



# HHS Public Access

Author manuscript

*Mol Ecol.* Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

*Mol Ecol.* 2016 November ; 25(21): 5377–5395. doi:10.1111/mec.13866.

## Global Genetic Diversity of *Aedes aegypti*

Andrea Gloria-Soria<sup>\*</sup>, Diego Ayala<sup>†,φ</sup>, Ambicadutt Bheecarry<sup>‡</sup>, Olger Calderon-Arguedas<sup>§</sup>, Dave D. Chadee<sup>\*\*</sup>, Marina Chiappero<sup>††</sup>, Maureen Coetzee<sup>‡‡</sup>, Khouaili bin Elahee<sup>‡</sup>, Ildefonso Fernandez-Salas<sup>§§</sup>, Hany A. Kamal<sup>\*\*\*</sup>, Basile Kamgang<sup>†††</sup>, Emad I. M. Khater<sup>†††</sup>, Laura D. Kramer<sup>§§§</sup>, Vicki Kramer<sup>\*\*\*\*</sup>, Alma Lopez-Solis<sup>§§</sup>, Joel Lutomiah<sup>††††</sup>, Ademir Martins Jr<sup>††††</sup>, Maria Victoria Micieli<sup>§§§§</sup>, Christophe Paupy<sup>†,φ</sup>, Alongkot Ponlawat<sup>\*\*\*\*\*</sup>, Nil Rahola<sup>†,φ</sup>, Syed Basit Rasheed<sup>†††††</sup>, Joshua B. Richardson<sup>\*</sup>, Amag A. Saleh<sup>†††</sup>, Rosa Maria Sanchez-Casas<sup>§§§§§</sup>, Gonçalo Seixas<sup>\*\*\*\*\*</sup>, Carla A. Sousa<sup>\*\*\*\*\*</sup>, Walter J. Tabachnick<sup>†††††</sup>, Adriana Troyo<sup>§</sup>, and Jeffrey R. Powell<sup>\*</sup>

<sup>\*</sup>Yale University, 21 Schem Street, New Haven, CT 06520-8105 USA <sup>†</sup>Laboratory MIVEGEC, Institut de Recherche pour le Développement, Montpellier, 34394 Montpellier, France <sup>φ</sup>Centre International de Recherches Médicales de Franceville, Franceville, Gabon <sup>‡</sup>Vector Biology and Control Division, Ministry of Health and Quality of Life, Mauritius <sup>§</sup>Centro de Investigación en Enfermedades Tropicales, Facultad de Microbiología, Universidad de Costa Rica. San José, Costa Rica <sup>\*\*</sup>Department of Life Sciences, University of the West Indies, St. Augustine, Trinidad, W. I <sup>††</sup>Instituto de Diversidad y Ecología Animal. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y Universidad Nacional de Córdoba. Av. Vélez Sarsfield 299. X5000JJC Córdoba, Argentina <sup>‡‡</sup>Wits Research Institute for Malaria, School of Pathology, University of Witwatersrand, Johannesburg, South Africa <sup>§§</sup>Centro Regional de Investigación en Salud Pública INSP, Tapachula, Chiapas, México <sup>\*\*\*</sup>Dallah Establishment, Pest Control Projects, Jeddah, Kingdom of Saudi Arabia <sup>†††</sup>Research Unit Liverpool School of Tropical Medicine, Organisation de Coordination pour la lutte contre les Endemies en Afrique Centrale, Yaounde, Cameroon <sup>††††</sup>Department of Plant Protection. College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia <sup>§§§</sup>Wadsworth Center, New York State Department of Health, School of Public Health, State University of New York at Albany, Albany NY <sup>\*\*\*\*</sup>California Department of Public Health, Vector Borne Disease Section, Sacramento, CA <sup>††††</sup>Arbovirus/Viral Hemorrhagic Fever Laboratory, Center for Virus Research, Kenya Medical Research Institute (KEMRI), P. O. Box 54628-00200, Nairobi, Kenya <sup>†††††</sup>Laboratório de Fisiologia e Controle de Artrópodes Vetores, IOC-FIOCRUZ, Rio de Janeiro, Brazil <sup>§§§§</sup>Centro de Estudios Parasitológicos y de Vectores, CONICET, La Plata, Buenos Aires, Argentina <sup>\*\*\*\*\*</sup>Department of Entomology, USAMD-AFRIMS, Bangkok, Thailand <sup>†††††</sup>Department of Zoology, University of Peshawar, Peshawar, 25120 Pakistan <sup>§§§§§</sup>School of Veterinary Medicine, Escobedo, Centro de Investigación y Desarrollo en Ciencias de la Salud, Monterrey, Nuevo León, México <sup>\*\*\*\*\*</sup>Global Health and

Corresponding author: Andrea Gloria-Soria, 21 Schem Street, Room 168 ESC, Yale University, New Haven, CT 06520-8105 USA, andrea.gloria-soria@yale.edu, Telephone: 203-432-3886, Fax: 203-432-7394.

**Data accessibility:** Raw allele frequencies are available at VectorBase.org, Population Biology Project ID: VBP0000138.

**Author contributions:** Conceived and designed the experiments: AGS JRP. Performed the experiments: AGS, JBR. Analyzed the data: AGS JRP. Contributed reagents/materials, performed field collections: DA, AB, OC-A, DDC, MCh, MC,JbE, IF-S, HAK, BK, EIMK, LDK, VK, AL-S, JL, AMJr, MVM, CP, AP, NR, SBR, AAS, RMS-C, GS, CAS, WJT, and AT. Wrote the paper: AGS JRP.

Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 100, 1349-008 Lisbon, Portugal +++++Florida Medical Entomology Laboratory Department of Entomology and Nematology University of Florida, IFAS, Vero Beach, FL

## Abstract

Mosquitoes, especially *Aedes aegypti*, are becoming important models for studying invasion biology. We characterized genetic variation at 12 microsatellite loci in 79 populations of *Ae. aegypti*, from 30 countries in six continents and used them to infer historical and modern patterns of invasion. Our results support the two subspecies *Ae. aegypti formosus* and *Ae. aegypti aegypti* as genetically distinct units. *Ae. aegypti aegypti* populations outside Africa are derived from ancestral African populations and are monophyletic. The two subspecies co-occur in both East Africa (Kenya) and West Africa (Senegal). In rural/forest settings (Rabai District of Kenya) the two subspecies remain genetically distinct whereas in urban settings they introgress freely. Populations outside Africa are highly genetically structured likely due to a combination of recent founder effects, discrete discontinuous habitats, and low migration rates. Ancestral populations in sub-Saharan Africa are less genetically structured, as are the populations in Asia. Introduction of *Ae. aegypti* to the New World coinciding with trans-Atlantic shipping in the 16<sup>th</sup> to 18<sup>th</sup> Centuries was followed by its introduction to Asia in the late 19<sup>th</sup> Century from the New World or from now extinct populations in the Mediterranean Basin. *Aedes mascarensis* is a genetically distinct sister species to *Ae. aegypti* s.l.. This study provides a reference database of genetic diversity that can be used to determine the likely origin of new introductions that occur regularly for this invasive species. The genetic uniqueness of many populations and regions has important implications for attempts to control *Ae. aegypti*, especially for methods using genetic modification of populations.

## Keywords

*Aedes aegypti*; global population genetics; microsatellites; history; *Aedes mascarensis*

## INTRODUCTION

Non-native invasive species are of great concern for ecology, conservation biology, agriculture, and epidemiology. Invasive species can alter ecosystems by competing for resources with endemic counterparts in the same feeding guild, preying on native species, or transmitting pathogens and disease. Invasive species can also destabilize an ecosystem by displacing native populations while not taking over their ecological services (Mack et al. 2000). In the case of arthropod vectors of disease agents, an invasion can lead to the introduction of a novel disease to a naïve population or a more efficient spread of local pathogens. Mosquitoes are insect invaders of major relevance because of their connection to human disease (Lounibos 2002). Most medically important mosquitoes live in close proximity to humans and arrive to new territories with human migrations. Adaptation to human habitats almost invariably leads to the evolution of preference for the most available blood source: humans. Pathogens originally from non-human hosts (usually other primates)

then enter the human-mosquito cycle of transmission and can have major public health consequences.

*Aedes aegypti*, is the invasive mosquito that has caused the most human casualties worldwide, initially as the vector of devastating yellow fever epidemics; hence its common name “the yellow fever mosquito”. Today, *Ae. aegypti* continues to plague humans as the primary vector of viruses that cause dengue fever, chikungunya, and Zika. Because of its ease of rearing in the laboratory as well as its major epidemiological role, *Ae. aegypti* is the best known mosquito from all aspects of its biology (Christophers 1969; Clemens 1992; 1999) and has become a model organism over the last 15 years, thanks to an increase of molecular studies focused on disease vectors. The present report adds significantly to understanding the population genetics of this species, elucidates global invasion pathways, and informs emerging management options.

Three subspecies of *Ae. aegypti* have been described. The type subspecies is *Ae. aegypti aegypti* (abbreviated Aaa) that has spread throughout the tropical and subtropical world by humans (Powell and Tabachnick 2013), is highly anthropophilic (prefers human blood-meals) (McBride et al. 2014) and is adapted to breed in human habitats (“domestic”). The ancestral form of the species in sub-Saharan Africa, *Ae. aegypti formosus* (Aaf), breeds in nonhuman-disturbed habitats such as forests and vegetated ecotones (Lounibos 1981), and prefers non-human blood meals (McBride et al. 2014). These subspecies were originally described based largely on their geographic distribution, color, and scaling patterns, the forest form Aaf being blacker with less white scaling than the brownish domestic form Aaa. However, populations are highly variable for scaling pattern (McClelland 1974, Jupp et al. 1991), so morphology does not always reflect the major ecological distinction between the two subspecies (Powell and Tabachnick 2013; and see below). A third subspecies, *Ae. aegypti queenslandensis*, was named by Theobald (1901) and described as a variety of *Ae. aegypti* with golden brown scales in the thorax. Mattingly (1967) suggested that populations in the Mediterranean Basin and parts of Australia and Eastern Africa conform to this subspecies or variety. It is unclear whether members of this subspecies can still be found.

Given the ambiguity of morphology matching ecological and behavioral traits, especially in sub-Saharan Africa, the precision and usefulness of subspecific designations have been questioned (Powell and Tabachnick 2013). We use the terms Aaa and Aaf here largely for historical continuity recognizing the limitations and sometimes ambiguity of trying to apply this dichotomy. As McClelland (1967) correctly pointed out, *Ae. aegypti* s.l. is more accurately viewed as a highly polymorphic rather than a polytypic species.

Here we present the latest results of our ongoing studies on the genetic diversity of *Ae. aegypti*. While several previous studies have addressed large-scale genetic patterns of *Ae. aegypti* population structure and historical movements (e.g., Brown et al. 2011a; 2014, Bennet et al. 2016), we have genotyped a larger array of samples encompassing much of the *Aedes aegypti* geographic range, including six continents (Figure 1). This extended sampling provides higher resolution of the genetic structure at the regional scale and allows more accurate tests of hypotheses regarding the historical movement of *Ae. aegypti* out of Africa, since each region is better represented in the dataset. What we present here in no way

contradicts the earlier work, but strengthens the conclusions of those studies and adds more detail. We emphasize five aspects of these comprehensive data: (1) The distinct genetic differentiation patterns observed between populations of *Ae. aegypti* in the ancestral range of Africa and populations outside Africa; (2) what the data imply about the historical spread of *Ae. aegypti*; (3) how these data can serve as a reference panel for determining the origin of new introductions; (4) the validity of *Ae. mascarensis* as a genetically distinct sister species to *Ae. aegypti* s.l.; and (5) the importance of recognizing the extensive genetic variation in controlling diseases vectored by this mosquito.

