



Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Climate associated size and shape changes in *Aedes aegypti* (Diptera: Culicidae) populations from Thailand

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ARTICLE INFO

Article history:

Received 6 September 2009

Received in revised form 24 December 2009

Accepted 11 January 2010

Available online 1 February 2010

Keywords:

Aedes aegypti

Climate

Size

Shape

Relative humidity

Thailand

Dengue

ABSTRACT

In spite of the adult body size variability of *Aedes aegypti* (Linnaeus) and its likely association with life history and vectorial capacity, the causes of size variation itself have been only partially identified. In particular, possible important factors such as climatic variation have not received much attention.

The objective of this 2-year study was to describe from field collections the relationship of *Ae. aegypti* metric properties with available climatic data. The study took place in a dengue hyperendemic area of Thailand. Fourth instar larvae (L_4) and pupae were collected from the same breeding places allowing the comparisons between seven successive collections, four in 2007 and three in 2008. Climatic data were relative humidity (RH) and temperature (T). They were considered for the periods covering either the pre-imaginal development or, assuming heritability of size, the previous generation. The pre-imaginal period was further subdivided into embryonic and larval phases of development. Size was estimated by traditional and geometric techniques, the latter based on 18 landmarks collected at the intersections of veins also allowing estimation of shape.

The shape variation of the wing followed similar patterns as for size and was shown to be a passive allometric change. No significant correlation of size or shape could be disclosed with T . In contrast, significant correlation with RH was found during two periods of examination: (i) the period affecting the generation previous to the time of collection, suggesting possible selective mechanisms on genitors, and (ii) the one occurring during pre-imaginal development. The subdivision of the latter into embryonic and larval phases allowed to evidence a possible selecting effect on embryonic development. The selection would act through the resistance to water loss which is known to depend on the relative surface of the cuticle.

In conclusion, our data highlight the importance of the emerged period of *Ae. aegypti* eggs as a critical time for the size of future adults, and point to the relative humidity as the likely selecting factor.

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1. Introduction

The mosquito *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) is the primary vector of three important viral diseases, namely dengue, yellow fever and chikungunya, and is capable of transmitting a number of others. In the present study, we examined the seasonal variation of wing metric properties in specimens collected from an active focus of dengue disease in central Thailand. Both size and shape of the wings were considered.

Size, understood as the global size of the insect, has been associated with many fitness components in the genus *Aedes*

(Nasci, 1986, 1990; Packer and Corbet, 1989; Nasci and Mitchell, 1994; Renshaw et al., 1994; Xue et al., 1995a,b; Sumanochitrapon et al., 1998; Bosio et al., 1998; Frankino and Juliano, 1999; Scott et al., 2000; Blackmore and Lord, 2000; Briegel and Timmermann, 2001; Armbruster and Hutchinson, 2002; Maciel de Freitas et al., 2007; Leisnham et al., 2008), as well as in many other insects (Dujardin, 2008; Honek, 1993).

Several authors claimed that the size of adult mosquitoes may determine their effectiveness as a vector. According to Sumanochitrapon et al. (1998), larger *Ae. aegypti* could be more involved in dengue transmission than smaller ones. In the genus *Aedes*, larger size has been associated with higher blood-feeding frequency (Xue et al., 1995a), longer survival (Nasci, 1986; Packer and Corbet, 1989; Lounibos et al., 1990; Briegel and Timmermann, 2001) and higher fecundity (Lyimo and Takken, 1993; Blackmore and Lord, 2000; Lounibos et al., 2002), all factors liable to affect vectorial

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capacity. However, contrary to previous reports, longevity and dispersal of *Ae. aegypti* have not been necessarily associated with larger sizes (Maciel de Freitas et al., 2007), and smaller *Ae. aegypti* have been shown to blood-feed more successfully (Nasci and Mitchell, 1994), and more frequently (Scott et al., 2000; Xue et al., 1995b), inferring that small mosquitoes may be better vectors than larger ones (Maciel de Freitas et al., 2007). Moreover, experimental infection protocols using laboratory lines could not establish any relationship between body size and infection rate of *Ae. aegypti* (Bosio et al., 1998; Schneider et al., 2007), and a recent study on *Aedes albopictus* failed to detect commonly reported association of body size with blood-feeding frequency and survival (Leisnham et al., 2008).

Regardless to the kind of relationships size may have with vectorial capacity or mosquito dispersal, the understanding of the likely causes of size changes in natural conditions is an important chapter in the biology of an insect (Day and Ramsey, 1990; Janousek and Olson, 2006; Jirakanjanakit et al., 2007).

