Am. J. Trop. Med. Hyg., 78(6), 2008, pp. 895–903

Population Genetics and Phylogeography of *Aedes aegypti* (Diptera: Culicidae) from Brazil

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Abstract. Population genetic analyses were conducted using samples of *Aedes aegypti* from 14 localities in the north, southeast, northeast, and central regions of Brazil. An 852-bp region of the mitochondrial DNA cytochrome oxidase I (*COI*) gene was used in the analyses. Ten haplotypes were observed, and cluster analyses revealed 2 groups (lineages) separated by 8 fixed mutations, suggesting that the Brazilian *Ae. aegypti* populations probably came from East and West Africa, with evidence of multiple introductions, one related to Group 1 and two related to Group 2. Considering all samples, genetic and geographic distances were significantly correlated ($r^2 = 0.332$; $P = 0.038$), supporting the isolation by distance (IBD) model, but no correlation was detected for any particular region, which is consistent with human migrations and trade exchanges. Genetic distances (pairwise F_{ST} and *Nm* values), AMOVA, and cluster analyses indicated a deep genetic structure for the Brazilian *Ae. aegypti*, probably resulting from several factors: multiple introductions associated with distinct lineages, geographic differentiation (IBD), passive dispersal patterns, control activities, extinction and recolonization events, and genetic drift.

INTRODUCTION

Aedes (*Stegomyia*) *aegypti* (Linnaeus) is the most important vector of the dengue and urban yellow fever viruses. Although dengue viruses are currently the most important arboviruses affecting humans, no vaccine has yet been developed, and the most important tool for its prevention is the use of integrated vector control measures.[1–3] Many factors have contributed to the spread of dengue: human population growth, rapid and unplanned urbanization along with a decline in socioeconomic conditions, inadequate health care services, increased use of nonbiodegradable products (bottles, plastic, cans, tires, etc.), quick movement of people and commodities via travel and trade, a highly domesticated mosquito vector, and insecticide-resistant mosquito populations, among others factors, creating greater challenges for effective prevention and control.[3]

In Brazil, *Ae. aegypti* was considered eradicated in 1955,[4] as a result of the eradication program adopted by the Rockefeller Foundation in 1916 and continued by the Pan American Health Organization from 1940 through 1960, with the goal of eradicating the urban yellow fever in the Americas. Unfortunately, this program was discontinued in 1960, and some neighboring South American countries (Suriname, the Guianas, and Venezuela), the southern United States, and some Caribbean Islands did not achieve complete eradication of *Ae. aegypti*.[5] As a consequence, local re-infestations by this vector occurred in northern Brazil, but they were quickly eliminated.[5,6] However, *Ae. aegypti* was soon after detected again in 2 Brazilian states, Bahia and Rio de Janeiro, in 1976 and 1977, respectively.[7] In the early 1980s, the first outbreak of dengue fever (DF) occurred in Boa Vista (State of Roraima), with serotypes DENV-1 and DENV-4,[8,9] although some studies state that the first dengue cases described in Brazil, based on clinical criteria, were reported in 1923.[6] In 1986, other outbreaks occurred in the states of Rio de Janeiro (southeast region) and Ceará and Alagoas (northeast region), with serotype DENV-1.[10] Three years later, 8 states had reported dengue epidemics.[9] The state of Amazonas was one of the last Brazilian states infested by *Ae. aegypti*. It was first discovered there in the capital, Manaus, in 1996; subsequently, 2 large dengue epidemics occurred in 1998 and 2001, with 13,873 and 18,504 recorded cases, respectively.[10–12]

Since 1998, *Ae. aegypti* is present in all Brazilian states; despite programs designed to control the mosquito vector, its density remains high and it has not been possible to prevent dengue outbreaks in different urban centers.[10–12] In Brazil, the largest number of dengue cases (794,219) was recorded in 2002, with 2,714 cases of dengue hemorrhagic fever (DHF), causing 150 deaths,[13] followed by reductions in the 3 subsequent years.[11,12] In 2006, there was a new increase in dengue cases (345,922), with 628 DHF and 77 deaths cases reported.[12] In 2007 (January through September), there was a 49.77% increase compared with the same period of 2006, resulting in 121 deaths.[14] In the state of Amazonas, DHF cases were reported in 2001 (55), 2003 (59), and 2007 (94).[12,14–16] Currently, DENV-1, DENV-2, and DENV-3 serotypes are cocirculating in 24 of the 27 states of Brazil.[12] The risk of DHF will increase even more if serotype DENV-4 is introduced, and it may become an important cause of death in Brazil.[17]

Population genetic studies have been conducted with *Ae. aegypti* from different regions across the world, using multimarkers.[18–24] These studies have provided information on population structure and dispersion rates at the micro- and macrogeographic levels, showing that environmental and social factors and human interventions (urbanization, control activities) affect the population structure of this vector.[25,26] In Brazil, population genetic studies carried out primarily with isozymes and random amplified polymorphism DNA (RAPD) markers have indicated differentiation among populations.[5,22,27–30] Recently, a study using the mitochondrial gene nicotinamide adenine dinucleotide dehydrogenase subunit 4 (*ND4*) was conducted with American (including Brazilian), African, and Asian populations.[31] Here, we analyzed the population structure and the dispersal patterns, via gene flow, of 14 *Ae. aegypti* samples from 4 regions of Brazil, using

