the distance between them is about 70 km , As well as the occurrence of Baghdad, Diyala province, on the same latitude and longitude . According to Gaston (2000) several mechanisms are said to be determinant of biological diversity under the influence of environmental variables correlated with latitude. Within the Order Hymenoptera, environmental variation clearly is the most important determinant of phenotypic variation (Castanheira, 2005 and Owen, 2009). In recent years, human interventions are also playing key roles in the distribution and maintenance of species (Zeder *et al*., 2006 and Venturieri, 2009). The results of this study are consistent with the results of (Combey *et al*., 2013) this study showed that the different environmental conditions have an impact on the phenotypic for African bees. In another study carried out by (Nunes, *et al*., 2012) that differing site presence honeybees in terms of latitude and longitude have a clear impact on the shape and size of the wing and body and a basket of pollen grain.

Conclusion

In this study the results showed that the lack of variation in the shape and size right front wing of the Iraqi honeybee worker collected from the provinces of Baghdad, Diyala, using the Geometric Morphometric of wing, and this shows that it belonged to one species because of the convergence of the environmental conditions of asymmetry in both provinces, and Geometric Morphometric is a powerful tool for the classification of insects and The discovery of variation between species of different environmental conditions, And also is an easy way to classify the species belonging to the one genus. And also can by Geometric Morphometric discovery degree of convergence among the many genus belonging to the same family, order and draw a family tree. This method is inexpensive material compared with molecular methods.

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