## MATERIALS AND METHODS

### Mosquito collections

**Aedes aegypti**—Adults, larvae or eggs were received from 79 geographic locations worldwide (Table 1 and Figure 1). Allele frequencies from some of the earlier collections have been previously reported in Brown et al. (2011a), Gloria-Soria et al. (2014), and Monteiro et al. (2014); because all these studies were performed in the same laboratory at Yale University, designation of alleles is consistent across this and all previous work reported from this lab. Mosquitoes arrived as either eggs from oviposition traps, larvae or adults in 70–100% ethanol or on silica gel. Eggs were hatched at the Yale School of Epidemiology and Public Health insectary, reared to adults, and preserved in 100% ethanol at –20°C until DNA extraction. Most mosquitoes included in this study came directly from the field, when possible; in a few cases they were passed through one or two generations in the laboratory prior to genetic analyses: Tahiti (French Polynesia) and Pijijiapan (Mexico) -- one generation; Bolivar (Venezuela), Zulia (Venezuela), Rayong (Thailand), and Prachuabkhirikhan (Thailand) -- two generations. These laboratory colonies were established in large cages by several hundred to over 1000 mosquitoes from the field in an attempt to be as representative as possible of field populations (Brown et al. 2011a). Specimens from Rabai, Kenya were sampled as larvae from water stored in large clay vessels inside huts (“Rabai-in”) and from the forests (“Rabai-out”) a few hundred meters distant to the village. Previous studies have shown behavioral, morphological, and genetic differentiation among these populations (Trpis and Hausermann 1975; Tabachnick et al. 1979; Brown et al. 2011a, Brown et al. 2014, McBride et al. 2014) and thus we kept them separate for the purposes of this work.

A strain presumed to be *Ae. ae. queenslandensis* were obtained from Professor David Severson (University of Notre Dame) in ethanol. This strain originated from Surabaya, Indonesia and had undergone several generations of artificial selection for the *queenslandensis* scale pattern (Tsuda et al. 2003). *Aedes mascarensis* specimens were collected in the region known as Le Dauge, in the suburb of Port-Louis, Capital of the Republic of Mauritius, in 2014.

### DNA extraction and microsatellite genotyping

Total nucleic acids were extracted from 3,682 individual *Ae. aegypti* and 26 *Ae. mascarensis* mosquitoes using the DNeasy Blood and Tissue kit (Qiagen) according to manufacturer instructions, with an additional RNase A (Qiagen) step. Samples were stored at –20°C until

further analysis. Individual mosquitoes were genotyped as described in Brown et al. (2011a). The microsatellite loci analyzed were: A1, B2, B3, A9 (tri-nucleotide repeats), and AC2, CT2, AG2, AC4, AC1, AC5, AG1, and AG4 (di-nucleotide repeats) (Brown et al. 2011a; Slotman et al. 2008). Polymerase chain reactions were conducted as 10 $\mu$ l reactions using the Type-it Microsatellite PCR Master Mix (Qiagen), 25 nM of each forward primer, 250 nM of each reverse primer, and 500 nM of a fluorescently labeled M13 primer to allow multiplexing (Oetting et al., 1995; Brown et al., 2011a). Thermocycler conditions were: 94°C  $\times$  10', 35  $\times$  (94°C  $\times$  30", 54°C  $\times$  30", 72°C  $\times$  30"), and 72°C  $\times$  5'. Microsatellite primer sequences, multiplex pairings and fluorescent primers are as described in Brown et al. 2011a. PCR products were run for fragment analysis on an Applied Biosystems 3730xl DNA Genetic Analyser with a GS 500 Rox internal size standard (Applied Biosystems) at the DNA Analysis Facility at Science Hill at Yale University. Microsatellite alleles were scored using GeneMapper v4.0 (Applied Biosystems). Raw allele frequencies are available at VectorBase.org, Population Biology Project ID: VBP0000138.

### Genetic diversity

All microsatellite loci were analyzed for within-population deviations from Hardy-Weinberg equilibrium (HWE) using the exact test (Weir and Cockerham 1984) with complete enumeration as implemented in GENEPOP v.4.1.0 (Raymond and Rousset 1995; Rousset 2008). Linkage disequilibrium (LD) among pairs of loci was estimated using the same software. Both tests were run with 10,000 dememorizations, 1,000 batches, and 10,000 iterations per batch. Bonferroni corrections were applied to the resulting matrices of both HWE and LD. Allele numbers, allelic frequencies, and average observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities were estimated using GenAlEx (Peakall and Smouse 2012). Allelic richness (AR) and private allelic richness (PAR) were calculated in HPRARE (Kalinowski 2005), which uses rarefaction to correct for unequal sample sizes (N=200 for regional richness and N=100 for individual population richness estimation). Pairwise genetic distances ( $F_{ST}$ ) were calculated in Genodive 2.0b.27 (Meirmans and Van Tienderen, 2004).

### Population structure

Geographic and temporal population structure was evaluated via the Bayesian clustering method implemented by the software STRUCTURE v. 2.3 (Pritchard et al. 2000), which identifies genetic clusters and assigns individuals to these clusters with no a priori information of sample location. The most likely number of clusters (K) was determined by conducting 20 independent runs from each K=1 to 5 at the subspecies/species and global scale, and from K=1 to 10 for the subsequent analysis of each of the population subgroups. Each run assumed an admixture model and correlated allele frequencies using a burn-in value of 100,000 iterations followed by 500,000 repetitions. The optimal number of K clusters was determined both following the guidelines of Pritchard et al. (2000) and the Delta K method from Evanno et al. (2005) with the online version of STRUCTURE HARVESTER v. 0.6.94 (Earl and vonHoldt 2012). Plots of the most biologically informative number of clusters were generated with the program DISTRUCT v.1.1 (Rosenberg 2004). In most cases these plots correspond to the optimal K value as identified by the Evanno method (Evanno et al. 2005) but exceptions are noted in the figures. Discriminant analysis of Principal Components (DAPC; Jombart et al. 2010) were performed on allele frequencies of the same

groups evaluated by STRUCTURE and plotted with the ADEGENET package (Jombart 2008) in R v. 3.2.2. (R Core Team 2013).

### Scale of geographic genetic differentiation

Correlation between the geographic distance (Euclidean distance in km) and genetic distance ( $F_{st}$ ) matrices (often referred to as “isolation by distance”) was performed on each of the following population groups: Africa (*Ae. formosus* only), Asia, continental North America, South America, and the Caribbean islands (including the islands from the Florida Keys). Central America was excluded from the analysis, since only one sample from Costa Rica was sampled from this region. Distance matrices were generated in GENEPOP v.4.1.0 (Raymond and Rousset 1995; Rousset 2008), geographic distances were created from geographic coordinates using the Geographic Distance Matrix Generator v.1.2.3 (Ersts 2016). Mantel tests were conducted with the *ade4* package available for (R Core Team 2013), using 9999 permutations and plotted with the same software.

### Estimates of demographic parameters and population history

Inferences of effective population sizes and introduction history were made using approximate Bayesian computation methods (ABC: Beaumont et al. 2002) as implemented by DIYABC v.2.0.4 (Cornuet et al. 2014). Four plausible scenarios of migration among continents were tested, and a separate analysis compared six scenarios of the origin of the indoor Aaa from Rabai, Kenya. An estimated number of ten generations per year was chosen based on a life cycle of ~22 days from hatch to hatch of *Ae. aegypti* reared under ideal constant temperature (28°C) and humidity conditions (85%) in our insectary, with non overlapping generations. This would result in ~16 continuous generations/year. If we consider that in the field temperature is not constant, neither are nutrients or humidity, that predators are abundant and that life span is shortened by human intervention, the number of generations a year will be lower than under ideal conditions. Additionally, in some locations (eg. Georgia, USA), weather conditions prevent mosquito breeding during the winter months. Other independent studies have estimated that *Ae. aegypti* can undergo 9–37 generations a year in the laboratory based on its thermal requirements (Beserra et al, 2006; Marinho et al., 2016). Thus, we consider that a mean of 10 generations a year is a good but conservative estimate. The origin of the Asian populations was modeled by randomly subsampling 200 individuals from each of the Africa (excluding Rabai - Kenya and Goudiry - Senegal), America & Caribbean, and Asia (including the Australian populations) datasets. The origin of Rabai (Kenya) Aaa was subsequently investigated in a similar manner, by randomly drawing 32 individuals of each group to match the number of individuals available from Rabai (Kenya). Aaa from Rabai (Kenya) used for this analysis were exclusively individuals with the majority of Aaa ancestry, as determined by q values > 50% in the STRUCTURE analysis (Pritchard et al. 2000). A mutation rate ranging from  $9 \times 10^{-6}$  to  $1 \times 10^{-5}$  was used based on rates reported in the literature for other Diptera species (Schug et al. 1997; Pfeiler et al. 2013). Additional details of the DIYABC analysis can be found in Tables S1 and S2.

## RESULTS

We have sampled and analyzed 79 populations from six continents, across much of *Aedes aegypti*'s geographic range, (Figure 1). Details of these populations can be found in Tables 1 and S3. Some localities have been sampled more than once and we report patterns of temporal stability/instability of these populations elsewhere (Gloria-Soria et al. 2016). All samples came directly from the field with the exception of six, which were reared no more than two generations in the laboratory (see Materials and Methods and Table S3). Mean and median sample sizes are 46 and 48 individuals respectively.

Table 2 shows the allelic richness, private allelic richness, and genetic distance ( $F_{ST}$ ) estimates per continent. Allele richness in Africa is ~11 alleles, whereas the mean allele richness across continents outside Africa is 6.7 (North America, South America, Caribbean, Asia, and Pacific). The number of private alleles in Africa is 3.4, more than an order of magnitude higher than the values for continents outside Africa. These differences are statistically significant by Wilcoxon rank sum tests at  $p < 0.001$ . Population genetic statistics for individual locations are reported in Tables S4 and S5.

A total of 79 out of 925 (8.5%) population-by-locus comparisons deviate significantly from Hardy-Weinberg expectations ( $p < 0.05$ , HW exact test) as indicated by  $F_{IS}$ , after sequential Bonferroni correction (Table S4). Such small deviations from HW are common for microsatellites most often due to rare null alleles. Out of the 79 significant deviations, 67 had a positive  $F_{IS}$ , consistent with the excess of homozygotes expected in the presence of null alleles. A total of 313 out of 5,414 (6.0%) locus-by-locus tests for LD were significant at the 5% level after multiple test correction, with no locus pair consistently correlated across all populations. Each of the 12 microsatellites reported in this study are located on different supercontigs of the current *Ae. aegypti* genome assembly (*AaegL3* in VectorBase - Giraldo-Calderón et al. 2015). The low level of LD reported here (6.0% of tests significant at the 5% level) is consistent with the 12 loci being independent.

### Global scale patterns of population structure

Bayesian clustering analysis in STRUCTURE (Pritchard et al. 2000) and DAPC (Jombart et al. 2010) on the full dataset of 79 *Ae. aegypti* collections from around the world (Figure 1), support the existence of two major genetic clusters of *Ae. aegypti* s. l. (Figure 2). The “*Ae. queenslandensis*” strain was excluded from these analyses for reasons described below. Generally, the two genetic clusters correspond to the two subspecies designations: *Aedes aegypti formosus* (Aaf) in sub-Saharan Africa and *Aedes aegypti aegypti* (Aaa) spread around the tropical and subtropical world outside Africa. Admixture between the two named subspecies was detected in four countries: Senegal (localities 2 and 4 in Figure 2), Kenya (locality 15), Argentina (localities 27–30), and the USA (locality 59).

### Differentiation within and outside Africa

Figure 3 shows the Bayesian clustering analysis of the African and out-of-Africa populations separately. Populations outside of Africa are hierarchically genetically subdivided, first into three major groupings roughly representing North America, South America, and Asia plus

the Pacific (Fig 3A). Caribbean populations are heterogeneous displaying a combination of affinities to N. America and S. America, with the exception of Dominica that clearly groups with S. America (locality 62 in Fig 3A). The Pacific region, which includes the two Australian populations, Tahiti (French Polynesia), and Hawaii (USA), clusters with Asia. Madeira, the only European sample in this dataset, has both Asian and S. American genetic affinities (locality 79 in Fig 3A).

The strong population structure observed outside Africa contrasts to the relatively weak structure observed within Africa (Fig 3B). Interestingly, while there is no indication of individual population distinctness, there is indication that Senegal populations are different from the rest of Africa (excluding the Rabai indoor collection; Fig 3B). This difference in the degree of genetic structure within Africa compared to outside of Africa is confirmed by analyses of genetic differentiation by distance (often called isolation by distance), in Fig 4A. Populations separated by the same distance in the New World (Americas and the Caribbean) are more genetically differentiated than those in Africa. The case of Asia is discussed in the next section.

### **Genetic structure within continents/regions outside Africa**

When each continent is analyzed individually using STRUCTURE, finer geographical genetic structure is detected. North and South America generally split from each other forming two large genetic groups, with Exeter (USA) and Chetumal (Mexico) being exceptions (Figure 5). The single Central American sample (Siquirres, Costa Rica) shows genetic affinities to both South and North America, as expected from its transitional geographic location. However, because we only had a single population sample from Central America, we could not perform more detailed analyses of the region.