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TABLE 1
Collection sites of *Aedes aegypti*

Location code	Locality	State	Region	Coordinates	Sample size
CO*	Coroado	Amazonas	North	3° 05′ S, 59° 58′ W	17
PC*	Praça 14 de Janeiro	Amazonas	North	3° 07′ S, 60° 00′ W	16
CP*	Compensa	Amazonas	North	3° 06′ S, 60° 03′ W	12
TN*	Tancredo Neves	Amazonas	North	3° 03′ S, 59° 56′ W	12
MC	Manacapuru	Amazonas	North	3° 17′ S, 60° 37′ W	7
CR	Coari	Amazonas	North	4° 05′ S, 63° 07′ W	16
HU	Humaitá	Amazonas	North	7° 30′ S, 63° 01′ W	7
BL	Belém	Pará	North	1° 26′ S, 48° 29′ W	11
BV	Boa Vista	Roraima	North	2° 52′ N, 60° 39′ W	13
RB	Rio Branco	Acre	North	9° 58′ S, 67° 48′ W	11
RP	São José do Rio Preto	São Paulo	Southeast	20° 48′ S, 49° 22′ W	10
TB	Taubaté	São Paulo	Southeast	23° 01′ S, 45° 34′ W	10
CG	Campina Grande	Paraíba	Northeast	7° 13′ S, 35° 52′ W	10
CB	Cuiabá	Mato Grosso	Central	15° 36′ S, 56° 06′ W	11
Total					163

* Four urban neighborhoods of Manaus city.

sequences of the mitochondrial DNA cytochrome oxidase subunit I (*COI*) gene. We also analyzed 4 samples from different neighborhoods of the city of Manaus, to investigate the local gene flow pattern. These 4 neighborhoods had recorded DHF cases.

MATERIALS AND METHODS

Mosquito collection in the field. *Ae. aegypti* samples were collected in fourteen cities, from April 2003 to December 2006. The geographic localizations are shown in Table 1 and Figure 1. These cities are located in 4 regions of Brazil: northern, southeastern, northeastern, and central. The northern region includes Manaus (capital) and three other cities—Manacapuru, Coari, and Humaitá—in the state of Amazonas; Belém, the capital of the state of Pará; Boa Vista, the capital of Roraima; and Rio Branco, the capital of Acre. In Manaus, 4 different urban neighborhoods were sampled: Coroado, Praça 14 de Janeiro, Compensa, and Tancredo Neves. Coroado and Tancredo Neves are situated in the eastern sector of Manaus, Praça 14 de Janeiro in the southern, and Compensa in the western part of the city (Figure 1). A total of 7 locations were analyzed in the state of Amazonas, the largest of all Brazilian states. The climate conditions of the northern region are characterized by high temperature and humidity provided by the tropical climate and the tropical rainforest, with a pronounced rainy season (December through May). In the southeastern region, samples from 2 cities were collected: São José do Rio Preto and Taubaté, both in the state of São Paulo. São José do Rio Preto is situated in the north of the state, where the climate is arid and hot, and Taubaté is situated in the southeast of the state, where the climate is also arid but alternates between hot (November through March) and cold (April through August). In the northeastern region, the samples were collected in Campina Grande, state of Paraíba. Given the difficulties in collecting samples at this location, we also included five individuals from Boqueirão, a locality situated approximately 40 km from Campina Grande. The climate of this region is hot and arid throughout the year, with a short rainy season from May to July. In the central region, the samples were obtained in Cuiabá, capital of the state of Mato Grosso, where the climate is also hot and arid, and the rainy season goes from December to March. Of all these cities, Manaus and Belém are the most populous, with about 1,688,000 and 1,428,000 inhabitants, respectively.

Larvae and pupae were collected from a variety of artificial recipients near dwellings and also by using traps and oviposition traps (ovitraps). From 25 to 40 breeding sites widespread over different neighborhoods were sampled in each city, the geographic distances between sites ranging from ~20

FIGURE 1. Collection sites of *Aedes aegypti*. On the top map, the 4 urban neighborhoods of the city of Manaus are plotted. For abbreviations of localities, see Table 1.

m to 5 km. The specimens collected at each breeding site were transported separately in bottles to the laboratory and then reared to adulthood. When we collected eggs, the same procedures were used. Each positive ovitrap was immersed in an individual tray containing water for hatching eggs and then reared to adulthood. For analyzing the 4 urban neighborhoods of Manaus, we sampled 20–30 breeding sites in each neighborhood, widespread over streets and avenues, up to ~1 km from each other. The specimens collected were reared to adulthood, using the same procedures mentioned above. All collected samples were transported to the Laboratory of Population Genetics of Malaria and Dengue Vectors at the National Institute of Research of the Amazon (Instituto Nacional de Pesquisas da Amazônia, INPA), in Manaus (AM, Brazil), identified as in Forattini,[32] and stored in a freezer at –80°C. For all localities, 1 or 2 individuals from each breeding site were used in the analyses.

