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Genetic structure of Latin American *Aedes aegypti*
Estructura genética de Aedes aegypti *latinoamericano*

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SUMMARY

The mosquito *Aedes aegypti* is the main Dengue vector in Latin America. This study investigated the genetic structure of this vector using samples collected in Venezuela, Colombia, Peru, Mexico, Argentina, Puerto Rico, and French Guiana. We examined the distribution of a 246-basepair region of the NADH dehydrogenase subunit 4 mitochondrial gene among a total of 369 *Ae. aegypti* from all the populations. This gene was amplified by the polymerase chain reaction and tested for variation using single strand conformation polymorphism analysis. Twelve haplotypes were detected among all the countries sampled and grouped into two clades. Significant differentiation was detected among the populations studied and these were not genetically isolated by distance.

Keywords: Dengue, *Aedes aegypti*, ND4, haplotypes, differentiation, genetic.

RESUMEN

El mosquito Aedes aegypti *es el principal vector del Dengue en los países latinoamericanos. En este estudio se investigó la estructura genética de este vector en muestras colectadas en Venezuela, Colombia, Perú, Mexico, Argentina, Puerto Rico y Guyana Francesa. Nosotros examinamos la distribución de una región de 246 pares de bases del gen mitochondrial de la subunidad 4 de la NADH deshidrogenasa entre un total de 369* Ae. aegypti *de todas las poblaciones. Este gen fue amplificado por la reacción en cadena de la polimerasa y la variación se determinó usando el análisis de polimorfismos de conformación de cadena simple. Doce haplotipos se detectaron entre todos los países y se repartieron en dos clados. Una diferenciación significativa se detectó entre las poblaciones y estas no se encontraron genéticamente aisladas por distancia.*

Palabras clave: *Dengue,* Aedes aegypti*, ND4, haplotipos, diferenciación, genética.*

INTRODUCCIÓN

Dengue is the most important viral disease transmitted to human by *Aedes aegypti* mosquitoes in Venezuela and other Latin American countries, and its incidence and prevalence are rising annually (San Martin *et al.*, 2010). *Aedes aegypti* is the principal vector for Dengue viruses and it is able to maintain the four serotypes in an urban transmission cycle. In addition, this mosquito is also a vector for other viruses like Chikungunya and Zika which were responsible for a serious outbreaks of those disease in the Americas recently (Vorou, 2016; WHO, 2014).

Aedes aegypti populations may differ in biting behaviour, vectorial capacity, and other characteristics of epidemiological importance which may be detected through molecular markers (Tabachnick, 1991, Getis *et al.*, 2003, Harrington *et al.*, 2005). In addition, these markers could be used to determine the relatedness of geographic populations and associate this information with vector movements. This will help to analyze the risk of disease transmission (Getis *et al.*, 2003, Liotta *et al.*, 2005, Bracco *et al.*, 2007).

Single strand conformation polymorphism (SSCP) analysis offers a simple, sensitive, and cheap method of detecting DNA polymorphisms, therefore, SSCP has become one of the most used means for determining genetic variation (Orita *et al.*, 1989). SSCP is based on the principle that changes

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in the nucleotide sequences of a singlestrand DNA molecule alter its three-dimensional conformation. Point mutations could thereby change strands migration through polyacrylamide gels. SSCP detected 99% of base changes in 100 to 300 bp DNA fragments and 89% of point mutations in 300-450 bp molecules (Sunnucks *et al.*, 2000).

The understanding of genetic variability, population structure, and migration dynamics of the mosquito *Ae. aegypti* is a major issue because of the risk associated with invasive new mosquito strains in a region that could cause the emergence and spread of Dengue (Manguin & Boete, 2011). The persistent increase in air and sea traffic between American countries increases the probability of introducing new *Ae. aegypti* strains into the region. These vector invasions are particularly accentuated in regions where vector surveillance is poor for lack of routine entomological surveys.

The aim of this study was to investigate the population genetic structure of *Ae. aegypti* from Colombia, Peru, Mexico, Argentina, Puerto Rico and French Guiana, using ND4 mitochondrial DNA markers and compared these sequences with the haplotype sequences obtained from Venezuela in a previous work (Herrera *et al.*, 2006).

MATERIALS AND METHODS

Mosquito collection and DNA extraction – Eggs from mosquitoes collected during the period 2008-2010 in six countries (Fig. 1) were sent to the insectary in Maracay, Aragua, Venezuela where they were reared to adults and then stored at -70ºC awaiting DNA extraction. The DNA was extracted from individual specimens using a phenol/chloroform method, resuspended in 60 µl of sterilized water and stored at -80ºC (Rivero *et al.*, 2004). From the eight localities of the collections in Venezuela, it was selected the locality of La Esperanza because its haplotype distribution resembles more the total population than the others populations. The list of country of origin, localites, geographic coordinates and sample sizes are indicated in Table I.