South America is further divided in three genetic groups: the north including Colombian, Venezuelan, and Northern Brazil populations, a southern Brazil group, and Argentina (Figure 5A). Mexico has four genetic groups that correspond geographically to the north, south, central, and Caribbean regions of the country (Figure 5C). Northern Mexican populations along the border with the US are genetically close to Texas, Arizona, and southern California (Fig. 5A). Within the US, the Southeast (Louisiana, Florida, and Georgia) groups together (Fig 5A and 5B) and is distinct from the southwest (Texas and Arizona) and northern California. DAPC plots for the Americas and the American regions are shown in Figure S1.

Caribbean populations are also quite distinct, although we have only sampled four locations plus the Florida Keys, USA (Fig 6A and 6B). Dominica is strongly differentiated from the rest of the group and has strong affinities to S. America (Fig 3A and Fig 6A–B). Excluding Dominica, the Florida Keys (USA) break from all other islands.

Asia appears to have a similar rate of genetic differentiation by distance as Africa (Fig. 4A). However, Bayesian clustering analysis indicates a stronger genetic structure within Asia than within the African continent, similar to the genetic structure seen in the New World. When Asia and the Pacific populations are analyzed together, the Pacific clusters with Pakistan and Saudi Arabia at  $K=2$ , while Thailand, Vietnam, and the Philippines form another group (Fig



6C). More resolution is obtained by analyzing the STRUCTURE plot at  $K=5$ , with most major regions being genetically distinct (Fig 6C). DAPC on Asian and Pacific populations are in agreement with the STRUCTURE results at  $K=5$ , highlighting the distinctiveness of the Australian populations and of Hanoi, Vietnam (Figure 6D).

### Gene flow estimates

The patterns of genetic relatedness among the continents and the Caribbean are summarized in Figure 4B. The thickness of the lines is based on  $F_{ST}$ , a measure of genetic differentiation that can also be interpreted as level of gene flow among populations ( $4N_e m$  for populations at equilibrium, which is unlikely to be the case for most of these relatively young populations). The data suggest that the Caribbean and N. America are highly connected, and that there is also significant gene flow between the Caribbean and S. America. Africa, as expected, is the most genetically isolated, i.e., all lines connecting Africa are narrow. The Pacific (Australia plus Tahiti –French Polynesia and Hawaii) is also quite distinct, only showing some affinity with Asia.

### History of *Ae. aegypti*

We use ABC (Approximate Bayesian Computation; Beaumont et al. 2002) to investigate the origin of *Ae. aegypti* outside Africa by testing the four plausible migration scenarios shown in Figure 7: (1) Africa to America to Asia, (2) Africa to Asia to America, (3) 1st Africa to America/2nd Africa to Asia, and (4), 1st Africa to Asia/2nd Africa to America. The best-supported scenario was the one where the New World was founded from Africa and Asia was colonized from the New World (Scenario 1:  $p=0.9921$  in Fig. 7 and Table S1). Alternative scenarios were poorly supported by the analysis ( $p<0.001$ ; Fig. 7 and Table S1). The estimated mutation rate under the best-fit scenario was  $9.5\times 10^{-6}$ , and falls within the range of microsatellite mutation rates estimated for other Diptera (Schug et al. 1997; Pfeiler et al. 2013). The time estimated for the founding of Asian populations assuming an average of 10 generations per year is 1280–1770 generations or ~150 years ago (Table S1), setting priors for leaving Africa at 4500–6000 generations ago (~500 years ago) in agreement with historical records, suggesting the New World populations were founded by trade between the New World and Old World in the 15<sup>th</sup> and 16<sup>th</sup> Centuries.

ABC analyses to determine the origin of the indoor Aaa from Rabai, Kenya explored six scenarios involving a founder effect from Africa, New World, or Asia. These scenarios are described in Table S2 and Figure S2. The analyses assigned moderate posterior probabilities to multiple colonization hypotheses: (a) direct colonization from the Americas after colonization of Asia (Fig S2, Scenario 3,  $p=0.56$ ), (b) direct colonization from Asia (Scenario 1,  $p=0.24$ ) and (c) colonization from the Americas prior to colonization of Asia (Scenario 4,  $p=0.20$ ). The scenario with highest support, Rabai being founded from the Americas after Asia (Scenario 3), predicts that the indoor Rabai Aaa populations are between 10–300 generations, or less than 30 years old (Table S2).

### Other taxa

In addition to the samples of Aaf and Aaa, we had access to one laboratory strain labeled *Ae. aegypti queenslandensis* that was originally collected in Surabaya, Indonesia in 2000.

This strain underwent several rounds of selection for the scaling pattern assumed to be characteristic of the *queenslandensis* subspecies (Tsuda et al. 2003). The observed heterozygosity of this population was lower than any of the *Ae. aegypti* populations ( $H_o = 0.276$ ). DAPC shows this strain as genetically distinct from all other *Ae. aegypti* (Fig S3).

We also analyzed 26 wild-caught *Ae. mascarensis*, the most closely related living relative of *Aedes aegypti* (sister taxon or species), and successfully genotyped all 12 microsatellite loci. Microsatellite diversity in this species does not overlap with populations of *Ae. aegypti* s. l. (Fig. 8).

## DISCUSSION

### Global Patterns

From a global perspective, *Aedes aegypti* s.l. is divided in two major genetic units (Figure 2) that for the most part, fall into the classically defined subspecies: *Ae. aegypti formosus* in Africa and *Ae. aegypti aegypti* outside Africa. These *Ae. aegypti* subspecies are distinct from each other and inhabit different geographic ranges, consistent with Mayr's definition of subspecies (Mayr 1963). The distinctness is genetic, morphological (scaling patterns and cuticle coloration), behavioral (host and oviposition choice), and ecological (larval habitats) as well as in geographic distribution (Trpis and Hausermann 1975). However, these distinctions are not absolute and may be breaking down in recent years.

Aaa and Aaf have both been reported in parts of coastal East Africa as far back as the 1950s based on morphology and habitat (Mattingly 1957) and later corroborated by genetic studies (Tabachnick et al. 1979; Brown et al. 2011a). Interestingly, in the city of Mombasa, Kenya where both subspecies co-occur, there is free mixing among forms (locality 15 in Fig 2A, 2D, and Fig 3B). In contrast, in the Rabai District of Kenya, located just 20 km northwest of Mombasa, the two forms are not interbreeding even though adults can sometimes be found together indoors, or at least they remain genetically distinct (contrast locality 15 with locality 21 in Fig 2A, 2D, and Fig 3B). The Rabai District is rural consisting on small villages with mud wall huts surrounded by farmland and then forest. The samples we studied were larvae taken from water stored in large clay vessels inside huts ("Rabai-in") and from the forests ("Rabai-out") a few hundred meters distant (also analyzed in Tabachnick et al. 1979, Brown et al. 2011a; McBride et al. 2014). The genetic differentiation of these populations has remained stable over nearly four decades of study in Rabai (Tabachnick et al. 1979). Considering the geographic closeness of Mombasa to Rabai, it is likely that ecology plays a major role on whether these subspecies freely interbreed. Mombasa is urban whereas the Rabai District is rural with small villages adjacent to the forest. [See Lounibos (2003) for details of the ecology of mosquitoes in the Rabai District of Kenya.]

Senegal (SN) also harbors populations with affinities to domestic populations outside Africa (Goudiry and N'goye: localities 2 and 4 in Fig. 2A). This likely represents a recent introduction through shipping or other human activities (Brown et al 2011a). These collections from Senegal come from cities or towns, and like in urban Mombasa in East Africa, the two forms are freely interbreeding, again contrasting to the rural Rabai District of

Kenya. Huber et al. (2008), Sylla et al. (2009), and Paupy et al. (2010) have previously made similar observations on *Ae. aegypti* in Senegal.

The apparent recent Aaf ancestry in Argentinian populations is harder to explain (localities 27–31 in Fig. 2A). The presence of recent African ancestry may extend to populations of southern Bolivia and Paraguay (Rondan Duenas et al. 2009; Paupy et al. 2012; Llinas and Gradenal 2012), regions not sampled by us. This genetic result is consistent with observations of *Ae. aegypti* breeding in tree holes in Argentina, a typical Aaf larval breeding habitat (Mangudo et al 2015). In attempting to interpret this, it is important to note that *Ae. aegypti* was declared eradicated in much of South America including Argentina, Bolivia, and Paraguay by 1970 (Tonn et al. 1982; Schatzmayr 2000) and recolonized since that time. Although analyses of Brazilian populations were consistent with a complete eradication in that country (Monteiro et al. 2014), it is conceivable that, given the remoteness of the region, eradication was never fully achieved. The domestication event leading to Aaa likely occurred before *Ae. aegypti* migrated from Africa (see below). Thus, if there was a relict population in Argentina that escaped eradication efforts, it should have been Aaa. Furthermore, eradication was never achieved in many other parts of the New World including northern S. America, Caribbean, and the US (Gubler 1998) and yet there is no indication of recent Aaf ancestry today in these non-eradicated regions. An alternative explanation is that human trade or migration has recently introduced African Aaf to Argentina and the adjacent regions to the northwest and is now interbreeding with local Aaa populations.

### History of *Ae. aegypti* introductions

Data reported here, as well as much previous work, supports the ancestral status of the African populations (Brown et al. 2011a, Brown et al. 2014, Bennett et al. 2016). The elevated number of alleles and especially private alleles found in African populations compared to outside Africa is the strongest genetic evidence in favor of an ancestral African type (Table 2).

Movement of *Ae. aegypti* from the Old World likely occurred with trans-Atlantic travel beginning in the 1500s (Tabachnick 1991; Powell and Tabachnick 2013), with populations outside of Africa being monophyletic (Brown et al. 2011a, 2014; Bennett et al. 2016). Our interpretation of all populations outside Africa having a single origin contrasts with interpretation of mtDNA diversity. Moore et al. (2013) suggested a dual origin of Aaa populations outside Africa based on the diversity of mtDNA haplotypes. However, their data are also consistent with populations outside Africa originating from a single sample of two mtDNA lineages from ancestral Africa. If Aaa originated as a response to the expanding Sahara Dessert (as hypothesized in the next paragraph), these original proto-domestic populations were likely large enough to have captured multiple mtDNA lineages from the ancestral populations south of the Sahara.

It is well established that Aaa arrived in Asia after its arrival in the New World, likely in the 1890s (Powell and Tabachnick 2013). Our data supports a scenario where the colonization of Asia occurred from the New World (Scenario 1: in Fig. 7 and Table S1). However, an alternative colonization scenario not covered by our analysis is plausible. It has been

hypothesized that the domestication of *Ae. aegypti* was the result of the Sahara Desert expansion (4–6,000 years ago). Human habitats would have been the only reliable water sources north of the desert, so *Ae. aegypti* evolved to breed in human-generated containers and to take blood meals from humans (Peterson 1977, Tabachnick 1991; Powell and Tabachnick 2013). *Aaa* persisted in the Mediterranean Basin until about 1950 (Curtin 1967; Holstein 1967). It is conceivable that these populations were the original proto-*Aaa* form pre-adapted to surviving aboard ships traveling from the Mediterranean (Spain and Portugal) to the New World. These Mediterranean populations could be the source of Asian *Ae. aegypti*, their introduction aided by the opening of the Suez Canal in 1869 (Tabachnick 1991). The time estimated by the ABC analysis for the founding of Asia is remarkably consistent with the historical record ~150 years ago in the late 19<sup>th</sup> Century (Table S1). Urban dengue fever was first recorded in Asia in the 1890s and *Ae. aegypti* was the only Asian urban vector of dengue at that time (Smith 1956). Mediterranean populations, therefore, could represent a missing “ghost taxon” (*sensu* Norell 1996) that would change the outcome of the ABC results, if included.

We also tested hypotheses concerning the origin of the indoor-collected populations of *Aaa* in Rabai (Kenya), most likely a re-introduction from outside Africa (Fig. S2, Table S2). Interestingly, the scenario with highest support, Rabai being founded from the Americas after Asia, predicts a very young age for the Rabai indoor populations, 10–300 generations or less than 30 years ago (Table S2). This result is inconsistent with the observation that *Aaa* has been in coastal Kenya for at least 65 years (Mattingly 1957). It is possible that, like for the New World and Asia, the now extinct Mediterranean Basin populations may have been the immediate source of the Rabai *Aaa* indoor form, especially given the proximity of the Suez Canal connecting the Kenyan coast to the Mediterranean.

