Quantitative Genetic Diagnosis of Medical and Forensic Insect Species in Diyala Governorate Using Geometric Wing Morphometrics

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Abstract: A morphometric analysis was conducted on the right wing of adult males of some forensic insects, such as *Chrysomya megacephala*, and medical insects such as *Sarcophaga carnaria* and *Musca domestica*, collected from different areas in Diyala Governorate. The aim of this research was to differentiate between male medical and forensic insects based on the shape and size of the right wing central centroid. The analysis of the right wings in this work was carried out from adult males of the species *S. carnaria*, M. domestica, and *C. megacephal*. Samples were collected using light traps, and 19 marks were identified on each wing and placed at the ends of the longitudinal veins and between the intersections of the veins with the longitudinal veins. Significant differences were found in the mean right wing centroid and wing shape between adult males of medical and forensic insects. The results showed that the mean right wing centroid was 1576.52, 1633.33, and 1126.15 for *C. megacephala* and *S. carnaria* and *M. domestica*, respectively, when comparing *C. megacephala* with *S. carnaria* (f = 3.49, p = 0.03 and t = 1.13, p = 0.27 and AD = 56.81), comparing *C. megacephala* with *M. domestica* (f = 2.19, t = 0.15 and t = 15.8, p = 1.77 and AD = 450.35), and comparing *S. carnaria* with *M. domestica* (f = 7.65, p = 0.00 and t = 10.82, p = 1.61 and AD = 507.17). When comparing the right wing morphology between males of *C. megacephala*, *S. carnaria* and *M. domestica*, a significant difference was observed (f = 0.00, p = 0.99). A significant difference in wing size (f = 0.18, p = 1.00) was also observed between species.

1 INTRODUCTION

All flies part of the order Diptera are of criminal, medical and veterinary medicine Significance, as they are good mechanical vectors of many diseases that threaten human life throughout the world, especially in developing countries[1, 2]. Flies feed on numerous plant species and animal remains and are known disease vectors throughout history [3]. Diptera insects are distinguished from other insects by possessing a well-developed pair of forewings used for flight, while the second pair of wings is modified into structures known as balance pins (halters) [4]. Some of these insects are considered medically important pests as they transmit dangerous diseases affecting human and animal health. The impact of medically significant pests, such as *M. domestica* (housefly), is exacerbated in developing countries due to neglect in public health measures [5]. Additionally, insects play a crucial role in forensic veterinary entomology, as they are often the first living organisms to colonize a corpse immediately after death. This can help estimate the post-mortem interval by identifying the age of larvae from the first batch of eggs laid on the victim's body [6]. Insects contribute significantly to crime scene investigations, and forensic entomology has evolved into a specialized field within entomology, highlighting their role in forensic investigations [7]. Some insect species feed directly on corpses, such as the metallic blue blowflies from the Calliphoridae family and flesh flies from the Sarcophagidae family, both belonging to the order Diptera [8]. Therefore, an effective, efficient, and prompt method for identifying that insects is essential. This brings up an important subject: the taxonomic identification of fly species through wing geometry, which serves as a valuable tool for entomologists and forensic entomologists [9]. The geometric morphometric system for determining wing shape and size is an advanced technique used in quantitative genetics to measure significant variations in wing morphology. This method enhances the accurate identification of forensically important species [10].

Extensive studies on medically important insects have shown that the analysis of geometric morphometric of wings of insect has a high capacity for detecting variations among geographically isolated populations within the same species or different species with great accuracy [11]. The primary goal of employing this quantitative genetic technique is to determine whether the analysis of geometric morphometric of insect wings be able to serve as an alternative or complementary tool to molecular markers in studying insect populations with medical and forensic significance. Consequently, this technique plays a vital role in accurately identifying the origin of any collected specimens. Furthermore, landmark-based geometric morphometric analysis is widely used for species identification due to its ease of application and low cost [12]. The geometric morphometric (GM) technique is a new tool in the field of biological research that is used to distinguish species with phenotypic affinity and to study the extent of morphological variations of organisms in different environments, is a new approach [11], and is frequently was accustomed to detect species in beings with analogous morphology and to study morphological differences in dissimilar surroundings particularly, with homologous insect species [13]. Recently, GM's landmark-based approach has been Serves to distinguish three genera of mosquitoes Aedes albopictus, Aedes Aegypti, and Aedes Scutellaris in Thailand and he discovered that this technique is very accurate in classifying species [14].

The current research aims to use a landmark-based genetic engineering approach to efficiently discriminate on three types of flies *C. megacephala*, *S. carnaria, M. domestica* that are criminally and medically important.