Mitochondrial gene amplification - A 246-bp region of the ND4 gene was amplified using the oligonucleotides and reaction conditions of Herrera *et al.* (2006) but with a high annealing temperature (55°C) to limit the amplification of Nuclear Mitochondrial Elements (NUMTs) which are present in the *Ae. aegypti* genome (Black & Bernhardt, 2009). The PCR amplifications were carried out in 50 µL reaction volumes using 1 µL of template DNA in a PTC-100 thermal cycler (MJ Research, Inc., Watertown, MA). Negative controls (all reagents except template) were run to detect possible contamination. The amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide.

SSCP analysis - The PCR product (10 µL: 40-50 ng) was mixed with 8 µL of loading buffer (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol), centrifuged and heated to 95ºC for 10 min on a thermal cycler, then transferred immediately into ice. Samples were loaded onto 27 × 20 cm, 1 mm thick, 7% polyacrylamide gels. Gels were run at 4ºC for 20 h at a constant 8 milliamps and silver stained to visualize DNA fragments (Black & DuTeau, 1997). ND4 PCR products from 3-6 individuals of each haplotype were sequenced in both directions using the PCR primers. Sequencing reactions were performed with ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit on an Applied Biosystem Model ABI 3130XL.

Statistical analysis of mitochondrial haplotype frequencies- Analysis of molecular variance

Fig. 1. Map showing the geographic collection sites.

(AMOVA) (Excoffier *et al.*, 1992) was conducted on the resulting haplotypes within and among regions using Arlequin versión 3.1 (Excoffier *et al.*, 2005). The significance of the variance components was computed using a non-parametric permutation test (Excoffier *et al.*, 1992). The DNA sequences were aligned using the Clustal W software package (Thompson *et al.*, 1994). The nucleotide sequence and the frequency of each haplotype for each population were analyzed using DnaSP version 5.10 (Librado & Rozas, 2009). The number of polymorphic sites, the average number of nucleotide differences (k) (Tajima, 1983), the nucleotide diversity (π1) and the nucleotide diversity with the Jukes and Cantor correction (π2) (Nei 1987) were estimated. Effective migration rates (Nm) were calculated from FST. Transformed FST / (1- FST) were regressed on the natural logarithm of pairwise geographic distances among populations to test for isolation by distance (Slatkin, 1993). The Mantel test was performed using FORTRAN program MANTEL (William C. Black IV, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO). Genetic distance matrices were used to construct a cladogram among all populations by means of unweighted pair-group method with arithmetic averaging analysis in the NEIGHBOR procedure of PHYLIP 3.63 (Felsentein, 2004).

Phylogenetic relationships among haplotypes-MEGA (version 4.0) was used to perform phylogenetic analyses using maximum parsimony (Tamura *et al.*, 2007). A bootstrap analysis with 1000 replications was done to assess the consistency with which the dataset supported the resolved phylogenies. ND4 homologous regions of *Anopheles darlingi* and *Ae. albopictus* were used as outgroups.

RESULTS

Haplotypes frequencies

A total of six samples, representing 324 wild specimens of *Ae. aegypti* collected in Colombia, Peru, Mexico, Argentina, Puerto Rico and French Guiana (Table I) were analyzed. Twelve haplotypes were detected by SSCP analysis and sequencing. The haplotype sequences were aligned with ND4 sequences from Venezuela (Herrera *et al.,* 2006). All these haplotypes sequences, including haplotypes from Venezuela, were denoted from A-L and are already registered in the GenBank (KR349202-KR349213). The frecuencies of these haplotypes in each country, including the previous reported in Venezuela, are shown in Table II.

The haplotype A was shared among Peru, Mexico, Puerto Rico, French Guiana and Venezuela populations. Except Mexican population, its frequency was > 40%. Haplotype H was the most frequent in the Colombia (96.22%) and Argentina (90.74%) populations. Haplotype J was present in the Mexico and French Guiana populations and its frequency varied from 98.14 to 32.07 % respectively. Haplotype L was found only in the population from Puerto Rico at a high frecuency (57.89%). The rest of the haplotypes (I and K) occurred at low frequencies. The haplotype sequences were also aligned with other ND4 sequences registered in the GenBank. The haplotypes HH from Colombia and Argentina and HI from Colombia have identical sequences to the ones reported in Brazil for haplotypes H1 and H2, respectively (Sousa & Scaparssa, 2009). Several haplotypes from the coast of northeastern Mexico (Gorrochotegui-Escalante *et al.*, 2000) have

