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LARGE GENETIC DIFFERENTIATION AND LOW VARIATION IN VECTOR COMPETENCE FOR DENGUE AND YELLOW FEVER VIRUSES OF *AEDES ALBOPICTUS* FROM BRAZIL, THE UNITED STATES, AND THE CAYMAN ISLANDS

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Abstract. We conducted a population genetic analysis of *Aedes albopictus* collected from 20 sites in Brazil, the United States (Florida, Georgia, and Illinois), and the Cayman Islands. Using isoenzyme analysis, we examined genetic diversity and patterns of gene flow. High genetic differentiation was found among Brazilian samples, and between them and North American samples. Regression analysis of genetic differentiation according to geographic distances indicated that *Ae. albopictus* samples from Florida were genetically isolated by distance. Infection rates with dengue and yellow fever viruses showed greater differences between two Brazilian samples than between the two North American samples or between a Brazilian sample and a North American sample. Introductions and establishments of new *Ae. albopictus* populations in the Americas are still in progress, shaping population genetic composition and potentially modifying both dengue and yellow fever transmission patterns.

INTRODUCTION

Aedes albopictus is a mosquito species native to Southeast Asia[1] with a wide distribution in the Oriental region, including New Guinea, most islands in the Indian Ocean, and some islands in the Pacific region such as Hawaii and Guam.[2] The distribution of *Ae. albopictus* has expanded recently in the Americas; it was established in Houston, Texas in 1985,[3] probably arriving in shipments of used tires from Japan.[4] Soon afterwards. South America was infested by *Ae. albopictus*; in 1986, it was detected in southeastern Brazil.[5] Infestations in Brazil seemed to have originated from tropical Asia.[4] This species is now established in 23 states in the United States[6] and in 22 states in Brazil (Brazilian Ministry of Health, unpublished data). *Aedes albopictus* is frequent found in the southeastern and southern states of Brazil, but its pattern of distribution is scattered in coastal northeastern and central Brazil; it is essentially absent in the Amazon region and in the inland dry areas of northeastern Brazil.

In regions where the distribution of *Ae. albopictus* and *Ae. aegypti* overlap, species segregation has shown that *Ae. aegypti* predominate in urban zones with high concentration of humans, while *Ae. albopictus* predominates in periphery of cities and semi-rural localities (Braks M and others, unpublished data). The colonization history of *Ae. albopictus* can be compared with that of *Ae. aegypti*, which originated from Africa.[7] Both species have spread worldwide because of their ability to breed in human-made containers.[8] Unlike *Ae. aegypti, Ae. albopictus* has developed a photoperiodic egg diapause and freezing tolerance,[4] allowing colonization in temperate zones. In the Americas, the introduction of *Ae. albopictus* has been associated with a decrease in the abundance of *Ae. aegypti*.[9,10] For example, in Texas, *Ae. albopictus* is three times more abundant than *Ae. aegypti*,[3] but the distribution of native mosquitoes such as *Ae. triseriatus*, a treehole mosquito, is not affected.[11] However, in Southeast Asia, *Ae. aegypti* has displaced *Ae. albopictus*.[12] Typically, *Ae. albopictus* prefers suburban and rural areas where it breeds in natural containers such as tree holes, leaf axils, bamboo internodes, and artificial containers such as tin cans and tires.[13]

In the Americas, control programs for the eradication of urban yellow fever, which were initiated in 1916 by the Rockefeller Foundation, and then continued by the Pan American Health Organization from 1940 to 1950, led to the disappearance of *Ae. aegypti* from several countries in the late 1960s.[14] Urban yellow fever has not been reported from the Americas since 1954.[15] However, jungle yellow fever, which is transmitted by sylvan mosquitoes, e.g., *Haemagogus janthinomys*, has increased in Bolivia, Brazil, Columbia, Ecuador, and Peru.[16] After the relaxation of the control program in the early 1970s, *Ae. aegypti* has re-infested most American countries,[17] colonizing more areas than before the eradication campaign.[18] Thus, reinvasions by *Ae. aegypti* of cities pose the threat of re-urbanization of yellow fever.[19,20]

Dengue became a serious public health problem in the Americas in 1981 with an outbreak in Cuba caused by the dengue type 2 virus serotype, and resulted in 340,000 cases and 158 deaths, mostly due to dengue hemorrhagic fever (DHF).[21] Since then, many countries in the Americas have become endemic regions for dengue, with sporadic cases of DHF generally related to *Ae. aegypti*.[22] The introduction and establishment of *Ae. albopictus* in the United States and Brazil has potentially serious medical implications because it is also a vector of dengue[1] and other arboviruses.[23] *Aedes albopictus* has been incriminated as a dengue vector in Japan, Indonesia, the Seychelles, Thailand, Malaysia,[13] and more recently in 2001 in Hawaii (Reiter P, unpublished data). In the continental Americas, *Ae. albopictus* has never been implicated in a dengue epidemic,[24] but it has been found naturally infected with dengue virus in Mexico[24] and Brazil.[25] Because of its lack of ecologic specialization, it is suspected to be involved in the transfer of enzootic forest viruses into inhabited areas in Southeast Asia.[26] Thus, it has been assumed that *Ae. albopictus* could introduce urban yellow fever in South America by linking the sylvatic habitat occupied by the *Haemagogus* spp. mosquitoes with the urban environment occupied by *Ae. aegypti*.[27]

Aedes albopictus in North America has been shown to be an efficient experimental vector of dengue viruses[28,29] and yellow fever virus.[28] In this study, we addressed the following questions: 1) do Brazilian populations of *Ae. albopictus* carry yellow fever and dengue viruses as efficiently as North American populations, 2) are Brazilian mosquito populations

genetically differentiated from North American populations, and 3) what are the implications of our findings for transmission of dengue and yellow fever?