DNA extraction and *COI* gene amplification. Genomic DNA was extracted from individual whole mosquitoes as described by Collins and others.[33] An 852-bp region of the mitochondrial *COI* gene was amplified using a pair of oligonucleotide primers. Primer sequences and amplification conditions are described in Joy and Conn.[34] A negative control was used in all analyses. PCR products were checked on agarose gels, purified, and sequenced in a MegaBACE 1000 DNA sequencer. All individuals were sequenced in both directions.

Statistical analyses. The sequences were aligned using the BioEdit[35] and Chromas softwares. Genetic variability measurements as shown in Table 3 and neutrality tests—such as Tajima's *D* test, Fu and Li's *D* and *F* tests, Fu's F_S test, and Strobeck's *S* statistic—were analyzed using DnaSP software.[36] The Tajima[37] and Fu and Li[38] tests were used to test the hypothesis that all mutations are selectively neutral.[39] Tajima's *D* test is based on the differences between the number of segregating sites and the average number of nucleotide differences.[37] The *D* and *F* tests, proposed by Fu and Li, are based on molecular polymorphism data.[38] Fu's F_S test[40] and Strobeck's *S* statistic[41] assess the haplotype structure based on the haplotype frequency distribution and were used as additional neutrality tests. The estimated genealogical relationships of the haplotypes were obtained using the TCS software.[42] Two previous studies analyzed the *COI* gene in *Ae. aegypti* populations,[23,43] but we did not compare our haplotypes to those of Beebe and others[23] because the overlap region was only 167 bp. We also did not achieve an alignment between our sequences and those of Mousson and others,[43] which prevented comparison with the haplotypes described in those studies. Otherwise, our sequences were aligned to *Ae. aegypti* sequences deposited in the GenBank, with accession numbers AF380835 (Moyo-R strain, origin from Kenya, East Africa), AY056596 (Liverpool strain; origin from West Africa), and AY056597 (formosus strain).[44] Genetic differentiation (F_{ST} and *Nm* values), hierarchical analysis (AMOVA), and Mantel test were estimated using the Arlequin software.[45] The significance level of F_{ST} values was determined by a permuting test between localities (10,100 permutations). AMOVA analyses were performed at several levels: within Manaus, within the state of Amazonas, within the northern region, between the northern/northeastern and southeastern/central regions, and among all samples (nongrouped). In the cases with a single sample within the group, AMOVA was not performed. Isolation by distance (IBD) was estimated by Mantel's test, using the correlation between genetic and geographic distances by the regression of $F_{ST}/(1 - F_{ST})$ on the natural logarithm (ln) of straight-line geographic distance,[46] and the significance level was tested using 1000 permutations. Straight-line geographic distances between the sites were obtained using Google Earth and GPS. The dendrogram containing the haplotypes of this study plus 3 haplotypes from GenBank was based on the neighbor-joining (NJ) method using Mega software,[47] following the Tamura–Nei genetic distance model. The average number of nucleotide differences (*K*) and nucleotide divergence (average number of substitutions per sites = *D*) calculated between 2 groups of haplotypes were obtained with the DnaSP software.[36] Two *Aedes albopictus* individuals from Manaus were also sequenced and used as outgroups in this analysis. When multiple tests were used, the levels of significance were adjusted by the Bonferroni correction.[48] Haplotypes are deposited in GenBank under the accession numbers EU625264 to EU625273. This study was reviewed and approved by the Research Board of the National Institute of Research of the Amazon and by the Brazilian Ministry of Science and Technology.

RESULTS

Analyses were carried out on 852 bp of the mtDNA *COI* sequences from 163 *Ae. aegypti* individuals from the 4 regions of Brazil: northern, southeastern, northeastern, and central. We identified 18 variable sites (2.11%), of which 13 (1.52%) were phylogenetically informative and 5 were singleton sites. All substitutions found were transitions. Table 2 and Figure 2

TABLE 2
Haplotype frequencies in the *Aedes aegypti* populations analyzed*

	Locality														
Haplotype	CO	PC	CP	TN	MC	CR	HU	BL	BV	RB	RP	TB	CG	CB	Total
1	6	5	9	–	2	7	–	–	7	–	–	–	–	–	36
2	5	8	3	5	1	7	7	5	–	11	4	–	5	2	63
3	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
4	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
5	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
6	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1
7	–	–	–	–	–	1	–	–	–	–	–	–	–	–	1
8	6	1	–	7	3	–	–	–	4	–	–	–	2	–	23
9	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
10	–	2	–	–	–	–	–	6	–	–	6	9	3	9	35
Total	17	16	12	12	7	16	7	11	13	11	10	10	10	11	163

* For abbreviations of localities, see Table 1.