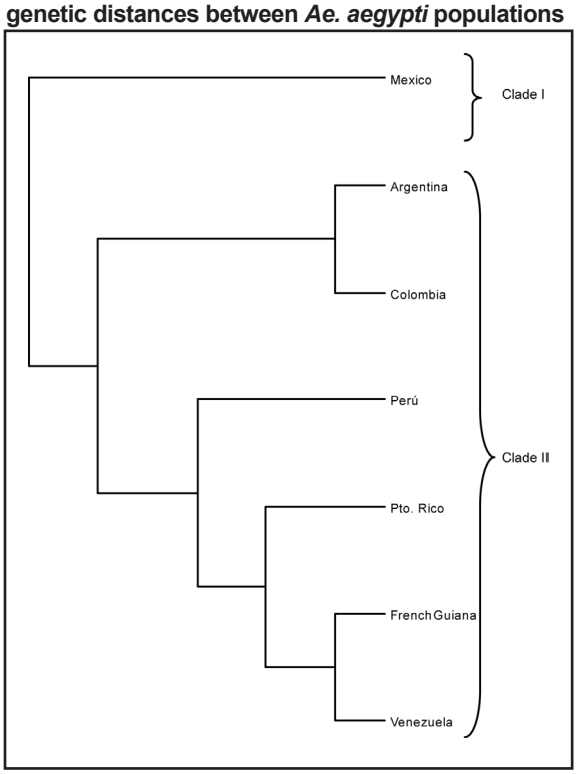
Table I. Countries, locations per region, geographic coordinates, and sample size of *Aedes aegypti* populations analized.

Country	Locality	Latitude	Longitude	N° individuals
Venezuela	La Esperanza	10º2'22"N	70º50'35"W	49
Colombia	Barranquilla	10°59'10"N	74°46'13"W	53
Peru	Lima	11º81' S	77º07' W	53
Mexico	Cuernavaca	18°56'68"N	99°13'33"W	54
Argentina	Córdoba	31º22'S	64 º12'W	54
Puerto Rico	San Juan	18º23'47"N	66°04'51"W	57
French Guiana	Baduel/Cayenne	04º55'53"N	52°18'17"W	53
Total	7			373

Table II. Present Haplotypes and their percentage per country.

Country	Haplotypes	% of Haplotypes
Venezuela	A	58.68
	B	0.78
	C	24.29
	D	2.80
	E	11.89
	F	0.37
	G	1.19
Colombia	H	96.22
	I	3.78
Peru	A	100
Mexico	A	1.86
	J	98.14
Argentina	H	90.74
	K	9.26
Puerto Rico	A	42.11
	L	57.89
French Guiana	A	67.92
	J	32.07

Fig. 2. Unweighted pair-group method with arithmetic averaging cluster analysis of pairwise FST/(1-FST) genetic distances between *Ae. aegypti* populations



similarities to the haplotypes reported in this work: haplotype-9b is 99% similar to the haplotype HJ from Mexico and French Guiana, haplotype 3a-3c is 98% analogous to HK from Argentina, haplotype-6b corresponds to 99% of HL from Puerto Rico and haplotype-10a is 99% similar to the haplotype HA found in Peru, Mexico, Puerto Rico, French Guiana and Venezuela. HA is also 97% analogous to one haplotype detected in Kenya (Burugu *et al.*, 2008).

Genetic heterogeneity

Geographical analysis of variation in ND4 haplotype frequencies was conducted by AMOVA (Table III). Most of the variation (70.6%) arose among populations while 29.5% arose among mosquitoes in populations. The average FST was 0.705 indicating a very high genetic structure among populations. In addition, the effect of the distance on levels of gene flow was estimated by regressing linearized FST values on geographic distance. This analysis indicated that significant correlation was not detected between genetic and geographic distances neither among all populations nor among populations in any particular subgroup of these countries (data not shown).

A distance matrix containing all the pairwise linearized FST among populations was collapsed using the UPGMA option in NEIGHBOR and the rectangular cladogram option on PHYLIP (Fig. 2). Two main clades were detected. Clade I contained only the Mexican population while clade II contained all of the other populations. This last clade was again divided into two subgroups: one with Argentina and Colombia populations and the other with Peru, Puerto Rico, French Guiana and Venezuela populations.

Haplotype diversity

The number of polymorphic sites and the diversity indices for each population and for all mosquitoes are listed in Table IV. The majority of populations had similar numbers of polymorphic sites (5-7) except for Peru (0), Argentina (1) and Venezuela (10). In relation to values for the mean number of nucleotide differences (k) and nucleotide diversity (π), populations from Venezuela, French

Table III. Analysis of molecular variance in the frequency of ND4 haplotypes among *Aedes aegypti* populations in seven Latin American countries.

Source of Variation	Degrees of freedom	Variance Components	Variation (%)	Fixation index	P
Among populations	6	0.28099	70.54		
Within populations	413	0.11738	29.46	FST = 0.705	$< 10^{-4}$
Total	419	0.39837			

Fixation index: FST, correlation among haplotypes within collections relative to the correlation of random pairs drawn from the whole sample.

Table IV. Variability estimates in the mitochondrial genome among *Aedes aegypti* populations in seven Latin America countries.

