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Magda Clara Vieira da Costa-Ribeiro, Ricardo Lourenço-de-Oliveira,* and Anna-Bella Failloux

Laboratório de Transmissores de Hematozoários, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil; Insectes et Maladies Infectieuses, Institut Pasteur, Paris, France

Abstract. We present a population genetic study of *Aedes aegypti* in Brazil using isoenzyme markers. Four polymorphic loci were used to examine 11 mosquito collections at four periods in 2003. Samples from a dengue-endemic area (southeastern region) and a dengue-free area (southern region) connected by an important network of roads and railways were analyzed. The degree of genetic differentiation observed between populations is consistent with limited gene flow between them. There was no evidence of passive dispersion of *Ae. aegypti* by vehicles among the different routes linking metropolitan areas.

INTRODUCTION

It is believed that *Aedes aegypti* was reintroduced in Brazil in the late 1970s after nearly two decades of absence.[1,2] Because vector control was not well implemented, dengue epidemics started to be reported in the early 1980s. *Aedes aegypti* is now present in all Brazilian states[2–4] and approximately 80% of all dengue cases registered in the Americas are reported in Brazil,[5] most of them acquired in the southeastern and northeastern regions.[6] In the southeastern Brazil, Rio de Janeiro is considered as the most important point for the entry and dissemination of dengue viruses into the country.[2,5] The city is connected to numerous localities in other southeastern states such as Minas Gerais, Espírito Santo, and São Paulo through an important network of roads and railways.

Because *Ae. aegypti* is usually a poor flyer (approximately 10–800 meters during its entire life),[7,8] passive migration through human transportation have been described and could explain mosquito dispersal and gene flow over long distances.[9] Knowledge concerning migration and gene flow between *Ae. aegypti* populations can provide information about the evolution of the species and understanding about the spread of *Ae. aegypti* traits that impact the epidemiology of *Ae. aegypti*-borne pathogens. This information can help in the design of more effective vector control strategies.

In the states of Rio de Janeiro, Minas Gerais and Espírito Santo, dengue epidemics are annually reported all over the region; in the state of São Paulo, dengue transmission occurs mainly in the inland regions and sporadically along the coast.[10] Although these economically developed and dengue-endemic southeastern states have intensive commercial trade with the southern state of Rio Grande do Sul where dengue has never been reported, despite the presence of *Ae. aegypti* since 1995.[11]

A high degree of genetic differentiation of *Ae. aegypti* has been demonstrated in Rio de Janeiro.[2] The breeding season of the mosquito extends throughout the year. However, mosquito densities decrease during the dry season and increase during the rainy season, coinciding with a period of high dengue incidence. In this study, we compared samples from two areas, dengue-endemic and dengue-free areas to characterize the geographic and seasonal structure of *Ae. aegypti* populations and to evaluate the role of passive migration in gene flow among *Ae. aegypti* populations from different cities in southeastern and southern Brazil connected to Rio de Janeiro by roads and railways.

MATERIALS AND METHODS

Mosquito samples. *Aedes aegypti* was sampled in 11 Brazilian localities (Table 1) from four southeastern states: Rio de Janeiro state (Barra Mansa, 25 de Agosto, Parque Duque, Nova Iguaçu, Paraíba do Sul, Três Rios), São Paulo (Potim), Minas Gerais (Belo Horizonte), and Espirito Santo (Consolação and Cariacica), and one in the southern state of Rio Grande do Sul State (Porto Alegre). Sampled localities are separated by a minimum distance of 1.2 km and a maximum distance of 1,538.6 km, and were all connected by ground transportation to Rio de Janeiro (Figure 1 and Table 1). Mosquito collections were performed at three-month intervals from March 2003 to December 2003 using 20 ovitraps[12] per locality during two consecutive weeks to avoid collections of descendents from a small number of females. The first collection was carried out in March 2003 (at the end of rainy season), the second in June (at the beginning of the dry season), the third in September 2003 (at the end of the dry season), and the fourth in December 2003 (at the beginning of the rainy season). Mosquitoes were reared until adult stage (F_0 generation) in insectaries under standardized conditions (25 ± 1°C, relative humidity of 80 ± 10%, and a 12-hour light/dark cycle) and subsequently stored at -80°C for isoenzyme assays. When the number of F_0 adults was too low (less than 20 individuals), adults from the F_1 generation were used; this was the case for the third collections in POTI, TRRI, and PARS.

Electrophoresis. Mosquitoes were individually grounded in 25 μL of distilled water and centrifuged (12,000 × *g* for 3 minutes at 4°C). The supernatant containing 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.[13] A total of 48 adults from each sample (Table 2) were analyzed for 10 enzyme systems: glucose phosphate isomerase (Gpi, EC 5.3.1.9.), glutamate oxaloacetate transaminases (Got1 and Got2, EC 2.6.1.1.), glycerol phosphate dehydrogenase (Gpd, EC 1.1.1.8.), hexokinases (Hk1, Hk2, and Hk3, EC 2.7.1.1.), malate dehydrogenase (Mdh, EC 1.1.1.37.), malic enzyme (Me, EC 1.1.1.40.), and phosphoglucomutase (Pgm, EC 2.7.5.1.) according to Failloux and others[14] and

* Address correspondence to Ricardo Lourenço de Oliveira, Laboratório de Transmissores de Hematozoários, Instituto Oswaldo Cruz-Fiocruz, Avenida Brasil 4365, 21045-900-Rio de Janeiro, RJ, Brazil. E-mail: lourenco@ioc.fiocruz.br

TABLE 1
Characteristics of districts where *Aedes aegypti* was sampled in Brazil (four collection dates from March to December 2003)

