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A comparative quantitative genetic study between two populations of a fly *Calliphora vicina* (R-D,1830; Diptera: Calliphoridae) in Iraq

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ABSTRACT

The quantitative genetic relationship between two populations of the fly, *Calliphora vicina* was studied, specimens of which were collected from some areas of Waist and Southern Iraq. In this study, the geometric morphometric system for wing shape and size was used to detect quantitative genetic variations in the shape, size and area of the right wing of the fly specimens. Central size of the right wing was 1412.4-1423.7 microns for specimens from the Waist and southern regions of Iraq, respectively. The results of the statistical analysis using the F and T tests showed that there were no significant differences in right wing central size between the specimens of the study areas, except for one significant difference found in one of the insect specimens of the southern region (the specimens of the Waist region). It was also noted that there were very few significant differences in the shape and size of the right wing of some insect specimens for that area due to their impact on the different environmental conditions in the areas from which the specimens were collected.

Keywords: *Calliphora vicina*, Geometric morphometric, Landmarks, Calliphoridae.
Article type: Research Article.

INTRODUCTION

The *Calliphora vicina* fly is one of the most important forensic fly species (Sanei- Dehkordi *et al.* 2014). It is the first insect colonizing human and animal corpses (Byrd & Castner 2009). As it is an important decomposition factor that facilitates the fast decomposition of any corpse, it has been used in many studies of forensic entomology and toxicology in India in order to know the post-mortem period (Amendt 2010). The dispersal and development of this species of insect is related to the availability of a suitable environment and a good food, thus the fast growth and evolution of the larvae. The presence of a species of toxins was observed in the decomposing tissues, as it appeared to be of great importance in the complete growth of larvae in those tissues (Clark *et al.* 2006; Salehi *et al.* 2021; Almuhsin Ahmed *et al.* 2022; Abdul Hussein *et al.* 2023). One of the scientific studies that is being conducted to study the quantitative genetic variations in the shape and size of the wing of any insect is the use of the geometric morphometric system for the apparent form of success, since it shows the extent of the differences that will occur between individuals of the same species or different species. It is a new method for specialized studies in the field of quantitative genetics. The main advantage of this system is the use of data on the shape and size of the wing through landmarks that are installed on certain places in the insect's wing. By means of these data, the variation in the shape and size of the wing of the different species is analysed (Brereton 2015). The work of the geometric morphometric of wing based on placing certain landmarks on the intersections of the veins of the insect wings and taking coordinated digital images of them starting from the base of the wing and extending along the intersections of the veins with each other and the intersections of the veins with the edge of the wing (Rohlf 2013a). Through this landmarks, a comparison is made between all species belonging to the same order to

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find the central size of the wing and thus knowing the discrepancy between them (Rohlf 2013b). The wing shapes of adults *C. vicina* were tested for the purpose of quantitative genetic comparison between individuals of the above species, which were collected from two geographical areas in Diyala and Maysan. The aim of the study was to identify the most important genetic variations in quantitative terms resulting from the effect of feed and environment on the individuals of this species of insects by comparing the wings of the specimens and thus benefiting from them in the field of forensic investigations and forensic medicine in Iraq.

MATERIALS AND METHODS

Specimens processing

In this study, 15 right wings of adult insects collected from the two study areas: medial, i.e., Diyala and south, i.e., Maysan were used for the purpose of studying the quantitative genetic variations in the shape and size of the insect wing in these areas and distinguishing between the populations of them in the two regions, where following the Karagoli method (2013) in preparing the glass slides for the wings. Fifteen adult specimens were isolated for each area in transparent plastic cups with tightly covers and left without feeding in order to perdition and dry perfect. Afterward, the right wing of each insect was removed by microdissection forceps and very carefully in order to preserve the wing from breaking. Then we placed the wing between two glass slides and tied the two edges of the slides firmly with G2100 paper tape. The information for each model was recorded, which included: the specimen number, the collection area to which the model belongs, at the edge of the slide. After completing the preparation of the glass slides, they were photographed using a digital microscope connected to a laptop computer provider with a 1.3-megapixel digital camera provider with UV rays.