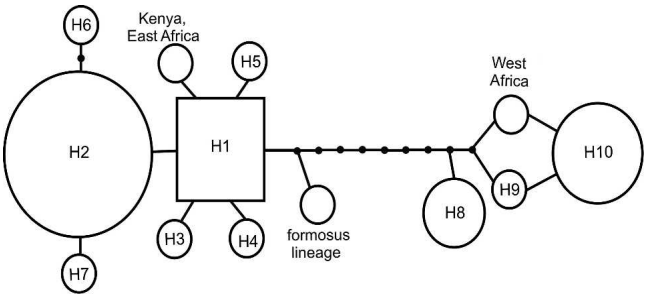

FIGURE 2. Genealogical relationships among 10 haplotypes of *Aedes aegypti* from Brazil. Solid circles represent single mutational events.

show the frequencies and network of the 10 haplotypes observed, respectively. Haplotype H2 was the most common and occurred in all localities, except in BV and TB. H1, the second most frequent and probably the oldest, occurred in 5 locations in the state of Amazonas and in BV. Both haplotypes are separated by a single mutational step. H8 was found in 4 of the 7 localities of the state of Amazonas, in BV, and in CG. This haplotype is separated from H1 and H2 by 8 and 9 mutational events, respectively. H10 was the most frequent in the southeastern and central regions and in BL; it was less frequent in CG and rare in PC. H10 is separated from H1 and H2 by 10 and 11 mutational steps, respectively. H3, H4, H5, H6, H7, and H9 were restricted to a single locality and were therefore considered more recently derived.

The average haplotype diversity (h) was 0.740 ± 0.017, ranging from 0.200 ± 0.154 (TB) to 0.810 ± 0.130 (MC). The average nucleotide diversity (π) was 0.00651 ± 0.0003, ranging from 0.00023 ± 0.0002 (TB) to 0.00793 ± 0.0010 (CG). The average number of nucleotide differences (K) was 5.546, with the highest values for BL, RP, and CG (Table 3).

For CO, TN, BL, RP, and CG, Tajima's D and Fu and Li's D and F neutrality tests showed positive and significant values, rejecting the neutral model and suggesting a possible balancing selection or population subdivision (Table 4). Fu's F_S test, which is more powerful for detecting population expansion, showed negative values for CR and TB but was not significant. Strobeck's S test was positive but not significant for all samples. Thus, none of the tests indicated population expansion. In the combined analyses of the Manaus samples, Fu and Li's D and F neutrality tests showed positive and significant values, indicating that molecular polymorphism cannot be explained by the neutral model.

Genetic differentiation showed a large range (F_{ST} = –0.099–0.992; Nm = ∞ to 0.004); however, most of the pairwise comparisons were not significant after Bonferroni correction (Table 5). The sample from TB showed the highest number of significant comparisons of all (F_{ST} = 0.596–0.992; Nm = 0.3 to 0.004), with 4 exceptions (TB and BL, TB and RP, TB and CG, and TB and CB), followed by the samples from CB (F_{ST} = 0.442–0.800; Nm = 0.6 to 0.1) and RB (F_{ST} = 0.405–0.717; Nm = 0.7 to 0.2). Genetic and geographic distances were significantly correlated (r^2 = 0.332; P = 0.038), but no correlation was detected for any particular region (data not shown).

The 5 AMOVA tests were statistically significant for all

TABLE 4

Summary of statistical analyses of the molecular polymorphism in *Aedes aegypti*

Population	Statistical test				
	Tajima's D	Fu and Li's D	Fu and Li's F	Fu's F_S	Strobeck's S
CO	2.291*	1.414*	1.911**	6.809	0.008
PC	0.040	1.124	0.949	3.699	0.090
CP	0.540	0.752	0.787	0.735	0.725
TN	2.474**	1.433*	1.933**	8.709	0.003
MC	1.704	1.264	1.500	2.107	0.351
CR	–0.387	–1.122	–1.060	–0.798	0.887
HU	NA	NA	NA	NA	NA
BL	2.600**	1.480**	1.991**	9.482	0.002
BV	1.045	0.693	0.900	3.504	0.110
RB	NA	NA	NA	NA	NA
RP	2.298*	1.487**	1.900**	8.858	0.003
TB	–1.112	–1.243	–1.347	–0.339	0.914
CG	2.139*	1.499**	1.866**	6.147	0.018
CB	–0.180	1.476**	1.195	6.822	0.014
Total	2.008	–0.951	0.226	6.420	0.005
Manaus† (N = 57)	1.589	1.513*	1.821*	8.653	0.001

Note: NA, not analyzed; N, number of sequences analyzed. For abbreviations of localities, see Table 1.

† Combined analysis of 4 urban neighborhoods of Manaus: *P < 0.05; **P < 0.02.

TABLE 3

Genetic variability in *Aedes aegypti* populations from Brazil

Population	Haplotypes observed	NS	K	Haplotype diversity (h) ± SD	Nucleotide diversity (π) ± SD
CO*	**H1**, H2, **H8**	10	4.809	0.706 ± 0.042	0.00564 ± 0.0008
PC*	H1, **H2**, H8, H10	13	3.958	0.675 ± 0.085	0.00464 ± 0.0016
CP*	**H1**, H2	1	0.409	0.409 ± 0.133	0.00048 ± 0.0002
TN*	H2, **H8**	10	5.303	0.530 ± 0.076	0.00622 ± 0.0009
MC	H1, H2, H6, **H8**	11	5.905	0.810 ± 0.130	0.00692 ± 0.0012
CR	**H1**, **H2**, H4, H7	3	0.783	0.650 ± 0.075	0.00092 ± 0.0002
HU	H2	0	0 (NA)	0 (NA)	0 (NA)
BL	H2, **H10**	12	6.545	0.545 ± 0.072	0.00768 ± 0.0010
BV	**H1**, H3, H5, H8	11	4.461	0.654 ± 0.106	0.00470 ± 0.0010
RB	H2	0	0 (NA)	0 (NA)	0 (NA)
RP	H2, **H10**	12	6.400	0.533 ± 0.095	0.00751 ± 0.0013
TB	H9, **H10**	1	0.200	0.200 ± 0.154	0.00023 ± 0.0002
CG	**H2**, H8, H10	13	6.760	0.689 ± 0.104	0.00793 ± 0.0010
CB	H2, **H10**	12	3.930	0.327 ± 0.153	0.00461 ± 0.0022
Average		18	5.546	0.740 ± 0.017	0.00651 ± 0.0003