Sample	Designation	Human density/km^2	Total no. of dengue cases	
			2002	2003
Southeastern region				
BARM	Barra Mansa, Rio de Janeiro state	311.9	3,198	262
BELH	Belo Horizone, Minas Gerais state	6,763.8	11,653	3,759
CARI	Cariacica, Espírito Santo state	1,158.2	1,761	4,929
CONS	Consolação, Espírito Santo state	3,509.5	4,287	6,411
DCPD	Parque Duque, Rio de Janeiro state	1,669.1	13,226	192
DC25	25 de Agosto, Rio de Janeiro state	1,669.1	13,226	192
PARS	Paraiba do Sul, Rio de Janeiro state	64.4	795	781
POTI	Potim, São Paulo state	304.6	9	–
NOVI	Nova Iguaçu, Rio de Janeiro state	1,757.2	8,383	212
TRRI	Três Rios, Rio de Janeiro state	221.8	227	17
Southern region				
PORT	Porto Alegre, Rio Grande do Sul state	2,738.5	–	–

Huber and others.[15] A laboratory strain of *Ae. aegypti* known as Paea (collected in 1994 in Tahiti, French Polynesia) was used as a mobility control for isoenzyme polymorphism.[16,17]

Genetic analysis. Hardy-Weinberg proportions were compared using the GENEPOP software (version 3.4).[18] Deviations were based on an alternative hypothesis (H_1 = deficits or excess) using an exact test procedure.[19] Linkage disequilibrium was tested between pairs of loci for each sample using Fisher's exact test on rank × column contingency tables. $F_{IS,}$ the inbreeding coefficient, and $F_{ST,}$ the fixation index, were estimated as described by Weir and Cockerham.[20] Genetic differentiation between populations or groups of populations was tested using Fisher's exact test on R × C contingency tables for each locus. An unbiased estimate of the exact prob-

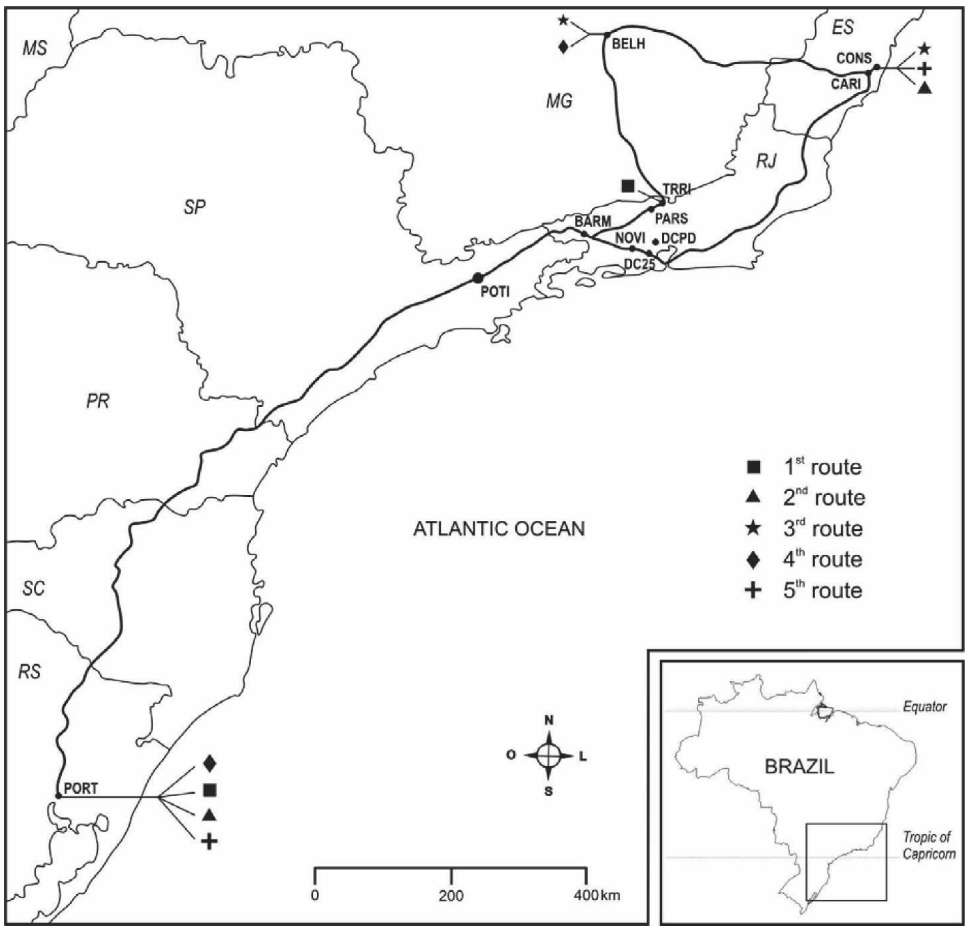

FIGURE 1. *Aedes aegypti* samples collected in southern and southeastern Brazil in 2003. District abbreviations are shown in Table 1. Routes correspond to roads or railways connecting cities. MS = Mato Grosso do Sul; MG = Minas Gerais; ES = Espirito Santo; RJ = Rio de Janeiro; SP = São Paulo; PR = Parana; SC = Santa Catarina; RS = Rio Grande do Sul.

ability was obtained with a Markov chain method.[18] Significance levels for multiple testing were corrected using sequential Bonferroni's procedures.[21] Genetic isolation by geographic distance was tested by estimating rank correlations between $F_{ST}/(1 - F_{ST})$ calculated between pairs of samples and Ln distances.[22]