MATERIALS AND METHODS

Mosquito samples. *Aedes albopictus* were collected from 10 sites in Brazil, 9 sites in the United States, and 1 site in the Cayman Islands (Table 1 and Figure 1). In Brazil, *Ae. albopictus* was intentionally sampled from localities in its northern (São Luís [SAO]) and southern (Três Passos [RGS]) regions, where the climate, range of temperature, and biotope type vary considerably. We chose sites with variable urbanization levels and human densities, i.e., from rural or semi-urban areas with a preserved natural environment and low human densities (Represa do Cigano [RCI], Tinguá [TIN], and RGS), urban centers with medium (Moquetá [MOQ] and Paranaguá [PAR]) and high (SAO, Salvador [SAL], and Florianópolis [FLO]) human densities, and a slum (Comandados Soares [CSO]) with a lack of urban services and very high human density and environmental degradation. Mosquitoes from Brazil were sampled in March–April 2001 using around 20 ovitraps containing 10% hay infusion in tap water[30] per locality. Mosquitoes from North America were collected in 2000–2001 and composed of larvae and adults, except for the sample from Vero Beach (VER), where collections were also made with ovitraps.[30] Samples were reared to obtain adults (F_0 generation for all samples and F_1 for the West Palm Beach [WPB] North American sample), which were fed on mice to obtain eggs (F_1/F_2 generation). The F_0/F_1 adults were kept at -80°C until use and when possible, F_1/F_2 females were infected with dengue 2 virus and yellow fever virus (see Table 1 for more details).

Isolation of virus. The Bangkok strain of dengue type 2, provided by Dr. Leon Rosen (Institut Pasteur, Paris, France), was isolated in 1974 from a serum sample of a DHF patient from Bangkok, Thailand.[31] This virus had been passed only in mosquito species by intrathoracic inoculation: two passages in *Ae. albopictus* and two passages in *Toxorhynchites amboinensis*. Our dengue 2 virus stocks were prepared on *Ae. albopictus* C6/36 cells. We used a final titer of $10^{8.2}MID_{50}$ (50% mosquito infectious dose for *Ae. aegypti*)/mL for mosquito infections.

The yellow fever virus strain (FIOCRUZ 74018/MG/01) was isolated on C6/36 cells in 2001 from the serum of a 39-year-old fatal human case of yellow fever from Bom Despacho in the State of Minas Gerais in Brazil.[32] The titer used in the infectious meal was $10^{8.7}MID_{50}$/mL.

Experimental infection of F_1 females and statistical analysis. For experimental infection with dengue type 2 virus, we performed one or two assays per sample depending on the number of available females. For mosquito infections with yellow fever virus, only one assay was carried out for each sample. Five to ten days after emergence, females were deprived of sucrose solution 24 hours before exposure to virus. The infectious meal contained two-thirds washed rabbit erythrocytes, one-third virus suspension, and 5×10^{-3} M ATP. After 20 minutes of feeding, fully engorged females were kept at 28°C for 14 days. To detect infected females (i.e., with dengue or yellow fever viruses in nervous tissues), we used an indirect immunofluorescence assay on head squashes.[33] Viral infection was also tested in the Paea colony of *Ae. aegypti* (collected in 1994 in Tahiti, French Polynesia), whose infection rate for dengue virus is known.[34]

We conducted analysis to compare infection rates (i.e., the proportion of females becoming infected by dengue and yellow fever viruses 14 days after infection). Independence of rows and columns in an R × C contingency table was tested using Fisher's exact test.[35]

TABLE 1
Aedes albopictus collected in Brazil, the Cayman Islands, and the United States in 2000–2001*

No.	Sample	City	State or country	Date of collection	Number of F_0 adults	Generation tested	
						Experimental infection	Isoenzyme analysis
Brazil							
1	CAR	Cariacica	Espírito Santo	May 2001	69	F_1	F_0
2	CSO	Comandados Soares	Rio de Janeiro	Mar 2001	54	F_1	F_0
3	FLO	Florianópolis	Santa Cantarina	May 2001	108	F_1	F_0
4	MOQ	Moquetá	Rio de Janeiro	Mar 2001	91	F_1	F_0
5	PAR	Paranaguá	Paraná	May 2001	840	F_1	F_0
6	RCI	Represa do Cigano	Rio de Janeiro	Apr 2001	828	F_1	F_0
7	RGS	Três Passos	Rio Grande do Sul	May 2001	27	F_1	F_0
8	SAL	Salvador	Bahia	Apr 2001	228	F_1	F_0
9	SAO	São Luís	Maranhão	Apr 2001	33	F_1	F_0
10	TIN	Tinguá	Rio de Janeiro	Apr 2001	1,575	F_1	F_0
Caribbean							
11	CAY	Georgetown	Cayman Islands	Aug 2000	26	–	F_0
United States							
12	COT	Cottondale	Florida	Jan 2001	20	–	F_0
13	HIA	Hiawassee	Georgia	Oct 2000	20	–	F_0
14	JAC	Jacksonville	Florida	Jan 2001	20	–	F_0
15	KRO	Miami	Florida	Oct 2000	20	–	F_0
16	NOR	Norcross	Georgia	Oct 2000	19	–	F_0
17	PAH	Pahokee	Florida	Sep 1999–Oct 2001	513	–	F_0
18	STL	East Saint Louis	Illinois	Oct 2000	22	–	F_0
19	VER	Vero Beach	Florida	Sep 2001	345	F_1	F_0
20	WPB	West Palm Beach	Florida	Sep 2001	850	F_2	F_1

* = not determined.