Country	N	S	k	π_1	π_2	Tajima's D
Venezuela	49	10	3.11300	0.01526	0.01555	1.09860
Colombia	53	5	0.37010	0.00182	0.00185	-1.59002
Peru	53	0	0	0	0	0
Mexico	54	7	0.25926	0.00127	0.00130	-2.16671
Argentina	54	1	0.17100	0.00084	0.00084	-0.28956
Puerto Rico	57	6	2.97740	0.01460	0.01489	3.19629
French Guiana	53	7	3.10900	0.01524	0.01560	2.65638
Total samples	373	15	4.68816	0.02309	0.02365	2.38142

N: number of samples; S: polymorphic sites; k: average number of nucleotide differences; π_1: nucleotide diversity; π_2: nucleotide diversity with Jukes and Cantor correction.

Guiana and Puerto Rico had higher values of k and π, in which values were at least 8-18 times higher than the k and π from Argentina, Mexican and Colombian populations. The values of k and π from the Peruvian population were 0. Tajima's D test gave a statistically significant positive result for the total sample (Table IV).

Phylogenetic analysis

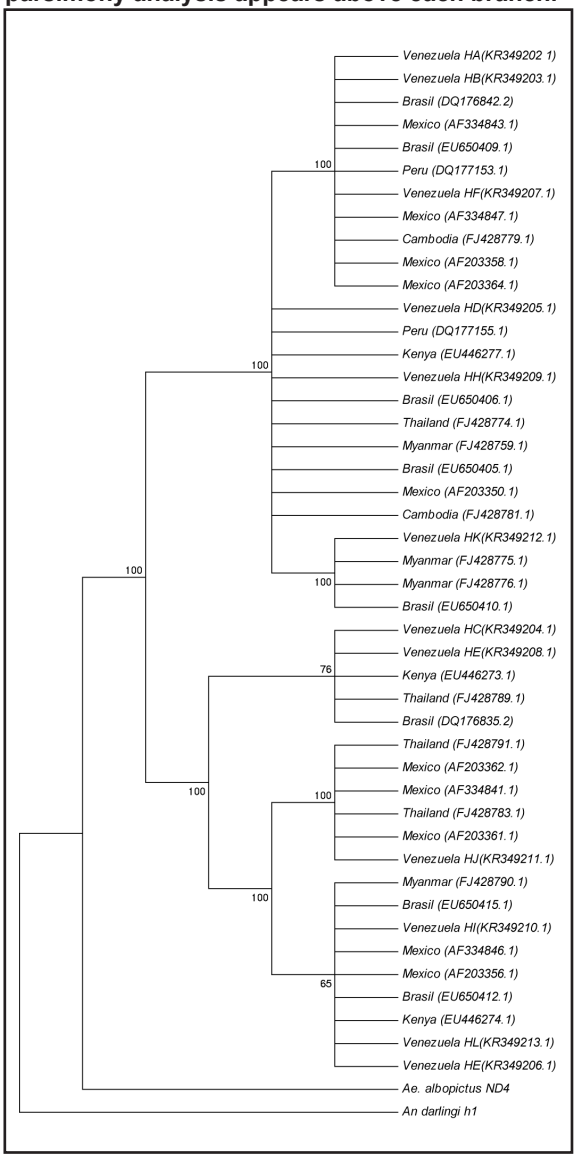
Phylogenetic analysis, based upon use as outgroups of one related subgenus *Stegomyia* species (*Aedes albopictus*) and other species of the genus *Anopheles* (*An. darlingi*), provided a well-supported phylogeny with two maternal lineages (Fig 3). The Clade I contained all the haplotypes found in the total population while the Clade II missed haplotypes from Argentina and Peruvian populations. The Clade I divided into clearly three sub-branches with 100% bootstrap support while Clade II divided into two branches with ≥ 76% support. In addition, both clades contained haplotypes that have been found independently in several studies from America (Brazil, Mexico, and Peru), Asia (Thailand, Myanmar, Cambodia) and Africa (Kenya).

DISCUSSION

Aedes aegypti populations from different countries were genetically differentiated. This suggests a restricted level of interchange of genes among populations. This genetic differentiation did not arise by geographic distance. Similar results were reported in Mexico (Gorrochotegui-Escalante *et al.*, 2000), Thailand (Bosio *et al.*, 2005), Peru (Costa da Silva *et al.*, 2005) and Brazil (Da Costa-Ribeiro *et al.*, 2007, Twerdochlib *et al.*, 2012). The existence of geographic barriers (Caribbean Sea and the Andean Mountain Range) among populations might account for our findings.

Another possibility that could explain the results may be genetic drift arising from a strong reduction in the effective population size in collections caused by insecticide applications (Sharma *et al.*, 2009). The presence of only few major haplotypes, unique haplotypes or haplotypes with very small frequencies in some countries could arise due to major population bottlenecks caused by insecticide treatment. A similar hyphotesis has been proposed by Gonçalves da Silva *et al.* (2012).