RESULTS

Hardy-Weinberg proportions. Six (Gpd, Got2, Hk1, Hk3, Me, and Pgi) of 10 loci investigated were monomorphic or displayed limited polymorphism in all samples. Genetic analysis was therefore based on the four remaining polymorphic loci: Pgm, Mdh, Hk2 and Got1 (Table 2). Significant deviations from Hardy-Weinberg equilibrium were associated with heterozygote deficit in 16 tests and heterozygote excess in 3 tests (Table 2). Global tests considering all loci for each sample showed significant heterozygote deficits in six samples in BARM (F_{IS} = 0.3357) in the first collection; CARI (F_{IS} = 0.2116), DC25 (F_{IS} = 0.2954), NOVI (F_{IS} = 0.4780), POTI (F_{IS} = 0.2698), and TRRI (F_{IS} = 0.5468) in the fourth collection; and significant heterozygote excesses in eight samples: DC25 (F_{IS} = -0.0577), DCPD (F_{IS} = -0.1834) and POTI (F_{IS} = -0.1877) in the first collection; BARM (F_{IS} = -0.0581), CONS (F_{IS} = -0.1408), DC25 (F_{IS} = -0.0839), POTI (F_{IS} = -0.1770), and TRRI (F_{IS} = -0.3313) in the second collection. No deviation from Hardy-Weinberg equilibrium was observed at the third collection.

Temporal genetic differentiation. At each period of collection, samples showed a significant differentiation ($P \leq 10^{-6}$). The highest F_{ST} value was observed at the fourth collection, i.e., at the beginning of the rainy season (Table 3). When considering each sample at two periods (rainy season versus dry season), five of eight collections had F_{ST} values smaller during the rainy season than during the dry season: CONS, DC25, NOVI, PARS, and POTI (Table 4).

Geographic genetic differentiation. Samples were pooled according to geographic localization. Mosquito samples were divided into two groups: localities inside the Rio de Janeiro state separated by 1.2–118.3 km (BARM, DC25, DCPD, NOVI, PARS, and TRRI) and localities outside Rio de Janeiro state from other states in the southeastern and southern regions separated by 16.2–1,538.6 km (BELH, CONS, CARI, PORT, POTI) (Table 5). Differentiation evaluated by estimating F_{ST} values was highly significant in all samples and collection dates. When considering samples from Rio de Janeiro state, the highest values of F_{ST} were obtained at the end of the dry season (F_{ST} = 0.1043, $P < 10^{-6}$), and collections from the other states (BELH, CONS, CARI, PORT, POTI) showed higher differentiation at the beginning of the dry season (F_{ST} = 0.1705, $P < 10^{-5}$) (Table 5).

Genetic isolation by distance. When testing hypothesis of isolation by distance for samples connected by roads or railways (Figure 1), no significant correlation ($P > 0.05$) was showed between F_{ST} values and geographic distances: route 1 (P = 0.1258), route 2 (P = 0.1080), route 3 (P = 0.1638), route 4 (P = 0.2118), and route 5 (P = 0.2509).

Statistical independence. Genotypic associations between pairs of loci were analyzed for each sample. Random association was rejected by Bonferroni's sequential test ($P < 0.05$) in 2 of 215 combinations when taking into account multiple tests: Mdh-HK2 in BARM (first collection) and Pgm-Pgi in PORT (fourth collection). When we used the statistics of Ohta[23] statistics for each sample-loci combination, it was demonstrated that gametic associations were caused by genetic drift ($D_{IS} < D_{ST}$ and $D_{IS} > D_{ST}$), which ruled out the effect of selection acting on pairs of loci.

DISCUSSION

We demonstrated that *Ae. aegypti* populations are highly differentiated, the pattern of genetic differentiation varies according to the period of the year, and *Ae. aegypti* populations sampled along the main routes connecting cities in southeastern and southern Brazil are highly differentiated. A total of 84% of Hardy-Weinberg deviations concerned heterozygote excess, which were mostly found in samples collected at the beginning of the dry season (second collection). An explanation could be related to the small number of breeders producing the next generation, which leads to differences in allele frequencies in male and female parents because of binomial sampling error.[24] A total of 16% of Hardy-Weinberg deviations corresponded to heterozygote deficits that occurred mainly at the beginning of the rainy season (at the fourth collection); inbreeding by positive assortative mating or by pooling population with different allele frequency (the Wahlund effect) are the common explanations. Inbreeding could be excluded because deficits would be expected in all loci. Fragmented mosquito populations begin to restore larger panmictic units with the arrival of rain.

Because mosquitoes occupy transient habitats where water availability varies greatly across seasons, populations are subdivided into distinct patches that are subjected to temporal fluctuations. Thus, distribution of genetic variation often depends on the pattern of insect oviposition. If a female tends to deposit eggs in different sites, a same area could be visited by numerous females and thus variance of genotypes will be minimal. Conversely, if a female tends to deposit all its eggs in a same area, there will be different patches occupied by different females and variance of genotypes among patches will be high. Among factors that may affect differentiation (and gene flow) in insect populations, dispersal behavior is the most intuitively obvious. Thus there is a positive correlation between the extent of dispersal and levels of gene flow. Moreover, infectious agents such as viruses can be strongly dependent on the genetic diversity found in the host population (i.e., the vector population).[25] Diseases are thought to spread more easily among genetically similar individuals.

Differentiation evaluated by estimating F_{ST} values was highly significant in all samples and collection dates. A plausible explanation would be a low level of migration in *Ae. aegypti* populations from southeastern and southern regions. This can be attributed to the high availability of larval containers in most areas in the country because of low efficiency of control measures, which reduces dispersal of *Ae. aegypti* for the search of oviposition sites. High genetic differentiation of *Ae. aegypti* has been currently observed in Brazil[2,26–29] and other countries,[30,31] which suggests low gene flow among populations in macro-regional and micro-regional scales.