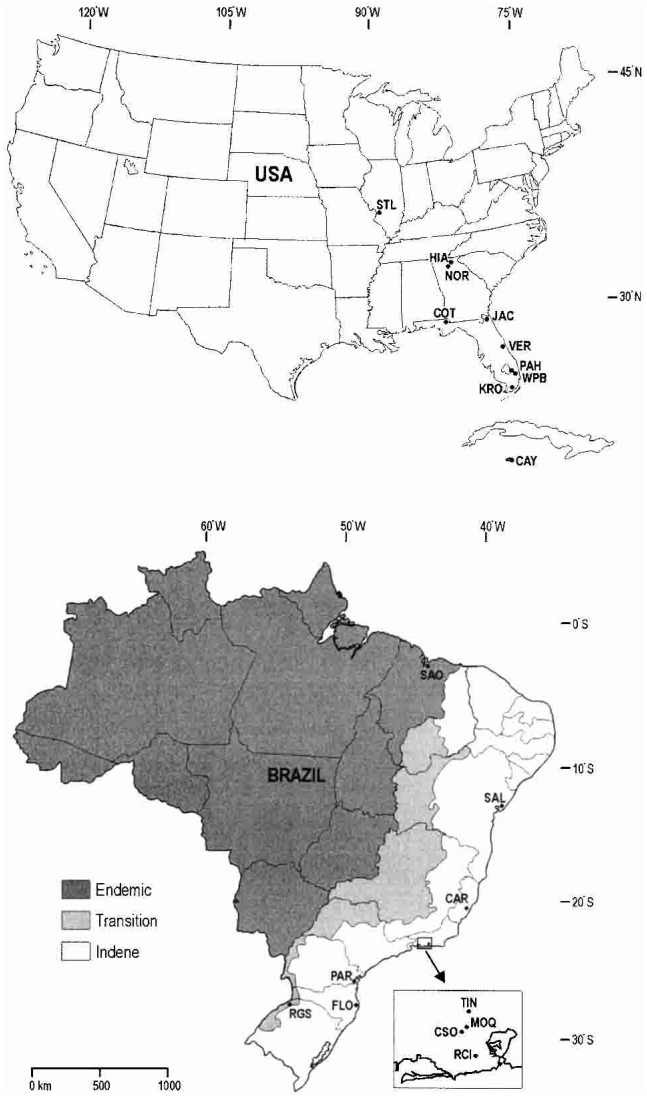

FIGURE 1. Location of *Aedes albopictus* sampled in 2000–2001 in the United States, the Cayman Islands, and Brazil. STL = East Saint Louis; HIA = Hiawassee; NOR = Norcross; COT = Cottondale; JAC = Jacksonville; VER = Vero Beach; PAH = Pahokee; WPB = West Palm Beach; KRO = Miami; CAY = Georgetown; SAO = São Luís; SAL = Salvador; CAR = Cariacica; TIN = Tinguá; MOQ = Moquetá; CSO = Comandados Soares; RCI = Represa do Cigano; PAR = Paranaguá; FLO = Florianópolis; RGS = Três Passos; Indene = a zone with no circulation of yellow fever virus.

Isoenzyme electrophoresis and genetic analysis. Each mosquito was ground in 25 μL of distilled water and after a low-speed centrifugation, the supernatant containing the soluble proteins was loaded onto a 12.8% starch gel in Tris-maleate-EDTA (pH 7.4) buffer and subjected to electrophoresis for 4–5 hours. The most polymorphic loci encoding enzymes were chosen: glutamate oxaloacetate transaminases (Got-1 and Got-2), glycerol-3-phosphate dehydrogenase (Gpd), hexokinases (Hk-1 and Hk-2), malate dehydrogenase (Mdh), malic enzyme (Me), phosphoglucoisomerase (Pgi), and phosphoglucomutase (Pgm).

For genetic analysis, the GENEPOP software (version 3.1) developed by Raymond and Rousset[35] was used. Departure from Hardy-Weinberg equilibrium was measured using the F_{IS}[36] and tested using an exact test procedure.[37] For each sample, linkage disequilibrium between pairs of loci was tested using Fisher's test on R × C contingency tables. Genetic differentiation was measured using the F_{ST}.[36] Isolation by geographic distance[38] was tested by estimating rank correlations between $F_{ST}/(1 - F_{ST})$ calculated between pairs of samples and Ln distances.

RESULTS

Infection rates for dengue type 2 virus. The infection rates for dengue type 2 virus in 12 *Ae. albopictus* samples ranged from 22.5% (CSO replicate a) to 80.0% (RGS replicate a) (Table 2). When infection rates between control replicates were compared, two significant differences were found in the CSO ($P = 0.006$) and TIN replicates ($P = 0.043$). Replicates b of CSO and TIN were not considered for further analysis because they corresponded to assays with the lowest infection rates obtained in the controls. When infection rates were compared with the corresponding control, only one sample was not significantly different (PAR replicate a; $P = 0.087$). The SAO sample showed a significant difference ($P < 0.05$) between replicates. When samples were grouped according to country, a significant difference was detected among samples collected in Brazil ($P < 0.0001$), whereas two samples from the United States were not significantly different ($P = 0.355$). The two samples from the United States (VER and WPB) showed infections rates similar to those of Cariacica (CAR) replicate a and SAO replicate b.