Data collection

After completing the imaging of the wings, the data for each image were collected separately using the ready-made program. Collecting landmarks for identification and characterization, for the geometrical analyses of the data of the wings was performed from the landmarks, which are anatomical points placed on the intersections of the longitudinal veins with the transverse veins and the ends of the longitudinal veins (as shown in Fig. 1). To identify between populations of the same genus and species (Bookstein 1991), 15 landmark were used on the intersections of the longitudinal veins with the transverse veins, as numbered points were placed between these intersections through the COO unit within the program, which is a unit for setting coordinate landmarks. The connections between the features installed on each wing gave us polygon shapes that were used in many analyses. It included a comparison between the shape and size of the wing for each specimen in the study for the purpose of revealing the variance between the different population communities of the specimens, as well as to clarify the variance also within the same population group. Models were compared with each other in order to compare the populations of the fly and to know the extent of similarity and contrast between them. Then the data was transferred to the MOG unit, through which the translation, scaling and rotation operations were carried out. After these three operations, we got the central size of each wing, the partial warp, the relative warp, as well as the shape variances for each wing. In this study, the central size of the right wing was depended on the populations of the two study areas.

Software

The landmarks were fixed to the wing and all obtained from the COO unit. These data included central size, partial warp as well as relative warp. The other data were analysis data for the basic compounds, Principle Component Analysis, which was obtained from the MOG unit. In the case of the data for discriminant analysis, it was obtained from the PAD unit, as well as the central volume variance data obtained from the COV unit and the wing shape symmetry data obtained from the ASI unit. In analysing the data of this study, Principle Analysis was used to reveal the variance between populations of the fly, as well as using Discriminate Analysis to detect the variance between members of one population group for this insect and these two analyses were present in the ready program.

RESULTS AND DISCUSSION

The quantitative genetic variations of wing shape and size in *C. vicina* fly were studied for specimens taken from some areas of Waist and Southern Iraq using the geometrical morphometric of wing shape and size. It was carried out by fixation the coordinates of the landmarks between the intersections of the longitudinal veins with the transverse veins of the insect wing and by which the central size of each wing was calculated. Thus, it was concluded that these specimens of this species belong to one origin despite the phenotypic differences between them and for the purpose of benefiting from them in forensic medicine and investigations into the causes of death. Fig. 2 shows the right wing of the whole fly and its group specimens from middle Iraq (Diyala), on which the coordinates of the landmarks are fixed between the intersections of the longitudinal veins with the transverse veins and the ends of the longitudinal veins. Fig. 3 represents the right wing of the specimens collected from Southern Iraq (Maysan) as well as the coordinates of the landmarks between the intersections of the longitudinal veins with the transverse veins and the ends of the longitudinal veins.

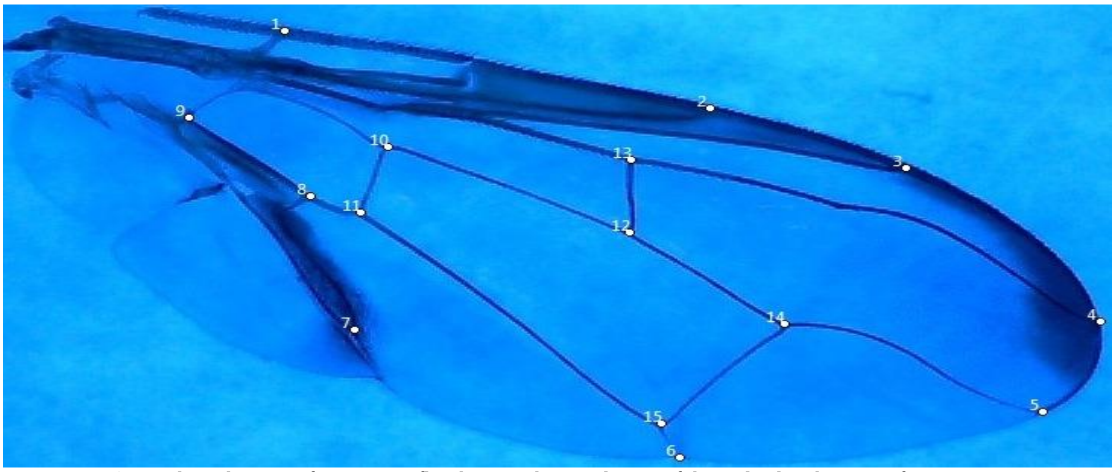


Fig. 1. The right wing of a *C. vicina* fly, showing the coordinates of the 15 landmarks, magnification 45X.