Note: NS, number of variable sites; K, average number of nucleotide differences; NA, not analyzed. In boldface type are shown the most frequent haplotypes of each population. For abbreviations of localities, see Table 1.

* The 4 urban neighborhoods of Manaus.

TABLE 5

Genetic distance and gene flow, based on F_{ST} and *Nm* values, respectively, for 14 *Aedes aegypti* populations

Population	CO	PC	CP	TN	MC	CR	HU	BL	BV	RB	RP	TB	CG	CB
CO	–	5	8	5	75	368	594	1288	656	1152	2270	2694	2707	1447
PC	0.028 (17.5)	–	4	10	69	364	589	1293	660	1147	2268	2693	2711	1445
CP	0.232 (1.6)	0.081 (5.7)	–	13	66	360	588	1297	658	1144	2272	2697	2715	1448
TN	0.026 (18.3)	0.216 (1.8)	0.500 (0.5)	–	78	373	599	1284	654	1157	2271	2695	2705	1450
MC	–0.085 (Inf.)	0.076 (6.0)	0.398 (0.8)	–0.077 (Inf.)	–	293	538	1360	675	1085	2286	2716	2773	1449
CR	0.258 (1.4)	0.075 (6.2)	0.0381 (12.6)	0.517 (0.5)	0.422 (0.7)	–	378	1653	810	830	2371	2818	3037	1488
HU	0.266 (1.4)	0.094 (4.8)	0.668 (0.2)	0.468 (0.6)	0.392 (0.8)	0.264 (1.4)	–	1739	1170	592	2076	2533	2995	1171
BL	0.145 (2.9)	0.191 (2.1)	0.476 (0.6)	0.110 (4.0)	0.092 (4.9)	0.496 (0.5)	0.430 (0.7)	–	1434	2336	2141	2403	1538	1774
BV	–0.045 (Inf.)	0.044 (10.8)	0.223 (1.7)	0.090 (5.1)	–0.052 (Inf.)	0.283* (1.3)	0.341 (1.0)	0.182 (2.2)	–	1621	2884	3290	2967	2097
RB	0.321 (1.0)	0.144 (3.0)	0.717* (0.2)	0.532 (0.4)	0.483* (0.5)	0.320 (1.0)	0.000 (Inf.)	0.500 (0.5)	0.405* (0.7)	–	2307	2769	3524	1414
RP	0.192 (2.1)	0.254 (1.5)	0.544 (0.4)	0.133 (3.2)	0.129 (3.4)	0.564* (0.4)	0.501 (0.5)	–0.099 (Inf.)	0.228 (1.7)	0.571 (0.4)	–	463	2088	915
TB	0.660* (0.2)	0.745* (0.2)	0.972* (0.01)	0.596* (0.3)	0.691* (0.2)	0.951* (0.02)	0.990* (0.005)	0.368 (0.8)	0.712* (0.2)	0.992* (0.004)	0.315 (1.1)	–	2030	1374
CG	0.013 (38.7)	0.088 (5.2)	0.388 (0.8)	–0.011 (Inf.)	–0.043 (Inf.)	0.407 (0.7)	0.338 (1.0)	–0.063 (Inf.)	0.062 (7.6)	0.413 (0.7)	–0.041 (Inf.)	0.486 (0.5)	–	2389
CB	0.442* (0.6)	0.529* (0.4)	0.775* (0.1)	0.344 (1.0)	0.394 (0.8)	0.782* (0.1)	0.759 (0.2)	0.074 (6.3)	0.479 (0.5)	0.800* (0.1)	0.016 (30.1)	0.070 (6.6)	0.192 (2.1)	–

Note: Geographic distances (in km) and F_{ST} values are above and below the diagonal, respectively; *Nm* values are in parentheses; Inf., Infinity.
* $P < 0.0005$, after Bonferroni correction; 10,100 permutations. For abbreviations of localities, see Table 1.

hierarchical levels (Table 6). Within Manaus, state of Amazonas, and the northern region, the most variation (~76.35–82.86%) occurred within samples; nevertheless, there were significant differences (F_{ST} = 0.171–0.236) among samples. Between the northern/northeastern and the southeastern/central regions, ~45.83% (F_{CT} = 0.458; $P < 0.01$) of the total variance were due to differences between them. Among samples within 2 regions, a smaller (~11.25%) but highly significant proportion of the variance was found. Again, the most variance was found within samples (~61.80%) when all samples (nongrouped) were considered. The haplotype dendrogram based on the NJ method identified 2 well-supported groups (Figure 3) separated by 8 fixed mutations. Between the 2 groups, the average number of nucleotide differences (K) was 10.879, whereas the nucleotide divergence (D) was 0.013.