When grouping was analyzed according to climate/biotope type and human population densities, significant differences ($P < 0.05$) was obtained for all categories except for the two samples collected in the rural outskirts of Rio de Janeiro (RCI and TIN; $P = 0.5217$). Plotting geographic distances

TABLE 2
Infection rates of *Aedes albopictus* with dengue-2 virus*

Sample	Replicate	% infected females (n) Assay	Control	P†
Brazil				
CAR	a	47.02 (151)	98.21 (56)	**<0.0001**
CSO	a	22.5 (40)	98.55 (69)	**<0.0001**
FLO	a	67.86 (56)	100 (56)	**<0.0001**
	b	56.06 (66)	100 (54)	**<0.0001**
P†		0.195	1.0	
MOQ	a	68.42 (57)	96.82 (63)	**0.0001**
	b	50.0 (60)	90.0 (70)	**<0.0001**
P†		0.060	0.172	
PAR	a	72.90 (107)	85.0 (60)	0.087
RCI	a	71.14 (149)	96.67 (60)	**<0.0001**
RGS	a	80.0 (75)	100 (56)	**0.0001**
	b	75.0 (36)	100 (54)	**0.0001**
P†		0.625	1.0	
SAL	a	31.09 (119)	93.33 (45)	**<0.0001**
SAO	a	63.08 (65)	100 (56)	**<0.0001**
	b	41.67 (72)	100 (54)	**<0.0001**
P†		**0.015**	1.0	
TIN	a	66.18 (68)	98.11 (53)	**<0.0001**
P†		**<0.0001**		
United States				
VER	a	38.6 (127)	85.2 (115)	**<0.0001**
WPB	a	44.6 (112)	85.0 (60)	**<0.0001**
P†		0.355		

* n = number of females tested. The mosquito control is the *Ae. aegypti* Paea strain from Tahiti in French Polynesia. For definitions of samples, see Table 1.
† Probability of homogeneity by Fisher's exact test. Significant values ($P < 0.05$) are in **bold**.

separating samples against probabilities of homogeneity calculated when the corresponding infection rates were compared showed that 53 of 66 comparisons were highly significant ($P < 0.05$), among which 36 corresponded to comparisons between Brazilian samples (Figure 2).

Infection rates for yellow fever virus. The infection rates for yellow fever virus in 12 *Ae. albopictus* samples ranged from 3.48% (SAO) to 30.95% (RGS) (Table 3). The VER sample the United States showed a rate similar to those of CSO and TIN, and the rate for WPB was similar to those of PAR and SAL. When samples were compared within a country, a significant difference ($P < 0.05$) was demonstrated among the 10 Brazilian samples ($P < 0.0001$), whereas two North American samples were similar ($P = 0.171$).

When grouping was done according to climatic characteristics, significant differences ($P < 0.05$) were found, whereas when groupings were analyzed according to human population densities, all infection rates were similar ($P > 0.05$), except for the RGS sampled ($P = 0.0002$). When geographic distances were analyzed according to probabilities of homogeneity estimated when comparing infection rates, 25 of 66 comparisons were highly significant ($P < 0.05$), mostly corresponding to comparisons between Brazilian samples (i.e., 20 of 25 tests) (Figure 2).

Population differentiation. When testing Hardy-Weinberg equilibrium, we conducted 91 tests and the results of 29 were significant ($P < 0.05$). Eighteen tests showed a heterozygote excess and nine tests showed a heterozygote deficit. When Hk-1 and Hk-2 were removed from the data set, only 10 test results remained significant ($P < 0.05$), with eight showing a heterozygote deficit (Cottondale [COT]/Got-2, Jacksonville [JAC]/Got-2, Miami [KRO]/Got-2, Norcross [NOR]/Got-2, CAR/Me, FLO/Me, Hiawassee [HIA]/Me, and East Saint Louis [STL]/Me) and two showing a heterozygote excess (CAR/Pgm and Pahokee [PAH]/Pgm) (Appendix 1). Genotypic associations between pairs of loci were also tested. Among 142 combinations of loci, only one (Pgi-Mdh at PAR) remained significant when the Bonferroni sequential test was taken into account. According to Ohta,[39] this result was due to genetic drift rather than selection.

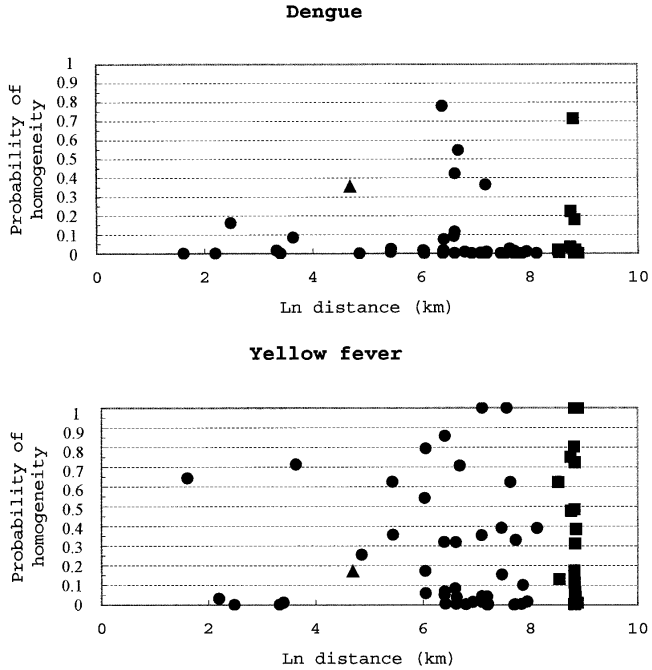

FIGURE 2. Geographic distances separating pairs of *Aedes albopictus* samples plotted against the probability of homogeneity between infection rates tested using Fisher's exact test. Dots correspond to comparisons between two Brazilian samples, squares to comparisons between one Brazilian sample and one North American sample, and triangles to comparisons between two North American samples.

TABLE 3
Infection rates of *Aedes albopictus* with yellow fever virus*

Sample	% infected females (n) Assay	Control
Brazil		
SAO	3.48 (115)	50.0 (60)
SAL	18.42 (114)	55.93 (59)
CAR	6.30 (127)	49.15 (59)
CSO	13.51 (37)	43.33 (60)
MOQ	16.67 (96)	44.07 (59)
RCI	7.5 (120)	48.33 (31)
TIN	14.28 (119)	60.53 (38)
PAR	19.01 (121)	56.14 (57)
FLO	9.65 (114)	41.67 (60)
RGS	30.95 (84)	29.81 (104)
P†	**<0.0001**	
United States		
VER	14.2 (134)	49.1 (116)
WPB	21.4 (112)	56.1 (97)
P†	0.171	

* n = number of females tested. The mosquito control is the *Ae. aegypti* Paea strain from Tahiti in French Polynesia. For definitions of samples, see Table 1.
† Probability of homogeneity by Fisher's exact test. Significant values ($P < 0.05$) are in **bold**.