2 MATERIALS AND METHODS

2.1 Specimen Collection and Identification

Samples of the species *C. megacephala*, *S. carnaria* and *M. domestica* were gathered from different farms and in several areas of Diyala Governorate (muqdadiya) by installing fly traps and putting poison on animal waste, then the samples were kept in plastic bottles with the addition of naphthalene to prevent rotting of the samples [15], and then the sample was sent to the Natural History Museum at the University of Baghdad for the purpose of identifying and classifying the sample using classification keys.

2.2 Specimen Preparation and Landmark Collection

In this study, a total of 45 right wings (15 per species) were used to investigate the quantitative genetic diversity in wing size and shape among three medical and forensic insect species from Diyala Governorate: *C. megacephala*, S. *carnaria*, and M. *domestica*. The research employed the geometric morphometric (GM) technique.

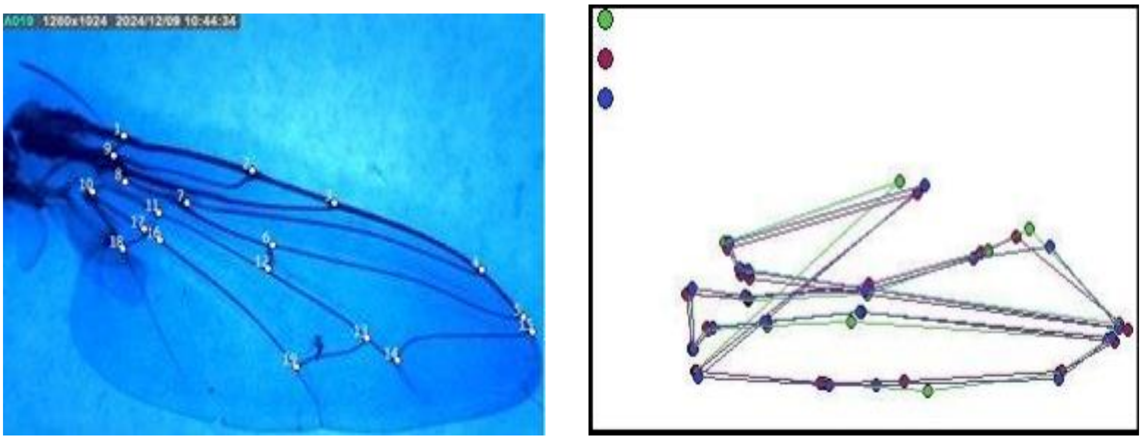
For the preparation, 15 male samples were isolated for each species. A technique followed [16] was used to prepare slide-mounted specimens. The right wing of each insect was removed and placed between two glass slides. The edges of the slides were securely sealed using G2100 adhesive paper tape. Each slide was labeled with the species, sex, and sample number.

After preparation, the slides were visualized using a digital microscope at a magnification power of x50. The intersections of the longitudinal wing veins and cross-veins were learned (Fig. 1). Specialized statistical analyses were then employed to assess differences between the species, including:

- Centroid size analysis.
- Discriminant analysis.
- Shape and size analysis.
- Mahalanobis distance analysis.

2.3 The Analysis of Geometric Morphometric

In wing size examination, the mean central size (MCS) wsas calculated as a means of estimating right wing size in medical and forensic insects, which is calculated via the square root of the sum of squares between the center of the landmark formation and every individual Anatomical point [17]. Then, statistical tests for wing size among males in each Non-parametric permutation tests (1000 cycles) with Bonferroni correction at a significance level of $P < 0.05$ were used to analyze the fly species. Similarly, wing shape analysis for *C. megacephala*, *S. carnaria* and M. *domestica*, where shape variables were evaluated by principal component analysis (PCA) of partial warp points (also known as relative warp), which were calculated after performing a generalized regression analysis of coordinates to assess variation in wing shape among species [18].



(a) (b)

Figure 1: Landmark configuration and morphological variation in wing venation among fly species: a) The locations of numbered landmarks on the ends of longitudinal and transverse veins. b) Histogram of the distribution of mean landmark configuration in each fly species. Green dot: *C. megacephala*, Red dot : S. *carnaria*, Blue dot: M. *domestica*.

2.4 The Software of Geometric Morphometric

The Collection of Landmarks for Identification and Characterization package (CLIC), release 97, was utilized to digitize landmarks and perform geometric morphometric analysis[1].