### New World population structure

In interpreting New World population structure for *Ae. aegypti*, it is important to consider that this species *was* officially eradicated from much of this area in the 1950s and 60s, with re-colonization starting in the 1970's. The genetic data available in Monteiro et al. (2014) was interpreted as consistent with Brazil being colonized by *Ae. aegypti* from two directions, from S. American countries to the north and from the Caribbean, regions where eradication was never attained. Frequencies of mtDNA haplotypes (Braco et al. 2007) and alleles at the insecticide resistance gene, *kdr*, are consistent with these two major groups in Brazil (Linss et al. 2014). Argentina, the third genetic group is an enigma in being the only region outside Africa with evidence of recent ancestry of mosquitoes from Africa (discussed previously).

The genetic pattern observed in Mexico is consistent with previous work using collections taken about 20 years prior to our sampling (Gorrochotegui-Escalante et al. 2000), attesting to temporal stability of *Aaa* population genetic patterns. Similarly, in the USA, the genetic break in *Ae. aegypti* approximately at the border between Louisiana and Texas was documented ~40 years ago using allozymes (Powell et al. 1980; Wallis et al. 1983), suggesting that this division is temporally stable, at least for that time frame. California populations, some of which are thought to be quite recently established (Gloria-Soria et al.

2014), are complex and are the subject of ongoing independent analyses that will be reported in the near future.

### Aaf and Aaa population structure and introgression

Aaf in Africa are less genetically differentiated and structured compared to Aaa populations outside Africa (Figures 3 and 4). This pattern could be explained by the fact that populations outside Africa are young, have experienced founder effects, inhabit highly discontinuous habitats, and active migration in this species is restricted (Reiter 2007). Being the ancestral region, African populations are much older and there is evidence of historical admixture events within the region during the Holocene (1000 – 12,000 ya), when the forest expanded from glacial refugia (Bennett et al. 2016) and likely led to the relative homogenization of gene frequencies.

It is also important to note that this relatively low level of genetic differentiation among African populations belies the greater habitat diversity now occupied by African populations of *Ae. aegypti*. Historically, African populations bred almost exclusively in forests. Today, populations of *Ae. aegypti* in Africa can be found in urban habitats (Paupy et al. 2008; Kamgang et al. 2013) even if they fall genetically into the Aaf group, for example localities 5 (Dakar, Senegal), 8 (Franceville, Gabon) and 13 (Yaounde, Cameroon) in Fig 2A. There is evidence that this urban and forest African populations are genetically differentiated to certain degree (Paupy et al. 2008), yet remain within the large Aaf genetic group.

Interestingly, one might expect the younger Asian populations to be similarly differentiated as New World populations, but this is not the case with regard to overall genetic differences as measured by  $F_{ST}$  (Figure 4). The observed genetic homogeneity of Asian populations relative to the New World might be due to different time and patterns of passive migration due to human commerce. From the time of the first appearance of Aaa in Asia in the late 19<sup>th</sup> Century, trade by rail, sea, and overland routes in Asia was much more developed compared to commerce in the New World of the 16<sup>th</sup> to 18<sup>th</sup> Centuries (e.g., Tracy 1990) when *Ae. aegypti* populations were first established there (Powell and Tabachnick 2013).

Given the genetic, morphological, ecological, and behavioral differences between Aaa and Aaf, it is perhaps surprising that there is little or no evidence of reproductive isolation between these forms. Hybrids and backcrosses are fertile and viable (Moore 1979). In areas where the subspecies coexist (e.g., Senegal, Kenya, and Argentina), they seem to freely interbreed, as indicated by the vast majority of loci being in H-W proportions. For example localities 2, 3, 4, 15, 27, 28, and 29 in Figure 2 have less than two loci deviating from H-W expectations at the 5% significance level (Table S4) similar to the number of deviations observed for all population samples, which we attribute to the presence of rare null alleles (see Results). Even in Rabai, Kenya, where Aaa and Aaf retain genetic distinctness in sympatry, no pre- or post-mating isolation can be detected (Moore, 1979). [Low fertility has recently been reported in  $F_1$  offspring between laboratory colonies derived from Senegal Aaf and laboratory colonies of Aaa (Dickson et al. 2016)].

Introgression among divergent populations resulting from multiple introductions could lead to increased invasiveness due to cumulative genetic variation. This mechanism has been

postulated for other invasive taxa (reviewed in Bock et al. 2015), as well as for the invasive mosquito *Culex pipiens* (Fonseca et al., 2009). From the data presented here, it appears as if the two classically defined subspecies Aaa and Aaf are increasingly coming into contact and hybridizing (e.g., Senegal, Kenya, and Argentina). Will this lead to increased invasiveness of *Ae. aegypti* in these localities? There is presently no information on this.

### Other taxa

It is unclear whether the third named subspecies, *Ae. aegypti queenslandensis*, is supported by genetic data. We had access to one laboratory strain that had been labeled *Ae. aegypti queenslandensis* and was originally collected in Surabaya, Indonesia in 2000. This strain underwent several rounds of selection for the scaling pattern assumed to be characteristic of that subspecies (Tsuda et al. 2003). The strain is genetically distinct from all other *Ae. aegypti* populations (Fig S3), but its low heterozygosity suggests that this could be due to the long laboratory rearing and/or artificial selection performed on this single line (Tsuda et al. 2003). Rasçic et al. (2016) recently showed that specimens corresponding morphologically to *Ae. ae. queenslandensis* collected in Australia and Singapore were not genetically distinct from specimens collected in the same locality that corresponded morphologically to *Ae. ae. aegypti*. With regard to morphology, Mattingly (1957) states that *queenslandensis* "...does not differ notably from specimens from many other parts of the world." As far as known, there are no extant populations of this dubious subspecies.

*Aedes mascarensis* is the closest living relative of *Aedes aegypti* (sister taxon or species). Its distribution is restricted to a single island in the Indian Ocean, Mauritius (McGregor 1924). This species can form fertile F<sub>1</sub> hybrids with *Ae. aegypti*, although hybrids breakdown in further generations (Hartberg and Craig 1970). In fact, this species is so closely related to *Ae. aegypti* that Delatte et al. (2011) suggested it should be considered simply a "form" or subspecies of *Ae. aegypti*, based on mtDNA data. Our results however, indicate strong genetic differentiation not overlapping with the genetic diversity within Aaa and Aaf (Figure 8). Identical findings were made by Brown et al. (2014) using 1,504 SNPs. These data, therefore, suggest that *Ae. mascarensis* is a genetically distinct species, and not simply another "form" within *Ae. aegypti* s.l..

### Implications for disease control

These results suggest that microsatellite data can serve to identify the likely origin of new introductions of *Ae. aegypti* that place resident human populations at risk for diseases transmitted by this mosquito. Populations of *Ae. aegypti* vary considerably in efficiency of transmitting disease-causing viruses (Tabachnick et al. 1985; Black et al. 2002; Sim et al. 2013). A recent study on *Ae. albopictus* highlights the importance of population genetic differentiation of mosquitoes and their involvement in transmission of pathogenic viruses. Only certain genotypes of *Ae. albopictus* can preferentially transmit the chikungunya virus variant that led to the massive epidemic in La Reunion Island (Vazeille et al. 2016). Likewise, populations vary in their degree of resistance to insecticides (e.g., Montella et al. 2007; Linss et al. 2014). Recognizing the source of the introduction allows assessment of the level of health risks associated with the invasion and can guide control measures.

As an example of how analysis of hierarchical genetic structure can narrow down the origin of a new invasion, we consider the Caribbean (Fig 6A). Assume an initial analyses on the total data set indicated the new introduction is Aaa (Fig 2), subsequent regional analysis then points to the New World, and finally to the Caribbean (Figs 3 and 6). When the Caribbean alone is considered, using K=2 in STRUCTURE analyses, Dominica can be distinguished from all other samples of the region (Fig 6A). Removing Dominica from the analysis allows, first, to separate the Florida Keys from the other islands. Then at K=3, Trinidad stands out as genetically distinct from the other populations, with Puerto Rico and Carriacou starting to differentiate. Differentiation between these two latter islands however becomes more obvious at K=4. So, while our data do not always allow precise identification of a single population, it does allow identification to a reasonably narrow geographic range. Microsatellites were used in a similar manner to identify the origin of a 2010 temporary introduction into The Netherlands (Brown et al. 2011b) and a presumed recent introduction into California (Gloria-Soria et al. 2014), similar to studies of colonization routes of other pest species such as the hemlock woolly adelgid from Japan to North America (Havill et al., 2016).

The accuracy of such assignments depends on two factors. One is thoroughness of sampling. This points to the importance of adding more samples to this database in the future. Secondly, how long a database like this is reliable in determining origin of new introductions depends on the temporal genetic stability of populations. We addressed temporal genetic stability/instability of *Ae. aegypti* populations in a previous study (Gloria-Soria et al. 2016) and found that some populations remain quite stable at least over 2–7 years, whereas others change. However, almost all temporal changes are minor compared to spatial stability and do not distort the geographic patterns used to identify the origin of new introductions. In earlier sections of this paper, we also discussed the evidence that genetic patterns in Rabai Kenya, the Southern US, and Mexico have been stable for at least 20–40 years.

Our results should be considered in any attempts to use genetic modification of populations to control disease transmission (McGraw and O'Neill 2013). The genetic distinctness of populations, especially outside Africa, means that ease with which introduced genes can be driven into a population may vary depending on the genetic composition of the target population. Different genetic strategies may need to be designed for genetically distinct target populations. Genetic modification using a method based on standing genetic variation in populations (e.g. Powell and Tabachnick 2014) will need to be specific to the population. Finally, the low rates of migration implied by the high genetic structuring of *Ae. aegypti* means that released genetic material will not rapidly spread beyond the local population, i.e., migration rates and distances are highly restricted. This evidence for low dispersal based on genetic patterns is also supported by direct behavioral and ecological studies (Reiter 2007).

## Conclusions

We have documented the great degree of genetic diversity and genetic structure of *Aedes aegypti*. In the laboratory, no signs of reproductive isolation between the *Aedes aegypti* subspecies or between any populations have been observed. The evident free interbreeding in African cities where the domestic form has been introduced (Senegal and Mombasa,

Kenya) is evidence that there is no reproductive isolation under field conditions. Thus, *Aedes aegypti* can be considered a single genetic species.

In addition to the domestication event that led to *Ae. aegypti*'s spread out of Africa ~500 years ago, it is clear that additional independent domestication events are occurring in Africa, probably in response to expanding urbanization. A prime example is Yaounde, Cameroon, (locality 13 in Fig 2A) where populations were collected in an urban environment yet are genetically much more similar to forest-breeding populations typical of Aaf, than to Aaa outside Africa. These recent ecologically domestic types in Africa have retained the black cuticle typical of forest-breeding populations. Mirroring this shift of classically defined Aaf from forest to domestic breeding are classically defined Aaa domestic populations that revert to breeding in natural sites such as rock holes, bromeliads, plant axials, and tree holes, best documented in the Caribbean (Chadee et al. 1998).

This mosquito may be the most genetically diverse species of insect ever studied. The rapid and highly successful adaptation to human habitats and its subsequent spread is proof of the adaptive flexibility this genetic variation confers. This adaptability presents a challenge to control populations of *Aedes aegypti* in efforts to decrease their impact on human health.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

Generous colleagues assisted in providing samples for this study; we thank: J. Pinto, R. Butlin, M. Boots, D. Severson, R. Kelly, K. McCarley, Y. Lourenco, R. Martinez, Dr E. Nchoutpouen, S. Klüh, L. Irby, M. Garcia, A. Scalzo, S. Mulligan, J. Holeman, R. Gay, N. Zahiri, B. Weber, and D. J. Gubler. David A. Kellner and Robert Hallberg provided technical assistance in the lab.

Financial support for was provided by NIAID RO1 AI101112 awarded to JRP. A. Gloria-Soria was supported by NIAID 3R01AI091646-04S1. G. Seixas was funded by a PhD fellowship of Fundação para a Ciência e Tecnologia/MCTES (SFRH/BD/98873/2013). EIK and AAS were funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award number (BIO1483) to EIK.