When genetic divergence among all 20 samples was examined, a high and significant differentiation was detected ($F_{ST} = +0.249$, $P < 0.0001$). Brazilian samples were highly differentiated ($F_{ST} = +0.136$), although less differentiated than North American samples ($F_{ST} = +0.289$). When the relationship between $F_{ST}/(1\text{-}F_{ST})$ and Ln distance was estimated, the correlation was positive ($b = +0.039$) and significant ($P = 0.002$), indicating a slight tendency to an increase in genetic differentiation with geographic distances (Figure 3). This was more easily detected for small distances (within 1,000 km), as among the five samples collected in Florida. An isolation by distance was observed for samples collected along the Florida peninsula (JAC, KRO, PAH, VER, and WPB) ($b = +0.633$, $P = 0.018$).

DISCUSSION

We showed that differences in infections rates for both dengue and yellow fever viruses were greater between Brazilian populations of *Ae. albopictus* than between a Brazilian and North American populations. In addition, based on our samples, Brazilian populations tended to be more differentiated than North American samples. Evidence of isolation by distance was detected among samples collected along the Florida peninsula, which showed that species tend to less dispersal using human modes of transportation such as roads.

Aedes albopictus has been incriminated as a vector of dengue in several regions.[2] Many suspected cases of dengue have been imported into the United States. However, the first dengue outbreak due to *Ae. aegypti* occurred in Texas in 1980.[40] In Brazil, dengue outbreaks have been associated with *Ae.*

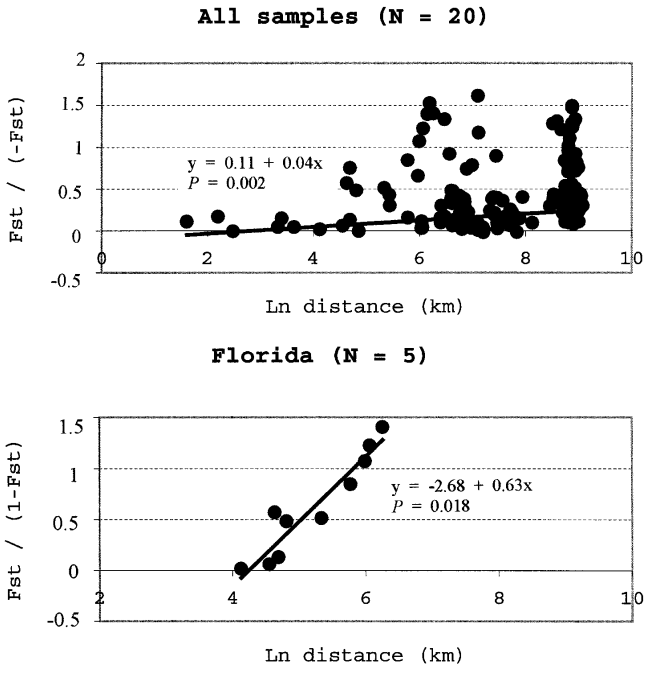

FIGURE 3. Genetic differentiation of *Aedes albopictus* in Brazil, the United States, and the Cayman Islands according to geographic distances separating samples.

aegypti, and the role of *Ae. albopictus* in virus transmission in nature remains to be confirmed.[41] However, dengue viruses have been isolated from larvae of *Ae. albopictus*.[25] Urban yellow fever has not been reported from the Americas since 1954, except for a small outbreak recently reported in Santa Cruz, Bolivia.[19] However, the high infestation index of *Ae. aegypti* in cities and the recent introduction of *Ae. albopictus* increase the threat of urbanization of yellow fever transmission in South America.[32] Our results showed that Brazilian *Ae. albopictus* were as efficient as North American populations in being infected with dengue and yellow fever viruses. Using the same titer of dengue virus type 2 in the infectious meal, we observed infected rates that were lower than those estimated by Mitchell and others,[28] who used F_2/F_3 generations of a the Houston strain established from 48 females. It has been demonstrated that infection rates tend to increase with the number of laboratory generations of mosquitoes.[42,43] Using a different method of virus titration and a colony of *Ae. albopictus* from Cariacica, Brazil, Miller and Ballinger[44] demonstrated a lower infection rate for dengue virus type 2 (38%) and higher rates for yellow fever virus (36–57%) than we found using F_1 females from the same locality (47.0% and 6.3%, respectively). In addition, studies using a lower virus titer (10^6 MID_{50}) and a long-term laboratory colony of *Ae. albopictus* from Rio de Janeiro found lower infection rates for dengue virus (35.9–48%) (Castro M and others, unpublished data). These results highlight the importance of using the same experimental conditions (generation of mosquito used, incubation period, virus titer, passage history of the viral strain) when comparing infection rates. Moreover, laboratory-colonized strains of *Ae. aegypti*, such as the Paea strain, when used as controls were chosen based on their homogenous infection rates. This has been confirmed for a dengue virus infection,[34] but is more difficult to obtain for a yellow fever virus YF infection.[45]

In addition, differences in infection rates were greater between two Brazilian samples than between some Brazilian samples and the two North American samples tested. Based on our results, we can assume that as successive introductions of *Ae. albopictus* populations continue to occur in other geographic regions (likely through used tire imports), established mosquito populations will undergo genetic changes that may alter vector competence.

