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Effect of imipramine on structures and shapes of wings of the blow fly Calliphora vicina (Diptera : Calliphoridae)

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Abstract

Geometric morphometric of wings was used to identify and know the variation in the size and the shape of the wings of two groups of blow fly species *Calliphora vicina* , one group composed of wings of adult flies resulted from larvae previously reared beef liver spiked with imipramine 25mg drug and the second group were the wings of adult flies emerged from larvae reared on untreated beef liver as a control group . The average centroid size of the left wing were 1361.15 for the treated resulted group and 1388.66 for the control group. Each groups were reared at same environmental conditions of humidity 37.16% RH and temperature 27.78 C˚ . The results showed that there were no significant differences in the average of the centroid size for the left wing for the blow fly *C. vicina* of the two groups , which probably means that the specimens of the treated group did not affected by the given drug- treated (imipramine – treated) diet .

Key words : *Calliphora vicina* , Geometric Morphometric , Imipramine 25 mg , Forensic Entomology .

Introduction :

Calliphora vicina is one of the most forensically important blow flies [1] this species considered as one of the primary colonizing insects of human and animal dead bodies [2] so that it is an important decomposition agent that facilitates the rapid degradation of the dead bodies . This blow fly species used in many studies in forensic entomology and in entomotoxicology in order to make an accurate correlation of post mortem interval (PMI) [3]. This insect has a wide distribution in several countries. In general the development of the blow fly related with natural and synthetic diet and with the competition between larvae , also it had noticed that the presence of toxins in decomposing tissues from natural diets demonstrated effects on the developmental rates of blow flies[4]. The shape variations of wings of the resulted adult *C. vicina* were tested by Geometric Morphometric system. Previous studies used this technique for the comparison of wings of population communities of the same species of insects , such as the study of [5] of the comparison of the shape and structure of honey bee workers *Apis mellifera L.* collected from two geographical areas Baghdad and Babylon . The present study focused on the effect of drug-treated diet (beef liver) spiked with the lethal dose of imipramine 25 mg (an antidepressant drug) on the morphology of the wings of adult blow flies resulted from larvae were previously reared on imipramine treated beef liver using geometric morphometric in order to the comparison between the two groups of wings of the blow fly insect *C. vicina* reared on different diet.

Materials and methods :

The insect species *C.vicina* was previously bred by using a cattle lung to attract the blowflies. After ovipositing of egg the latter were collected and put on a piece of beef liver inside a plastic container until egg hatching .The larvae were divided into two groups, first group was used as the treatment with 20mg of beef liver spiked with 0.2mg of the antidepressant drug imipramine 25mg as it is the equivalent amount to the lethal dose in human liver [6] that used as powdered

tablets and the second group was reared on untreated media as control that composed of 20mg of beef liver without drug spiking . After larvae breeding and obtained the adult flies. In this study it had used sixteen insect sample of each group and the left wing was taken off to study the variation in the shape and size of the wings by geometric morphometric of wings . Method of [7] was carried out in the preparation of wings samples, resulted *C. vicina* were isolated in a plastic containers and left without feeding until it died and dried , after drought the left wing of each sample was taken off by a fine tweezers carefully in order to keep wings without fractioning . The wing preserved between two slides with tightening the ends of the slide by an adhesive ribbon . The information of each sample were recorded on one end of the slides . The slides then photographed by a digital microscope linked with a computer provided with a digital camera with 1.3 megapixel the camera provided with an ultra violet light (UV) , after photographing . The picture were preserved in a special folder . The statistical analysis ANOVA test followed by T and F tests was used .

Data collecting :

After photographing of wing samples of each treated and untreated groups , data of each photograph or picture were collected separately by using the available program Collecting Landmarks for Identification and Characterization, this program is specialized in geometrical analyses landmarks , landmarks are known as anatomical points put on wings at the points of intersections of longitudinal with transverse veins or at the ends of longitudinal veins which uses to the discrimination between one individual and another [8] in this study (11) landmark were appointed on the intersections of longitudinal with transverse veins . Numerated points were put at these intersections by the unit (COO) specialized in appointing landmarks for the coordinates . Links among the eleven landmarks that appointed on each wing gave as a polygonal shapes these polygonal shapes use in a lot of analyses such as the comparison between size and shape of the wing for each sample used in the study to explain the variation between the two groups of *C. vicina* . It is possible to know the variation inside the same group , the obtained information transferred to the next unit in this program (TET) which is specialized in data merge in order to the comparison between the treated and untreated resulted groups of wings and knowing the identification and variation. After data merge it transfered to (MOG)unit by this unit processes of analyses on landmark coordinates are accomplished such as Translation , Scaling and Rotation to know the centroid size for each wing , partial warp , relative warp and shape variation of each wing . The centroid size of wing is an equal amount of wing it is calculated from the square root of summation square of distances between the polygon center and each landmark of the landmarks were appointed on the wing [9] , and used in the comparison of the treated and untreated groups of the resulted flies .

