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Comparison of wing geometry data and genetic data for assessing the population structure of *Aedes aegypti*

Paloma Oliveira Vidal, Lincoln Suesdek *

Instituto Butantan – Seção Parasitologia, Av. Vital Brazil, 1500 São Paulo, SP, Brazil
Biologia da Relação Patógeno-Hospedeiro, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil

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ABSTRACT

Aedes aegypti is the most important vector of dengue viruses in tropical and subtropical regions. Because vaccines are still under development, dengue prevention depends primarily on vector control. Population genetics is a common approach in research involving *Ae. aegypti*. In the context of medical entomology, wing morphometric analysis has been proposed as a strong and low-cost complementary tool for investigating population structure. Therefore, we comparatively evaluated the genetic and phenotypic variability of population samples of *Ae. aegypti* from four sampling sites in the metropolitan area of São Paulo city, Brazil. The distances between the sites ranged from 7.1 to 50 km. This area, where knowledge on the population genetics of this mosquito is incipient, was chosen due to the thousands of dengue cases registered yearly. The analysed *loci* were polymorphic, and they revealed population structure (global $F_{ST} = 0.062$; $p < 0.05$) and low levels of gene flow (Nm = 0.47) between the four locations. Principal component and discriminant analyses of wing shape variables (18 landmarks) demonstrated that wing polymorphisms were only slightly more common between populations than within populations. Whereas microsatellites allowed for geographic differentiation, wing geometry failed to distinguish the samples. These data suggest that microevolution in this species may affect genetic and morphological characters to different degrees. In this case, wing shape was not validated as a marker for assessing population structure. According to the interpretation of a previous report, the wing shape of *Ae. aegypti* does not vary significantly because it is stabilised by selective pressure.

1. Introduction

Aedes (*Stegomyia*) *aegypti* (L.) is native to afrotropical areas and has been spread worldwide by human activities (Belkin, 1962). Its presence and dispersal are considered a public health concern because this species is recognised as the main vector for yellow fever, chikungunya and dengue viruses. Approximately 50 million cases of dengue occur annually worldwide (WHO, 2011), and approximately 80% of the cases in the Americas were reported in Brazil during the 1990s (Schatzmayr, 2000).

Reducing *Ae. aegypti* population is the primary way to fight dengue viruses because no efficient therapies are available and a vaccine for dengue is still under development. Strategies to control mosquitoes have been developed for decades, including chemical and biological insecticides, and, more recently, transgenic mosquitoes (Speranca and Capurro, 2007; Yakob et al., 2008).

Although *Ae. aegypti* was eradicated in Brazil in the 1950s, it was re-introduced in the 1970s (SUCEN, 2011). Since then, it has become the principal vector for dengue viruses in this country. This mosquito is currently thoroughly distributed across the entire country (SVS, 2010). Further aggravating this situation in Brazil, cases of insecticide resistance and high vectorial capacity for dengue viruses have also been reported for these mosquitoes (Braga et al., 2004; Da-Cunha et al., 2005; Macoris et al., 2003, 2007). Moreover, disordered urbanisation and human transit facilitate the spread of vectors and the associated viruses (Gubler, 1998; Herrera et al., 2006).

Understanding the dispersal of *Ae. aegypti* is a central question in surveys focusing on controlling the vector because vector dispersal is a major determinant of the spread of pathogens (Wang et al., 2001; Costa-Ribeiro et al., 2006; Urdaneta-Marquez and Failloux, 2011). Equally important is the quantification of the exchange of individuals between demes. Studies carried out in Brazil have shown that imagoes do not disperse widely. The males and females usually travel up to 100 and 500 m, respectively, if sufficient blood sources and oviposition sites are available. However, in the absence of such resources, they are capable of flying longer distances (Maciel-de-Freitas and Lourenço-de-Oliveira, 2009).

Dispersal can also be indirectly estimated by population genetics approaches. Microevolutionary patterns may be informative for

* Corresponding author at: Instituto Butantan – Seção Parasitologia, Av. Vital Brazil, 1500 São Paulo, SP, Brazil. Tel.: +55 11 3726 7222.
E-mail address: linrocha@butantan.gov.br (L. Suesdek).

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estimating gene flow between locations of epidemiological relevance (Lourenço-de-Oliveira et al., 2004; Costa-Ribeiro et al., 2007). The description of the population structure and genetic dissimilarities within this anthropophilic and synanthropic species permits the evaluation of the influence of human activities on the movement of mosquitoes (Costa-Ribeiro et al., 2006; Scarpassa et al., 2008).

Among the classical genetic markers used in population characterisation are SSRs (simple sequence repeats), which are also known as microsatellites. These microsatellites are often sensitive to genetic fluctuations that have occurred within short periods or over small geographical scales. Another promising biological marker is wing morphology. Wing shape in insects is heritable, is evolutionarily informative and can be described by morphometrics (Bitner-Mathé and Klaczko, 1999; Dujardin, 2008). Using wing morphologies to determine population structure has been encouraged by some authors who consider this method to be a low-cost alternative for the preliminary estimation of population structure (Dujardin, 2008; Morais et al., 2010).