Brazilian as well as North American populations of *Ae. albopictus* were highly differentiated, even those that were geographically close to each other (e.g., in Rio de Janeiro, 5–38 km apart). Samples of *Ae. albopictus* were more differentiated within a city than those collected on a larger geographic scale. This pattern was also observed using a random amplified polymorphic DNA technique, which showed the high genetic variation observed in local Brazilian populations.[46] One explanation could be that drastic reductions occurred in founding populations that are likely due to insecticide treatments.[47] Over distances of 90–250 km, populations along the northeastern coast of Mexico were isolated by distance.[48] This was also observed in populations collected along the Pacific coast and the Yucatan peninsula in Mexico.[49] In such cases, the dispersal of *Ae. albopictus* may occur primarily through flight. For this species, the maximum dispersal distance is estimated to be 800 meters.[50] However, the maximum range of dispersal during adult life is usually 200 meters.[13] Human movement is known to facilitate mosquito dispersal (e.g., eggs, larvae, or adults) along commercial routes and consequently attenuate genetic divergence between geographically distant populations.[51] However, *Ae. albopictus* populations in Florida tended to become more differentiated as geographic distances separating them increased. This result confirms the small range migration ability of this species, which does not tend to disperse through human displacements. These populations had colonized this area for a sufficient time to approach equilibrium between dispersal and genetic drift, and for isolation by distance to become apparent.[38] This pattern was not detected in populations collected within lower distances, as in the State of Rio de Janeiro. This could confirm that *Ae. albopictus* invaded the area more recently than it did in Florida, suggesting that the Brazil and United States populations were derived from independent sources.[52]

In the Americas, *Ae. albopicus* populations are still arriving from different geographic regions. The distinction between the North and the South American populations was based on egg diapause and photoperiod sensitivity. Our findings showed that *Ae. albopictus* from these areas were not differentiated with respect to vector competence for dengue and yellow fever viruses. Based on these findings, *Ae. albopictus* populations are still evolving, as is their vector competence. Since *Ae. albopictus* shows a relatively high susceptibility to dengue type 2 virus and a high capacity to ensure vertical transmission,[53] we can assume that the present expansion of this species is a disturbing threat to dengue control in Brazil.

When *Ae. albopictus* and *Ae. aegypti* were sampled together in the same Brazilian site, we conducted experimental infection assays simultaneously with F_1 females of both species for yellow fever virus. Infection rates for *Ae. aegypti* have been previously reported,[31] and those for *Ae. albopictus* are shown in Table 3. Samples of *Ae. albopictus* from four of seven localities were more susceptible to yellow fever virus than those of *Ae. aegypti*: SAL (18.4% versus 6.3%, respec-

tively), MOQ (16.6% versus 7.6%), CSO (13.5% versus 0.9%), and TIN (14.2% versus 4.9%). The highest infection rate for yellow fever virus in *Ae. albopictus* from Brazil (30.9%) was found in RGS (Table 3), which is located in the transition area of sylvatic yellow fever (Figure 1), where monkeys were found harboring the virus in 2001. In addition, *Ae. albopictus* is widespread and often more abundant than *Ae. aegypti* in the western regions of southeastern and southern Brazilian states, such as in Minas Gerais, which is close to foci of sylvatic yellow fever and where human yellow fever outbreaks have been recently reported (Brazilian Ministry of Health, unpublished data). Although less susceptible to yellow fever virus than several *Ae. aegypti* samples tested from Brazil,[31] *Ae. albopictus* may continue to colonize and become more abundant in rural areas and in the forest fringe in endemic and transition areas for sylvatic yellow fever, thus becoming a real problem since it could become the link between the jungle and urban cycles of yellow fever.

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APPENDIX 1

Allele frequencies, F_{IS}, inbreeding coefficient, and probability of homogeneity (P) in *Aedes albopictus* populations collected in 2000–2001 in Brazil and United States*

	Gpd									Got-1											
Samples	1	2	3	4	5	6	N	F_{IS}	P	1	2	3	4	5	6	7	8	9	N	F_{IS}	P
Brazil																					
1 CAR	0.000	0.000	0.156	0.000	0.844	0.000	32	+0.068	0.564	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	32	–	–
2 CSO	0.000	0.000	0.167	0.000	0.833	0.000	6	–0.111	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	6	–	–
3 FLO	0.000	0.000	0.000	0.000	1.000	0.000	18	–	–	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	42	–	–
4 MOQ	0.000	0.000	0.063	0.000	0.938	0.000	24	–0.045	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	24	–	–
5 PAR	0.000	0.000	0.240	0.000	0.760	0.000	48	–0.306	0.044	1.990	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48	–	–
6 RCI	0.000	0.000	0.109	0.000	0.891	0.000	23	–0.100	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48	–	–
7 RGS	0.000	0.000	0.091	0.000	0.909	0.000	11	–0.053	1	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	11	–	–
8 SAL	0.000	0.000	0.135	0.000	0.865	0.000	48	–0.146	0.576	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48	–	–
9 SAO	0.000	0.000	0.313	0.000	0.688	0.000	8	–0.400	0.487	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	8	–	–
10 TIN	0.000	0.000	0.000	0.000	1.000	0.000	30	–	–	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	78	–	–
Caribbean																					
11 CAY	0.000	0.000	0.000	0.000	0.962	0.038	26	+1	0.020	0.981	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	26	–	–
United States																					
12 COT	0.000	0.000	0.000	0.000	1.000	0.000	20	–	–	0.800	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.200	20	+0.088	1
13 HIA	0.000	0.000	0.000	0.000	1.000	0.000	22	–	–	0.889	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.056	9	–0.032	1
14 JAC	0.000	0.000	1.000	0.000	0.000	0.000	20	–	–	0.975	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	20	–	–
15 KRO	0.000	0.000	0.000	0.000	1.000	0.000	20	–	–	0.975	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	20	–	–
16 NOR	0.000	0.000	0.000	0.000	1.000	0.000	18	–	–	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	18	–	–
17 PAH	0.000	0.000	0.000	0.000	1.000	0.000	48	–	–	0.979	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	48	–0.011	1
18 STL	0.000	0.000	0.000	0.000	1.000	0.000	22	–	–	0.909	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	22	–0.077	1
19 VER	0.000	0.000	0.000	0.000	0.948	0.052	48	–0.044	1	0.802	0.000	0.188	0.000	0.010	0.000	0.000	0.000	0.000	48	–0.222	0.33
20 WPB	0.000	0.000	0.000	0.000	1.000	0.000	48	–	–	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	48	–	–
All samples								–0.1118	0.602											–0.096	0.99

