

DISCRIMINATION OF *Aedes aegypti* (DIPTERA: CULICIDAE) LABORATORY LINES BASED ON WING GEOMETRY

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Abstract. Recent techniques of geometric morphometrics were applied to the study of four Thailand laboratory lines of the dengue vector *Aedes aegypti*. These lines differed by their geographic origin and the number of generations spent in the laboratory. Using a phase contrast microscope, a set of sixteen landmarks of the wings could be identified. A subsequent Procrustean superposition analysis produced the corresponding size and shape variables which were statistically analyzed. Size showed a significant decrease with the number of generations spent in the laboratory. Shape allowed an almost perfect reclassification of the specimens. This tool is able to accurately distinguish different laboratory lines and will be useful in field studies related to entomological surveillance and vector control.

INTRODUCTION

Aedes aegypti (Linneus) is a main vector of arthropod-borne viruses in tropical and subtropical regions. In Thailand, dengue fever, caused by the dengue virus, is transmitted by *Aedes* mosquitoes and presents a serious public health problem. Although the production of dengue vaccine is ongoing with satisfactory results (Sabchareon *et al*, 2002), 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 (Dujardin *et al*, 1997).

It has been suggested that the metric properties of the wing provide precise quantitative information for the identification of species complexes (Calle *et al*, 2002; Villegas *et al*, 2002), as well as within-species variation in different geographical areas (Dujardin *et al*, 2003). In the present study, we applied a landmark-based geometric morphometric analysis of the wings

of *Ae. aegypti* mosquitoes. Wings are bi-dimensional rigid structures well suited for morphometric work. 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. The objective of our study was to explore the technique of geometric morphometrics in relation to strain distinctiveness.

MATERIALS AND METHODS

Female *Aedes aegypti* mosquitoes from 4 different localities in Thailand (Bangkok: BK; Chanthaburi: CB; Chiang Mai: CM; and Ratchaburi: RB Provinces) were used in the present study. About 150 larvae were maintained in 28 x 34 x 8 cm plastic trays half-filled with clean water. The adult mosquitoes from BK, CB, CM, and RB were reared in insectariums at 28-30°C, 80% relative humidity for 95, 564, 260, and 17 generations, respectively, at the Department of Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Mosquito wings were detached from the thorax, placed on a clean microscopic slide and then secured with mounting solution (Euparal) under a cover slip. 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 16 landmarks as shown in Fig 1.

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Fig 1—Landmarks on the *Aedes aegypti* mosquito wing used for geometric morphometric analysis. (TpsDig software, Rohlf JF, available at: life.bio.sunysb.edu/morph).

The coordinates of the landmarks were digitized using TPSdig (Rohlf J, Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794-5245, available at <http://life.bio.sunysb.edu/morph/>). Collection of the raw coordinates of each landmark was performed on the computer screen in units of pixels for further 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 (Rohlf, 1990). Centroid size, an isometric estimator of size, was derived from the square root of the summation of the squared distances between the center of the objects and each landmark. The centroid size was therefore compared between lines using the Wilcoxon non-parametric test and Bonferroni correction. After log-transformation, it was also regressed on the number of generations spent in the laboratory by each line.

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 (Bookstein, 1991; Rohlf and Marcus, 1993; Rohlf, 1996). Procrustes superimposition, centroid sizes, and partial warps were computed using MOG (Dujardin JP, available at <http://life.bio.sunysb.edu/morph>) and TPSregr softwares (Rohlf J, Department of Ecology and Evolution, State

University of New York, Stony Brook, NY 11794-5245, available at <http://life.bio.sunysb.edu/morph>). The shape variables were computed pooling the four laboratory lines and were used as inputs for discriminant analysis (DA). A reclassification of the individuals was computed on this base. The relationship between shape divergence and differences in laboratory lifetime was tested by linear regression, in the same way as those for size variation. Independence between shape and size was estimated by linear regression of individual discriminant scores against corresponding sizes. The statistical significance of each DA and corresponding generalized distance was estimated by 1,000 permutation tests: at each permutation, randomly chosen individuals were exchanged between lines and the discriminant analysis was re-computed. Statistical analyses were done using PAD software (Dujardin JP, available at <http://life.bio.sunysb.edu/morph>), JMP® (SAS Institute, 1997), and Stata® (StataCorp 2001).