Fig. 3. Maximum parsimony tree showing phylogenetic relationships among individual haplotypes. Bootstrap support using maximum parsimony analysis appears above each branch.



They argued that recent bottlenecks have ocurred in Peru, Venezuela, Mexico-North America, and Brazil probably due to vector control practices through the use of insecticides.

Contrary to these results, some authors have found isolation by distance among samples from Brazil and other countries (Monteiro *et al.*, 2014). They have suggested that in Brazil first ocurred a complete eradication of *Ae. aegypti* (late 50's) followed by re-colonization (in the 1970's), probably from mosquitoes from neighboring countries like Venezuela. It could be argued that in this time span the lineages that may have initially re-invaded Brazil are now exchanging genes and perhaps merging.

The genetic diversity found in this work is high and comparable to another similar study (Bracco *et al.*, 2007). This caused a very high genetic structure with large FST (~ 1.0) values among populations. Similarly, Ayres *et al.* (2004) have reported that Brazilian populations of *Ae. aegypti* showed high levels of genetic differentiation in areas most frequently treated with chemical insecticides. Other investigators suggested that insecticide pressure is probably the major cause of genetic diversity in *Ae. aegypti* from highly populated urban areas (Paupy *et al.*, 2003, Ocampo & Wesson, 2004, Twerdochlib *et al.*, 2012). The use of insecticides has also facilitated the appearance of insecticide resistance in *Ae. aegypti* (Bisset *et al.*, 2001, Rodríguez *et al.*, 2001, Álvarez *et al.*,. 2008, Mourya *et al.*, 2015). This phenomenom appears to be happening in these populations as the statistically significant Tajima's D values were positive for all populations, indicating that there are haplotypes under positive selection (Tajima, 1983).

The populations were divided into two major clades, according to their genetic distance. The populations of Argentina, Mexico and Colombia, grouped in the first clade, were more genetically close than those of Venezuela, French Guiana, Puerto Rico and Peru, grouped in the second.

Phylogenetic analysis of haplotype sequences demonstrated two lineages. These did not contain haplotypes from a particular region since Asian, African and American haplotypes were found in both lineages. The haplotypes with greater frequencies (HA and HH) in the total population were located only in the lineage I. At least one of them was present in all subpopulations and together they constituted over 50% of the total haplotypes. Thus, HA was found in 5 of 7 populations and, except for Mexico, its frecuency varied from 42.1 to 100%. It can be argued, therefore, that this lineage is the oldest. Probably HA and HH have spread to all subpopulations through migration of individuals that are best adapted to environmental changes (insecticide pressure) which has allowed their high frequencies (Sousa & Scarpassa, 2009). The presence of two phylogenetic lineages that

subdivide the *Ae. aegypti* populations is consistent with earlier research (Gorrochotegui-Escalante *et al.*, 2002, Bosio *et al.*, 2005, Herrera *et al.*, 2006, Bracco *et al.*, 2007, Paduan & Ribolla, 2008, Sousa & Scarpassa, 2009, Fraga *et al.*, 2013, Moore *et al.*, 2013).

Populations from Puerto Rico, French Guiana and Venezuela had at least two haplotypes with frecuencies ≥ 25%, therefore their nucleotide diversity (π) and the mean number of nucleotide differences (k) were higher than the corresponding ones to the others populations with only one dominat haplotype ≥ 90,7%. In addition, all the populations with the highest level of genetic diversity contained HA in a major frecuency which strengthen the idea that HA is an ancient haplotype with a nucleotide differentiation already fixed.

The genetic variability found in these american countries (AC) was higher than those from Thailand (T) (k~1,6 and π~2,9 times) and the island of São Luís in the Brazilian state of Maranhão (BM) (k~2 and π~3 times) with an equal number of polymorphic sites (S =15) (Bosio *et al.*, 2005, Fraga *et al.*, 2013). It could be explained because AC have more haplotypes than T (12 vs 7) and than BM (12 vs 10). On the contrary, other comparison with populations from Brazilian Amazon (BA) resulted in πAC > πBA (2 times) while k value and haplotype number were in a similar range. This could mean that the more frequent BA haplotypes are more similar to each other than those of AC (Sousa & Scaparssa, 2009). The amount of polymorphic sites varied among the populations: 7-10 for Venezuela, México and French Guiana, 5-6 for Colombia and Puerto Rico, 1 for Argentina and 0 for Perú. This last result arose because Peru had only one haplotype. At the same time, it can also be noted that even though existed a striking difference between the populations of Colombia and Argentina (the first had 5 times more polymorphic sites than the second), both populations had a common dominant haplotype (Colombia 96.22%, Argentina 90.74%). This result agrees with the observed distribution of Colombia (HH and HI) and Argentina (HH and HK) haplotypes in different and same lineages respectively. This means that the nucleotide divergence of Argentina haplotypes that share a common ancestor, is smaller than those of Colombia.

