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GENETIC STRUCTURE OF NATURAL POPULATIONS OF *AEDES AEGYPTI* AT THE MICRO- AND MACROGEOGRAPHIC LEVELS IN BRAZIL

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ABSTRACT. Genetic variation in 13 populations of *Aedes aegypti* from 3 regions of Brazil was compared using variation at 10 isozyme loci. Heterozygosities varied from 0.050 ± 0.027 to 0.280 ± 0.120, and a large genetic differentiation (F_{ST} = 0.144) was observed among all populations. The largest within-regions differences were found between populations from the urban areas of northeast Brazil (F_{ST} = 0.152). Ecological conditions are likely having an impact on the population structure of *Ae. aegypti* in the different regions of Brazil.

KEY WORDS *Aedes aegypti,* allozymes, genetic structure, control programs, Brazil

INTRODUCTION

The *Aedes aegypti* mosquito is the single dengue and urban yellow fever vector known in Brazil. Its presence in the country has been recorded since colonial times, when it was probably introduced through the slave trade (Consoli and Oliveira 1994). Due to its importance as a yellow fever vector, a first eradication campaign was conducted in 1940 and the species was considered eradicated in 1955 and 1973. Reinfestations occurred after each eradication period. The first dengue epidemics (caused by dengue virus [DENV] serotypes 1 and 4) occurred in 1982 in the Amazonian state of Roraima (Osanai et al. 1983). In 1986, the DENV serotype 1 was detected in the state of Rio de Janeiro, in southeast Brazil, and thereafter it became widely distributed in most Brazilian states (Nogueira et al. 1988).

Dengue virus type 2 was isolated in 1990, in the city of Niterói, Rio de Janeiro (Nogueira et al. 1990), and was followed by the first cases of dengue hemorrhagic fever (DHF). In spite of the creation in 1996 of the *Ae. aegypti* Eradication Program (PEAa; Brasil 1996) the number of municipalities infested by the vector has continued to increase. Pontes et al. (2000) observed that dengue epidemics occurred mainly in the periods when the control programs were less intensive, demonstrating that, without intervention strategies, the situation could get worse. In December of 2000, DENV serotype 3 was isolated in the state of Rio de Janeiro (Nogueira et al. 2001), extending the risk of sequential infections by different serotypes and consequently increasing the possibility of further cases of hemorrhagic dengue (Halstead 1997). In 2002, the country dealt with the worst dengue epidemics seen in Brazil, with more than 780,000 cases, including 2,607 cases of DHF, and in 2003, 248,487 cases were reported as of July, with a mortality rate of ca. 6.5% (FUNASA 2003).

Aedes aegypti is one of the better-studied mosquito species with regard to its population genetics. Extensive genetic homogeneity has been observed among samples of *Ae. aegypti,* in cities in Mexico that are 90–250 km apart (Gorrochotegui-Escalante et al. 2000), between island populations in French Polynesia (Failloux et al. 1995), and within the city of New Orleans (Tabachnick 1982). Populations in the Caribbean showed between-island variation in heterozygosity related to population size (Tabachnick and Wallis 1985). In east Africa, domestic populations of *Ae. aegypti* are panmictic, with little apparent substructure within a village. In contrast, significant differences in gene frequency are found between villages that are less than 2 km apart from each other (Tabachnick and Powell 1978). Levels of genetic structure between *Ae. aegypti* populations, therefore, can vary according to geographic area and colonization history. Consequently, they have to be estimated, independently, for each region.

Studies using random amplified polymorphic DNA (RAPD) patterns have indicated that Brazilian populations of *Ae. aegypti* could be highly divergent both at the micro- and macrogeographical levels (Ayres et al. 2003). The detection of genetically distinct vector populations within the country of Brazil could have important public health implications, as there could be an association with variations in the epidemiological characteristics demonstrated by the vector (Tabachnick and Black 1995). In this study, we use 10 allozyme loci to investigate the level of polymorphism and population genetic structure of mosquitoes from different macrogeographic regions of Brazil.