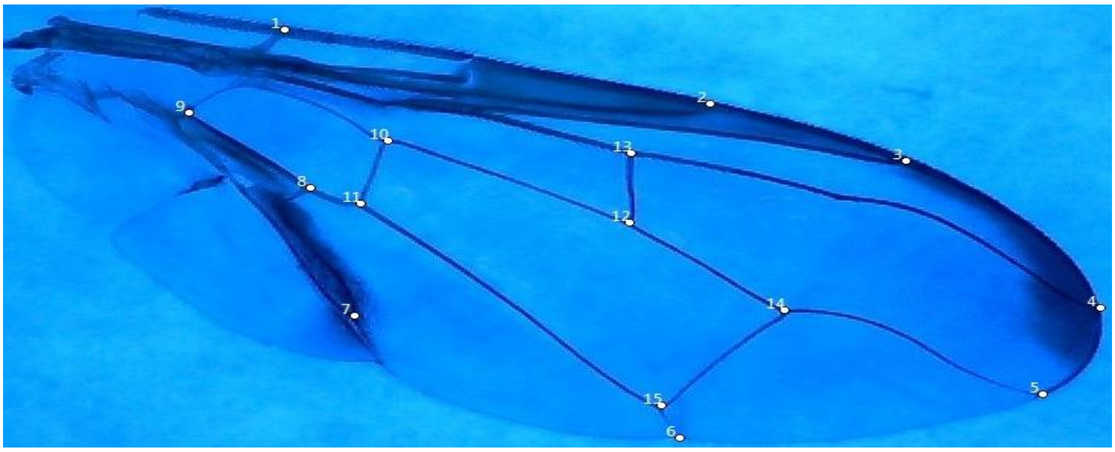


Fig. 2. The right wing of a *C. vicina* fly from a population in medial Iraq, showing the coordinates of the 15 landmarks, magnification 45X.

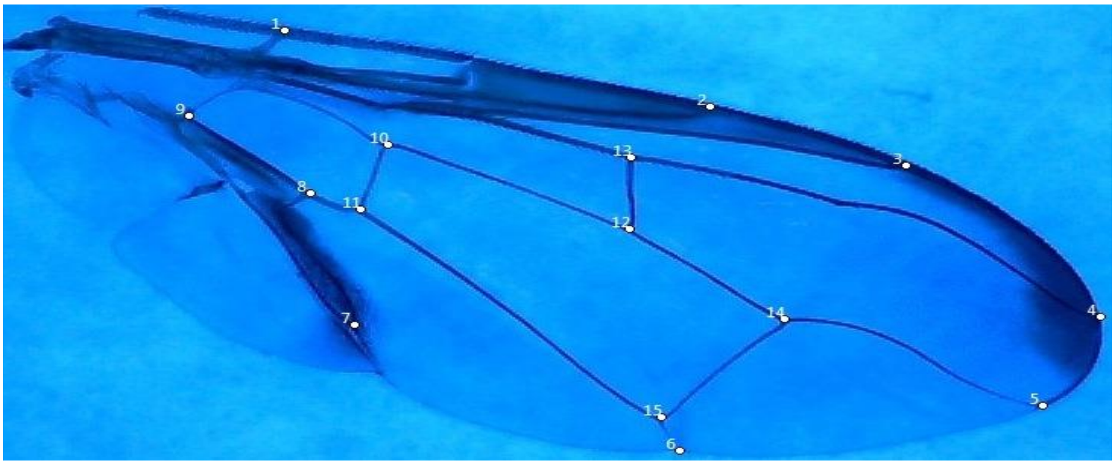
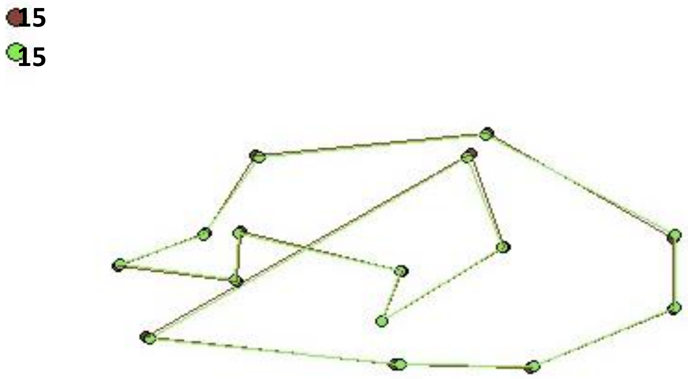