The concomitant use of genetic data for validating the shape metrics in population structure investigations may be useful in preliminary surveys. Wing shape variation has been increasingly documented in *Ae. aegypti* (Dujardin et al., 2010; Henry et al., 2010); however, comparisons including genetic data obtained from the same sample sets are still scarce. Among the few attempts to decipher the association between morphological and molecular variations, Paupy et al. (2010) showed that body peculiarities of *Ae. aegypti formosus* do not reflect the population genetic structure. Morphological variability can be measured by the Q_{ST} index. In some cases, the values approximate the genetic F_{ST} index. Despite the usefulness of the Q_{ST} index, employment of this index is quite rare.

To evaluate the potential of wing morphometric analysis for population genetic studies of *Ae. aegypti* on a microgeographic scale, we compared the morphometric output to the genetic information yielded by analysis of five microsatellite *loci*. Both analyses were performed for the same population samples collected from four sites that ranged from 7.1 to 50 km away from each other in São Paulo, Brazil. This city is a large urban agglomeration with 8974 registered autochthonous dengue cases in 2010 (CVE, 2011).

2. Materials and methods

2.1. Mosquito samples

Brazilian samples of *Ae. aegypti* mosquitoes were collected from four sites in the greater São Paulo area (Fig. 1 and Table 1): Butantã (BUT), a district situated to the west of the city of São Paulo, and Guarulhos (GUA), Osasco (OSA) and Suzano (SUZ), which are three municipalities situated in the metropolitan area of São Paulo. These four locations were chosen due to their epidemiological relevance. Autochthonous cases of dengue in 2010 numbered 189, 1206, 316 and 3 at each site, respectively (SMS, 2011; CVE, 2011). The two closest sites are BUT and OSA, which are separated by a distance of 7.1 km. The maximal distance was 50 km between OSA and SUZ. Despite the long distances between some locations, all of them are placed in a single urban patch and are interconnected by a dense and homogeneous street network. To avoid sampling sibling individuals at the different sites, larvae and pupae were collected from houses that were least 200 m apart. In each home, at least two domestic water containers were sampled when available. Even without this precaution, the likelihood of collecting a sibling-enriched sample was low because females tend to avoid laying all of their eggs in the same container when others are available (Colton et al., 2003). Containers consisted of artificial water receptacles that were naturally filled with approximately 500 ml of rainwater. Immature stages of the mosquitoes were kept in the laboratory under standard conditions of temperature and humidity (25 ± 1 °C; 80 ± 10%), and the emerging adults were species-identified and stored in liquid nitrogen. All individuals subjected to genetic analysis (n = 116) were also subjected to morphometric analysis (n = 210; Table 1).

Fig. 1. Partial geographic map of Brazil (left) and the greater São Paulo (right) showing the borders of some municipalities. Black circles indicate locations where *Ae. aegypti* populations where collected and letters represent the respective toponyms: **B**utantã, **G**uarulhos, **O**sasco, **S**uzano.

2.2. Geometric morphometric analysis

Wings were detached from the thorax and mounted with Entellan (Sigma, St. Louis, MO, USA) on a microscope slide with a coverslip. Images of wings were captured with a Leica 320 digital camera coupled to a Leica S6 stereoscope with plain optics, which eliminated image aberrations. On these pictures of Brazilian mosquitoes, 18 landmarks (Fig. 2) were digitised using the TPSdig V.1.40 software (Rohlf, 2006). Additional wing pictures of *Ae. aegypti* specimens from other countries, including the United States of America (USA), Colombia (COL) and Thailand (THA), were taken from the CLIC image bank (http://www.mpl.ird.fr/morphometrics/; Henry et al., 2010), and their landmarks were digitised and included in some analyses.

Standard procedures for geometric morphometric analysis were employed as follows. Global wing size was assessed from the isometric estimator centroid size (Bookstein, 1991) that was derived from the coordinates, obtained using the TpsRelw 1.44 program (Rohlf, 2006). The sizes were then statistically compared between samples using a parametric ANOVA test. The generalised least-squares Procrustes superimposition algorithm (Rohlf, 1996) was used to produce shape variables (partial warps), and the principal components (relative warps; Bookstein, 1991) were used to compare population samples. To assess the degree of similarity between populations, pairwise Mahalanobis distances between populations were calculated using PAD software (Dujardin, 2002) and plotted in neighbour-joining trees using the PHYLIP package (Felsenstein, 2005). To statistically validate the comparisons, the significance of the metric disparity of the partial warps between populations (Brazilian and foreign samples) and the Q_{ST} (quantitative differentiation) estimates were tested by nonparametric permutation tests (2000 iterations each) using COV software (Dujardin and Slice, 2006).

Pooled individuals of Brazilian samples were reclassified according to their similarity to each group using the Mahalanobis distances as estimators of metric distance. Distances were computed on discriminant axes estimated without the individual (wing) to be classified. The individual was only introduced afterwards (validated classification, PAD software Dujardin, 2002). Voucher specimens were deposited in the Butantan Institute insect collection (São Paulo, Brazil), and wing images were deposited in the CLIC image bank.