Nevertheless, strong local differences in the type of breeding sites and their pupal productivity, use of personal protection against mosquitoes (insecticide sprays, repellents), and vector control implemented may explain the variable levels of

TABLE 2

F_{IS} and deviations from Hardy-Weinberg expectations observed at four polymorphic loci in *Aedes aegypti* samples collected in Brazil in 2003*

Samples	Pgm								Mdh				
March	70	80	90	100	120	F_{IS}	P	N	100	110	F_{IS}	P	N
BARM	0.052	0.906	0.042	0.000	0.000	–0.0655	1	48	0.656	0.344	0.1744	0.3220	45
BELH	0.000	0.729	0.125	0.146	0.000	–0.0519	0.0922	48	0.422	0.578	0.4623	**0.0024**†	45
CARI	0.000	0.906	0.000	0.094	0.000	0.1521	0.3377	48	0.552	0.448	–0.2116	0.1584	48
CONS	0.000	0.958	0.000	0.042	0.000	–0.0330	1	48	0.426	0.574	–0.0337	1	47
DC25	0.000	0.854	0.000	0.135	0.010	0.1009	0.0999	48	0.553	0.447	0.1497	0.3790	47
DCPD	0.000	0.906	0.000	0.094	0.000	0.3959	0.0397	48	0.531	0.469	–0.3714	0.0189	48
NOVI	0.000	0.781	0.000	0.219	0.000	0.2177	0.1952	48	0.344	0.656	0.1330	0.5200	48
PARS	0.000	0.936	0.000	0.064	0.000	–0.0575	1	47	0.670	0.330	0.1921	0.2035	47
PORT	0.000	0.554	0.446	0.000	0.000	–0.1773	0.2497	46	0.511	0.489	0.1218	0.5503	45
POTI	0.000	0.840	0.000	0.160	0.000	–0.1795	0.5784	47	0.667	0.333	0.1665	0.3295	48
TRRI	0.000	0.760	0.000	0.240	0.000	–0.0759	0.7086	48	0.330	0.670	–0.0964	0.7404	47

Samples	Hk2						Got1					All loci	
March	80	100	110	F_{IS}	P	N	60	100	F_{IS}	P	N	F_{IS}	P
BARM	0.521	0.479	0.000	0.9583	**0.00001**†	47	0.188	0.812	–0.2208	0.1812	48	0.3357	**0.00001**†
BELH	0.573	0.427	0.000	–0.0538	0.7728	48	0.319	0.681	0.1293	0.5006	47	0.1213	0.0165
CARI	0.468	0.532	0.000	–0.2719	0.0817	47	0.375	0.625	–0.2345	0.1300	48	–0.1981	0.0602
CONS	0.351	0.649	0.000	0.4020	0.0093	47	0.319	0.681	–0.1646	0.3245	47	0.0639	0.1695
DC25	0.389	0.611	0.000	–0.4403	**0.0043**‡	45	0.115	0.885	0.0864	0.4753	48	–0.0577	**0.0151**‡
DCPD	0.245	0.745	0.011	–0.3151	0.0552	47	0.156	0.844	–0.0166	1	48	–0.1834	**0.0097**‡
NOVI	0.000	1.000	0.000	–	–	48	0.031	0.969	–0.0217	1	48	0.1560	0.5994
PARS	0.174	0.826	0.000	–0.2000	0.3175	46	0.202	0.798	–0.2432	0.1704	47	–0.0482	0.3408
PORT	0.122	0.878	0.000	–0.1282	1	45	0.553	0.447	0.1497	0.3790	47	0.0124	0.6574
POTI	0.500	0.500	0.000	–0.5577	**0.0003**‡	46	0.083	0.917	–0.0805	1	48	–0.1877	**0.0117**‡
TRRI	0.000	1.000	0.000	–	–	48	0.219	0.781	0.2177	0.1952	48	0.0046	0.6017

Samples	Pgm							Mdh				
June	80	90	100	110	F_{IS}	P	N	100	110	F_{IS}	P	N
BARM	0.719	0.000	0.281	0.000	–0.2786	0.0742	48	0.792	0.208	0.3775	0.0174	48
BELH	0.750	0.094	0.156	0.000	0.1343	**0.0148**†	48	0.292	0.708	0.0021	1	48
CARI	0.927	0.000	0.073	0.000	–0.0682	1	48	0.781	0.219	0.2177	0.1952	48
CONS	0.979	0.000	0.021	0.000	–0.0108	1	48	0.583	0.417	0.3237	0.0368	48
DC25	0.865	0.000	0.125	0.010	0.4802	**0.0017**†	48	0.415	0.585	–0.2609	0.1298	47
DCPD	0.875	0.000	0.125	0.000	–0.1325	1	48	0.372	0.628	–0.2188	0.2094	47
NOVI	0.802	0.000	0.198	0.000	–0.2368	0.1718	48	0.729	0.271	0.0610	0.7199	48
PARS	0.750	0.000	0.250	0.000	–0.1007	0.7019	48	0.750	0.250	0.0105	1	48
POTI	0.875	0.010	0.115	0.000	0.2562	0.2036	48	0.479	0.521	–0.4839	**0.0013**‡	47
TRRI	0.906	0.000	0.094	0.000	0.1521	0.3377	48	0.531	0.469	–0.7098	**0.00001**‡	48

Samples	Hk2						Got1					All loci	
June	80	100	110	F_{IS}	P	N	60	100	F_{IS}	P	N	F_{IS}	P
BARM	0.010	0.990	0.000	0.0000	–	48	0.198	0.802	–0.2368	0.1718	48	–0.0581	**0.0099**‡
BELH	0.000	0.958	0.042	–0.0330	1	48	0.375	0.625	–0.2345	0.1300	48	–0.0418	0.1302
CARI	0.130	0.870	0.000	–0.1392	1	46	0.385	0.615	–0.0009	1	48	0.0297	0.9165
CONS	0.500	0.500	0.000	–0.5672	**0.0001**‡	47	0.188	0.812	–0.2208	0.1812	48	–0.1408	**0.0005**‡
DC25	0.000	1.000	0.000	–	–	48	0.209	0.791	–0.2537	0.1657	43	–0.0839	**0.0023**‡
DCPD	0.000	1.000	0.000	–	–	48	0.128	0.872	–0.1358	1	47	–0.1775	0.7927
NOVI	0.000	1.000	0.000	–	–	48	0.177	0.823	0.0812	0.6207	48	–0.0271	0.5267
PARS	0.000	1.000	0.000	–	–	48	0.240	0.760	–0.1906	0.2515	48	–0.0926	0.7481
POTI	0.000	1.000	0.000	–	–	48	0.043	0.957	0.4860	0.0642	47	–0.1770	**0.0012**‡
TRRI	0.000	1.000	0.000	–	–	48	0.281	0.719	–0.0717	0.7299	48	–0.3313	**0.00001**‡