Data analysis :

Principle Component analysis is used to know the variation between the two groups of the same species (treated and untreated resulted flies), while Discriminate Analysis used to know the variation inside each group separately.

Soft Ware :

Unites involved in the geometric morphometric program were used . each unit has its own job , COO this unit is used to appoint the landmarks and the anatomical dots on wings , MOG is used to know the centroid size data , partial warp ,and relative warp. principle component analysis and all discrimination analyses are obtained by the unit PAD , data of variation analysis of centroid size of wings were collected from the unit COV . The unit ASI used for data identical analysis shape and size of the wing .

Results and Discussion :

Geometric morphometric system based on landmark coordinates between the intersections of longitudinal with transverse veins of the wing .The centroid size of each wing was calculated .Variations of the shape and size of the wing for the resulted adult flies of each imipramine 25 mg -treated and untreated groups were calculated . The results showed a non significant variation in wing shapes and size between these two groups, figure (1) and (2) refer to the left wing of the blow fly species *C.vicina* marked by the landmark coordinates by using geometric morphometric system of shape and size of wings . The main usefulness of this system is the use of all information available for the shape from the landmark coordinates and then determination of shape variations [10] . It had compared the wings of two groups of this insect by using discriminate analysis depending on the centroid size of each wing , figure (3) refer to the average or mean of landmark coordinates of (16) left wing of *C. vicina* , each group of (11) landmark , violet spots represent the control resulted group and the green spots represent the drug- treated resulted group. Figure (4)represent the output of consensus configuration of the control and treated groups while the figures (5) and (6) respectively , represented the variation of the centroid size of the left wing of *C. vicina* for the two groups , each box represent the median group distributed between the 10^{th} and the 90^{th} quartiles , and between 25^{th} and 75^{th} quartiles. The results showed a non significant variation in the centroid size among individuals of the two groups of this species , the centroid size of the left wing for the control group was 1388.66 while the centroid size of the left wing for the drug- treated group was 1361.15. These groups were compared using T and F tests their values were 1.53 and 7.23 respectively. the probability value (P) was 0.0004 , while the absolute difference was 27.51 , thus there were not significant differences between the wings of individuals of these two groups of the same species . The variations may be as a result of number of causes , one of these causes is the physiology, it considered as the main cause of metric differences appeared in differential growth , variation can be also caused by pathological causes such as toxins that may affect the morphogenesis of some individuals but generally morphometric is not always required to detect these changes since they produce obvious visible.In addition the presence of toxins in decomposing tissues from natural diets had demonstrated affecting the developmental rate of blow flies [4] and these developmental rates of flies are used in estimation of the post mortem interval (PMI)in homicide investigations in the first few weeks after death [11] .The use of multiple generations of the same species of the blow fly that reared on different diets such as drug treated media is necessary to study the wings variations in order to knowing the effect of drug on forensically important blow flies .

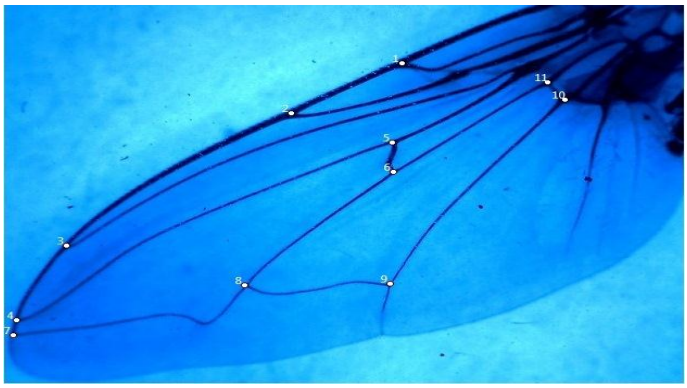


Fig.1. Eleven landmarks on the *C. vicina* left wing Control in geometric analysis