	Got-2									Mdh								
Samples	1	2	3	4	5	6	N	F_{IS}	P	1	2	3	4	5	6	N	F_{IS}	P
Brazil																		
1 CAR	0.000	0.000	1.000	0.000	0.000	0.000	32	–	–	0.000	0.000	0.000	0.000	0.984	0.016	32	–	–
2 CSO	0.000	0.000	1.000	0.000	0.000	0.000	6	–	–	0.000	0.000	0.000	0.000	1.000	0.000	6	–	–
3 FLO	0.000	0.000	1.000	0.000	0.000	0.000	42	–	–	0.000	0.000	0.000	0.000	0.944	0.056	18	–0.030	1
4 MOQ	0.000	0.000	1.000	0.000	0.000	0.000	24	–	–	0.000	0.000	0.000	0.000	1.000	0.000	24	–	–
5 PAR	0.000	0.000	0.990	0.000	0.000	0.000	48	–	–	0.000	0.000	0.010	0.000	0.896	0.094	48	+0.346	0.064
6 RCI	0.000	0.000	1.000	0.000	0.000	0.000	48	–	–	0.000	0.000	0.000	0.000	0.896	0.104	48	+0.117	0.406
7 RGS	0.000	0.000	1.000	0.000	0.000	0.000	11	–	–	0.000	0.000	0.000	0.000	1.000	0.000	11	–	–
8 SAL	0.000	0.000	0.990	0.000	0.000	0.000	48	–	–	0.000	0.000	0.010	0.000	0.948	0.042	48	+0.589	0.002
9 SAO	0.000	0.000	1.000	0.000	0.000	0.000	8	–	–	0.000	0.000	0.000	0.000	1.000	0.000	8	–	–
10 TIN	0.000	0.000	1.000	0.000	0.000	0.000	78	–	–	0.000	0.000	0.000	0.000	1.000	0.000	30	–	–
Caribbean																		
11 CAY	0.000	0.000	0.460	0.000	0.540	0.000	25	+0.453	0.042	0.000	0.000	0.000	0.000	0.904	0.096	26	–0.087	1

APPENDIX 1
(Continued)

	Got-2									Mdh								
Samples	1	2	3	4	5	6	N	F_{IS}	*P*	1	2	3	4	5	6	N	F_{IS}	
United States																		
12 COT	0.000	0.000	0.900	0.000	0.000	0.000	20	+1	**0.002**	0.000	0.000	0.000	0.000	0.900	0.100	15	−0.077	1
13 HIA	0.100	0.000	0.071	0.000	0.786	0.143	7	−0.125	1	0.000	0.000	0.000	0.000	1.000	0.000	22	–	–
14 JAC	0.000	0.000	0.813	0.000	0.188	0.000	16	+1	**0.001**	0.000	0.000	0.000	0.000	1.000	0.000	20	–	–
15 KRO	0.000	0.000	0.175	0.000	0.625	0.200	20	+0.554	**0.003**	0.000	0.000	0.053	0.000	0.895	0.053	19	+0.478	0.055
16 NOR	0.000	0.000	0.889	0.000	0.111	0.000	18	+1	**0.003**	0.000	0.000	0.083	0.000	0.417	0.500	12	−0.128	0.021
17 PAH	0.000	0.000	0.935	0.000	0.043	0.022	46	+0.308	0.016	0.000	0.000	0.000	0.000	0.771	0.229	24	+0.195	0.556
18 STL	0.000	0.000	0.795	0.000	0.205	0.000	22	+0.045	1	0.000	0.000	0.000	0.000	0.476	0.524	21	−0.315	0.206
19 VER	0.000	0.000	0.989	0.000	0.011	0.000	47	–	–	0.000	0.000	0.000	0.000	0.522	0.478	45	−0.461	0.003
20 WPB	0.000	0.000	0.938	0.000	0.063	0.000	48	−0.056	1	0.000	0.000	0.000	0.000	0.862	0.138	47	+0.029	1
All samples								+0.428	**<0.0001**								−0.045	**0.002**

