Temporal variation of wing geometry in Aedes albopictus

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Although native to the tropical and subtropical areas of Southeast Asia, Aedes albopictus is now found on five continents, primarily due to its great capacity to adapt to different environments. This species is considered a secondary vector of dengue virus in several countries. Wing geometric morphometrics is widely used to furnish morphological markers for the characterisation and identification of species of medical importance and for the assessment of population dynamics. In this work, we investigated the metric differentiation of the wings of Ae. albopictus samples collected over a four-year period (2007-2010) in São Paulo, Brazil. Wing size significantly decreased during this period for both sexes and the wing shape also changed over time, with the wing shapes of males showing greater differences after 2008 and those of females differing more after 2009. Given that the wings play sex-specific roles, these findings suggest that the males and females could be affected by differential evolutionary pressures. Consistent with this hypothesis, a sexually dimorphic pattern was detected and quantified: the females were larger than the males (with respect to the mean) and had a distinct wing shape, regardless of allometric effects. In conclusion, wing alterations, particularly those involving shape, are a sensitive indicator of microevolutionary processes in this species.

Key words: geometric morphometrics - microevolution - Culicidae - mosquitoes - Aedes albopictus

Aedes (Stegomyia) albopictus (Skuse 1894) (Diptera: Culicidae), commonly known as the Asian tiger mosquito, originated in the forests of Southeast Asia and has now spread to America, Africa, the Middle East and Europe, largely due to human activities (Gratz 2004, WHO 2012). This species is an epidemiologically important vector for the transmission of many viral pathogens, such as dengue fever, yellow fever virus, West Nile virus and St. Louis encephalitis virus (Fernández et al. 2012). This mosquito was also confirmed to have been the primary vector for Chikungunya virus, a pathogen originally from Africa, during the 2007 outbreaks in Italy and Gabon (Paupy et al. 2009).

The spread of Ae. albopictus throughout the world began in the 1970s (Benedict et al. 2007) and this mosquito was first recorded in Brazil in the 1980s (Forattini 1986). This species is currently considered the most invasive mosquito in the world and control is difficult owing to the rapid reproduction of the species and its capacity to adapt to different environments.

The efficacy of the control procedures for Aedes spp depends on many contextual features, with microevolution representing one relevant limiting factor. For instance, the evolutionary changes within a species may produce populations that are resistant to insecticides (Braga et al. 2004, da-Cunha et al. 2005, Gómez et al. 2011, Fontoura et al. 2012) or may alter their vectorial capacity (Almeida et al. 2005, Freitas 2010). Although microevolution has been investigated in the congeneric dengue vector Aedes aegypti (Lourenço-de-Oliveira et al. 2004, Costa-Ribeiro et al. 2007, Vidal & Suesde 2012), the knowledge of microevolution in Ae. albopictus remains anecdotal.

Although the traditional approach to investigating the microevolution of culicids is based on genotypic markers (Lenormand et al. 1999, Lenormand & Raymond 2000, Corley 2005), there is currently an increasing tendency to use phenotypic characters, primarily wing shape, to describe microevolutionary patterns (Dujardin 2008). The wings of culicids have been geometrically characterised using the low-cost “geometric morphometrics” method (Jirakanjanakit et al. 2008, Dujardin et al. 2009, Henry et al. 2010, Devicari et al. 2011, Vidal et al. 2011). Microevolutionary studies of the wings are feasible because of the strong heritability of the relevant phenotypic traits. Moreover, the wings are nearly bidimensional and can be used for geometrical and multivariate analyses.

Given that wing geometry is a powerful microevolutionary marker and that microevolutionary studies of Ae. albopictus have not been conducted, our primary objective was to investigate the temporal variation in wing size and shape in samples of this species collected from the same location over a four-year period.

MATERIALS AND METHODS

Mosquitoes - Larvae of Ae. albopictus were collected from traps consisting of four open quarter-litre water containers (for a total volume of 1,000 mL). A group of four containers (with 1 ovitraps per container) was placed in a one square metre patch in each of two locations in a park in São Paulo (SP), Brazil (23.566°S 46.719°W), with a 200 m distance between each. Four chronological samples were collected during the rainy seasons from...
2007-2010 and were maintained in the laboratory under standard temperature and humidity conditions (25 ± 1°C; 80 ± 10%). The emerging adults were identified at the species level (Forattini 2002) and were preserved in 70% ethanol. We analysed the left wings of 22 individuals collected in 2007, 53 individuals collected in 2008, 70 individuals collected in 2009 and 49 individuals collected in 2010.

**Geometric data acquisition** - The left wing of each individual was detached from the thorax and mounted with Canada balsam (Sigma, St. Louis, MO, USA) between a slide and a coverslip. Images of the wings were obtained using a Leica S6 stereoscopic microscope equipped with plain lenses, which diminish image distortion. The coordinates of 18 landmarks represented by vein intersections (Fig. 1) were obtained using TpsDig version 1.4 (Rohlf 2006).

**Morphometric analyses** - Chronological samples were compared with respect to wing size and shape to evaluate the morphological variation during the study period. Owing to the sexual dimorphism of the wings, the males and females were analysed separately.

To assess the overall wing sizes, the isometric estimator known as centroid size (Bookstein 1991) was computed from the landmark coordinates with the MOG V. 82 programme (Dujardin 2010). The scores of the centroid sizes were compared among samples with a parametric ANOVA and a post hoc Tukey test or T-test using Statistica 7.0 software (StatSoft, Tulsa, OK, USA).

The generalised least-squares Procrustes superimposition algorithm (Rohlf 1990) was used to produce shape variables (partial warps) and the canonical variations were used to examine the differences in the shape of the left wing of samples collected in the different years. The graphics were obtained using MorphoJ software (Klingenberg 2011) and the most influential landmarks were obtained with the COV programme (Dujardin 2010).