In addition, the data presented here could have important implications for Dengue, Zika or Chikungunya transmission since the genetic variability in *Ae. aegypti* from these countries may cause differences in their susceptibility to these viruses. A correlation between population differentiation and heterogenous patterns of vector competence in *Ae. aegypti* has been suggested for other authors (Tran *et al.*, 1999, Vazeille-Falcoz *et al.*, 1999, 2001, Paupy *et al.*, 2003, Black WC *et al.*, 2002, García-Franco *et al.*, 2002, Lourenço-de-Oliveira *et al.*, 2004, Ocampo & Wesson, 2004). A population genetic analysis and an evaluation of susceptibility to Dengue 2 virus were conducted among *Ae. aegypti* samples from different states of Brazil (Laurenço-de-Oliveira *et al.*, 2004). It was demonstrated that Brazilian *Ae. aegypti* were genetically differentiated within most of the regions, and that their infection rates towards DENV2 were heterogenous. Other study has found a correlation between genetic distances of *Ae. aegypti* populations and their infections rates (GarcíaFranco *et al.*, 2002). Recently, it was observed considerable variation in vector competence within and between Brazilian populations analyzed from short spatial and temporal collections (Gonçalves *et al.*, 2014).

This study suggests that the vector control programme from these countries may induce recurrent emergence of insecticide resistant populations. Moreover, the rapid fixation of haplotypes conferring insecticide resistance could take place; the resistant populations may be spread at a larger scale as the migration of resistant mosquitoes could be facilitated over the one of susceptible insects. *Ae. aegypti* resistant to insecticides represent a great problem in Dengue, Chikungunya or Zika control. The next step could be to look for insecticide resistance in the studied populations to confirm the involvement of selection effects in the actual genetic structure of their *Ae. aegypti* populations.

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REFERENCIAS

Álvarez G., Oviedo A. & Briceño A. (2008). Evaluación de Temephos 50% CE sobre poblaciones de *Aedes aegypti* (Diptera: Culicidae) en Trujillo, Venezuela. *Rev. Colomb. Entomo.* **34:** 188-191.

Ayres C., Melo-Santos M., Prota J., Solé-Cava A., Regis L. & Furtado A. (2004). Genetic structure of natural populations of *Aedes aegypti* at the micro- and macrogeographic levels in Brazil. *J. Am. Mosq. Control Assoc.* **20:** 350-356.

Bisset J., Rodriguez M., Molina D., Díaz C. & Soca L. (2001). High esterases as mechanism of resistance to organophosphate insecticides in *Aedes aegypti* strains. *Rev. Cubana Med. Trop.* **53:** 37-43.

Black W., Bennett K., Gorrochotegui-Escalante N., Barillas-Mury C., Fernandez-Salas I., Muñoz M., *et al.* (2002). Flavivirus Susceptibility in *Aedes aegypti*. *Arch. Med. Res.* **33:** 379–388.

Black W. & Bernhardt S. (2009). Abundant nuclear copies of mitochondrial origin (NUMTs) in the *Aedes aegypti* genome. *Insect Mol. Biol.* **18:** 705-713.

Black W. & Du Teau N. (1997). *RAPD-PCR and SSCP analysis for insect population genetic studies*. pp. 361-373. In: The Molecular Biology of Insect Disease Vectors: A Methods Manual. Eds. Crampton J., Beard C.B., Louis C. Chapman and Hall. New York, U.S.A.

Bosio C., Harrington L., Jones J., Sithiprasasna R. & Norris D. (2005). Genetic Structure of *Aedes aegypti* populations in Thailand using Mitocondrial DNA. *Am. J. Trop. Med. Hyg.* **72:** 434-442.

Bracco J., Capurro M., Lourenco-de-Oliveira R. & Mureb Sallum M. (2007). Genetic variability of *Aedes aegypti* in the Americas using a mitocondrial gene: evidence of multiple introductions. *Mem. Inst. Oswaldo Cruz.* **102:** 573-580.

Burugu M., Sang R., Kamau L. & Kenya E. (2008). *Genetic structure of* Aedes aegypti *populations in coastal and inland Kenya using mitochondrial DNA*. Submitted Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya. EMBL/GenBank/ databases.

Costa da Silva A., Capurro M. & Bracco J. (2005). Genetic lineages in the yellow fever mosquito *Aedes (Stegomyia) aegypti* (Diptera: Culicidae) from Perú. *Mem. Inst. Oswaldo Cruz.* **100:** 539-544.

Da Costa-Ribeiro M., Lourenco-de-Oliveira R. & Failloux A. (2007). Low Gen Flow of *Aedes aegypti* between Dengue-Endemic and Dengue-Free areas in Southeastern and Southern Brazil. *Am. J. Trop. Med. Hyg.* **77:** 3003-309.