MATERIALS AND METHODS

Samples

Two hundred and forty mosquitoes from 13 populations from 9 Brazilian states, representing the 3

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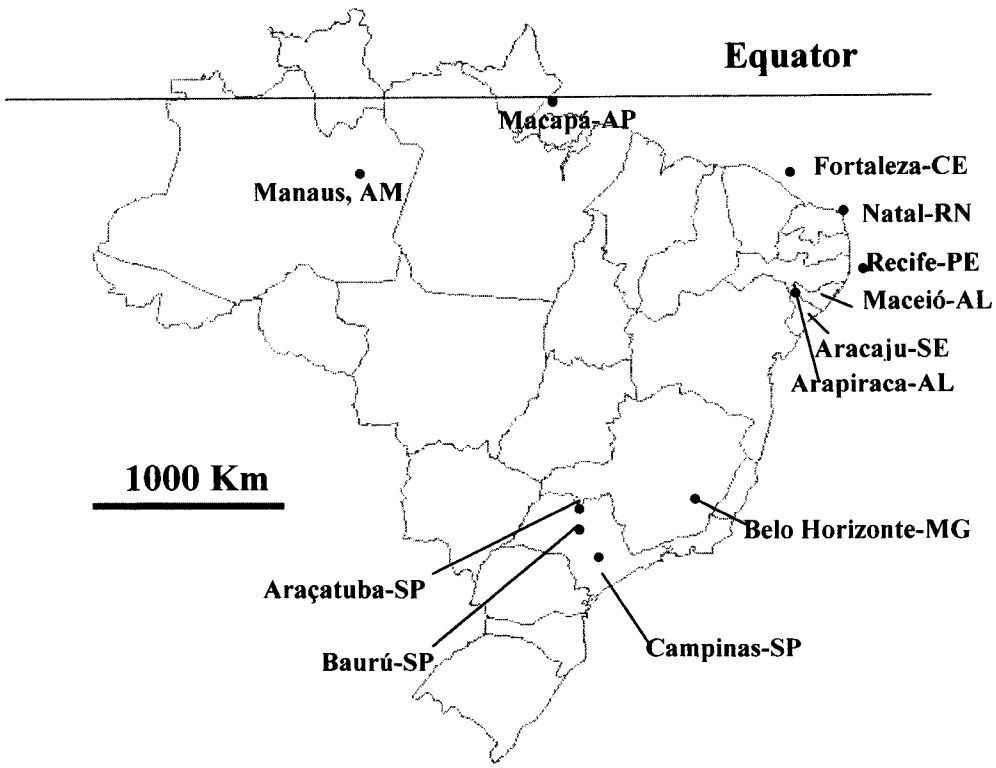

Fig. 1. Map of Brazil showing the collection sites of *Aedes aegypti.*

macroregions of 5 (northern, northeastern, and southeastern) were analyzed (Fig. 1; Table 1). These macroregions are characterized by different climatic, topographic, and socioeconomic conditions. According to the Brazilian Institute of Geography and Statistics (IBGE), in the northeast region, ca. 20% of the houses are not provided with a sewerage system, while this situation affects only 5% and less than 2% in the northern and southeast regions, respectively. The collection of garbage covers only 59.7% of the houses in northeast versus 81% in the northern region and more than 90% in the southeast. These deficiencies in minimal social facilities in the northeast region contribute to the increase of mosquito breeding sites. Samples were collected as eggs using oviposition traps or as larvae and pupae collected from natural breeding sites. The eggs, larvae, and pupae were maintained in an insectarium until adult emergence. Adults were subsequently frozen and stored at −20°C until analysis.