To explore the relationships among the *Ae. albopictus* samples collected over four years, pairwise Mahalanobis distances between the samples were calculated and compared using nonparametric permutation tests (10,000 randomisations) with MorphoJ software. To illustrate the morphological divergence among the samples, neighbour-joining phenograms were constructed using the PHYLIP - Phylogeny Inference Package version 3.6. (distributed by the author, J Felsenstein, 2005, Department of Genome Sciences, University of Washington, Seattle, WA).

Allometry was estimated with a multivariate regression of the Procrustes coordinates vs. the centroid size and the significance of the allometry was assessed by a permutation test with 10,000 randomisations using MorphoJ software. Reclassification tests were performed using the Mahalanobis distances as estimators of the metric distance. The distances were computed on discriminant axes that were estimated without the individual (wing) to be classified; the individual was only introduced afterwards (cross-validated classification, MorphoJ software (Klingenberg 2011). Voucher specimens were deposited in the entomological collection of the Butantan Institute, SP.

**RESULTS**

**Size variation** - The mean centroid sizes for the females (in mm) were 2.37 (in 2007), 2.67 (in 2008), 2.33 (in 2009) and 1.95 (in 2010) and those for the males were 2.33 (in 2007), 2.05 (in 2008), 1.96 (in 2009) and 1.78 (in 2010). Within each sex, all pairwise size comparisons indicated significant differences (ANOVA; p < 0.05), except for the transition between 2008-2009 for the males. The females were larger than the males in 2008 and 2009 (t test; p < 0.05); the descriptive statistics for size are presented in Fig. 2. In terms of allometry, the contribution of size to the shape variation was statistically significant (p < 0.001; both sexes): the proportion of the variance in shape explained by size was 6% for females and 5% for males. Although weak, the allometry was removed from the shape analyses.

**Shape variation** - After the removal of the allometry, a canonical variate analysis revealed differences in wing shape over the years 2007-2010 (Fig. 3). The greatest divergence occurred between 2009-2010 (for both sexes), whereas the least divergence occurred between 2007-2008 (for males).

The comparison of the wing shape consensus after the Procrustes superimposition revealed a landmark dis-
placement over the study period (Fig. 4). The most influential landmarks were #10 for the females and #2 for the males (Fig. 4).

The scores of the Mahalanobis distances between the yearly samples ranged from 3.04-5.33 (females) and from 3.42-5.00 (males). The phenograms of those pairwise distances (Fig. 5) corroborated the similarity patterns among the chronological groups observed in the canonical variate analysis (Fig. 3). Permutation tests revealed that the shape divergence was extremely significant (p < 0.0001) in all comparisons. The cross-validated reclassification accuracy based on the Mahalanobis distances ranged from 50-100% among the females and from 66.6-92.6% among the males (Table), with the scores increasing in a parallel fashion between the sexes during the four-year study period.

Wing shape sexual dimorphism was observed in all samples after the removal of the allometric effect of size, as shown by the morphospaces of canonical variables in Fig. 6 (only years 2009 and 2010 are depicted). Similar results were obtained if the same analysis was repeated without the removal of allometry (Fig. 6).

**DISCUSSION**

To our knowledge, this is the first description of phenotypic variation in a natural population of *Ae. albopictus* over time. The results showed that wing morphology in *Ae. albopictus* may change over periods as short as one year and that this process is continuous. These results led us to believe that wing geometry is sensitive to microevolutionary processes, an interpretation that is in accordance with that predicted by Dujardin (2008) for culicids. Similar observations have also been reported for hemipteran insects (Dujardin et al. 2009).

Of the main primary components of wing form, it is probable that the shape is more directly related to microevolution in the present study. Given that shape in *Aedes* spp has been shown to be genetically determined and

Fig. 3: morphological spaces of first two canonical variables (CV)1 and 2 originated from the comparison of wing shape across the years. Between brackets, the relative contribution of each CV.

Fig. 4: wing shape consensus after Procrustes superimposition in each year aligned on landmark 1. Comparisons illustrate shape transition between 2007-2008, 2008-2009 and 2009-2010. Arrows indicate the landmarks of most influence on wing variation.
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Fig. 5: neighbour-joining phenograms of Mahalanobis distances between each yearly sample, regarding wing shape. *Culex nigripalpus* was used as outgroup.

### TABLE

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<th>Comparison</th>
<th>Year</th>
<th>Female (%)</th>
<th>Male (%)</th>
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<tr>
<td>2007 vs. 2008</td>
<td>2007</td>
<td>50</td>
<td>66.6</td>
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<td></td>
<td>2008</td>
<td>73</td>
<td>73.3</td>
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<tr>
<td>2008 vs. 2009</td>
<td>2008</td>
<td>81.6</td>
<td>80</td>
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<td></td>
<td>2009</td>
<td>77.1</td>
<td>77.1</td>
</tr>
<tr>
<td>2009 vs. 2010</td>
<td>2009</td>
<td>91.4</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>100</td>
<td>92.6</td>
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Fig. 6: morphological spaces of first two canonical variables (CV)1 and 2 originated from the comparison of wing shape between 2009-2010. A: without remotion of allometry; B: after remotion of allometric effect. Between brackets, the relative contribution of each CV.
ful characteristic for detecting microevolutionary patterns and processes. Geometric morphometric analysis remains an effective and inexpensive tool to detect recent phenotypic and, arguably, genotypic changes. This tool is particularly useful for investigating *Ae. albopictus*, which is an invasive and plastic vector. As a result of this investigation, some new complex questions have arisen, confirming that this approach opens promising opportunities for further research.

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**REFERENCES**