## References

- Beaumont MA, Zhang W, Balding DJ. Approximate Bayesian Computation in Population Genetics. *Genetics*. 2002; 162(4):2025–2035. [PubMed: 12524368]
- Beebe NW, Ambrose L, Hill LA, Davis JB, Hapgood G, Cooper RD, Russell RC, Ritchie SA, reamer L, Lobo NF, Syafruddin D, den Hurk AF. Tracing the tiger: population genetics provides valuable insights into the *Aedes* (*Stegomyia*) *albopictus* invasion of the Australasian region. *PLoS Negl Trop Dis*. 2013; 7:e2361. [PubMed: 23951380]
- Bennett KL, Shija F, Linton Y-M, Misinzo G, Kaddumukasa M, et al. Historical environmental change in Africa drives divergence and admixture of *Aedes aegypti* mosquitoes: a precursor to successful worldwide colonisation? *Mol Ecol*. 2016; Available early online. doi: 10.1111/mec.13762
- Beserra EB, de Castro FP Jr, dos Santos JW, da Santos TS, Fernandes CR. Biology and thermal exigency of *Aedes aegypti* (L.) (Diptera: Culicidae) from four bioclimatic localities of Paraíba. *Neotrop Entomol*. 2006; 35(6):853–860. [PubMed: 17273720]



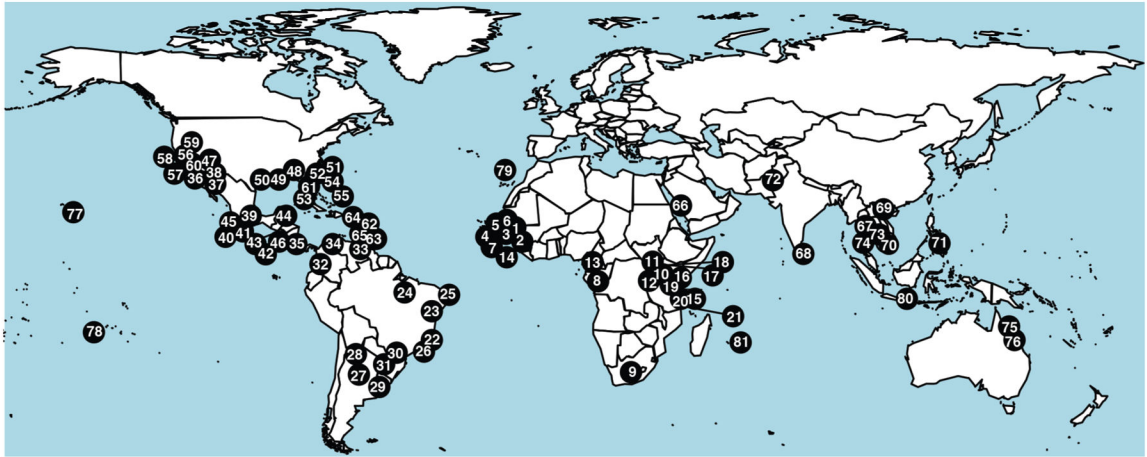
- Black WC, Bennett KE, Gorrochotegui-Escalante N, Barillas-Mury C, Fernandez-Sala I, Munoz MDL, Farfan-Ale JA, Olson KE, Beaty BJ. Flavivirus susceptibility in *Aedes aegypti*. *Arch Med Res*. 2002; 33:379–388. [PubMed: 12234528]
- Braco JE, Lourenco-de-Oliveira R, Salluym MA. Genetic variability of *Aedes aegypti* in the Americas using a mitochondrial gene: evidence of multiple introductions. *Mem Inst Oswaldo Cruz*. 2007; 102:573–580. [PubMed: 17710301]
- Bock, dG; Caseys, C.; Cousens, RD.; Hahn, MA.; Heredia, SM.; Hubner, S.; Turner, KG.; Whitney, KD.; Rieseberg, LH. What we still don't know about invasion genetics. *Mol Ecol*. 2015; 24:2277–2297. [PubMed: 25474505]
- Brown JE, McBride CS, Johnson P, Ritchie S, Paupy C, Bossin H, Fernandez-Salas I, Sylla M, Ponlawat A, Cornel AJ, Black WC, Gorrochotegui-Escalante N, Urdaneta-Marquez L, Slotman M, Walker C, Murray K, Powell JR. Worldwide patterns of genetic differentiation imply multiple “domestications” of *Aedes aegypti*, a major vector of human diseases. *Proc Royal Soc B*. 2011a; 278:2446–2454.
- Brown JE, Scholte E-J, Dik M, Hartog WD, Beeuwkes J, Powell JR. *Aedes aegypti* mosquitoes imported into the Netherlands, 2010. *Emerg Infect Dis*. 2011b; 17:2335–2337. [PubMed: 22172498]
- Brown JE, Evans B, Zheng W, Abas V, Barrera-Martinez L, Egizi A, Zhao H, Caccone A, Powell JR. Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. *Evolution*. 2014; 68:514–525. [PubMed: 24111703]
- Chadee DD, Ward RA, Novak RJ. Natural habitats of *Aedes aegypti* in the Caribbean—a review. *J Am Mosq Control Assoc*. 1998; 13:5–11.
- Christophers, R. *Aedes aegypti, the Yellow Fever Mosquito*. Cambridge University Press; Cambridge, UK: 1960.
- Clemens, AN. *The Biology of Mosquitoes. Vol. 1: Development, Nutrition and Reproduction*. Chapman and Hall; London: 1992.
- Clemens, AN. *The Biology of Mosquitoes Vol 2: Sensory Reception and Behaviour*. CABI, University Press; Cambridge: 1999.
- Cornuet J-M, Pudlo P, Pierre, Veyssier J, Dehne-Garcia A, Alexandre, Gautier M, Leblois R, Marin J-M, Estoup A. DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics*. 2014; 30:1187–2620. [PubMed: 24389659]
- Curtin TJ. Status of *Aedes aegypti* in the eastern Mediterranean. *J Med Entomol*. 1967; 4:48–50. [PubMed: 6074036]
- Delatte H, Bagny L, Brengue C, Bouetard A, Pauly C, Fontenille D. The invaders: Phylogeography of dengue and chikungunya viruses *Aedes* vectors on the southwest islands of the India Ocean. *Infect Genet Evol*. 2011; 11:1769–1781. [PubMed: 21827872]
- Dickson LB, Sharakhova MV, Timoshevskiy VA, Fleming KL, Carparly A, Sylla M, Black WC. Reproductive incompatibility involving Senegalese *Aedes aegypti* (L.) is associated with chromosome rearrangements. *PLoS Neg Trop Dis*. 2016; 10:e0004626.
- Earl SA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour*. 2012; 4:359–361.
- Ersts, PJ. [Accessed on Feb. 2, 2016] Internet 2016 Geographic Distance Matrix Generator(version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available from [http://biodiversityinformatics.amnh.org/open\\_source/gdmg](http://biodiversityinformatics.amnh.org/open_source/gdmg)
- Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*. 2005; 14:2611–2620. [PubMed: 15969739]
- Fonseca DM, Smith JL, Kim H-C, Mogi M. Population genetics of the mosquito *Culex pipiens pallens* reveals sex-linked asymmetric introgression by *Culex quinquefasciatus*. *Infect Genet Evol*. 2009; 9:1197–1203. [PubMed: 19584006]
- Giraldo-Calderón GI, Emrich SJ, MacCallum RM, Maslen G, Dialynas E, et al. VectorBase: an updated bioinformatics resource for invertebrate vectors and other organisms related with human diseases. *Nucleic Acids Res*. 2015; 43:D707–713. [PubMed: 25510499]

- Gloria-Soria A, Brown JE, Kramer V, Hardstone Yoshimizu M, Powell JR. Origin of the dengue fever mosquito, *Aedes aegypti*, in California. *PLoS Neglected Trop Dis*. 2014; 8(7):e3029.
- Gloria-Soria A, Kellner D, Brown JE, Gonzalez-Acosta C, Kamgang B, Luwama J, Powell JR. Temporal genetic stability of *Stegomyia aegypti* (= *Aedes aegypti*) populations. *Medical and Veterinary Entomology*. 2016; doi: 10.1111/mve.12153
- Gorochotegui-Escalante N, De Lourdes Munoz M, Fernandez-Salas I, Beaty BJ, Black WC. Genetic isolation by distance among *Aedes aegypti* populations along the northeastern coast of Mexico. *Am J Trop Med Hyg*. 2000; 62:200–209. [PubMed: 10813474]
- Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev*. 1998; 11:480–496. [PubMed: 9665979]
- Hartberg WK, Craig GB Jr. Reproductive isolation in *Stegomyia* mosquitoes. II. Hybrid breakdown between *Aedes aegypti* and *A. mascarensis*. *Evolution*. 1970; 24:692–703.
- Havill NP, Shiyake S, Lamb Galloway A, Footitt RG, Yu, et al. Ancient and modern colonization of North America by hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), an invasive insect from East Asia. *Mol Ecol*. 2016; 25(9):2065–2080. [PubMed: 26880353]
- Holstein M. Dynamics of *Aedes aegypti* distribution, density and prevalence in the Mediterranean area. *Bull Wld Hlth Org*. 1967; 36:541–543.
- Huber K, Ba Y, Dia I, Mathiot C, Sall AA, Diallo M. *Aedes aegypti* in Senegal: Genetic diversity and genetic structure of domestic and sylvatic populations. *Am J Trop Med Hyg*. 2008; 79:218–229. [PubMed: 18689628]
- Jombart T, Adegnet A. R package for the multivariate analysis of genetic markers. *Bioinformatics*. 2008; 24:1403–1405. [PubMed: 18397895]
- Jombart T, Devillard S, Balloux F. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*. 2010; 11:94. [PubMed: 20950446]
- Jupp PG, Kemp A, Frangos C. The potential for dengue in South Africa: morphology and the taxonomic status of *Aedes aegypti* populations. *Mosq Syst*. 1991; 23:182–190.
- Kalinowski ST. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol Ecol Notes* 2005. 2005; 5:187–189.
- Kamgang B, Ngoagouni C, Manirankiza A, Nakoune E, Paupy C, Kazanji M. Temporal patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and mitochondrial DNA analysis of *Ae. albopictus* in Central African Republic. *PLoS Negl Trop Dis*. 2013; 7:e2590. [PubMed: 24349596]
- Linss JG, Brito LP, Garcia GA, Araki AS, Bruno RV, Lima JBP, Valle D, Martins AJ. Distribution and dissemination of the Val1016Ile and Phe1534Cys Kdr mutation in *Aedes aegypti* Brazilian natural populations. *Parasites Vectors*. 2014; 7:25. [PubMed: 24428880]
- Linas GA, Gardenal CN. Phylogeography of *Aedes aegypti* in Argentina: Long-distance colonization and rapid restoration of fragmented relicts after a continental control campaign. *Vector-Borne Zoon Dis*. 2012; 12:254–261.
- Lounibos LP. Habitat segregation among African treehole mosquitoes. *Ecol Entomol*. 1981; 6:129–154.
- Lounibos LP. Invasions by insect vectors of human disease. *Ann Rev Entomol*. 2002; 47:233–266. [PubMed: 11729075]
- Lounibos, LP. Genetic-control trials and the ecology of *Aedes aegypti* in the Kenya coast. In: Takken, W.; Scott, TW., editors. *Ecological Aspects for Application of Genetically Modified Mosquitoes*. Kluwer Academic Publishers; Dordrecht, The Netherlands: 2003. p. 33–43.
- MacGregor ME. *Aedes (Stegomyia) mascarensis*, MacGregor: A new mosquito from Mauritius. *Bull Entomol Res*. 1924; 14:409–412.
- Mack, Richard N.; Simberloff, Daniel; Lonsdale, W Mark; Evans, Harry; Clout, Michael; Bazzaz, Fakhri A. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol Appl*. 2000; 10(3):689–710.
- Mangudo C, Aparicio JP, Gleiser RM. Tree holes as larval habitats for *Aedes aegypti* in urban, suburban and forest habitats in a dengue affected area. *Bull Entomol Res*. 2015; 105:679–684. [PubMed: 26193903]

