Describing Wing Geometry of *Aedes Aegypti* Using Landmark-Based Geometric Morphometrics

Judy P. Sendaydiego, Mark Anthony J. Torres, and Cesar G. Demayo

***Abstract*—Insect wing morphology has been used in many studies to describe variations among species and populations using traditional morphometrics and more recently, geometric morphometrics. This study was conducted to determine intraspecific divergence in wing shape and venation in *Aedes aegypti* using landmark-based geometric morphometrics. In the Philippines, *Ae. aegypti* has been identified as a common dengue vector species. With the increasing cases of dengue, mosquito control programs are faced with problems on vector species diversification and proper identification. Variation in wing geometry may provide relevant information on proper identification of species and in describing population diversity. In this study, the geometry of 30 wings of female *Ae. Aegypti* was described using 18 anatomical landmarks and subjected to Procrustes superimposition and relative warp analysis. Results of the relative warp analysis showed some intraspecific variation in the wing outline of *Ae. aegypti*. The observed morphological disparity in wing shape suggest a possible morphological divergence among populations of *Ae. aegypti*. Based from the results of the study, landmark-based geometric morphometrics is a good tool in describing quantitatively variations in wing shape of the mosquitoes.**

***Index Terms*—Procrustes, aedes, landmarks, dengue, landmarks.**

I. Introduction

Dengue is a mosquito-borne viral disease that has become a major international public health concern as it has led led to the global resurgence of epidemic dengue fever and emergence of dengue hemorrhagic fever (dengue/DHF). Also of maximum concern is the findings of the existence of hyperendemicity in many urban centers in countries like the Philippines and Thailand. Currently, emerging DHF cases are already causing increased dengue epidemics not only in southeast Asia but also in the Americas and other parts of Asia and has become a leading cause of hospitalization and death among children in these countries [1].

Aedes aegypti Linnaeus is a vector of arthropod-borne viruses worldwide especially in the areas where it has become a serious public health problem in recent years [2], [3]. Ae. *aegypti* is a mosquito that can spread the dengue fever, Chikungunya and yellow fever viruses, and other diseases. The mosquito can be recognized by white markings on legs and a marking in the form of a lyre on the thorax. The mosquito originated in Africa [4] but is now found in tropical and subtropical regions throughout the world [5]. Mosquito control programs are faced with the challenge of accurately identifying all possible vectors of the disease as other species have been associated also like *Aedes albopictus* [6]. Although the production of dengue vaccine is ongoing with satisfactory results [7], vector control and entomological surveillance remains an important issue in controlling the disease. A comprehensive knowledge of the morphological characteristics of the vector may help in describing the population diversity, and could then be useful for decision making during control campaigns [8].

Among insects, the use of geometric morphometric analysis to study wing venation has been useful in identification at the individual level [9]-[11] and in distinguishing sibling species [12]-[15]. Wings are excellent structures for studying morphological variation because the intersections of the wing veins provide many well-defined landmarks suitable for morphometrics and that the metric properties of the wing provide precise quantitative information for the identification of species complexes [14], [16] and within-species variations [10]. Since the morphology of insects is under genetic and environmental influences, variation in morphometric traits may provide relevant information on the many aspects of insect biology thus in the present study, we applied a landmark-based geometric morphometric analysis of the wings of *Ae. aegypti* mosquitoes.

Geometric morphometrics utilizes powerful and comprehensive statistical procedures to analyse shape differences of a morphological feature, using either homologous landmarks or outlines of the structure [17]-[19]. Landmarks are the points at which biological structures are sampled. These points produce an exact geometric description of the differences in shape of a structure. The approximated tangent space [20], [21] enables one to perform standard multivariate statistics on a data set of homologous landmarks (or *x,y* co-ordinates) of taxa being compared. The technique uses Procrustes distances to capture shape variation considered to be the most reliable method to determine geometric morphometric relationships among taxa [20]-[31]. Rohlf [32], [33] developed the tps series of programs which performs the statistics and visualizations of geometric morphometrics and were used in the current study. The objective of our study was to explore the technique of geometric morphometrics to determine if within *Ae. aegypti* variations can be described quantitatively and be understood as to where characters could be used later for studies on

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strains that serve as vectors of the dengue virus.