Excoffier L., Laval G. & Schneider S. (2005). Arlequin ver 3.0: An Integrated Software Package for Population Genetics Data Analysis. *Evol. Bioinform. online* **1:** 47-50.

Excoffier L., Smouse P. & Quattro J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics.* **131:** 479-491.

Felsenstein J. (2004). *PHYLIP, Phylogeny Inference Package.* Version 3.6C. Seattle, WA, University of Washington.

Fraga E., Oliveira D., Aragão D., Schneider H., Sampaio I. & Barros M. (2013). Genetic variability and evidence of two distinct lineages of *Aedes aegypti* (Diptera, Culicidae) on São Luís Island in Maranhão, Brazil. *The Open Trop. Med. J.* **6:** 11-18. **doi:** 10.2174/1874315301306010011.

García-Franco F., Muñoz L., Lozano-Fuentes S., Fernández-Salas I., García-Rejon J., Beaty B., *et al.* (2002). Large genetic distances among *Aedes aegypti* populations along the South Pacific coast of Mexico. *Am. J. Trop. Med. Hyg.* **66:** 594-598.

Getis A., Morrison A., Kenneth G. & Scott T. (2003). Characteristics of the Spatial Pattern of the Dengue

Vector, *Aedes aegypti*, in Iquitos, Peru. *Am. J. Trop. Med. Hyg.* **69:** 494–505.

Gonçalves da Silva A., Cunha I., Santos W., Ribolla L. & Fernando Abad-Franch F. (2012). Gene flow networks among American *Aedes aegypti* populations. *Evol. Appl.* **5:** 664–676. **doi:**10.1111/j.1752-4571.2012.00244.x

Gonçalves C., Melo F., Bezerra J., Chaves B., Silva B., .Silva L., *et al.* (2014). Distinct variation in vector competence among nine field populations of *Aedes aegypti* from a Brazilian Dengue-endemic risk city. *Parasites & Vectors.* **7:** 320-327.

Gorrochotegui-Escalante N., Gómez-Machorro C., Lozano-Fuentes S., Fernández-Salas I., Muñoz M., Farfan-Ale J., *et al.* (2002). Breeding structure of *Aedes aegypti* populations in Mexico varies by region. *Am. J. Trop. Med. Hyg.* **66:** 213-222.

Gorrochotegui-Escalante N., Munoz M., Fernandez-Salas I., Beaty B. & Black W. (2000). Genetic isolation by distance among *Aedes aegypti* populations along the northeastern coast of Mexico. *Am. J. Trop. Med. Hyg.* **62:** 200–209.

Harrington C., Scott T., Lerdthusnee K., Coleman R., Costero A., Clark G., *et al.* (2005). Dispersal of the Dengue vector *Aedes aegypti* within and between rural communities. *Am. J. Trop. Med. Hyg.* **72:** 209-220.

Herrera F., Urdaneta L., Rivero J., Zoghbi N., Ruiz J. & Carrasquel G. (2006). Population genetic structure of Dengue mosquito *Aedes aegypti* in Venezuela. *Mem. Inst. Oswaldo Cruz.* **101:** 625-633.

Librado P. & Rozas J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* **25:** 1451-1452.

Liotta D., Cabanne G., Campos R. & Tonón S. (2005). Molecular detection of Dengue viruses in field caught *Aedes aegypti* mosquitoes from northeastern Argentina. *Rev. Latinoam. Microbiol.* **47:** 82-87.

Lourenço-de-Oliveira R., Vazeille M., de Filippis A. & Failloux A. (2004). *Aedes aegypti* in Brazil: genetically differentiated populations with high susceptibility to Dengue and yellow fever viruses. *Tran. R. Soc. Trop. Med. Hyg.* **98:** 43-54.

Manguin S. & Boëte Ch. (2011). *Global Impact of Mosquito Biodiversity, Human Vector-Borne Diseases and Environmental Change.* pp. 27-50. In: The Importance of Biological Interactions in the Study of Biodiversity. Ed. López-Pujol J. Tech. Rijeka, Croatia.

Monteiro F., Shama R., Martins A., Gloria-Soria A., Brown J. & Pow J. (2014). Genetic Diversity of Brazilian *Aedes aegypti*: Patterns following an Eradication Program. *PLoS Negl. Trop. Dis.* **8:** e3460.

Moore M., Sylla M., Goss L., Burugu M., Sang R., Kamau L., *et al.* (2013). Dual African Origins of Global *Aedes aegypti s.l.* Populations Revealed by Mitochondrial DNA. *PLoS Negl. Trop. Dis.* **7:** e2175.