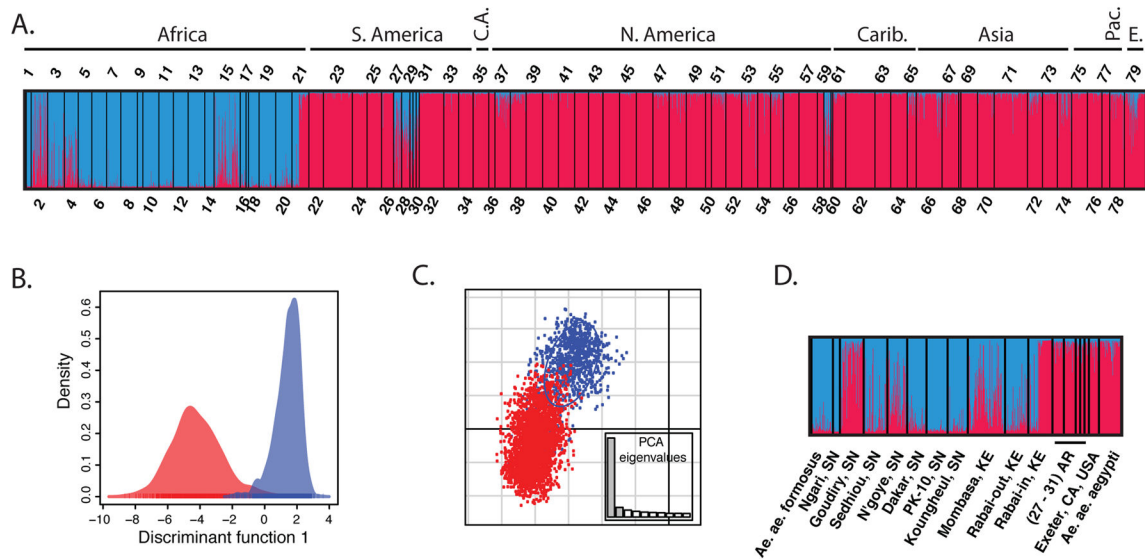
- Marinho RA, Beserra EB, Bezerra-Gusmão MA, de Porto VS, Olinda RA, et al. Effects of temperature on the life cycle, expansion, and dispersion of *Aedes aegypti* (Diptera: Culicidae) in three cities in Paraíba, Brazil. 2016
- Mattingly PF. Genetical aspects of the *Aedes aegypti* problem I. Taxonomy and bionomics. *Ann Trop Med Parasitol.* 1957; 51:392–408.
- Mattingly PF. Taxonomy of *Aedes aegypti* and related species. *Bull World Health Org.* 1967; 36:552–554. [PubMed: 4383544]
- Mayr, E. *Animal Species and Evolution.* Belknap Press; Harvard, Cambridge, MA: 1963.
- McBride CS, Baier F, Omondi AB, Spitzer SA, Lutomiah J, Sang R, Ignell R, Vosshall LB. Evolution of mosquito preference for humans linked to an odorant receptor. *Nature.* 2014; 515:222–227. [PubMed: 25391959]
- McClelland, GAH. Speciation and evolution in *Aedes*. In: Wright, JW.; Pal, R., editors. *Genetics of Insect Vectors of Disease.* Elsevier; Amsterdam, The Netherlands: 1967. p. 277–311.
- McClelland GAH. A worldwide survey of variation in scale pattern of the abdominal tergum of *Aedes aegypti* (L.) (Diptera:Culicidae). *Trans Roy Entomol Soc Lond.* 1974; 126:239–259.
- McGraw EA, O'Neill SL. Beyond insecticides: New thinking on an ancient problem. *Nature Rev Microbiol.* 2013; 11:181–193. [PubMed: 23411863]
- Meirmans PG, Van Tienderen PH. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes.* 2004; 4:792–794.
- Montella IR, Martins AJ, Viana-Medeiros PF, Pereira Lima JB, Braga IA, Valle D. Insecticide resistance mechanisms of Brazilian *Aedes aegypti* populations from 2001 to 2004. *Am J Trop Med Hyg.* 2007; 77:467–477. [PubMed: 17827362]
- Monteiro FA, Shama R, Martins A, Gloria-Soria A, Brown JE, Powell JR. Genetic diversity of Brazilian *Aedes aegypti*: Patterns following an eradication program. *PLoS Negl Trop Dis.* 2014; 8(9):e3167. [PubMed: 25233218]
- Moore DF. Hybridization and mating behavior of *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol.* 1979; 16:223–226. [PubMed: 537005]
- Moore M, Sylla M, Goss L, Burugu MW, Sang R, Kamau LW, Kenya EU, Bosio C, Munoz M, Sharakova M, Black WC. Dual African origins of global *Aedes aegypti* s.l. populations revealed by mitochondrial DNA. *PLoS Negl Trop Dis.* 2013; 74:e2175.
- Norell MA. Ghost taxa, ancestors, and assumptions: A comment on Wagner. *Paleobiology.* 1996; 22:453–455.
- Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers TA, King RA. Linkage Analysis with Multiplexed Short Tandem Repeat Polymorphisms Using Infrared Fluorescence and M13 Tailed Primers. *Genomics.* 1995; 30(3):450–458. <http://doi.org/http://dx.doi.org/10.1006/geno.1995.1264>. [PubMed: 8825630]
- Paupy C, Kamgang B, Herve J-P, Fontenille D, Simard F. Gene flow between domestic and sylvan populations of *Aedes aegypti* (Diptera:Culicidae) in north Cameroon. *J Med Entomol.* 2008; 45:391–400. [PubMed: 18533431]
- Paupy C, Brengues C, Ndiath O, Toty C, Herve J-P, Simard F. Morphology and genetic variability within *Aedes aegypti* in Niakhar, Senegal. *Infect Genet Evol.* 2010; 10:473–480. [PubMed: 20223297]
- Paupy C, Le Goff G, Brengues C, Guerra M, Revollo J, Simon ZB, Herve J-P, Fontenille D. Genetic structure and phylogeography of *Aedes aegypti*, the dengue and yellow-fever mosquito vector in Bolivia. *Infection Genet Evol.* 2012; 12:1260–1269.
- Peterson, JL. Ph.D dissertation. University of Notre Dame; Notre Dame, Ind: 1977. Behavior differences in two subspecies of *Aedes aegypti* (L.) (Diptera:Culicidae) in East Africa.
- Peakall RP, Smouse E. GenA1Ex 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 2012. 2012; 28:2537–2539.
- Pfeiler, E.; Flores-López, CA.; Mada-Vélez, JG.; Escalante-Verdugo, J.; Markow, TA. Genetic diversity and population genetics of mosquitoes (Diptera: Culicidae: *Culex* spp.) from the Sonoran Desert of North America. *Sci World J.* 2013. <http://dx.doi.org/10.1155/2013/724609>
- Powell JR, Tabachnick WJ. History of domestication and spread of *Aedes aegypti*. *Mem Inst Oswald Cruz.* 2013; 108(Suppl):11–17.

- Powell JR, Tabachnick WJ. Genetic shifting: A novel approach for controlling vector-borne diseases. *Trends Parasitol.* 2014; 30:282–288. [PubMed: 24794113]
- Powell JR, Tabachnick WJ, Arnold J. Genetics and the origin of a vector population: *Aedes aegypti*, a case study. *Science.* 1980; 208:1385–1387. [PubMed: 7375945]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000. 2000; 155:945–959.
- R Core Team. 2013. <http://www.R-project.org/>
- Raši G, Filipovi I, Callahan AG, Stanford D, Chan A, Lam-Phua SG, Tan HC, Hoffmann AA. The queenslandensis and the type form of the dengue fever mosquito (*Aedes aegypti* L.) are genomically indistinguishable. 2016; bioRxiv. doi: 10.1101/063792
- Raymond M, Rousset F. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *J Hered.* 1995; 86:248–249.
- Reiter P. Oviposition, dispersal, and survival in *Aedes aegypti*: implications for efficacy of control strategies. *Vector-borne Zoon Dis.* 2007; 7:261–273.
- Rondan Duenas JC, Llinas GA, Panzetta-Dutari gM, Gradenal CN. Two different routes of colonization of *Aedes aegypti* in Argentina from neighboring countries. *J Med Entomol.* 2009; 46:1344–1354. [PubMed: 19960679]
- Rousset F. Genepop '07: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour.* 2008; 8:103–106. [PubMed: 21585727]
- Rosenberg NA. DISTRUCT: a program for the graphical display of population structure. *Mol Ecol Notes.* 2004; 4:137–138.
- Schatzmayr HG. Dengue situation in Brazil by year 2000. *Mem Inst Oswaldo Cruz.* 2000; 95:179–181.
- Schug MDT, Mackay F, Aquadro CF. Low mutation rates of microsatellite loci in *Drosophila melanogaster*. *Nat Genet.* 1997; 15:99–102. [PubMed: 8988178]
- Sim S, Jupatanakul N, Rabirez JL, Kang S, Romero-Vivas CM, Mohammed H, Dimopoulos G. Transcriptomic profiling of diverse *Aedes aegypti* strains reveals increased basal-level immune activation in dengue virus-refractory populations and identifies novel virus-vector molecular interactions. *PLoS Negl Trop Dis.* 2013; 7(7):e2295. [PubMed: 23861987]
- Slotman MA, Kelly NB, Harrington LC, Kittahwee S, Jones JW, Scott TW, Caccone A, Powell JR. Polymorphic microsatellite markers for studies of *Aedes aegypti* (Diptera:Culicidae), the vector of dengue and yellow fever. *Mol Ecol Notes.* 2007; 7:168–171.
- Smith CEG. The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *Am J Trop Med Hyg.* 1956; 59:3–11.
- Sylla M, Bosio C, Urdaneta-Marquez L, Ndlaye M, Black WC. Gene flow, subspecies composition, and dengue virus-2 susceptibility among *Aedes aegypti* collections in Senegal. *PLoS Negl Trop Dis.* 2009; 3:e408. [PubMed: 19365540]
- Tabachnick WJ. Evolutionary genetics and the yellow fever mosquito. *Am Entomol.* 1991; 37:14–24.
- Tabachnick WJ, Munstermann L, Powell JR. Genetic distinctness of sympatric forms of *Aedes aegypti*. *Evolution.* 1979; 33:287–295.
- Tabachnick WJ, Wallis GP, Aitken THG, Miller BR, Amato GD, Lorenz L, Powell JR, Beaty BR. Oral infection of *Aedes aegypti* with yellow fever virus: Geographical variation and genetic considerations. *Am J Trop Med Hyg.* 1985; 34:1219–1224. [PubMed: 3834804]
- Theobald, FV. *Brit Mus (Nat Hist)*. Vol. I–V. London: 1901. Monograph of the Culicidae of the World.
- Tonn RJ, Figueredo R, Uribe LJ. *Aedes aegypti*, the yellow fever and dengue mosquito in the Americas. *Mosquito News.* 1982; 42:497–505.
- Tracy, JD., editor. *The Rise of Merchant Empires: Long Distance Trade in the Early Modern World*. Cambridge Univ. Press; Cambridge, UK: 1990.
- Trpis M, Hausermann W. Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera, Culicidae) in Africa by mark-release-recapture. *Bull Entomol Res.* 1975; 65:199–208.
- Tsuda Y, Yotopranoto S, Bendryman SS, Rossmanida, Dachlan YP, Takagi M. Seasonal changes in variation of dorsal scale pattern of *Aedes aegypti* (L.) (Diptera:Culicidae) in Surabaya, Indonesia. *Japan Soc Med Entomol.* 2003; 54:73–80.

- Vazeille, Zouache K, Vega-Rua A, Thiberge J-M, Caro V, Yebakima A, Mousson L, Piorkowski G, Dauga C, Vaney M-C, Manni M, Garperi G, de Lamballeri X, Failloux A-B. Importance of mosquito “quasispecies” in selecting an epidemic arthropod-borne virus. *Nature Sci Reports*. 2016; 6:29564.
- Wallis GP, Tabachnick WJ, Powell JR. Macrogeographic genetic variation in a human commensal: *Aedes aegypti*, the yellow fever mosquito. *Genet Res*. 1983; 41:241–258. [PubMed: 6884770]
- Weir BS, Cockerham CC. Estimating F-statistics for the analysis of population structure. *Evolution*. 1984; 38:1358–1370.

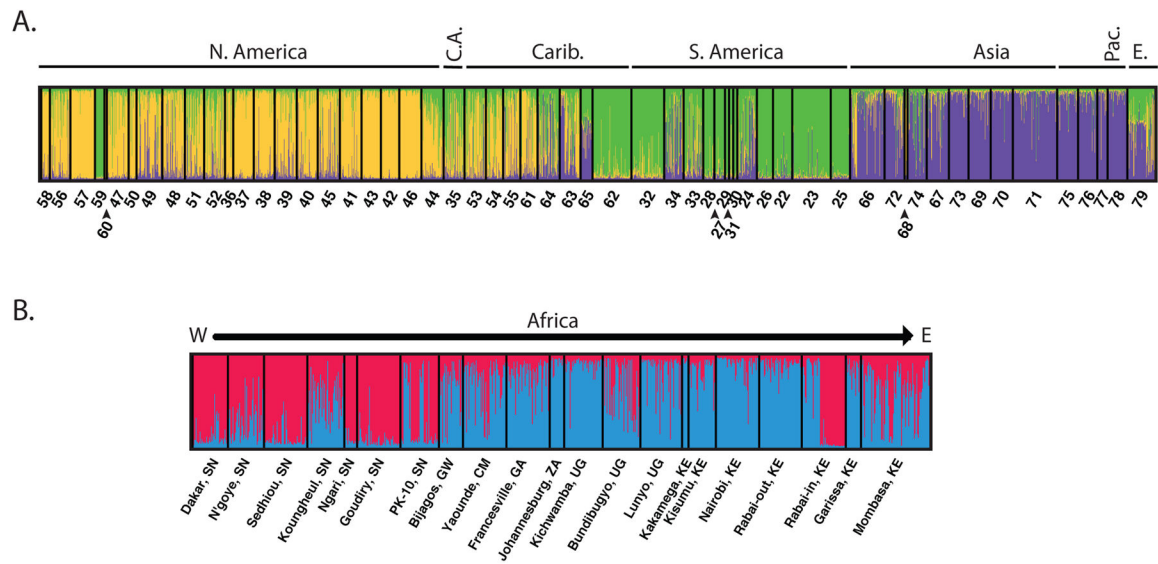


**Figure 1.** Sampling locations of *Aedes aegypti* and *Aedes mascarensis* collections used in this study. Population codes are as labeled in Table 1. Putative *Aedes aegypti queenslandensis* is indicated as population 80 and *Aedes mascarensis* as population 81. Approximate locations are displayed in order to accommodate all labels.