Allozyme electrophoresis

Individual adult mosquitoes were homogenized in 40 μl of distilled water, absorbed onto filter papers (2 × 7 mm), and submitted to 12.5% starch gel electrophoresis in a Tris-citrate buffer, pH 8.0 (0.25 M Tris, 0.06M citric acid), according to standard methodology (Harris and Hopkinson 1976). Twenty enzyme systems were investigated, of which 10 were selected for this work based on enzyme activity, reproducibility, and resolution: acid phosphatase (Acp, EC 3.1.3.2), glycerol-3-phosphate dehydrogenase (α-Gpd, EC 1.1.1.8), malic enzyme (Me, EC 1.1.1.40), malate dehydrogenase (Mdh, EC 1.1.1.37), alpha-esterases (Est, EC 3.1.1.1), phosphoglucose isomerase (Pgi, EC 5.3.1.9), phosphoglucomutase (Pgm, EC 2.7.5.1), phosphogluconate dehydrogenase (Pgd, EC 1.1.1.44), hexokinase (Hk, EC 2.7.1.1), and isocitrate dehydrogenase (Idh, EC 1.1.1.42). Allozyme loci and alleles were numbered as described in Tabachnick et al. (1979).

Data analysis

Allele frequencies, genetic variability parameters, and unbiased genetic distances (*D*; Nei 1978) between populations were estimated using the Biosys-1.7 (Swofford and Selander 1981) and the Tools for Population Genetic Analyses—TFPGA 1.3—programs (Miller 1997). Departures from Hardy–Weinberg equilibrium were tested for each locus and population using an exact test proposed by Haldane (1954). The genetic differentiation between populations was estimated for each locus or sample using the inbreeding index F_{ST} (Wright

Table 1. Sample size (N) and measure of genetic variability: observed mean heterozygosity (H_o) and expected (H_e), and percentage of polymorphic loci (P).

Region	State	Locality	N	$H_o \pm SE^1$	$H_e \pm SE^1$	P (%)
Northeast	Pernambuco (PE)	Várzea	20	0.136 ± 0.069	0.156 ± 0.080	30
		Casa Forte	20	0.131 ± 0.060	0.168 ± 0.075	40
	Alagoas (AL)	Maceió	20	0.087 ± 0.047	0.099 ± 0.054	30
		Arapiraca	15	0.073 ± 0.040	0.086 ± 0.038	40
	Ceará (CE)	Fortaleza	20	0.192 ± 0.070	0.208 ± 0.074	50
	Rio Grande do Norte (RN)	Natal	20	0.050 ± 0.027	0.074 ± 0.042	30
	Sergipe (SE)	Aracaju	20	0.145 ± 0.043	0.218 ± 0.064	60
Southeast	São Paulo (SP)	Baurú	20	0.230 ± 0.100	0.180 ± 0.076	40
		Araçatuba	20	0.117 ± 0.051	0.179 ± 0.076	50
		Campinas	15	0.129 ± 0.068	0.175 ± 0.079	40
	Minas Gerais (MG)	Belo Horizonte	20	0.159 ± 0.064	0.205 ± 0.069	50
North	Amazonas (AM)	Manaus	20	0.119 ± 0.048	0.174 ± 0.065	50
	Amapá (AP)	Macapá	10	0.280 ± 0.120	0.209 ± 0.073	50

[1] H, mean heterozygosity per locus.

1978, Weir and Cockerham 1984). Samples from neighborhoods and municipalities were named subpopulations and those from states were named populations. Possible associations between genetic differentiation (estimated as $F_{ST}/(1 - F_{ST})$) and log geographic distance were tested through a Mantel test (Sokal and Rohlf 1995). Significance values for F_{ST} were estimated nonparametrically through the use of 500 global permutations over all individuals, using the Genetix 4.02 program (Genetix 2001).

RESULTS

Genetic variability

Sixteen putative loci were initially identified from the analysis of 10 enzyme systems (Table 2). Est-2, Est-3, Me, Mdh-2, Idh-2, and Acp presented weak enzyme activity and were subsequently discarded from the analysis. Four out of the 10 loci thus selected for analysis were monomorphic in all collections (α-Gpd, Hk-1, 2, and 3) and the remainder were polymorphic in at least 1 of the populations, using the 95% criterion, where frequency of the most common allele does not exceed 95%. Average mean heterozygosities varied between 0.050 ± 0.027, in Natal (northeast Brazil), and 0.280 ± 0.120, in Macapá (the Amazonian region), and the percent polymorphic loci varied from 30 to 60% (Table 1). Two loci (Pgi at Aracaju and Est at Maceió) had genotype proportions significantly different, after sequential Bonferroni correction (Lessios 1992), from those expected for populations at Hardy–Weinberg equilibrium.