Samples	Pgm							Mdh					
September	80	90	100	110	F_{IS}	P	N	80	100	110	F_{IS}	P	N
BELH	0.864	0.114	0.023	0.000	0.2664	0.3235	22	0.000	0.750	0.250	–0.0678	1	22
CONS	0.896	0.000	0.104	0.000	–0.1059	1	48	0.000	0.688	0.312	0.2342	0.1738	48
DC25	0.979	0.010	0.010	0.000	–0.0054	1	48	0.000	0.750	0.250	0.0105	1	48
DCPD	0.938	0.000	0.062	0.000	–0.0562	1	48	0.000	0.594	0.406	0.3614	**0.0168**†	48
NOVI	0.727	0.000	0.114	0.159	0.0663	0.0863	44	0.000	0.381	0.619	0.4146	0.0780	21
PARS	1.000	0.000	0.000	0.000	–	–	47	0.000	0.448	0.552	–0.1270	0.3989	48
POTI	0.979	0.000	0.021	0.000	–0.0108	1	48	0.010	0.760	0.229	–0.0053	0.3023	48
TRRI	1.000	0.000	0.000	0.000	–	–	46	0.000	0.562	0.438	–0.1750	0.2536	48

TABLE 2
Continued

Samples	Hk2						Got1					All loci	
September	80	100	110	F_{IS}	*P*	N	60	100	F_{IS}	*P*	N	F_{IS}	*P*
BELH	0.023	0.977	0.000	0.0000	–	22	0.523	0.477	0.0212	1	22	0.0428	0.8946
CONS	0.000	1.000	0.000	–	–	48	0.281	0.719	–0.0717	0.7299	48	0.0511	0.6591
DC25	0.000	0.990	0.010	0.0000	–	48	0.000	1.000	–	–	48	0.0085	1
DCPD	0.000	0.990	0.010	0.0000	–	48	0.167	0.833	–0.1899	0.3228	48	0.1288	0.1076
NOVI	0.000	0.659	0.341	–0.0896	1	22	–	–	–	–	0	0.1167	0.1246
PARS	0.000	1.000	0.000	–	–	48	0.000	1.000	–	–	48	–0.1270	–
POTI	0.000	1.000	0.000	–	–	47	0.000	1.000	–	–	48	–0.0059	0.6639
TRRI	0.000	1.000	0.000	–	–	48	0.156	0.844	0.2985	0.0677	48	–0.0093	0.0870

Samples	Pgm						Mdh					
December	80	90	100	F_{IS}	*P*	N	80	100	110	F_{IS}	*P*	N
BELH	0.904	0.043	0.053	0.1722	0.0918	47	0.000	0.394	0.606	–0.2827	0.0693	47
CARI	0.870	0.043	0.087	0.4523	**0.0021**†	46	0.000	0.468	0.532	0.2411	0.1421	47
CONS	0.967	0.000	0.033	0.6617	**0.0330**†	46	0.000	0.413	0.587	–0.1550	0.3662	46
DCPD	0.917	0.000	0.083	–0.0805	1	48	0.000	0.641	0.359	0.0187	1	46
DC25	0.862	0.000	0.138	0.3843	**0.0283**†	47	0.000	0.523	0.477	0.2188	0.2202	43
NOVI	0.875	0.000	0.125	–0.1325	1	48	0.032	0.660	0.309	0.6432	**0.0000**†	47
PARS	0.964	0.000	0.036	–0.0250	1	42	–	–	–	–	–	0
PORT	0.585	0.404	0.011	–0.1954	0.1670	47	0.000	0.511	0.489	0.3285	**0.0394**†	47
POTI	0.883	0.000	0.117	0.0841	0.4834	47	0.000	0.521	0.479	0.2852	0.0779	47
TRRI	0.845	0.000	0.155	0.5534	**0.0030**†	42	0.000	0.793	0.207	0.4900	**0.0052**†	41

Samples	Hk2						Got1					All loci	
December	80	100	110	F_{IS}	*P*	N	60	100	F_{IS}	*P*	N	F_{IS}	*P*
BELH	0.000	1.000	0.000	–	–	47	0.479	0.521	0.0302	1	47	–0.0770	0.1199
CARI	0.000	1.000	0.000	–	–	47	0.468	0.532	0.0707	0.7699	47	0.2116	**0.0101**†
CONS	0.141	0.837	0.022	–0.1578	0.6881	46	0.318	0.682	–0.0361	1	44	–0.0741	0.2957
DCPD	0.000	1.000	0.000	–	–	48	0.156	0.844	0.2985	0.0677	48	0.0873	0.4954
DC25	0.282	0.718	0.000	0.6281	**0.0002**†	39	0.250	0.750	0.0255	1	46	0.2954	**0.0007**†
NOVI	0.000	1.000	0.000	–	–	48	0.104	0.896	0.7809	**0.0001**†	48	0.4780	**0.00001**†
PARS	0.190	0.810	0.000	0.3926	0.0240	42	–	–	–	–	0	0.3167	0.1135†
PORT	0.000	1.000	0.000	–	–	48	0.851	0.149	0.036	1	47	0.0555	0.1227
POTI	0.271	0.729	0.000	0.3761	**0.0223**†	48	0.085	0.915	0.1906	0.2768	47	0.2698	**0.0330**†
TRRI	0.000	1.000	0.000	–	–	42	0.110	0.890	0.6330	**0.0025**†	41	0.5468	**0.00001**†