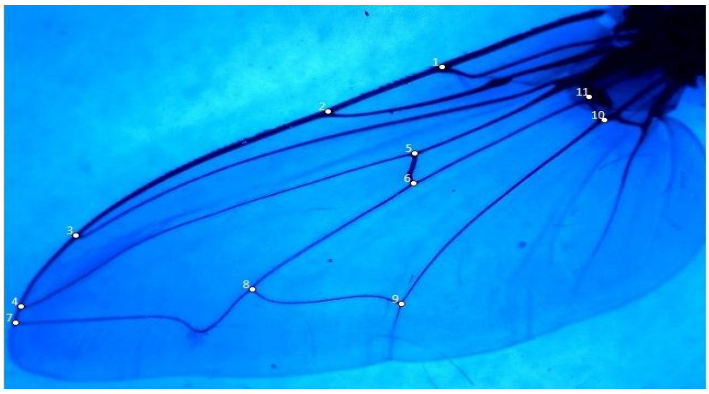
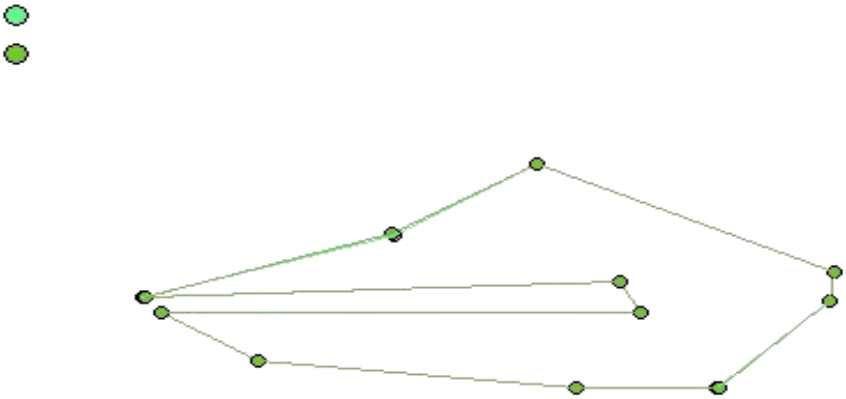
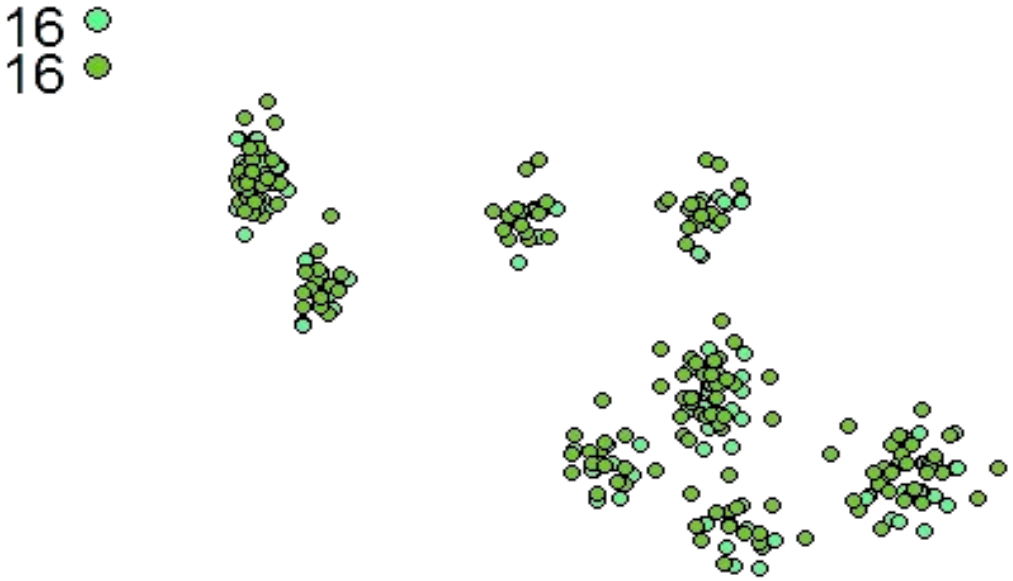


Fig.2. Eleven landmarks on the *C. vicina* left wing treatment used in geometric analysis



X= of -0.456 to 0.382 , Y = of -0.127 to 0.200

Fig. 3. Mean coordinates of landmark in the left wing of *C. vicina*, turquoise color represents Control specimens and Green color represents Treatment specimens.



X = of -570.420 to 609.580 , Y = of -424.420 to 365.580

Fig. 4. Output of consensus configuration by the GPA Procrustes superimposition method locating the 11 landmarks for each individual left wing of the two colonies. turquoise color represents Control specimens and Green color represents Treatment specimens.