3 RESULTS AND DISCUSSION

The geometric scale based on landmarks has been used to distinguish morphological genetic differences between the males of some medical and forensic insects namely *C. megacephala*, *S. carnaria* and *M. domestica* using a set of statistical analyses, which had separated into shape and size investigates, where the percentage of differences in the central size of the wings was very high, 1576.52, 1633.33 and 1126.15, *C. megacephala*, *S. carnaria* and *M. domestica*, respectively., When comparing *C. megacephala* with *S. carnaria* (f = 3.49, P = 0.03 , t = 1.13, P = 0.27, AD = 56.81) , and comparing Ch. *megacephala* with *M. domestica* (f = 2.19, p = 0.15 and t = 15.8, p = 1.77 and AD = 450.35) , and comparing *S. carnaria* with *M. domestica* (f = 7.65, p = 0.00 and t = 10.82, p = 1.61 and AD = 507.17 (Table 1). Comparisons of variation of wing mean Centroid Size between medical and forensic fly species (Figures 2 and 3). Wing shape and size in all species were different among males based on Bonferroni test ($p < 0.01$) (Tables 2 and 3). When comparing the shape of the right wing between males of forensic insects and medical insects, which includes *C. megacephala*, *S. carnaria*, *M. domestica* a significant difference was observed (f = 0.00, p = 0.99). A significant difference was also observed in wing size (f = 0.18, p = 1.00) between the species.

Each box represents the median for the species, distributed between the 25th and 75th percentiles. The blue lines represent the samples for each species. Numbers 1, 2, and 3 correspond to *C. megacephala*, S. *carnaria*, and M. *domestica*, respectively (Fig. 2). In Figure 3, the polygons show group distribution, demonstrating the degree of morphological overlap and distinction.

Increasing knowledge of disease-carrying insects is crucial to understanding vectors, which in turn leads to effective control. Accurately identifying fly genus and species is an important first step in studying them phenotypically and genetically. Although adult flies are relatively easy to genus and species by morphological methods, field specimens are sometimes incomplete, leading to misdiagnosis. The GM technique based on the numbered landmarks on the ends of the veins was used in this study to conduct a dimorphic analysis of three common fly species in Diyala Governorate/Iraq: *C. megacephala*, S. pulchra. Wing size analysis based on the wing central size ratio (MCS) found statistical differences between medical and criminal species. This study results are aligned with a group of previous studies, including a study conducted in Thailand Which used landmark-based morphometric analysis to distinguish twelve different species of flies *Chrysomya megacephala*, *Chrysomya chani*, *Chrysmya pinguis*, *Chrysomya rufifirina*, *Chrysomya villeneuvi*, *Cheysomya ligurriens*, and *Hermetia pulchra*. The study demonstrated that wing

[1] The CLIC package (release 97) is freely available at https://xyom.io

size can be employed for Classification of species [19]. Wing size is an inappropriate factor for differentiating between species within a genus and between genera within same order, as it easily changes according to environmental conditions, especially at breeding sites [19]. Wing shape is a common factor used to distinguish species within the same genus, genera within the same order, and sexual variation because wing shape traits are directly influenced by genetic factors and are less affected by ecological factors than wing size factors. [14]. In this study, shape and size analysis of right wing based on Mahalanobis distances revealed differences between males all species of flies. The geometric morphometric approach enhances the ability to identify dimorphism in fly wings. The geometric morphometric scale was used to distinguish and distinct genera and species, and to differentiate sexes among, twelve different fly species in Thailand: Boettcherisca Peregrine, B. Nathani, Lioproctia Pattoni, L. Ruficornis, L. Saprianovae, Parasarcophaga BreviCornis, P. Antilope, S. Multivillosa, S. Senior Whitei and Senior Whitea Princeps. [19]. The Geometric Morphometric method is also effective in distinguishing between males and females of the same genus in wings, especially in *C. megacephala*, which can be distinguished with over 89% accuracy. This is consistent with a previous study by Sontigun in Thailand, which found that males and females of *C. megacephala* could be easily distinguished from each other by wing size and shape (99% of females and 89% of males had acceptably categorized in compared with the biological sexes by wing size and shape across individuals [19]. This study demonstrated that a landmark-based geometric morphometric approach was effective in identifying and distinguishing *C. megacephala*, S. *carnaria*, and M. *domestica*, and is therefore an easy and inexpensive method to aid in discrimination in circumstances of imperfect Samples that are difficult to distinguied by geometric morphometric analysis. [19]. The research revealed that the geometric morphometric approach was effective and highly accurate in distinguishing between males of *S. carnaria M. domestica C. megacephala*, , and, which is one of the methods to quickly assist in differentiation in cases of incomplete specimens that cannot be classified by molecular methods.