**Figure 2.**

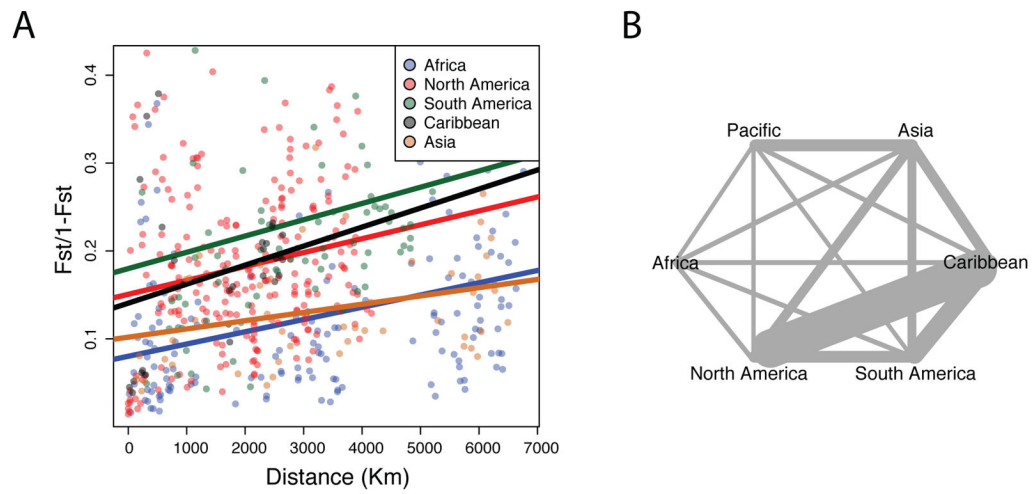
Global genetic structure of *Aedes aegypti*. A) STRUCTURE bar plot indicating genetic groupings of 79 geographic locations based on 12 microsatellite loci. Each vertical bar represents an individual. The height of each bar represents the probability of assignment to each of  $K = 2$  clusters as determined using the Delta K method. Each cluster is indicated by different colours: Aaa: red and Aaf: blue. Population code numbers are in Table 1. B) Discriminant Analysis of Principal Components (DAPC) on microsatellite allele frequencies showing two clear genetic clusters with minimal overlap; colors are as in A. C) Scatter plot of the first two principal components of the same data analysed in A and B. Groups corresponding to the Aaa and Aaf genetic clusters are plotted using the same colors as in A. Most of the variation is captured by the first and second PCA, as shown by the eigenvalue graph. D) STRUCTURE bar plot of those individual populations showing admixture in A, colors are consistent in A, B, and C.



**Figure 3.**

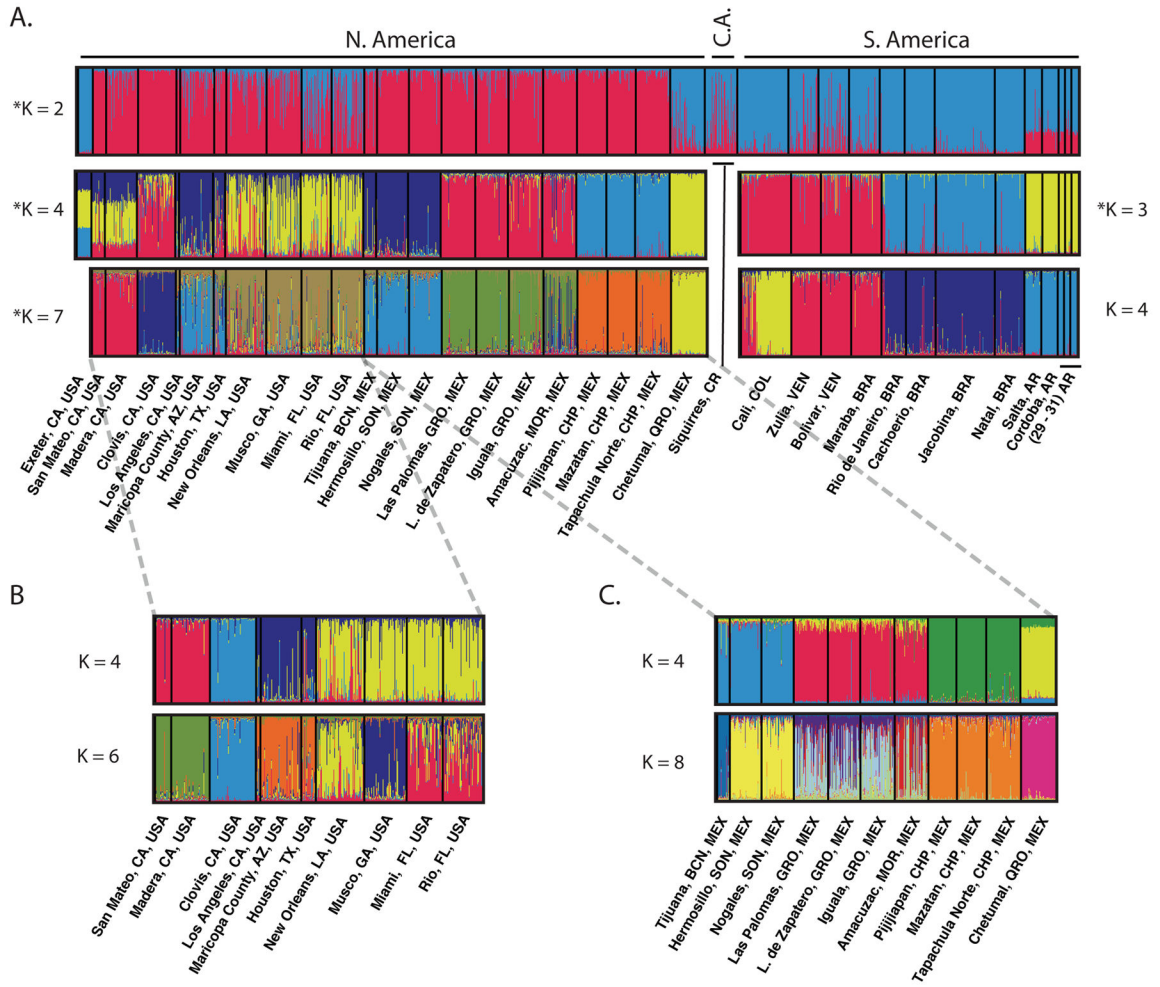
Genetic structure of *Aedes aegypti* A) out-of-Africa and B) within Africa. STRUCTURE bar plots indicating relatedness among geographic locations. Population codes in A are as labeled in Table 1. Abbreviations in A top: C. A. = Central America, E. = Europe, Pac. = Australia, Tahiti, and Hawaii. Populations are sorted by countries and by longitude (W: west to E: east).



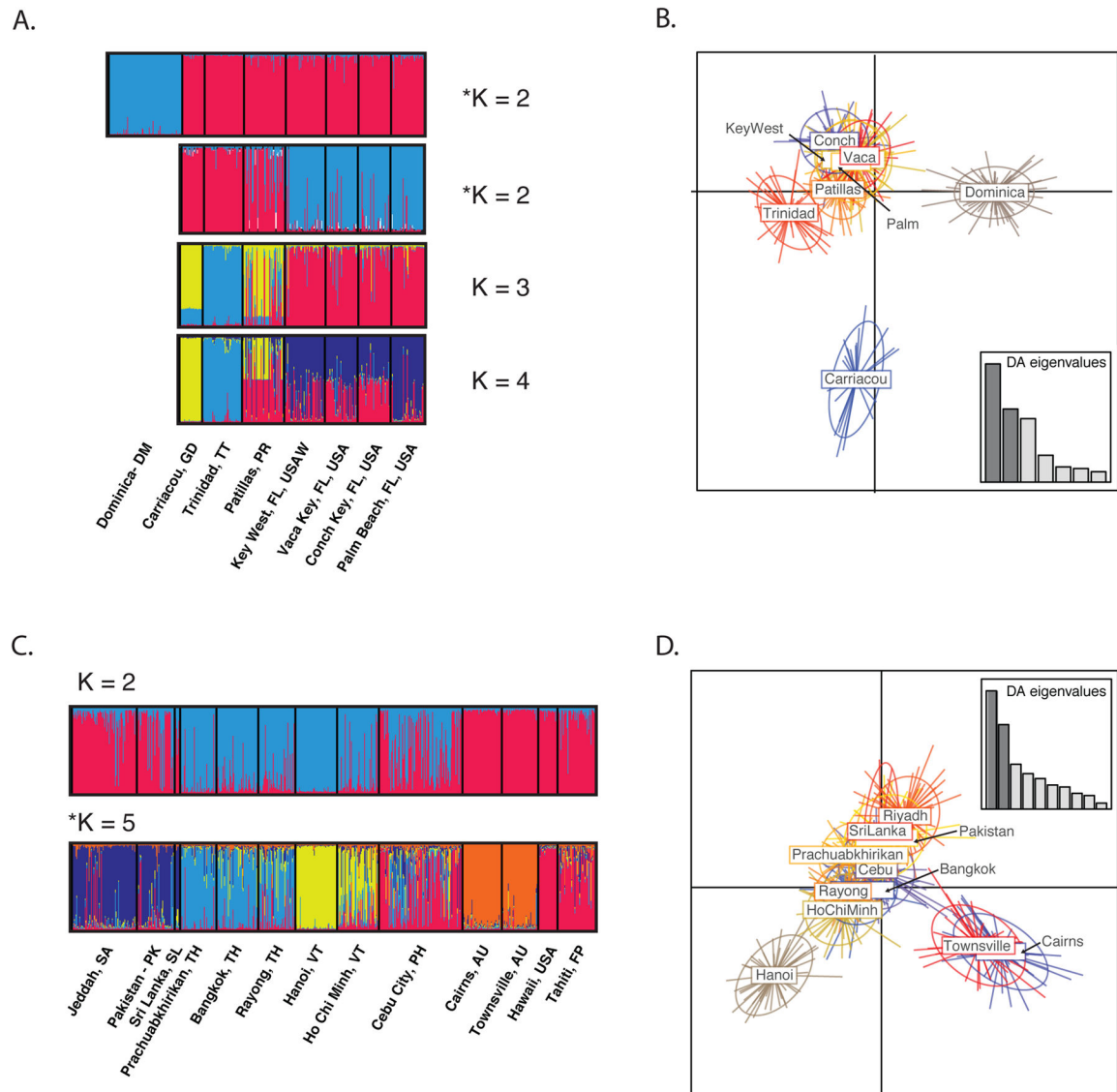


**Figure 4.**

Genetic differentiation of major geographic regions. A) Scale of geographic genetic differentiation. Genetic distance is given as the linearized  $F_{ST}$  ( $F_{ST}/(1-F_{ST})$ ) for the analysis of 12 microsatellite loci. Statistical significance was evaluated using a Mantel test and were all significant positive slopes ( $p < 0.05$ ) except for the Caribbean ( $p = 0.18$ ) and S. America ( $p = 0.07$ ) populations. B) Gene flow network between the continents or regions. The thickness of the lines is proportional to  $F_{ST}$ .