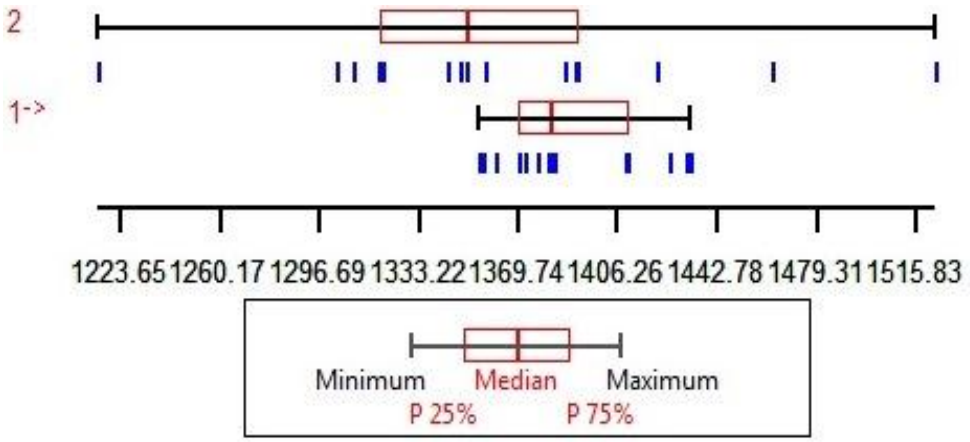


Fig. 5. Variation of the centroid size of left wings for *C.vicina* according to feeding, Each box shows the group median separating the 25 th and 75 th the quartiles. Vertical bars under the boxes represent the wings numbers 1 and 2 in the Fig. represent Control and Treatment specimens respectively. Units are pixels. P, percentile.

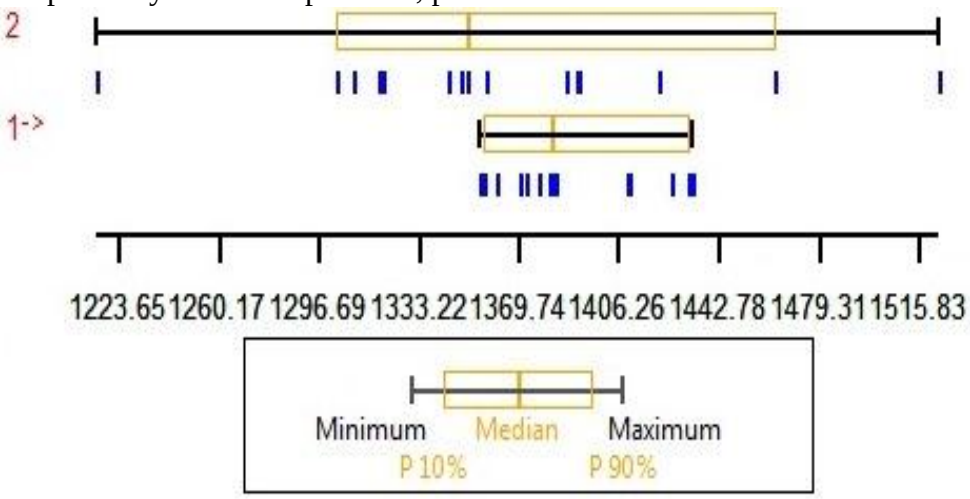
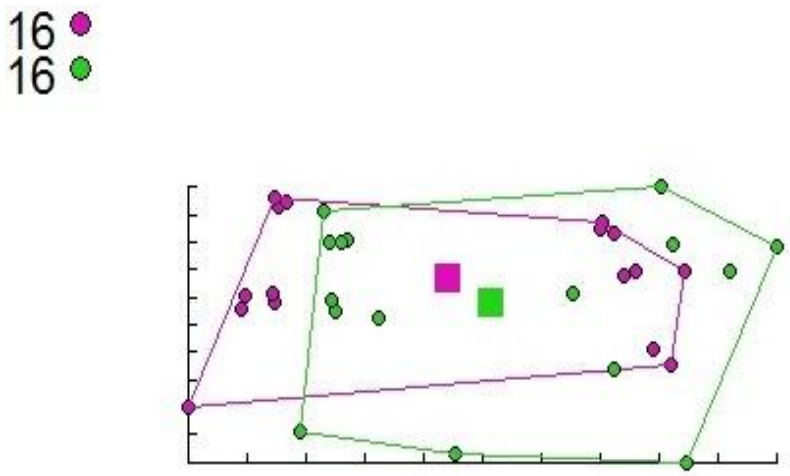


Fig. 6. Variation of the centroid size of left wings for *C.vicina* according to feeding, Each box shows the group median separating the 10th and 90th the quartiles. Vertical bars under the boxes represent the wings numbers 1 and 2 in the Fig. represent Control and Treatment specimens respectively. Units are pixels. P, percentile.