Fig. 3. The right wing of a *C. vicina* fly from a population in southern Iraq, showing the coordinates of the 15 landmarks, magnification 45X.

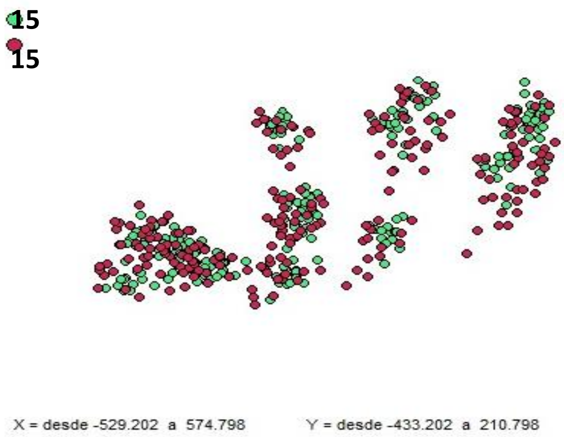
By using the geometric morphometric, the quantitative genetic heterogeneity was detected between the two populations of the studied areas, according to their locations, depending on the shape and size of the wing. The comparison was made between specimens by Principal Component analysis and Discriminant analysis, depending on the central size of the wing.



X = -0.325 to 0.402, Y = -0.129 to 0.189

Fig. 4. The rate coordinates of the landmarks for the right wing of the population of *C. vicina* fly, which are 15 landmarks for each wing, the green and red colours represent the population communities of middle and southern Iraq, respectively.

Fig. 4. shows the coordinates of the landmarks of the 15 right wings of the corpses fly. The green colour represents the rate coordinates of the landmarks for 15 right-wing flies from middle Iraq (Diyala). In the case of red colour, it represents the coordinates of the landmarks for 15 male right wings collected from Southern Iraq (Maysan). When comparing them using the geometrical morphometric for the shape and size of the wing by means of the MOG unit, which is included in the contents of the ready-made program available on websitehttp://www.mpl.ird.morphometrics/, it was found that the wings of flies in the two regions are largely identical in most of the indicators. However, Landmarks 1, 7, 8, 13 and 14 did not match up perfectly for all the wings. The variation in the congruence of the features fixed on the wings of insects for the Waist and southern regions may be due to several reasons, including the amount of food and the difference in environmental conditions in the areas from which the study specimens were collected. The annual temperatures during 2020 and 2022 in the regions of middle (Diyala) and south (Maysan) were 20.02 ºC and 22.05 ºC, respectively, while the annual rainfall for the two years in the two regions were 30.112 cm and 23.048 cm respectively. In the case of the relative humidity in both areas during the two years it was 36.22% and 39.34%, respectively. The results of this study are very much in agreement with the results of the preliminary study by Hall *et al.* (2014) on the use of the geometric morphometric of wing shape and size to determine populations of Old World screwworm fly. They compared the right wings of 22 insects pendent by small pins on a board in the British National Museum in London and 22 right wings belonging to the same species of insect. These latter specimens were collected in the form of mature or fully-grown larvae from the wounds of animals infected with this insect in some regions of the African continent to be bred in the laboratory. and then compared when they reached the role of the adults with the museum specimens. In that study, they identified phenotypic characteristics that were previously described by Hall (2008) identified in another study of the same species of fly. In the above study, the researchers observed the mismatch of some landmarks installed on the right wing of the museum insect specimens and the laboratory-bred insect specimens whose specimens were collected from some African countries. They mentioned that the complete mismatch between the specimens is mainly caused by the effect of the environmental conditions in the countries from which the insect specimens were collected.