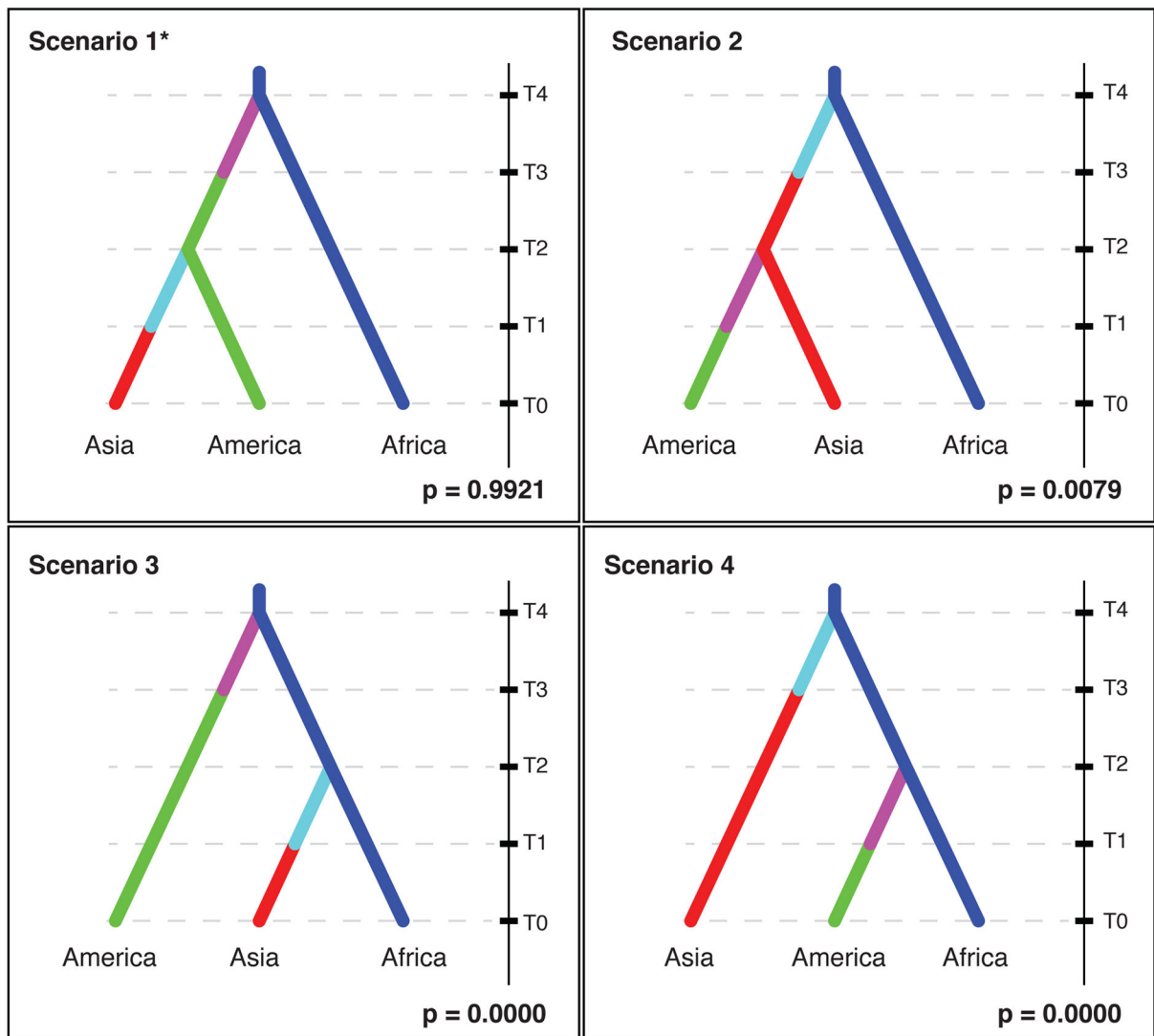


**Figure 5.** Genetic structure of *Aedes aegypti* within the American continent. Panels are A) All continental America, B) North America (excluding Exeter, California), and C) Mexico. STRUCTURE bar plots indicating relatedness among geographic locations. Plots representing the optimal K as determined by the Delta K method are indicated by an asterisk (\*). Discriminant Analysis of Principal Component plots for these data are shown in Fig S1.

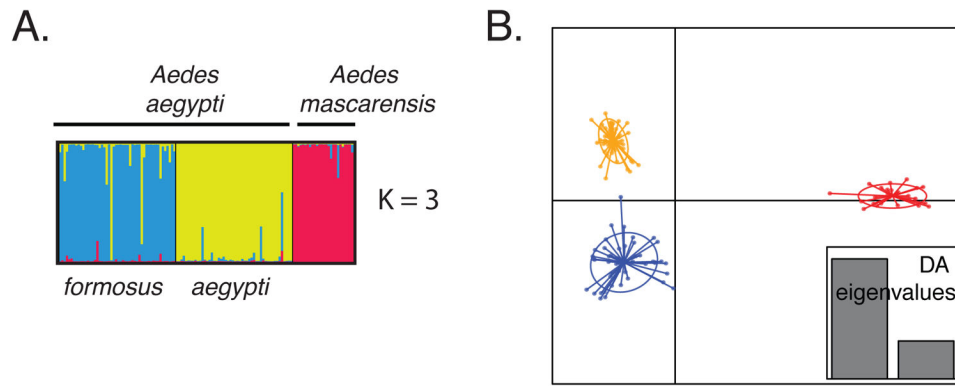


**Figure 6.**

Genetic structure of *Aedes aegypti* in the Caribbean and Asia/Pacific regions. A) STRUCTURE plots of Caribbean populations (including Florida Keys) with K number of clusters as indicated. Plots representing the optimal K as determined by the Delta K method are indicated by an asterisk (\*). B) Same populations in A in a Discriminant Analysis of Principal Components (DAPC). C) and D) same analyses as A) and B) for Asia and the Pacific (Australia, Tahiti, and Hawaii).

**Figure 7.**

Evolutionary scenarios of *Aedes aegypti* colonization of Asia, evaluated using Approximate Bayesian Computation inference as implemented by the DIYABC software (Cornuet et al. 2014). Scenarios include three populations: Africa, America, and Asia,  $N=200$  for each continent. T0 represents the most recent time point and increasing values of T go back in time. Scenario 1: Africa to America to Asia; Scenario 2: Africa to Asia to America; Scenario 3: Africa to America + Africa to Asia (after America colonization); and Scenario 4: Africa to America + Africa to Asia (before America colonization). Posterior probabilities are shown for each scenario. For more details see Materials and Methods and Table S1.



**Figure 8.**

Genetic structure among *Aedes aegypti* and *Aedes mascarensis* populations. A) STRUCTURE bar plots for the 26 *Ae. mascarensis* sampled and, to avoid sample size artifacts, fifty random individuals subsampled from the large Aaa and Aaf dataset, excluding those populations with large admixture levels (Fig 2D). B) Discriminant Analysis of Principal Components for the same samples depicted in the STRUCTURE plot.

TABLE 1

*AEDES AEGYPTI* COLLECTIONS INCLUDED IN THIS STUDY.

<sup>1</sup> Code	<sup>2</sup> Population	Region	<sup>3</sup> Year	<sup>4</sup> N
1	Ngari, SN	Africa	2012	16
2	Goudiry, SN	Africa	2012	54
3	Sedhiou, SN	Africa	2012	54
4	N'goye, SN	Africa	2007	45
5	Dakar, SN	Africa	2005	44
6	PK-10, Kedougou, SN	Africa	2006	48
7	Koungheul, SN	Africa	2006	46
8	Francesville - GA	Africa	2014	54
9	Johannesburg, ZA	Africa	2015	18
10	Lunyo, UG	Africa	2013	52
11	Bundibugyo, UG	Africa	2009	47
12	Kichwamba, UG	Africa	2009	48
13	Yaounde, CM	Africa	2014	54
14	Bijagos, GW	Africa	2009	30
15	Mombasa, KE	Africa	2012	85
16	Garissa, KE	Africa	2012	19
17	Kakamega, KE	Africa	2012	8
18	Kisumu, KE	Africa	2012	34
19	Nairobi, KE	Africa	2012	54
20	Rabai-out, KE	Africa	2006, 2009	53
21	Rabai-in, KE	Africa	2006, 2009	55
22	Cachoeiro, BR	South America	2012	47
23	Jacobina, BR	South America	2013	94
24	Maraba, BR	South America	2010	48
25	Natal, BR	South America	2010	47
26	Rio de Janeiro, BR	South America	2014	39
27	Cordoba, AR	South America	2003	26
28	Salta, AR	South America	2006, 2014	27
29	La Plata, AR	South America	2014	10
30	Iguazu, AR	South America	2014	10
31	Posadas, AR	South America	2014	10
32	Cali, CO	South America	2013	80
33	Bolivar, VEN	South America	2004	48
34	Zulia, VEN	South America	2004	47
35	Siquirres, CR	Central America	2014	51
36	Tijuana, BC, MEX	North America	2013	20
37	Hermosillo, SON, MEX	North America	2013	50
38	Nogales, SON, MEX	North America	2013	51
39	Las Palomas, GRO, MEX	North America	2012	54

<sup>1</sup> Code	<sup>2</sup> Population	Region	<sup>3</sup> Year	<sup>4</sup> N
40	Lomas de Zapatero, GRO, MEX	North America	2012	51
41	Amacuzac, MOR, MEX	North America	2014	53
42	Mazatan, CHP, MEX	North America	2012	45
43	Pijjiapan, CHP, MEX	North America	2008	47
44	Chetumal, QRO, MEX	North America	2013	54
45	Iguala, GRO, MEX	North America	2012	54
46	Tapachula Norte, CHP, MEX	North America	2012	54
47	Maricopa County, AZ	North America	2013	53
48	Musco, GA, USA	North America	2012	55
49	New Orleans, LA, USA	North America	2011	63
50	Houston, US	North America	2011	19
51	Miami, FL, USA	North America	2011	47
52	Rio, FL, USA	North America	2014	51
53	North Key West, FL, USA	North America	2013	52
54	Vaca Key, FL, USA	North America	2009	42
55	Conch Key, FL, USA	North America	2006	42
56	Madera, CA, USA	North America	2013	50
57	Clovis, CA, USA	North America	2013	60
58	San Mateo, CA, USA	North America	2013	21
59	Exeter, CA, USA	North America	2014	23
60	Los Angeles, CA, USA	North America	2014	6
61	Palm Beach, FL, USA	Caribbean	2006	42
62	Dominica - DM	Caribbean	2009	95
63	Trinidad - TT	Caribbean	2014	51
64	Patillas, PR	Caribbean	2014	54
65	Carriacou, GD	Caribbean	2015	29
66	Jeddah, SA	Asia	2012	84
67	Bangkok, TH	Asia	2013	54
68	Sri Lanka, SL	Asia	2014	7
69	Hanoi, VT	Asia	2013	54
70	Ho Chih Minh, VT	Asia	2013	54
71	Cebu City, PH	Asia	2013	108
72	Pakistan - PK	Asia	2010	49
73	Rayong, TH	Asia	2006	48
74	Prachuabkhirikan, TH	Asia	2009	47
75	Cairns, AU	Asia	2013	51
76	Townsville, AU	Asia	2009	47
77	Hawaii, USA	Pacific	2009	25
78	Tahiti, FP	Pacific	2010	48
79	Madeira, PO	Europe	2012	66
<i>Total</i>				<b>3632</b>

<sup>1</sup> Population identifier;

<sup>2</sup>Population: includes the ISO 3166 two-letter geographic country code;

<sup>3</sup>Year of collection;

<sup>4</sup>No. of individuals genotyped.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



**TABLE 2**  
**POPULATION GENETICS STATISTICS OF *Aedes Aegypti* POPULATIONS BY CONTINENT.**

Region	N	Ho	$\mu\text{He}$	AR (200)	PAR (200)	$F_{st}$ (intra)	Genetic distance ( $F_{st}$ )					
							vs. NA	vs. SA	vs. C	vs. As	vs. P	
Africa	918	0.591	0.738	10.97	3.41	0.114	0.119	0.118	0.114	0.135		
N. America	952	0.548	0.635	6.36	0.14	0.149	-	0.053	0.065	0.132		
S. America	562	0.489	0.611	7.37	0.3	0.181	-	0.034	0.069	0.13		
Caribbean	407	0.541	0.635	6.34	0.21	0.143	-	-	0.06	0.117		
Asia	603	0.551	0.643	7.87	0.58	0.121	-	-	-	0.052		
Pacific	73	0.557	0.597	4.75	0	0.123	-	-	-	-		

N: sample size; Ho: observed heterozygosity;  $\mu\text{He}$ : unbiased expected heterozygosity; AR and PAR: allele richness and private allelic richness estimated by rarefaction (N=200 genes);  $F_{st}$ (intra): average genetic distance between populations within the region; NA: North America; SA: South America; C: Caribbean; As: Asia; P: Pacific.