X = of -0.032 to 0.035 , Y = of -0.017 to 0.010

Fig. 7. Scatter plot of the principle component analysis of *C. vicina* specimens according to feeding based on Geometric Morphometric, Violet spots represent Control specimens and Green spots represents Treatment specimens, Violet square represent mean centroid size of the left wing for Control specimens = 1388.66 and Green square represent mean centroid size of the left wing Treatment specimens = 1361.15

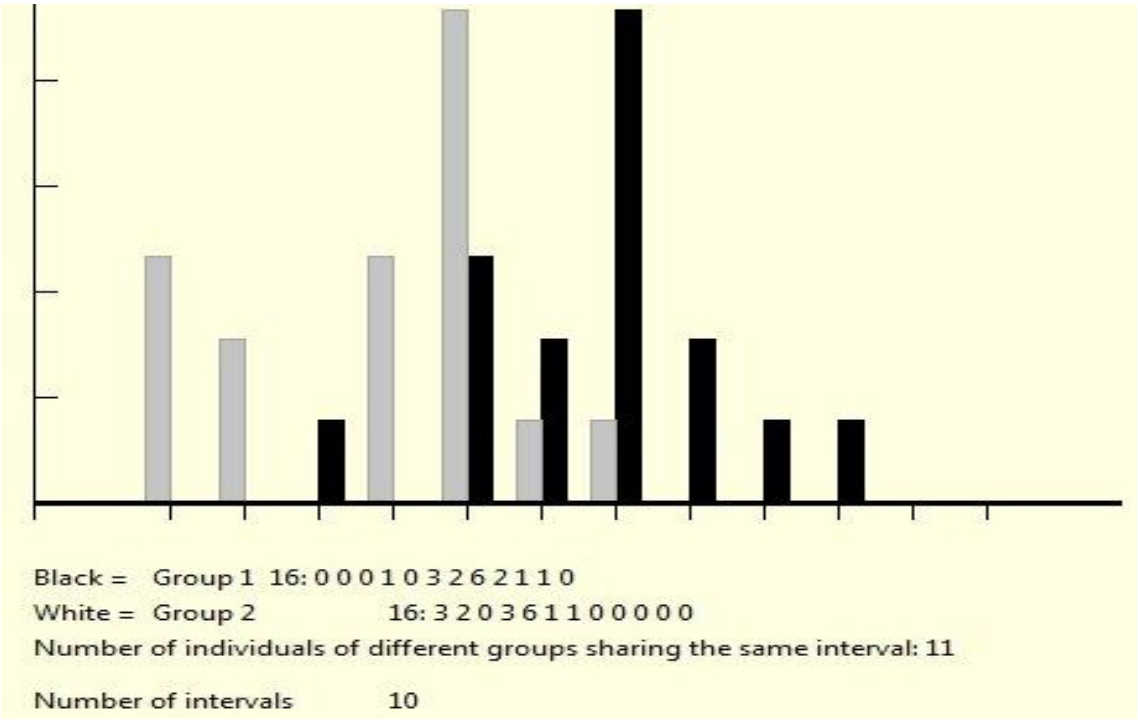


Fig. 8. Discriminate analysis of two groups for *C. vicina* **,**Mahalanobis distances between centroids size were as follows: Control specimens to Treatment specimens = 2.23, Black color in this figure represented to Control specimens and White color represented to Treatment specimens. Simple re-classification The classified individuals were part of the discriminate analysis First Correct assignments : Black group 15/16 = 93% , White group 15/16 = 93% .

Table 1. Compared the centroid size of the left wing for ***C. vicina*** between Control and Treatment specimens .

Group	M.CS.	St.D.	Va.	F	P	T	P	A.D.
Control	1388.66	25.12	630.93	7.23	0.0004	1.53	0.14	27.51
Treatment	1361.15	67.52	4558.97					

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

Table 2. Analysis of variance for asymmetry left wing size of *C. vicina* Control and Treatment specimens.

Source	SS	df	MS	F	Signification
Model	0.0000	3	0.000003	0.15	0.93
Individual	0.0000	1	0.000008	0.39	0.54
Side	0.0000	1	0.000000	0.02	0.89
Side*I	0.0000	1	0.000001	0.05	0.83
Residue	0.0005	24	0.000020		

Table 3. Analysis of variance for asymmetry left wing shape of *C. vicina* Control and Treatment specimens.

Source	SS	df	MS	F	Signification
Model	0.0017	54	0.00003	1.39	0.042
Individual	0.0008	18	0.00004	1.87	0.017
Side	0.0001	18	0.000004	0.19	0.99
Side*I	0.0009	18	0.00005	2.11	0.005
Residue	0.0100	432	0.00003		

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