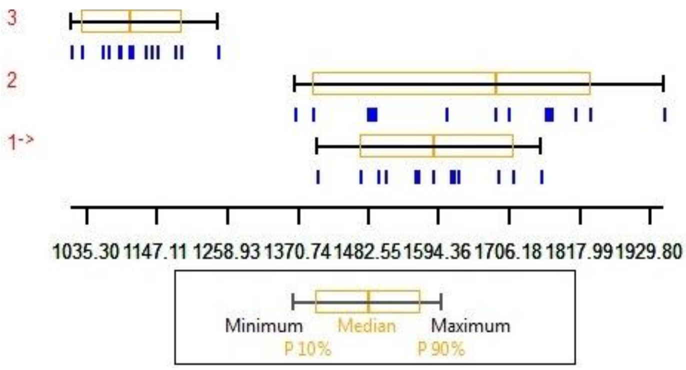


Figure 2: Variation in the central size of the right wing between male medical and forensic insects.

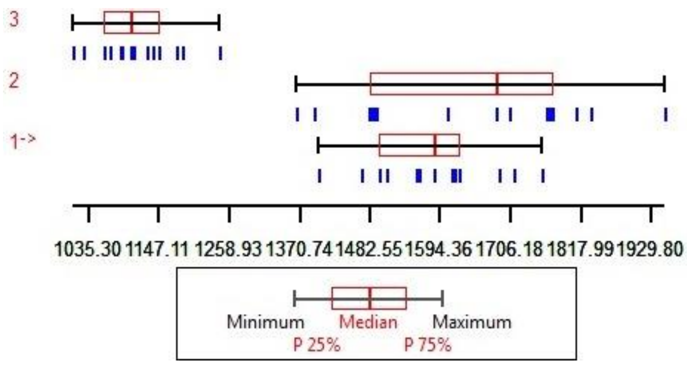


Figure 3: Discriminant analysis plot visualizing the differences in right wing shape among species based on Mahalanobis distances.

Table 1: Statistical analyses of mean of right wings Centroid Size differences between among *C. megacephala, S. carnaria and Musca domestica*.

Species	Mean central size	St.d.	Varience	F-test	p-value	t-test	p-value	A.d.
1 - *C.megacephala*	1576.52	91.27	8330.90	1 vs. 2 3.49	1 vs. 2 0.03	1 vs. 2 1.13	1 vs. 2 0.27	56.81
2 - *S.carnaria*	1633.33	170.59	29102.52	1 vs. 3 2.19	1 vs. 3 0.15	1 vs. 3 15.8	1 vs. 3 1.77	450.35
3 – *M.domestica*	1126.15	61.65	3801.48	2 vs. 3 7.65	2 vs. 3 0.00	2 vs. 3 10.82	2 vs. 3 1.61	507.17

Table 2: Statistical analysis of right-wing shape among species *C. megacephala, S. carnaria and M. domestica* using Model II oneway ANOVA (Arnqvist and Märtensson).

Source	Ss.	df.	Ms.	f.	*P-value*
Side	0.0001	1	0.000087	0.52	0.4744
Side*i	0.0000	2	0.000000	0.00	0.9978
Model	0.0001	5	0.000019	0.13	0.9844
Individual	0.0000	2	0.000009	0.06	0.9395
Residue	0.0053	36	0.000148		

Table 3: Quantitative Statistical analysis of right-wing size among *species C. megacephala, S. carnaria* and *M. domestica* using Model II oneway ANOVA (Arnqvist and Märtensson).

Source	Ss.	df.	Ms.	f.	*P-value*
Side	0.0007	34	0.000020	0.21	1.0000
Individual	0.0014	68	0.000021	0.22	1.0000
Model	0.0032	170	0.000019	0.20	1.0000
Side*i	0.0011	68	0.000017	0.18	1.0000
Residue	0.1167	1224	0.000095		

4 CONCLUSIONS

The findings of this study confirm that geometric morphometrics is a powerful and cost-effective tool for the quantitative genetic differentiation of medical and forensic fly species. By analyzing the right wing morphology of *Chrysomya megacephala*, *Sarcophaga carnaria*, and *Musca domestica*, significant differences in both wing shape and centroid size were detected, validating the reliability of landmark-based morphometric analysis for taxonomic and forensic applications.

The observed variations in Mahalanobis distances and centroid size metrics among the species suggest distinct genetic and phenotypic traits that are consistent across species boundaries. This supports the use of geometric morphometry not only for species identification but also for detecting interspecific and intraspecific morphological diversity, even when traditional morphological markers are inconclusive.

Furthermore, the ability to distinguish specimens using wing morphometrics is particularly valuable in forensic and medical entomology, where sample integrity is often compromised. This technique also provides a complementary or alternative method to molecular identification approaches, especially in resource-limited settings.

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