DISCUSSION

In the present study, the average genetic variability was moderately high. These results support the data reported by Mousson and others, who found that, in *Ae. aegypti*, the *COI* gene is more variable that the nicotinamide adenine dinucleotide dehydrogenase subunit *5* (*ND5*) and cytochrome *b* (*Cytb*) genes.[43] However, neither Mousson and others[43] nor Beebe and others[23] calculated the haplotype and nucleotide diversities. In studies on the *ND4* gene,[20,24,31] the nucleotide diversity values were higher than those obtained here, possibly because in *Ae. aegypti* the *COI* gene is more conservative than the *ND4* gene. The samples from 2 neighborhoods of Manaus (CO, PC) and from MC presented the highest h values. In Manaus, despite insecticide pressure, a recent study showed high density of this vector throughout the year.[49] Therefore, our results may reflect a large effective population size for these populations, likely due to multiple introductions, high gene flow with other populations, favorable social and environmental factors, intensive urbanization, and abundance of blood sources, fostering the proliferation of *Ae. aegypti*.[26,50,51] Using RAPD markers, Paduan and others[29] also found high variability in other Brazilian populations, suggesting that the introduction involved a large number of individuals.

In this study, samples from BL, RP, and CG showed the highest K and π (but not h) values, which may denote that these populations are older[52] and have not been submitted to a bottleneck effect for a long time, despite the use of insecticides. As another explanation, this result could also be due to the presence of genetically distant haplotypes in sympatry.

The presence of a single haplotype in the RB sample is consistent with either an introduction of this mosquito followed by reduced gene flow with the other localities analyzed except Humaitá, or a substantial recent bottleneck effect due to insect control activities. An identical result was also recently found for the *ND4* gene.[31] Furthermore, the sample from TB had the lowest h and π values, and this result is not related to geographic isolation. The city of Taubaté in the state of São Paulo is situated near a network of highways with an intense flow of people and trade activities connecting it to other cities and states. These factors could produce an increase of gene flow among subpopulations of *Ae. aegypti* from there, with subsequent increase in genetic variation; however,

TABLE 6
Hierarchical analysis of the genetic variation in the *Aedes aegypti* samples

Groups of samples	Source of variation	Degrees of freedom	Variation (%)	Fixation index†
Within Manaus	Among samples	3	17.14	F_{ST} = 0.171*
	Within samples	53	82.86	
Within the state of Amazonas	Among samples	6	21.49	F_{ST} = 0.212**
	Within samples	80	78.51	
Within the northern region	Among samples	9	23.65	F_{ST} = 0.236**
	Within samples	112	76.35	
Between northern/northeastern and southeastern/central regions	Between regions	1	45.83	F_{CT} = 0.458***
	Among samples and within regions	12	11.25	F_{SC} = 0.208**
	Within samples	149	42.92	F_{ST} = 0.571**
All (nongrouped)	Among samples	13	38.20	F_{ST} = 0.382**
	Within samples	149	61.80	

Note: Significance test = 1023 permutations;
† F_{ST}, fixation index within samples; F_{CT}, fixation index between regions; F_{SC}, fixation index among samples within regions; *P = 0.00664 ± 0.00263; **P = 0.00000 ± 0.00000; ***P = 0.00293 ± 0.00164.

this was not observed. This finding supports the hypothesis that a severe bottleneck effect occurred after the founding of this population, due to insecticide pressure or to periods of pronounced dry/cold weather. Alternatively, a small number of individuals may have established this population, or this low level of genetic variability could be due to the small size of TB, causing bias.

Tajima's D, and Fu and Li's D and F neutrality tests were positive and significant for CO, TN, BL, RP, and CG, suggesting population subdivision (Wahlund effect). The presence of 2 haplotype lineages within these localities could explain this result. It is consistent with the findings of Fraga and others,[28] who—on the basis of isozyme data—found population subdivision ($F_{IS} > F_{ST}$) in the samples from Manaus. This hypothesis, however, was not raised or discussed by those authors. Fu's F_S test did not show a clear sign of range expansion for the samples analyzed. Similarly, a study using the *ND4* gene did not indicate population expansion in most of these populations (R. S. Lima Junior and V. M. Scarpassa, unpublished data).

Within the city of Manaus (4–13 km), there was considerable gene flow for most samples (Nm = 1.6–18.3); nevertheless, AMOVA indicated significant genetic structure for these samples. This result may be explained by the fact that in CP only Group 1 haplotypes were sampled, whereas in the 3 other samples there were haplotypes of 2 groups, indicative of control efforts following genetic drift. However, it is possible that Group 2 haplotypes may be lower frequencies in the CP natural population compared with three other populations (CO, PC, and TN). This hypothesis may be better investigated collecting *Ae. aegypti* samples over several seasons. A similar pattern was found in Rio de Janeiro, Brazil,[5] and Cali, Colombia.[53] Within the state of Amazonas (4–599 km), most samples analyzed showed gene flow (Nm = 1.4 to ∞), due to the higher frequency of Group 1 haplotypes. The dispersal patterns of *Ae. aegypti* in the state of Amazonas may be related to the intense river traffic on the Amazon, Negro, and Solimões rivers, the main travel routes in this region. Our results also suggest that there is extensive gene flow among BV and localities in the state of Amazonas. The intensive human migration and trade exchange among these locations by means of highway BR-174 could explain these findings. Moreover, there was reduced gene flow between samples from BV and RB, which could be due to the difficulties of access between these locations by river or land routes.