X = from -529.202 to 574.798, Y = from -433.202 to 210,798

Fig. 5. The diffusive figure of the distribution of *C. vicina* specimens along the first and second Principal Component analysis (PCAs) for the shadow space coordinates derived from the coordinates of the original features of each wing, which numbered 15 landmarks (green and red represent the population communities of middle and southern Iraq, respectively).

Fig. 5. shows the method of distribution of *C. vicina* specimens using PCA of the coordinates of the landmarks installed on the right wings between the intersections of the longitudinal veins with the transverse veins and the ends of the longitudinal veins are 15 landmarks for each wing. The points in green represent the middle specimens and the points in red the southern specimens. In addition, we noted from the way the specimens are distributed that the specimens close to each other belong to one geographical origin. At the same time there was a variation in the shape and size of the wing in the specimens far from each other. The reason for the variation in the shape and size of the wings of the specimens in the studied areas far from each other may be due to the different environmental conditions in the areas from which the insect specimens were collected, which also affects the size of the insect. Among the universal studies in the field of using the geometric morphometric is the study conducted by Casper *et al.* (2010) using the geometric morphometric system to study the variation in the shape and size of the butterfly wing *Parage aegeria* (Linn.) and the effect of different environmental factors in its development. The data in that study was analysed by relying on ready-made programs that analyse the data of the features and convert them into numbers that are close to the required results by a large percentage. In that study, the researchers concluded that there is a heterogeneity in 4 out of a total of 8 specimens for a number of the studied populations. The reason for the variation in the shape and size of the insect wing is the difference in environmental conditions in the areas from which specimens of that insect were collected.

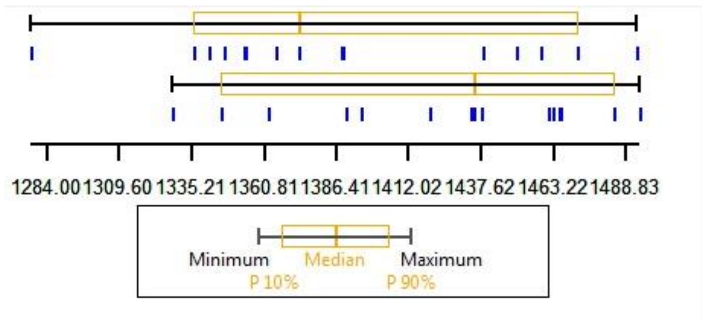


Fig. 6. The central size in the right wings of *C. vicina* fly according to the population it belongs to. Each box in the figure represents the middle group distributed between spring 10% to spring 90%, and the blue lines under each box represent the wings, while the numbers 1 and 2 in the figure represent the population communities of medial and southern Iraq, respectively.

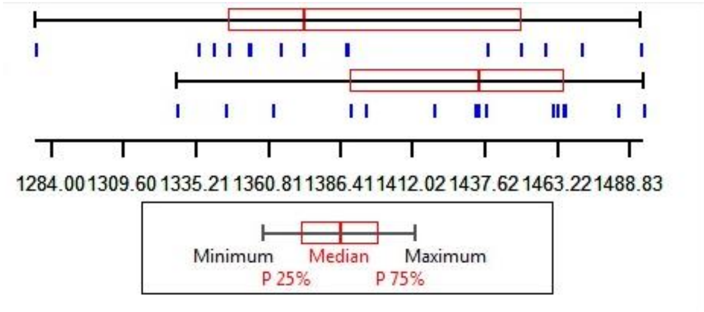


Fig. 7. The central size in the right wings of *C. vicina* fly according to the population it belongs to, as each box in the figure represents the median group distributed between spring 25% and spring 75%, and the blue lines under each box represent the wings, while the numbers 1 and 2 in the figure, they represent the population societies of medial and southern Iraq, respectively.