Mourya D., Kumar R., Barde P., Gokhale M. & Yadav P. (2015). Genetic Variation in *Aedes aegypti* mosquito populations along the West Cost of India and their susceptibility to insecticides and Dengue Virus. *Med. Sci.* **5:** 378-382.

Nei M. (1987). *Molecular Evolutionary Genetics*. Ed. Columbia University Press. New York, U.S.A.

Ocampo C. & Wesson D. (2004). Population dynamics of *Aedes aegypti* from a Dengue hyperendemic urban setting in Colombia. *Am. J. Trop. Med. Hyg.* **71:** 506-513.

Orita M., Iwahana H., Kanazawa H., Hayashi K. & Sekiya T. (1989). Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Natl. Acad. Sci. USA.* **86**: 2766 –2770.

Paduan K. & Ribolla P. (2008). Mitochondrial DNA polymorphism and heteroplasmy in populations of *Aedes aegypti* in Brazil. *J. Med. Entomol.* **45:** 59-67.

Paupy C., Chantha N., Vazeille M., Reynes J., Rodhain F. & Failloux A. (2003). Variation over space and time of *Aedes aegypti* in Phnom Penh (Cambodia):

genetic structure and oral susceptibility to a Dengue virus. *Genet. Res.* **82:** 171-82.

Rivero J., Urdaneta L., Zoghbi N., Pernalete M., Rubio-Palis Y. & Herrera F. (2004). Optimization of extraction procedure for mosquito DNA suitable for PCR-based techniques. *Intern. J. Trop. Insect Sci.* **24:** 266-269.

Rodríguez M., Bisset J., de Fernández D., Lauzan L. & Soca A. (2001). Detection of insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) from Cuba and Venezuela. *J. Med. Entomol.* **38:** 623-628.

San Martín J., Brathwaite O., Zambrano B., Solórzano J., Bouckenooghe A., Dayan G., *et al.* (2010). The epidemiology of Dengue in the americas over the last three decades: a worrisome reality. *Am. J. Trop. Med. Hyg.* **82:** 128-35.

Sharma A., Mendki M., Tikar S., Chandel K., Sukumaran D. & Parashar B. (2009). Genetic variability in geographical populations of Culex quinquefasciatus Say (Diptera: Culicidae) fron India based on random amplified polymorphic DNA analysis. *Acta Trop.* **112:** 71-76.

Slatkin M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution.* **47:** 264-279.

Sousa L. & Scaparssa V. (2009). Evidence of two lineages of the Dengue vector *Aedes aegypti* in the Brazilian Amazon, based on mitochondrial DNA ND4 gene sequences. *Genet. Mol. Biol.* **32:** 414-422.

Sunnucks P., Wilson A., Beheregaray L., Zenger K., French J. & Taylor A. (2000). SSCP is not so difficult: the application and utility of single-stranded conformation polymorphism in evolutionary biology and molecular ecology. *Mol. Eco.* **9:** 1699-1710.

Tabachnick W. (1991). The yellow fever mosquito: evolutionary genetics and arthropod-borne disease. *Am. Entomol.* **37:** 14–24.

Tajima F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics.* **105:** 437-460.

Tamura K., Dudley J., Nei M. & Kumar S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24:** 1596-1599.

Thompson J., Higgins D. & Gibson T. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673-4680.

Tran K., Vazeille-Falcoz M., Mousson L., Tran H., Rodhain F., Ngugen T., *et al.* (1999). *Aedes aegypti* in Ho Minh City (Viet Nam): susceptibility to Dengue 2 virus and genetic differentiation. *Trans R. Soc. Trop. Med. Hyg.* **93:** 581-586.

Twerdochlib A., Bona A., Leite S., Chitolina R., Westphal B. & Navarro-Silva M. (2012). Genetic variability of a population of *Aedes aegypti* from Paraná, Brazil, using the mitochondrial ND4 gene. *Rev. Bras. Entomol.* **56:** 249-256.

Vazeille-Falcoz M., Mousson L., Rodhain F., Chungue E. & Failloux A. (1999). Variation in oral susceptibility to Dengue type 2 virus of populations of *Aedes aegypti* from the islands of Tahiti and Moorea, French Polynesia. *Am. J. Trop. Med. Hyg.* **60:** 292-299.

Vazeille-Falcoz M., Mousson L., Rakatoarivony I., Villeret R., Rodhain F., Duchemin J. & Failloux A. (2001). Population genetic structure and competence as a vector for Dengue type 2 virus of *Aedes aegypti* and *Aedes albopictus* from Madagascar. *Am. J. Trop. Med. Hyg.* **65:** 491-497.

Vorou R. (2016). Zika virus, vectors, reservoirs, amplifying hosts, and their potential to spread world wide: what we know and what we should investigate today. *International Journal of Infectious Diseases.* **48:** 85-90.

WHO (2014). Epidemiological alert Chikungunya and Dengue fever in the Americas. Available at: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=27049+&Itemid=999999&.

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