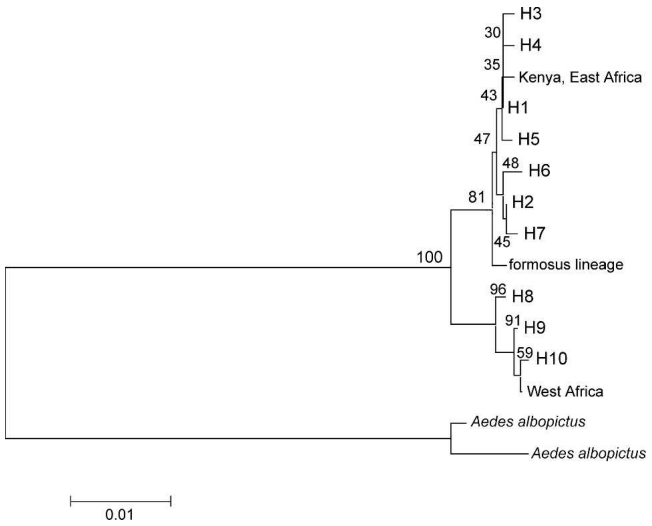

FIGURE 3. Haplotype dendrogram of *Aedes aegypti* from Brazil based on the neighbor-joining (NJ) method, according to the Tamura–Nei genetic distance model. Bootstrap support values are recorded above branches.

None of the comparisons involving the samples from BL and CG showed statistically significant results because they had haplotypes of the 2 groups, with similar frequencies. The results indicate extensive gene flow between BL and the populations from the state of Amazonas, probably due to the intense river traffic on the Amazon River, a major route of travel and trade exchange between these locations, contributing to the dispersal of *Ae. aegypti*. Gene flow was also extensive between BL and RP, which are separated by a distance of about 2141 km and share the same haplotypes. These data suggest reduced gene flow between BL and TB, separated by about 2403 km, but the reduction was not significant because both share H10. The large flow of persons circulating between these localities could influence dispersal of *Ae. aegypti*. Gene flow was also observed between CG and the state of Amazonas, CG and BL, and CG and RP. It was, however, limited, but not significant, between CG and RB and between CG and TB. Thus, BL and CG could represent an interface for gene flow between the northern/northeastern and southeastern/central regions, receiving migrants from both.[54] The sample from CB showed low differentiation with regard to

BL, the southeastern region, and CG, which is attributed to the higher frequency of Group 2 haplotypes. In these localities, human migration occurs mostly by land and air. The sample from TB showed the highest differentiation and reduced *Nm* values (0.004–0.3), indicating a very low dispersal rate[55] as a result of the high frequency of H10 and the presence of an exclusive haplotype (H9), both of Group 2. Although the sample size needs to be increased, this result suggests that TB is genetically differentiated from other populations, which may affect the vector competence of TB compared with other populations.

Aedes aegypti has a flight range of 50–800 m[56–58]; therefore, dispersal over long distances could be explained by passive migration of this vector. In this study, the correlation between genetic and geographic distances supported the IBD model, but no correlation was detected within the northern region (excluding RB) or within the southeastern/central plus northeastern regions. This pattern is consistent with human migration and commercial routes that—if allied with control activities—could be influencing the genetic structure of *Ae. aegypti* within regions.[59] AMOVA tests demonstrated significant genetic structure for all hierarchical levels; nevertheless, the most variance was found within samples of the Manaus, state of Amazonas, northern region, all populations, and between the northern/northeastern and southeastern/central regions. The 2 haplotype groups were sampled in all 4 regions of Brazil; however, Group 1 was predominant in the north, whereas Group 2 was the most frequent in the southeastern and central regions. The northeastern region (CG) had identical frequencies for both groups. This result explains the highest variance found between the northern/northeastern and southeastern/central regions and the very high F_{ST} values primarily involving the samples from RB, TB, and CB. It also explains that the greatest variation occurred within samples attributed primarily to both groups in sympatry. Taken together, these data suggest a deep genetic structure for *Ae. aegypti* populations, due to a combination of factors: multiple introductions associated with distinct lineages, geographic differentiation (IBD), passive dispersal patterns, control activities following extinction and recolonization events, and genetic drift.[20,54,59,60] Similarly, other studies conducted in Brazil have found highly structured populations of *Ae. aegypti*.[22,29]