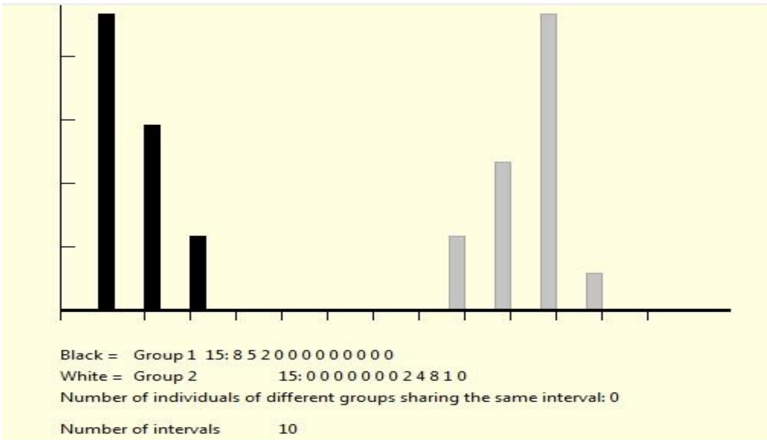
Table 1. Comparison of the central size of the right wing between the populations of *C.vicina* fly from medial and southern Iraq.

Specimens	MCs	SD	Va.	F	P	T	P	AD
C.vicina (m)	1423.7	48.5	2354.2	m-s= 2.3	m-s= 0.54	m-s= 1.8	m-s= 0.09	m-s= 34.5
C.vicina (s)	1412.4	57.4	3294.8					

Note: MCS: Mean centroid size: Mean centroid size of wings; SD: Standard Deviation; Va: Variance; F: the calculated F value; P: Probability; T: the calculated T value; AD: Absolute differences; M: Middle Iraq; S: South Iraq

Fig. 6. shows the results of the analysis of the variance in the central size of the right wing in whole species of *C.vicina* and the group from some regions of middle Iraq (Diyala) and south (Maysan). Each box represents as the middle group distributed between spring 10 and spring 90. The blue lines under the bottom of each box represent the right wings of the specimens included in the study. We note from the figure that there is a slight variation in the central size of the right wing of the fly according to the different regions from which the specimens were collected. Fig. 7 shows the results of the heterogeneity analysis in the central size of the right wing of this fly. However, in this figure the middle group is distributed between spring 25 and spring 75. We also note in this figure that there is a slight variation in the central size of the right wing of the fly according to the different regions from which the specimens of this insect were collected. Central sizes of the right wing, as shown in Table 1 were 1423.7 and 1412.4 microns for the populations in the middle and southern regions, respectively. The values of the variances in the central size of the right wing were 2354.2 and 3294.8 for the populations in the two study areas. In this study, the F and T tests were used to compare the insects of the two regions. When comparing the specimens of the middle region with the specimens of the southern one in the central volume of the right wing, it was found that the value of F = 2.3 and P = 0.54, while the value of T = 1.8 and P = 0.09. In the case of the absolute value of the difference AD, it was 34.5, as there were no significant differences between them. It is clear to us from the above that the specimens of the fly C. *vicina* from the middle and southern regions of Iraq belong to a single geographical origin. There have been many studies in this regard, including the study carried out by Zahiri *et al.* (2006) on the use of geometric morphometric of wing in studying quantitative genetic variation among population groups of rice stem borer mites *Chilo suppressalis* (Walker: Lepidoptera: Crambidae) in northern Iran. They used 15 landmarks placed on the intersections of the longitudinal veins with the transverse veins of the front wings, as well as 10 landmarks also placed on the intersections of the longitudinal veins with the transverse veins of the hind wings. In that study, a multivariate analysis was conducted to determine the quantitative genetic variation in 6 of the population groups of that insect by approaching the data at the level of the coordinates of the features. They used the mathematical geometric morphometric in the field of calculating the variance data in the shape. In addition, they transferred those data from the misleading field in the geometric morphometric to the linear field of all images. The 311 wings were for the front wings and 319 for the hind wings. The analysis showed that there are very significant differences in the shape and size of the wing and central size of the wing between the insect groups in the Guilan and Mazandaran provinces as well as the rest of the northern Iranian provinces from which the specimens were collected. The authors attributed that the existence of these differences is due to the geographical distance between those governorates and the difference in environmental conditions among the six population groups. Francoy *et al.* (2006) also studied quantitative genetic variation among honey bee populations

whose specimens were collected from three geographically separate regions, Italy, the American Carniolan State and South Africa. After comparing the three populations of the insect, they found a difference in the shape and size of the wing and rate the central size of the right wing between them. They mentioned that this is due to the different environmental conditions among the three areas from which the insect specimens were collected, such as temperature, annual rainfall and relative humidity in those areas.