II. Methodology

Adult mosquitoes were collected from housing units in a Subdivision in Cagayan de Oro City (Fig. 1), Misamis Oriental. Collection of adult mosquito samples was done using aerial nets. Sampling was done early in the morning and late afternoon. The collected adult mosquitoes were sorted and identified in the laboratory using a stereoscope. Only female *Ae. aegypti* adults were utilized in this study. Wings were detached from the thorax, placed on a glass slide and secured with a cover slip. The wing images were captured under Leica light microscope at 4x magnification using a digital camera. Eighteen landmarks were identified and tagged for geometric morphometric analysis following the method of Rohlf and Slice [29]. The landmarks identified are the intersections of wing veins with the wing margin, intersection of cross vein with major veins and some vein branch points. The description and locations of the identified landmarks are presented in Table I and Fig. 2.

Fig. 1. Map showing Cagayan de Oro.

The wing images were captured using a digital camera (2 mega pixels) under a phase contrast microscope (ocular 10x objective 10x). Our study was based on 18 landmarks as shown in Fig 2.

The coordinates of the landmarks were digitized using TPSdig software [33]. Thin-plate spline of relative warp analysis was carried out using the coordinates of all aligned wings [28], [34], [35]. The connections between 18 landmarks created polygons of comparative size and shape of mosquitoes' wings. The uniform component of shape variation was estimated by the Linearized Procrustes method [36]. In order to assess the local shape changes in the species, relative warp analysis was used. The reference used in this analysis is the average configuration of landmarks. The warps were computed with the alpha at zero, in order to weigh all landmarks equally [37]. Deformation grids were used to portray the resulting shape variations. The coordinates of the landmarks were digitized using TPSdig [34]. Collection of the raw coordinates of each landmark was performed on the computer screen in units of pixels for further analysis.

TABLE I: Description of Assigned Landmarks on Aedes Aegypti Wings.

Landmark	Description of the Landmark
1	intersection of Costa (C)
2	distal end of the Radius (R)
3	radial branch 2
4	radial branch 3
5	distal end of radial branches 4 & 5
6	distal end of M1 & 2
7	distal end of M3 & 4
8	distal end of cubital vein 1
9	distal end of cubital vein 2
10	Anal vein
11	origin of cubital 1
12	midpoint branch of cubital 3
13	medio-cubital cross vein
14	midpoint branch of medial vein
15	radio-sectoral vein
16	radio-medial cross vein
17	midpoint branch of radial vein
18	origin of radius branches 2 & 3

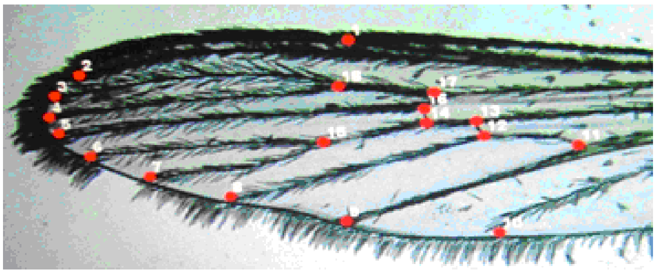

Fig. 2. Landmarks on Aedes aegypti wing used for geometric morphometric analysis.

The connections between the 16 landmarks provided polygons of comparative size and shape of the mosquitoes' wings. The raw coordinates of each landmark were superimposed using a Procrustes Generalized Least-squares (GLS) superimposition algorithm [34]. The superimposed coordinates were placed on to a square grid using thin-plate spline relative warps analysis to visualize the directional and quantitative change in shape [17], [28].

III. Results and Discussion

Ae. aegypti wings are flat rigid structures with an intrinsic venation pattern which differs in small detail from other species. The major vein branches and the intersection between veins were identified as anatomical landmarks. The landmarks were chosen for their easy identification and their ability to capture and contribute to the general shape of the wing. The presence of scales however, interfered in locating the exact position of some vein crosses.

Table II lists four relative warps that explain more than 80% of the total variation in wing venation pattern within this population of mosquito. Only four relative warps were included in the analyses as only these warps explain more than 5% variation. Of these four relative warps, the first explains 43.78% of the total wing shape variation.

TABLE II: TOP FOUR RELATIVE WARPS THAT EXPLAIN MOST OF THE VARIATION OBSERVED IN THE VENATION PATTERNS OF *AE. AEGYPTI*

RW	Eigenvalue	%	Cumulative
1	0.27470	43.78%	43.78%
2	0.13345	20.92	64.70%
3	0.10116	9.03%	73.72%
4	0.09164	6.86%	80.59

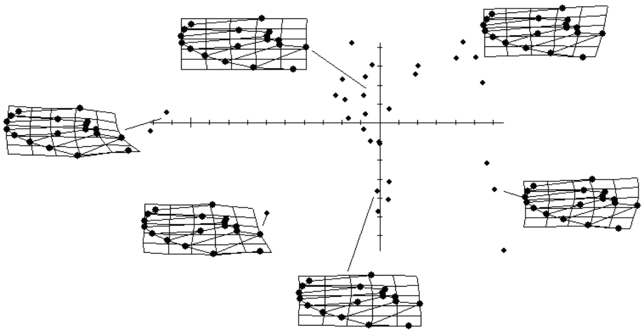

Fig. 3. Thin plate spline visualization grid of *Aedes egypti* wing shape pattern derived from relative warp axes 1 and 2.