RESULTS

Ae. aegypti is a small, black insect with white spots on the body and white rings on its legs. No area of the body can provide an obvious set of anatomical landmarks for geometric analysis, except the wings. The wings are flat, rigid structures presenting a stable pattern of venation, where crosses between veins represent well-located anatomical landmarks. The presence of scales, however, may represent a technical problem when one wants to localize the exact position of some vein crosses. Instead of trying to remove these scales which might have some interesting taxonomic properties, we used a phase contrast microscope which improved drastically the readability of the wing (Fig 1). The clearest set of the 16 landmarks was chosen from venation intersections and the junction of each vein to the wing outline (costa). The connections between the 16 landmarks provided a polygon which represented the comparative size and shape of the mosquitoes' wings. On the whole, size was significantly different between the lines (Wilcoxon test, $p < 0.001$), except between the BK and CM lines ($p > 0.05$). Its relationship with generations of each line was sig-

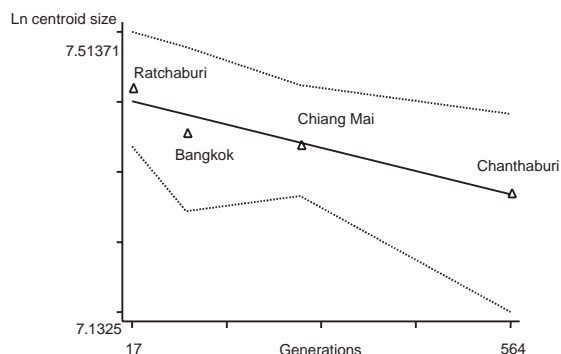


Fig 2—Relationship between wing sizes and number of generations of each mosquito line ($p=0.043$, $R^2=0.91$) Ln: natural logarithm

nificant (Fig 2), indicating a decrease in size with lifetime spent in the laboratory ($p=0.043$, $R^2 = 0.91$).

Shape, as described by geometric morphometrics, was able to accurately distinguish the four mosquito lines. All the discriminant analysis on shape was significant (permutation tests, $p<0.001$). The generalized distances indicated the oldest line (CB) as the most differentiated one, however we could not detect any correlation between shape divergence and generation differences ($p=0.88$, $R^2=0.00$). Reclassification of individuals was almost perfect ($Kappa = 0.96$, Table 1). Only one individual from each line was misclassified, except for the RB line with 2 misclassified wings, giving a total of 97% correct attribution. However, the discriminant factors (DF) on which the reclassification was based were not free of some allometric effects: size still contributed significantly to their variation (15% of DF1 variation, 11% and 18% for DF2 and DF3, respectively).

DISCUSSION

Although the size of a mosquito species has a genetic basis, environmental factors, such as temperature, nutrition, larval density and salinity also affect the size of individuals (Clements, 1992). Under laboratory conditions, generation time is as short as 2 weeks inside a small housing cage which could possibly affect the size of the mosquitoes. Application to field samples could also provide precise information on wing size, usually measured as wing length, an estimator of adult body size, and fecundity in *Aedes* mosquitoes (Blackmore and Lord, 2000; Armbruster and Hutchinson, 2002). Mosquito size may also play a role in whether the mosquito is as an effective pathogen carrier, as larger females can ingest greater volumes of blood. Morphometric measures are also best for weight determinations, which are often misleading in mosquitoes (Briegel, 2003).

Even if each line had a different geographic origin, we cannot favor the hypothesis of a geographic differentiation of *Ae. aegypti* in the present observation. The number of generations was indeed very high, so that effects on morphological variation due to genetic drift in the laboratory were likely. If simple isolation could generate such marked differences, we would expect natural populations to show a comparable level of divergence in the same area of the wings. Physical isolation is expected for geographic populations of *Ae. aegypti* because of its relatively low dispersal behavior, as females generally fly 100-500 m, depending on the availability of oviposition sites and blood meals (Trpis and Hausermann, 1986; Muir and Kay, 1988).

Table 1
Classification of mosquito lines according to the shape variables.

| Mosquito lines | Bangkok | Chanthaburi | Chiang Mai | Ratchaburi | Observed |
|----------------|---------|-------------|------------|------------|----------|
| Bangkok | 46 | 0 | 0 | 0 | 46 |
| Chanthaburi | 0 | 42 | 0 | 1 | 43 |
| Chiang Mai | 0 | 1 | 45 | 1 | 47 |
| Ratchaburi | 1 | 0 | 1 | 42 | 44 |
| Total | 47 | 43 | 46 | 44 | 180 |

Kappa measures the degree of agreement. Kappa 0.962949; Standard error 0.016336.

Variation in wing geometry between populations may provide insights into population structure, ecology and species complex. However, our results have delineated the existence of bio-geographical structures within the *Aedes* population. Further studies on field collected samples are in progress to clarify the differences among geographic strains. Considering the importance of mosquito wings in insect behavior and physiology, the differences could provide useful information on vector distribution and disease control.

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