* F_{IS} = inbreeding coefficient; N = sample size; *P* = probability for rejecting Hardy–Weinberg equilibrium: significant *P* values (< 0.05) are in **bold;** *Pgm* = phosphoglucomutase; *Mdh* = malate dehydrogenase; *Hk2* = hexokinase 2; *Got1* = glutamate–oxaloacetate transaminase *1*; – = not determined. Alleles are expressed in relative frequencies.
† Heterozygote deficit.
‡ Heterozygote excess.

genetic differentiation among sampling sites. Successive cycles of extinction and low rate of migration during the recolonization process may greatly increase random frequency drift in population.

In the southeastern and southern regions, the density of *Ae. aegypti* populations is generally dependent on rainfall,[32] with a peak occurring usually between January and March.[3,33] Nevertheless, local differences in the type of the most abundant and productive breeding sites are expected to vary between and also within cities. In those areas where water storage is unnecessary, the temporary water sites filled by rain-

TABLE 3
Differentiation of *Aedes aegypti* from Brazil according to collection period in 2003*

			F_{ST}†				
Comparison	N_s	N_i	Pgm	Mdh	HK2	GOT1	All
1st collection	11	440	0.1185	0.0493	0.1941	0.1148	0.1158
2nd collection	10	400	0.0450	0.1273	0.3740	0.0547	0.1139
3rd collection	8	320	0.0983	0.0647	0.2888	0.2176	0.1215
4th collection	10	400	0.1135	0.0533	0.1655	0.2780	0.1508

* F_{ST} = fixation index that measures the reduction in heterozygosity of a subpopulation due random genetic drift; 1st collection = BARM, BELH, CARI, CONS, DCPD, DC25, PARS, POTI, NOVI, TRRI, and PORT; 2nd collection = BARM, BELH, CARI, CONS, DCPD, DC25, PARS, POTI, NOVI, and TRRI; 3rd collection = BELH, CONS, DCPD, DC25, PARS, POTI, NOVI, and TRRI; 4th collection = BELH, CARI, CONS, DCPD, DC25, PaRS, POTI, NOVI, TRRI, and PORT; *P* = probability of homogeneity; N_s = no. of samples; N_i = total no. of individuals analyzed. For definitions of other abbreviations, see Table 2.
$P \leq 10^{-6}$.

TABLE 4
F_{ST} values for differentiation of *Aedes aegypti* at rainy and dry seasons in 2003, Brazil*

Collection	All samples	Rainy season†	Dry season‡
All	–	0.1402§	0.1180§
BARM	0.2044§	–	–
BELH	0.1441§	0.1939§	0.1449§
CARI	0.1053§	0.1313§	–
CONS	0.0911§	0.0181	0.1791§
DC25	0.1096§	0.0082	0.1745§
DCPD	0.0463§	0.0547§	0.0458
NOVI	0.1032§	0.1137¶	0.1687§
PARS	0.1112§	–0.0094	0.1919§
PORT	0.0567§	0.0567¶	–
POTI	0.1319§	0.0423#	0.1210§
TRRI	0.0887§	0.1798§	0.0345¶

* – = not possible.
† Collections 1 and 2.
‡ Collections 3 and 4.
§ $P < 0.00001$.
¶ $P < 0.0001$.
# $P < 0.05$.

TABLE 5

Differentiation of *Aedes aegypti* according to geographic localization (inside and outside the state of Rio de Janeiro), Brazil*

Comparison	Collection†			
	1st	2nd	3rd	4th
All samples				
N	11	10	8	10
F_{ST}	0.1158	0.1139	0.1215	0.1508
Inside Rio de Janeiro state				
N	6	6	5	5
F_{ST}	0.1030	0.0657	0.1043	0.0506
Outside Rio de Janeiro state				
N	5	4	3	5
F_{ST}	0.1039	0.1705	0.1300	0.1559

* F_{ST} = fixation index that measures the reduction in heterozygosity of a subpopulation due random genetic drift; Inside Rio de Janeiro state: BARM, DC25, DCPD, NOVI, PARS, and TRRI; Outside Rio de Janeiro state = BELH, CONS, CARI, PORT, and POTI; P = probability of homogeneity; N = no. of samples.
† $P \leq 10^{-6}$.

water become more important and result in increases in the mosquito population densities.[34] When these sites dry out during the dry season, there is a reduction in available oviposition sites that serve to stimulate dispersal for new sites and therefore gene flow. This explanation is consistent with our observations of low F_{ST} values. Less genetic differentiation was observed in samples collected during the dry season in Rio de Janeiro,[28] as well in three localities assessed in the present study: BELH, DCPD, and TRRI (Table 4). The house infestation index, a measure of *Ae. aegypti* densities, in BELH was usually higher during the rainy season when numerous water-filled larval sites were available.[35]

Conversely, samples from five sampling sites (CONS, DC25, NOVI, PARS, and POTI) showed a distinct pattern, with lower F_{ST} values during rainy season (i.e., increase in genetic differentiation during the dry season). Distinct patterns of temporal genetic variation in *Ae. aegypti* have been reported elsewhere, for example in Vietnam,[36] where investigators suggested that limited dispersion of *Ae. aegypti* during the dry season would be due to the nature of the most common breeding sites, which were usually found indoors and thus, not influenced by rainfall. Unfortunately, data on seasonal variation in densities of *Ae. aegypti*, insecticide treatments implemented, and pupal productivity of breeding sites are not available for most of our sites. Only few ecologic data are recorded for some sampled localities. As far as we know, most dwellings have running water and thus, intentional water storage is usually unnecessary in CONS, DC25, NOVI, PARS, and POTI. In DC25 and POTI, the most common of larval containers (70–85%) were water tanks, followed by indoors containers. Consequently, the *Ae. aegypti* densities were not correlated with rainfall in these areas[37,38] (Secretaria Municipal de Saúde de Duque de Caxias, unpublished data).