X (Factor I) = -8.783 to 5.589, Y (Factor II) = -2.859 to 4.447

Fig. 8. Discriminant analysis of the coordinates of the markers of the right wings of *C. vicina* (the black and white graphs represent specimens of middle and south Iraq, respectively).

Table 2. Comparison of distances Mahalanobis Distances between population specimens of *C. vicina* fly from middle and south Iraq.

Specimens	*C. vicina* (m)	*C. vicina* (a)
C. vicina (m)	0.00	
C. vicina (s)	10.52	0.00

Table 3. Analysis of variance (ANOVA) of right wing size symmetry in populations of *C. vicina* fly from middle and south Iraq.

Source	SS	DF	MS	F	Signification
Modal	0.0001	3	0.000024	0.63	0.6018
Individual	0.0000	1	0.000001	0.01	0.9065
Side	0.0000	1	0.000026	0.68	0.4189
Side*i	0.0000	1	0.000046	1.20	0.2834
Residue	0.0009	24	0.000038		

Table 4. Analysis of variance (ANOVA) symmetry of the right wing shape in the populations of *C. vicina* fly from middle and south Iraq.

Source	SS	DF	MS	F	Signification
Modal	0.0000	3	0.00003	0.06	0.9802
Individual	0.0000	1	0.00002	0.03	0.8541
Side	0.0000	1	0.00003	0.07	0.7973
Side*i	0.0000	1	0.00004	0.08	0.7820
Residue	0.0011	24	0.000046		

Fig. 8 shows the discriminatory analysis of the *C. vicina* fly communities in the two regions. This kind of analysis is used to compare individuals within a single population. The total number of right wings used in this analysis was 30 right wings, 15 of them were for the insects of the middle region and 15 for the insects of the south region. The results of the discriminant analysis in Table 2 showed that the Mahalanobis distance between the central size of the wings of insects in the middle region is 10.52. In addition, we reclassified based on the percentage of specimens from the central and southern region using the PAD unit available within the contents of the ready-made statistical program and based on the matching of the wings for the specimens of the two regions. The classification rate in the specimens of the middle region was 83%, while those of the southern region 97%, which means that the variation in the shape and size of the right wing in the specimens of the middle region is less than in the southern region. The variation may be due to the different environmental conditions, such as temperatures and annual rainfall rates, as well as the relative humidity rates in those areas first, and the geographical distance between the study areas from which the specimens were collected, which is equal to hundreds of kilometres, second. There have been many studies in this aspect, including the study by Ricardo *et al.* (2009), which included a comparison among a number of population groups of the bug that causes Chakas disease *Triatoma infestans* in different regions of Northwestern Argentina using the geometric morphometric for the shape and size of the wing. The discriminatory analysis of the coordinates of the landmarks attached to the right wings of 308 males of that insect showed little variation in the size of the wings within the same population. The analysis of variance in that study also showed that there is no complete match between two populations for specimens collected from the same area. They noticed that there is a heterogeneity between the specimens collected from goat stockade and those collected from pig stockade, including that there is a subspecies of the insect led to a difference in the central size of the right wing. In the present study, Tables 3 and 4 show the ANOVA test for the right wing size and, for corpse flies in the studied populations respectively. As the specimens of the two regions were compared by using the ASI unit. Within the components of the ready-made program, and through this unit, it was found that it is possible to know if there is a difference in the shape and size of the wing between the population communities or not. The results of the analysis of variance for wing size symmetry showed no significant differences in size, with a very small change in the symmetry of the shape of the wing for all communities. It was found that the proportion of symmetry in shape and size is 75%, and this large proportion of symmetry means that the insect whose specimens were collected from the middle and south regions belong to one progeny. The reason for the small variance between them may be because of to their effects on the environmental conditions in the areas from which the specimens were collected.

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