Fig. 3 shows thin-plate spline visualizations of the variations in the wing shape and venation patterns along the first two relative warp axes. The first relative warp, which explains nearly half of the total variation divides the population into at least three subgroups of mosquitoes that differ in the shapes of the wing base, specifically on the locations of the anal and the first cubital veins. The left-most group are mosquitoes with wings that have anal veins located proximal to the first cubital vein. Whereas the right-most group are mosquitoes with wings that have anal veins distant from the first cubital vein. The second relative warp further explains variation within each of the subgroups in terms of length-width (aspect) ratio and the shapes of the distal part of the wings. The wings described in the uppermost part of the second axis are characterized by wings that have more tapered apices. On the other hand, the lowermost part of axis 2 describes broader wings that have rounded wing apices.

A summary of the thin-plate spline visualization grids describing variation in wing shape and venation patterns is presented in figure 4. Relative warp 1 accounts for most of variation in wing pattern. The extension of landmarks 9, 10 and 11 which are tagged posteriorly in the cubital and anal veins results to a broader base. This posterior end of the wing is more labile as compared to the more rigid costo-radial boundary in the anterior side. Shape in the positive side reveals a wider wing span while in the negative side, wing shape is stretched resulting to a more tapered base and a slender apex. The change in wing shape pattern may affect flight performance as veins increase the mechanical rigidity of the wing [38].

Previous studies on mosquitoes have also shown that aside from wings, variations in the sizes of mosquitoes are influenced by an enumerable number of factors such as temperature, nutrition, larval density and salinity although genetics may also play a big role [39]. In controlled laboratory conditions, studies also pin-point to generation time as affecting sizes of mosquitoes.

Wing morphology in mosquitoes have also been studied as it relates to several life history traits such as fecundity in *Aedes* mosquitoes [40], [41], the capacity to serve as vectors of disease pathogens, and other characteristics such as weight [42].

The reasons for the observed variations in wing morphology in this population of mosquitoes are yet to be explored. As of now, we can only hypothesize that perhaps the mosquitoes studied may represent different genotypes and propably comes from one diverse gene pool kowing that they were sampled from one locality and that *Ae. aegypti* has relatively low dispersal behavior. However, studies have also shown that females of this species typically fly and disperses from 100 to 500 meters away from its origin depending on the availability of oviposition sites and blood meals [43], [44].

The importance of studying variations in wing geometry in mosquito populations belies on the fact that it can provide insight into the population structure, ecology and even the taxonomic identity of the mosquitoes as some have shown to exist as a species complex.. Considering the importance of mosquito wings in insect behavior and physiology, the differences could also provide useful information on vector distribution and disease control.

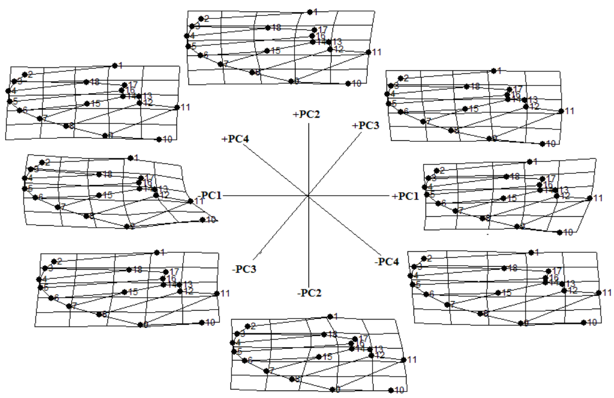

Fig. 4. Thin-plate spline grids that visualize shape variation in wings shapes in the population of *Aedes aegypti*.

Further studies should be done to determine whether variations in the wings of mosquitoes have genetic basis or are mere reflections of the existence of high phenotypic plasticity broght about by varied environmental condition during growth and development of the larvae [10], [45], [46]. The use of Geometric morphometrics should also be done to supplement researches using traditional morphometry and molecular markers. Other characters can also be analyzed such as the relative sizes of wing spots which was previously used to study populations of *Anopheles* [47], [48].

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