The shape of the wings of *Ae. aegypti* has seldom been considered (Jirakanjanakit and Dujardin, 2005). Significant changes have been produced experimentally through genetic drift, and they were shown to be independent from size variation (Jirakanjanakit et al., 2008). However, the modification of shape in relation to larval density and food concentration has been shown to represent a passive consequence of size variation, and it was different according to the cause of size change (Jirakanjanakit et al., 2007). To our knowledge, the effect of field climatic conditions on shape has never been analyzed.

Both size and shape were derived from the same set of anatomical landmarks. Traditionally, to estimate the size of adult mosquitoes, measurements of the wing length have been routinely used.¹ The “centroid size” (CS), as defined in geometric morphometrics (Bookstein, 1991), has been suggested as a more informative estimate of body size (Jirakanjanakit and Dujardin, 2005; Jirakanjanakit et al., 2007, 2008). The CS is able to detect size variation in different directions, as many as landmarks used to characterize the wing. Furthermore, the same data used to compute the CS of the wing can also be used to quantify its shape. This modern approach is a powerful and cheap characterizing tool for many organisms, including medically important insects (Dujardin and Slice, 2007). In the present study, to make the link between tradition and modernity, we used and compared both linear and geometric estimates of wing size.

Only two longitudinal studies (Scott et al., 2000; Tsuda and Takagi, 2001) examined the possible relationship between climatic parameters and metric properties of *Ae. aegypti*. These studies were limited to temperature, confirming a probable Bergmann's effect (negative correlation with size), and did not consider the relative humidity (RH). We examined *T* and RH across a 2-year longitudinal study. Our objective was to evaluate the relative influence of both factors on size, as well as on shape of natural populations of *Ae. aegypti*.

If the metric properties of *Ae. aegypti* have an impact on dengue virus transmission dynamics and/or on vector dispersal (Maciel de Freitas et al., 2007; Alto et al., 2008), it is epidemiologically relevant to understand the natural causes of their variation.

2. Materials and methods

2.1. Study area

This study was conducted in the sub-district Muang, the capital of Nakhon Ratchasima Province (Thailand), located 250 km

northeast of Bangkok (14°95'N 102°03'E). According the Annual Epidemiological Surveillance report of the Minister of Public Health (MOPH, <http://203.157.15.4/Annual/TotalAnnual.html>), Nakhon Ratchasima has an average dengue incidence of 108.14 cases/100,000 inhabitants (MOPH 2000–2006).

2.2. Mosquito collection

Three study sites were used, the same ones repeatedly surveyed in February, June, August and November 2007, February, June and November 2008. They were located in two different geo-referenced blocks at ca. 2 km apart. Each study site corresponded to larval sites found in and around houses where one or more family members had been reported with dengue hemorrhagic fever (DHF).

Fourth instar larvae (L4) and pupae were collected from natural and human made water containers. Collected larvae and pupae were transported together with the water and material residues from the larval habitat and maintained in an insectary until adult emergence.

2.3. Sample preparation and identification

The collected L4 and pupae were reared up to adult stage. Species identification followed the taxonomic keys of Huang (1979). Both wings of individual female *Ae. aegypti* were detached from the thorax, mounted on microscopic slide and secured with Euparal™. The slides were positioned on the phase contrast microscope with a 4× lens. A digital camera (2 mega pixels) was used to capture the wing images. The wings were always put at the center of the visual field to reduce the risk of peripheral optical distortion (Caro-Riaño et al., 2009) and left side only was used to avoid interference in the analyzes of within individual correlation.

2.4. Coordinates collection and related analyzes

A set of 18 landmarks covering the wing surface was selected (Fig. 1). For comparing overall wing size between groups, we used the isometric estimator known as “centroid size” (CS) derived from coordinates data. It is defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein, 1991). An additional estimate of size was provided by the distance between L7 and the proximal edge of the costal scales to estimate its length (*L*) (Fig. 1). Observed coordinates of landmarks were corrected for artifactual variation due to position and orientation of the wing, and scaled for size (Procrustes superposition). From these residual coordinates, shape variables (“partial warps”, or PW) were produced and their principal components (the so called “relative warps”, or RW) compared between groups (Rohlf, 1990). For each month, an “average form” was computed as the average relative warps (RW) of the corresponding left wings.

2.5. Climate variables and related analyzes

The mean temperature (*T*) and relative humidity (RH) were calculated using the mean daily *T* and RH recorded at a point-station in Muang district by the National Institute of Meteorology (NIM) during the following time periods (Fig. 2):

1. The previous generation: day –35 to day –15, i.e. a 20 days time period before the supposed oviposition day (–15).
2. The pre-imaginal period: day –15 to day 0, i.e. 15 days before collection (day 0).
3. The embryonic development period: day –15 to day –10, i.e. 5 days after oviposition when the eggs were supposed to be emerged.

¹ The wing length is commonly taken from the proximal edge of the costal scales to the most distal point on the lateral margin excluding the wing fringe.