As observed in previous studies,[20,22,24,29,31,59,61] our data indicate the existence of 2 genetic lineages within Brazil. These lineages likely evolved from the ancestral population, presumably in North Africa, and later on dispersed all over the world.[62] In this study, the haplotypes clustered in Group 1 show close relationships to the Moyo-R lineage (Kenya, East Africa), whereas the haplotypes included in Group 2 have a high level of genetic similarity to the Liverpool lineage (West Africa),[44] supporting the hypothesis of Powell and others.[18] On the basis of isozyme data, these authors suggested that the *Ae. aegypti* populations of South America, the United States, and the Caribbean are genetically related to those from East Africa. More recently, 2 studies[31,43] showed that the Brazilian *Ae. aegypti* populations are closely related to populations from Africa and Asia. Failloux and others[63] observed that *Ae. aegypti* from French Guiana and Southeast Asia had the highest level of genetic similarity, suggesting that probably this mosquito was introduced in the Americas coming from Asia.[63] Herein, we did not compare our samples with samples from Asia, and therefore it was not possible to infer the genetic relationships between them. We hypothesized that Group 1 might be ancestral, because it has older and more widespread haplotypes than Group 2. Furthermore, it is closely related to the formosus strain.[43] Nucleotide divergence between the 2 groups was much lower ($D = 0.013$) than that observed for the *ND4* gene ($D = 0.032$).[31] This difference could be explained either by the fact that the *COI* gene is more conservative than *ND4* or that Bracco and others[31] analyzed a larger range of samples including localities in the Americas, Africa, and Asia, and these populations probably had distinct dispersal patterns and colonization and/or recolonization events during their evolutionary histories. We speculated that H1, restricted to the state of Amazonas and BV, was introduced in the northern region of Brazil, probably via Boa Vista. H2 might have derived from H1 by recent mutation, which, when considering its position in the network, is compatible with recent expansion. However, the neutrality tests are not consistent with this hypothesis. To test this hypothesis, it will be necessary to investigate other samples across the geographic range of *Ae. aegypti*. H10 was probably introduced in the southeastern region of Brazil coming from other American countries that had not achieved eradication or from Africa, later on dispersing to other regions of Brazil. H8 may be a third lineage introduced into Brazil, probably via Boa Vista or harbor locations of the northern or northeastern regions. Mousson and others[43] observed that the samples from Boa Vista were more closely related to those from Guinea and the Ivory Coast (West Africa). Thus, the specimen from Boa Vista analyzed by those authors may belong to haplotype H8 of this study. Mousson and others[43] proposed that this sample was introduced during the past centuries and survived eradication programs. Our results support this hypothesis.[31,43] H1 and H8 may have been introduced in Brazil at different times, probably between the 16th and 18th centuries, whereas H10 could have been introduced later.

Multiple introductions have an enormous epidemiological impact on dengue, because gene flow may facilitate the spreading of transmission-related genes and thus enhance the vector competence for transmitting the dengue and urban yellow fever viruses. The city of Boa Vista in northern Brazil is situated on the border with other countries of northern South America, namely, Venezuela, the Guianas, and Suriname, representing an important entry site for new *Ae. aegypti* haplotypes and dengue serotypes and genotypes. The first dengue outbreak in Brazil occurred in Boa Vista in the early 1980s.[8,9] Later, a study compared the infection rates by DENV2 in samples of *Ae. aegypti*, and the results showed that the sample from Boa Vista had the highest susceptibility (~92–99%) out of 23 Brazilian samples analyzed.[5] Our data indicate high genetic similarity between samples from BV and the state of Amazonas (both share H1 and H8), implying that 1 or 2 mosquito introductions that became established in the state of Amazonas were from BV. Furthermore, in Manaus we found moderately high levels of genetic variability and evidence of multiple introductions. These factors, along with co-circulation of 3 serotypes, may explain the large number of DHF cases recorded in Manaus.[14–16] This situation enhances the need of constant surveillance by local and regional health authorities for dengue prevention by vector control.

The presence of genetically distinct lineages in Brazil could imply differences in the vector competence to transmit the dengue and urban yellow fever viruses and in the response to

vector control measures. Our findings can contribute to a better understanding of the epidemiological aspects of dengue and help improving the vector control measures, primarily the genetic control, to prevent or reduce the epidemic impacts in Brazil.

Received August 29, 2007. Accepted for publication February 29, 2008.

Acknowledgments: The authors thank Lúzia de Melo Mustafa and her team (SUSAM, Manaus, Amazonas) and the teams of the Secretaries of Health of Belém, Boa Vista, Coari, Cuiabá, and Rio Branco (Dulcinéia Barros de Aguiar, Agostinho Aroldo Limeira, Claudio Rodrigues de Souza, Oberdan F. Coutinho Lira, and Giovana B.M.L. Maciel); Dr. Gisela Marques and her team (SUCEN, Taubaté, São Paulo), Raimundo S. Lima Júnior and Fabio Medeiros da Costa (INPA, Manaus, Amazonas), and Carlos Rivero M. Fernandes (Universidade Estadual da Paraíba, Campina Grande, Paraíba) for providing facilities and logistic support during the field collections. We also thank Dr. Jan E. Conn, who kindly revised the English of the final version.

Financial support: Research funded by the Amazonas State Research Foundation (Fundação de Amparo à Pesquisa do Estado do Amazonas [FAPEAM], Manaus, AM, Brazil, grant number 905/2003, to V.M.S.), and by the Brazilian Ministry of Science and Technology/ INPA.

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