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Wing shape of dengue vectors from around the world.

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ABSTRACT

Wing shape is increasingly utilized in species identification and characterization. For dengue vectors *Aedes aegypti* and *Aedes albopictus*, it could be used as a complement for ensuring accurate diagnostic of damaged specimens. However, the impact of world migration on wing shape is unknown. Has the spread of these invasive species increased shape variation to the extent of producing interspecific overlapping? To answer this question, the geometric patterns of wing venation in *Ae. aegypti* and *Ae. albopictus* were compared between natural populations from the Pacific Islands, North and South America and South East Asia. The geometry of 178 female and 174 male wings were described at 13 anatomical landmarks, and processed according to Procrustes superposition, partial warps and subsequent multivariate analyzes. The variation of shape did not produce significant interspecific overlapping. Regardless of geographic origin, *Ae. aegypti* was recognized as *Ae. aegypti* and *Ae. albopictus* as *Ae. albopictus*. Some significant geographic differentiation was observed in Colombia for *Ae. aegypti* and in Thailand for *Ae. albopictus*. Globally, the morphology of these mosquitoes, for both size and shape, appeared well preserved. Strong canalizing mechanisms could account for the observed patterns of relatively uniform morphology, which could also be attributed to sporadic, recurrent mixing of populations, thwarting phenotypic drift.

1. Introduction

Aedes aegypti (Linnaeus) (Diptera: Culicidae) and *Ae. albopictus* (Skuse) (Diptera: Culicidae) are two of the most important mosquito vectors of human diseases. *Ae. aegypti* is the main vector of urban yellow fever, a disease that during the eighteenth and nineteenth centuries caused devastating epidemics in tropical America, Northern American coastal cites, and Europe (Staples and Monath, 2008). *Ae. aegypti* and *Ae. albopictus* are the primary and secondary vectors, respectively, of dengue virus (DF/DHF), possibly the most important arbovirus of the twenty first century (Gubler, 2002). They also transmit the chikungunya virus, a virus that originated from Africa, is re-emerging in India and Asia and has recently caused outbreaks in southern Europe, and Italy (Chastel, 2005; Josseran et al., 2006; Rezza et al., 2007).

Both mosquito vectors have undergone transcontinental migration (Gubler, 1997) and were likely exposed to large variations in environment. The species are morphologically close, and their respective adaptation to new continents could have affected their morphological distinction. In this study we explore empirically the shape canalization in *Ae. aegypti* and *Ae. albopictus* using geometric morphometrics on wing venation: were the canalization mechanisms strong enough to oppose external forces?

The anthropogenic worldwide migration of *Ae. aegypti* began a few centuries ago, while the spread of *Ae. albopictus* initiated within the last few decades. The domestic *Ae. aegypti* most likely differentiated from the darker sylvan form found in African tropical forests and after adapting to peridomestic life it migrated to the New World and to the South East Asian continents. It is believed to have reached the New World from West Africa aboard slave ships, starting in the seventeenth century (Gubler, 1997). Efforts to eliminate *Ae. aegypti* from the Americas to stop yellow fever started in 1915 by the International Health Commission of the Rockefeller foundation and then later in the 1940s by the Pan American Health Organization (PAHO). By the mid 1960s *Ae. aegypti* was eliminated from most countries in South America. The program was dismantled in the early 1970s before achieving complete eradication; Suriname, the Guyanas, Venezuela, the Southern USA, and some Caribbean Islands were still infested with *Ae. aegypti* (Lourenço-de-Oliveira et al., 2004). It has been suggested that all of Colombia was eradicated except for the city of Cucuta, close to the Venezuelan border (Groot, 1980). At the end of the decade many countries were reinfested and within a few years began to

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Table 1
Geographic origin and date of capture of *Aedes aegypti* and *Ae. albopictus*.

Species	F/M	Geographic location	Time of collection	Life stages captured
Ae. aegypti	30/32	Belen, Comuneros, C(C)	2008	Juveniles
	30/20	Clear Water, F(US)	2007–2008	Adults
	30/30	Nakhon Nayok, NN(T)	2008	Adults
Ae. albopictus	30/26	Pinellas, F(US)	2005–2008	Adults
	9/14	Vero Beach, F(US)	2009	Juveniles
	19/22	Honolulu, H(US)	2009	Adults
	30/30	Nakhon Nayok, NN(T)	2008	Adults

"juveniles" = collected as larvae and reared in breeding containers, "adults" = collected using traps with various attractants (see Section 2). F(US) = Florida (United States), H(US) = Hawaii (United States), NN(T) = Province of Nakhon Nayok (Thailand), C(C) = Municipality Cucuta (Colombia). F, females; M, males.

experience epidemic DF followed by the emergence of DHF (Gubler, 2005). Phylogenetic studies suggest that *Ae. aegypti* populations within South America are made up of multiple reintroductions and populations that survived the initial eradication programs (Mousson et al., 2005; Bracco et al., 2007).