Aedes aegypti density in Potim (in 2003) was higher in April (end of the rainy season) and lower in July and November–December (the dry and the beginning of the rainy season, respectively).[38] Considering that gravid *Ae. aegypti* females tend to lay eggs in different sites, dispersal was probably driven by the availability of water-filled outdoors temporary breeding sites (e.g., abandoned tires, cans, bottles).[37] This tends to reduce genetic differentiation. The flight range of *A. aegypti* in urban areas is oviposition-driven, making the dispersal and feeding frequency as functions of the availability of oviposition sites.[39] Nevertheless, it must be considered that genetic drift could also be responsible for or contributing to the observed temporal/seasonal variation in *Ae. aegypti* in southern and southeastern Brazil.

Ground transportation is considered a corridor for dissemination of both dengue viruses and their vector *Ae. aegypti.* The most densely populated and economically developed cities in Brazil are in the southeastern and southern regions, where commercial exchanges through a large network of roads and railways are very intense. From Rio de Janeiro, dissemination of *Ae. aegypti* populations that are highly dengue susceptible[2] and insecticide resistant[40,41] is a threat for dengue control.[42] However, high levels of genetic differentiation were observed when analyzing groups of samples obtained along the main routes linking metropolitan areas, which suggests low gene flow between mosquitoes from the sampled cities. Thus, mosquito genetic variation was independent of geographic distance separating cities and roads connecting cities. These results are in agreement with those obtained in Rio de Janeiro[28] and in Mexico.[30] Our results are consistent with the hypothesis that infected *Ae. aegypti* in this region of Brazil are unlikely to spread dengue virus over large distances whatever the time of year.

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Authors' addresses: Magda Clara Vieira da Costa-Ribeiro and Ricardo Lourenço-de-Oliveira, Laboratório de Transmissores de Hematozoários, Instituto Oswaldo Cruz-Fiocruz, Avenida Brasil, 4365, 21045-900-Rio de Janeiro, RJ, Brazil. Anna-Bella Failloux, Unité Génétique Moléculaire des Bunyaviridés, Institut Pasteur, 25–28 rue du Docteur Roux, 75724 Paris Cedex 15, France.

REFERENCES

1. Figueiredo LT, 1996. Dengue in Brazil I: history, epidemiology and research. *Virus Rev Res 1:* 9–16.
2. Lourenço-de-Oliveira R, Vazeille M, Filippis AM, Failloux AB, 2004. *Aedes aegypti* in Brazil: genetically differentiated populations with high susceptibility to dengue and yellow fever viruses. *Trans R Soc Trop Med Hyg 98:* 43–54.
3. Nogueira RM, Miagostovich MP, Schatzmayr HG, Flavia BS, Eliane SM, Ana Maria BF, Rogerio VS, Sonia Maris OZ, Cecilia N, Mary B, Gualberto TF, 1999. Dengue in the State of Rio de Janeiro, 1986–1998. *Mem Inst Oswaldo Cruz 94:* 297–304.
4. Lourenço-de-Oliveira R, Vazeille M, Filippis AM, Failloux AB, 2002. Oral susceptibility to yellow fever virus of *Aedes aegypti* from Brazil. *Mem Inst Oswaldo Cruz 97:* 437–439.
5. Schatzmayr HG, 2000. Dengue situation in Brazil by year 2000. *Mem Inst Oswaldo Cruz 95 (Suppl. 1):* 179–181.
6. Teixeira MG, Costa MC, Guerra Z, Barreto ML, 2002a. Dengue in Brazil: situation in 2001 and trends. *Dengue Bull 26:* 70–76.