	Me								Pgi								
Samples	1	2	3	4	5	N	F_{IS}	*P*	1	2	3	4	5	6	N	F_{IS}	*P*
Brazil																	
1 CAR	0.000	0.125	0.875	0.000	0.000	32	+1	**<0.0001**	0.000	0.000	0.000	0.000	1.000	0.000	32	–	–
2 CSO	0.000	0.000	1.000	0.000	0.000	6	–	–	0.000	0.000	0.000	0.000	0.833	0.167	6	−0.111	1
3 FLO	0.000	0.000	0.381	0.000	0.619	42	+1	**<0.0001**	0.000	0.000	0.000	0.000	0.958	0.042	36	−0.029	1
4 MOQ	0.000	0.000	1.000	0.000	0.000	24	–	–	0.000	0.000	0.000	0.000	1.000	0.000	24	–	–
5 PAR	0.000	0.000	1.000	0.000	0.000	48	–	–	0.000	0.000	0.010	0.000	0.958	0.031	48	−0.025	1
6 RCI	0.000	0.000	1.000	0.000	0.000	48	–	–	0.000	0.000	0.000	0.000	1.000	0.000	48	–	–
7 RGS	0.000	0.000	1.000	0.000	0.000	11	–	–	0.000	0.000	0.000	0.000	0.955	0.045	11	–	–
8 SAL	0.000	0.000	1.000	0.000	0.000	48	–	–	0.000	0.000	0.000	0.000	0.958	0.042	48	−0.033	1
9 SAO	0.000	0.000	1.000	0.000	0.000	8	–	–	0.000	0.000	0.000	0.000	1.000	0.000	8	–	–
10 TIN	0.000	0.000	1.000	0.000	0.000	78	–	–	0.000	0.000	0.000	0.000	1.000	0.000	78	–	–
Caribbean																	
11 CAY	0.000	0.000	1.000	0.000	0.000	26	–	–	0.000	0.000	0.000	0.000	0.769	0.231	26	+0.153	0.578
United States																	
12 COT	0.000	0.000	0.950	0.000	0.050	20	−0.027	1	0.000	0.000	0.000	0.000	1.000	0.000	20	–	–
13 HIA	0.000	0.000	0.864	0.136	0.000	22	+1	**0.001**	0.000	0.000	0.000	0.000	1.000	0.000	22	–	–
14 JAC	0.000	0.000	1.000	0.000	0.000	20	–	–	0.000	0.000	0.000	0.000	1.000	0.000	20	–	–
15 KRO	0.000	0.000	1.000	0.000	0.000	20	–	–	0.000	0.000	0.000	0.000	0.950	0.050	20	−0.027	1
16 NOR	0.000	0.000	1.000	0.000	0.000	18	–	–	0.000	0.000	0.000	0.000	0.778	0.222	18	+0.064	1
17 PAH	0.000	0.000	0.979	0.000	0.021	48	−0.011	1	0.000	0.000	0.000	0.000	0.938	0.063	48	−0.056	1
18 STL	0.000	0.000	0.909	0.000	0.091	22	+1	**0.002**	0.000	0.000	0.000	0.000	1.000	0.000	22	–	–
19 VER	0.000	0.000	1.000	0.000	0.000	48	–	–	0.000	0.000	0.138	0.000	0.862	0.000	47	+0.029	1
20 WPB	0.000	0.000	1.000	0.000	0.000	48	–	–	0.000	0.000	0.000	0.000	0.990	0.010	48	–	–
All samples							+0.902	**<0.0001**								−0.026	1

APPENDIX 1
(Continued)

Samples	Pgm										All loci	
	1	2	3	4	5	6	7	N	F_{IS}	*P*	F_{IS}	*P*
Brazil												
1 CAR	0.000	0.000	0.016	0.000	0.359	0.391	0.234	32	+0.214	**0.001**	+0.323	**<0.0001**
2 CSO	0.000	0.000	0.250	0.000	0.667	0.000	0.083	6	−0.290	1	−0.194	1
3 FLO	0.000	0.000	0.000	0.000	0.575	0.225	0.200	40	+0.148	0.563	+0.486	**<0.0001**
4 MOQ	0.000	0.000	0.000	0.000	0.521	0.229	0.250	24	−0.270	0.004	−0.234	**0.027**
5 PAR	0.000	0.000	0.000	0.362	0.383	0.000	0.255	47	−0.090	0.205	−0.080	0.061
6 RCI	0.000	0.000	0.000	0.000	0.500	0.431	0.069	29	−0.278	0.318	−0.132	0.664
7 RGS	0.000	0.000	0.000	0.000	0.591	0.182	0.227	11	+0.084	0.434	+0.048	0.796
8 SAL	0.000	0.000	0.000	0.000	0.510	0.271	0.219	48	−0.000	0.112	+0.021	**0.020**
9 SAO	0.000	0.000	0.000	0.000	0.875	0.063	0.063	8	−0.037	1	−0.273	0.837
10 TIN	0.000	0.000	0.000	0.000	0.487	0.276	0.237	78	+0.152	0.260	+0.152	–
Caribbean												
11 CAY	0.000	0.000	0.019	0.212	0.654	0.000	0.115	26	−0.329	0.261	+0.101	0.055
United States												
12 COT	0.000	0.000	0.000	0.400	0.500	0.000	0.100	20	+0.333	0.079	+0.299	0.065
13 HIA	0.000	0.000	0.000	0.000	0.114	0.159	0.727	22	−0.238	0.800	+0.138	**0.027**
14 JAC	0.000	0.000	0.000	0.000	0.100	0.175	0.725	20	+0.103	0.487	+0.405	**0.003**
15 KRO	0.000	0.000	0.000	0.000	0.079	0.026	0.895	19	−0.067	1	+0.356	**0.026**
16 NOR	0.000	0.000	0.000	0.222	0.583	0.000	0.194	18	−0.433	0.036	−0.045	**0.001**
17 PAH	0.000	0.000	0.000	0.000	0.219	0.188	0.594	48	−0.394	**0.001**	−0.148	**0.018**
18 STL	0.000	0.000	0.000	0.182	0.818	0.000	0.000	22	−0.200	1	−0.026	0.102
19 VER	0.000	0.000	0.032	0.032	0.223	0.213	0.500	47	−0.163	0.084	−0.218	**0.042**
20 WPB	0.000	0.000	0.010	0.000	0.354	0.083	0.552	48	+0.011	0.828	+0.007	0.999
All samples									−0.056	**0.001**	+0.043	**<0.0001**

* Gpd = glycerol-3-phosphate dehydrogenase; Got = glutamate oxaloacetate transaminase; Mdh = malate dehydrogenase; Me = malic enzyme; Pgi = phosphoglucoisomerase; Pgm = phosphoglucomutase; N = number of mosquitoes analyzed. The *P* values in **bold** correspond to the probability for rejecting Hardy-Weinberg equilibrium. For definitions of samples, see Table 1.