Aedes aegypti was probably introduced into coastal cities of South East Asia from East Africa around the nineteenth century via the shipping industry but not until World War II did it establish itself and spread to interior cities (Gubler, 1997; Failloux et al., 2002). Genetic analysis using mitochondrial genes revealed two distinct clusters in South East Asia, one cluster included *Ae. aegypti* strains from French Polynesia, Guinea, and Brazil, the other cluster contained strains from Martinique, Europa Island, and Northeast Amazonia (Mousson et al., 2005). A study looking at mitochondrial genetic diversity of *Ae. aegypti* within Thailand found that the most common haplotype is also present but rare in Mexico (Bosio et al., 2005).

Aedes albopictus is said to be native to South East Asia and only within the last three decades did it start its global spread. At least 28 countries have imported *Ae. albopictus*, largely due to the international trade of used tires (Benedict et al., 2007). It was imported to Hawaii in the early 1900s and it was first introduced into the Continental United States in the 1980s, where is has become widely dispersed (Reiter and Darsie, 1984; Effler et al., 2005). Global tire trade statistics and allozyme analysis revealed the origin of the continental US introduction was Japan (Hawley et al., 1987; Kambhampati et al., 1991). Mousson et al. (2005) analyzed the mitochondrial genes of *Ae. albopictus* from 13 different geographic locations revealing a generally low variability, which they attributed to the recent expansion of the species. Specimens from island populations such as Hawaii showed the highest rates of sequence evolution (Mousson et al., 2005).

It would be expected that processes such as different migration routes, passive dispersion at regional and continental scales, and repeated vector control efforts that disrupt populations, would create founder effects inducing genetic and subsequent morphological variation (Jirakanjanakit et al., 2008). Also one can anticipate differences in habitat environment for introduced populations would lead to local adaptation and morphological change (Jirakanjanakit et al., 2007; Caro-Riaño et al., 2009). Our study investigated the impact these processes had on metric properties.

2. Materials and methods

2.1. Insects

A total of 352 mosquitoes (178 females and 174 males) were analyzed from 4 geographical locations: Nakhon Nayok province of Thailand, Cucuta municipality of Colombia, and Florida and Hawaii in the United States (Table 1). Thailand mosquitoes were collected as adults during 2008 from the Muang district of Nakhon Nayok province, central Thailand. The BG sentinel trap, which uses a combination of lactic acid, ammonia, and fatty acids as attractants, and the Mosquito Magnet Liberty Plus, which utilizes CO2 as an attractant, were used. Mosquitoes from Hawaii were collected in Honolulu as adults in 2009, using similar traps as in Thailand. Adult mosquitoes from Florida were collected in Pinellas county between 2005 and 2008 using a John Hock miniature CDC light trap baited with CO2.The trap ran in 24-h intervals with the light turned off during the day. The Colombia specimens were collected during the end of 2008 from multiple breeding sites in Belen and Comuneros, two different locations in the Cucuta municipality. They were collected as pupae and 3rd and 4th instar larvae, reared to adults under laboratory conditions, and the progeny of multiple broods (F1) was used in the experiment. A subset of specimens from Florida were collected in Vero Beach in 2009 as pupae and 3rd and 4th instar larvae. They were obtained from multiple breeding habitats at distances between 1 to 100m apart and reared to adults (F0) in breeding containers at room temperature.

2.2. Data collection

Wings were digitized at 13 landmarks (LM), all of them of "type I" (venation intersections, see Bookstein, 1991) (Fig. 1). Other possible type I LM were not considered because they were less consistently visible. To avoid possible optical distortion at the periphery of optical lens, each wing was located at the center of the visual field. Different optical tools were used but each picture was calibrated to permit accurate size comparisons. The measurement error was estimated comparing two sets of digitization. It was computed as 1-R, with R the repeatability index as described by Arnqvist and M?artensson (1998), i.e. a Model II oneway ANOVA on repeated measures, where R is provided by the ratio of the

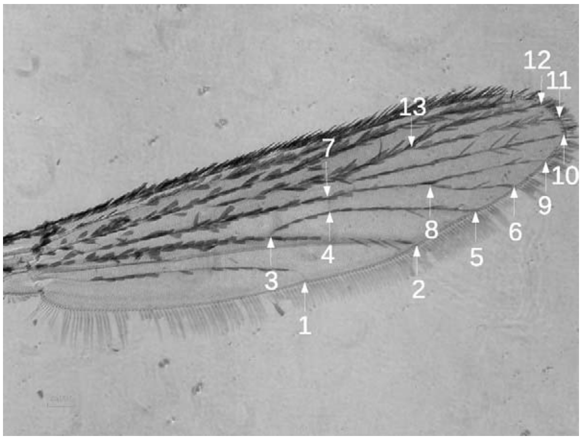

Fig. 1. Wing and landmarks positions. Arrows indicate the position of the landmark. Numbers refer to the order of collection, and are reproduced in Fig. 3. Scale equals .1 mm.