7. McDonald PT, 1977. Population characteristics of domestic *Aedes aegypti* (Diptera: Culicidae) in villages on the Kenya coast. I. Adult survivorship and population size. *J Med Entomol 14:* 42–48.
8. Trpis M, Hausermann W, 1986. Dispersal and other population parameter of *Aedes aegypti* in an African village and their possible significance in epidemiology of vector-borne diseases. *Am J Trop Med Hyg 60:* 1263–1279.
9. Tabachnick WJ, 1991. Evolutionary genetics and arthropod-borne disease. The yellow fever mosquito. *Am Entomol 37:* 14–23.
10. Nogueira RMR, Miagostovich MP, Schatzmayr HG, 2002. Dengue viruses in Brazil. *Dengue Bull 26:* 77–83.
11. Boletim Epidemiológico, 2004. *Vigilância Ambiental em Saúde do Rio Grande do Sul. Brasil*. Available from http://www.saude.rs.gov.br/cevs/boletim_epidemiologico/2004/n_04/boletim%20epidemiologico.pdf
12. Dibo MR, Chiaravalloti-Neto F, Battigaglia M, Mondini A, Favaro EA, Barbosa AA, Glasser CM, 2005. Identification of the best ovitrap installation sites for gravid *Aedes (Stegomyia) aegypti* in residences in Mirassol, state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz 10:* 339–343.
13. Pasteur N, Pasteur G, Bonhomme F, Catalan J, Britton-Davidian J, 1988. *Practical Isozyme Genetics*. Chichester, United Kingdom: John Willey and Sons/Ellis Horwood Ltd.
14. Failloux AB, Vazeille M, Rodhain F, 2002. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti. J Mol Evol 55:* 653–663.
15. Huber K, Loan LL, Hoang TH, Tien TK, Rodhain F, Failloux AB, 2002. Temporal genetic variation in *Aedes aegypti* populations in Ho Chi Minh City (Vietnam). *Heredity 89:* 7–14.
16. Paupy C, Vazeille-Falcoz M, Mousson L, Rodhain F, Failloux AB, 2000. *Aedes aegypti* in Tahiti and Moorea (French Polynesia): isoenzyme differentiation in the mosquito population according to human population density. *Am J Trop Med Hyg 62:* 217–224.
17. Tran Khanh T, Vazeille-Falcoz M, Mousson L, Tran Huu H, Rodhain F, Nguyen Thi H, Failloux AB, 1999. *Aedes aegypti* in Ho Chi Minh City (Vietnam): susceptibility to dengue 2 virus and genetic differentiation. *Trans R Soc Trop Med Hyg 93:* 581–586.
18. Raymond M, Rousset F, 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered 86:* 248–249.
19. Rousset F, Raymond M, 1995. Testing heterozygote excess and deficiency. *Genetics 140:* 1413–1419.
20. Weir BS, Cockerham CC, 1984. Estimating F-statistics for the analysis of population structure. *Evol Int J Org Evol 38:* 1358–1370.
21. Holm S, 1979. A simple sequentially rejective multiple test procedure. *Scand J Stat 6:* 65–70.
22. Slatkin M, 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evol Int J Org Evol 47:* 264–279.
23. Ohta T, 1982. Linkage disequilibrium due to random genetic drift in finite subdivided population. *Proc Natl Acad Sci U S A 79:* 1940–1944.
24. Pudovkin AI, Zaykin DV, Hedgecock D, 1996. On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics 144:* 383–387.
25. Hamilton WD, 1980. Sex *versus* non-sex versus parasite. *Oikos 35:* 282–290.
26. Ayres CF, Melo-Santos AV, Prota JR, Solé-Cava AM, Regis L, Furtado AF, 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.
27. Ayres CF, Melo-Santos MA, Sole-Cava AM, Furtado AF, 2003. Genetic differentiation of *Aedes aegypti* (Diptera: Culicidae), the major dengue vector in Brazil. *J Med Entomol 40:* 430–435.
28. Costa-Ribeiro MC, Lourenço-de-Oliveira R, Failloux AB, 2006. Geographic and temporal genetic patterns of *Aedes aegypti* populations in Rio de Janeiro, Brazil. *Trop Med Int Health 11:* 1276–1285.
29. Costa-Ribeiro MCV, Lourenço-de-Oliveira R, Failloux AB, 2006. Higher genetic variation estimated by microsatellites compared to isoenzyme markers in *Aedes aegypti* from Rio de Janeiro. *Mem Inst Oswaldo Cruz 101:* 917–921.
30. Garcia-Franco F, Munoz MD, Lozano-Fuentes S, Fernandez-Salas I, Garcia-Rejon J, Beaty BJ, Black WC IV, 2002. Large genetic distances among *Aedes aegypti* populations along the south Pacific coast of Mexico. *Am J Trop Med Hyg 66:* 594–598.
31. Costa-da-Silva AL, Capurro ML, Bracco JE, 2005. Genetic lineages in the yellow fever mosquito *Aedes* (*Stegomyia*) *aegypti* from Peru. *Mem Inst Oswaldo Cruz 100:* 539–544.
32. Teixeira MG, Barreto ML, Costa MC, Ferreira LD, Vasconcelos PF, Cairncross S, 2002b. Dynamics of dengue virus circulation: silent epidemic in a complex urban area. *Trop Med Int Health 7:* 757–762.
33. Honório NA, Cabello PH, Codeço CT, Lourenço-de-Oliveira R, 2006. Preliminary data on the performance of *Aedes aegypti* and *Aedes albopictus* immatures developing in tires placed under natural conditions in Rio de Janeiro. *Mem Inst Oswaldo Cruz 101:* 225–228.
34. Paupy C, Ngan C, Vazeille M, Reynes JM, Rodhain F, Failloux AB, 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:* 171–182.
35. Côrrea PR, França E, Bogutchi TF, 2005. *Aedes aegypti* infestation and occurrence of dengue in the city of Belo Horizonte, Brazil. *Rev Saude Publica 39:* 33–40.
36. Huber K, Luu Le L, Tran Huu H, Tran Khanh T, Rodhain F, Failloux AB, 2002. Temporal genetic variation in *Aedes aegypti* populations in Ho chi Minh City (Vietnam). *Heredity 89:* 7–14.
37. Forattini OP, Brito M, 2003. Household water reservoirs and control of *Aedes aegypti. Rev Saude Publica 37:* 676–677.
38. Serpa LL, Costa KV, Voltolini JC, Kakitani I, 2006. Seasonal variation of *Aedes aegypti* and *Aedes albopictus* in a city of southeastern Brazil. *Rev Saude Publica 40:* 1101–1105.
39. Reiter P, Amador MA, Anderson RA, Clark GG, 1995. Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. *Am J Trop Med Hyg 52:* 177–179.
40. Braga IA, Lima JBP, Soares SS, Valle D, 2004. *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe and Alagoas, Brazil. *Mem Inst Oswaldo Cruz 99:* 199–203.
41. Pereira-da-Cunha M, Lima JBP, Brogdon WG, Moya GE, Valle D, 2005. Monitoring of resistance to the pyrethroid cypermethrin in Brazilian *Aedes aegypti* (Diptera: Culicidae) populations collected between 2001 and 2003. *Mem Inst Oswaldo Cruz 10:* 441–444.
42. Herrera F, Urdaneta L, Rivero J, Zoghbi N, Ruiz J, Carrasquel G, Martínez JA, Pernalete M, Villegas P, Montoya A, Rubio-Palis Y, Rojas E, 2006. Population genetic structure of the dengue mosquito *Aedes aegypti* in Venezuela. *Mem Inst Oswaldo Cruz 101:* 625–633.