Differentiation and population structure

The overall level of genetic differentiation was high (F_{ST} = 0.088; Table 3) compared with other *Ae. aegypti* populations. Levels of genetic differentiation within each macrogeographic region were also very high: in the northeast, the F_{ST} was 0.152, in the southeast it was 0.139, and in the north it was 0.055. The indices of genetic distance between the populations varied between 0.003 and 0.150 (Table 4). No significant relationship was observed between population structuring and geographic distance (Mantel test; $P > 0.05$).

DISCUSSION

Our results show that the Brazilian populations of *Ae. aegypti* present high levels of genetic differentiation both locally (F_{ST} = 0.144) and nationwide (F_{ST} = 0.088). The highest population subdivision was observed in the northeast (F_{ST} = 0.152), where there is a combination of very high seasonal mosquito population densities, spatial heterogeneity, and intense, but fragmented, insecticide treatment. Over 50% of all cases of dengue notified in the year 2000 in Brazil occurred in the northeast. In that area, there are poor socioeconomic conditions and a precarious water supply. This has led the local human population to adopt the use of open receptacles to store water, which are ideal *Ae. aegypti* breeding habitats and have contributed to an increase of vector infestation and consequently an increase of dengue cases. Da Costa and Natal (1996) detected distinct ecological areas with respect to dengue distribution over the country. Incidences in these areas were closely related to factors considered by PAHO (1994) as dengue determinants.

Our data indicate that these ecological conditions likely affect the level of population structure of *Ae. aegypti*. Differences in the genetic structure of *Ae. aegypti* populations in relation to ecological conditions have also been observed in French Polynesia (Paupy et al. 2000), Thailand (Mousson et al. 2002), Vietnam (Huber et al. 2002), and northeast Brazil (Ayres et al. 2003). In all cases, the largest levels of genetic structure were detected in highly urbanized areas and the areas most frequently treated with chemical insecticides. Further evidence for an association between ecological heterogeneity and population structuring in *Ae. aegypti* comes

Table 2. Allelic frequencies observed at 10 loci for 13 *Aedes aegypti* samples from Brazil.

Region:	Northeast							Southeast				North	
State:	PE		AL		CE	RN	SE	SP			MG	AM	AP
Sites:	Várzea	C. Forte	Maceió	Arapiraca	Fortaleza	Natal	Aracaju	Baurú	Araçatuba	Campinas	B. Horizonte	Manaus	Macapá
α-Gpd													
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Est													
112	0.000	0.000	0.000	0.000	0.125	0.225	0.350	0.000	0.025	0.000	0.025	0.175	0.300
100	1.000	1.000	1.000	1.000	0.875	0.775	0.650	1.000	0.925	1.000	0.975	0.825	0.700
83	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
Hk-1													
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Hk-2													
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Hk-3													
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh													
113	0.421	0.667	0.737	0.100	0.447	0.000	0.325	0.425	0.444	0.429	0.605	0.316	0.500
100	0.579	0.333	0.263	0.900	0.553	1.000	0.675	0.575	0.556	0.571	0.395	0.684	0.500
Mdh													
125	0.333	0.550	0.350	0.200	0.275	0.050	0.425	0.300	0.450	0.333	0.650	0.450	0.150
100	0.667	0.450	0.650	0.800	0.725	0.950	0.575	0.700	0.550	0.667	0.350	0.550	0.850
Pgd													
105	0.000	0.031	0.025	0.067	0.375	0.000	0.175	0.000	0.000	0.100	0.050	0.000	0.000
100	1.000	0.969	0.975	0.933	0.625	1.000	0.825	1.000	1.000	0.900	0.950	1.000	1.000
Pgi													
105	0.000	0.050	0.050	0.000	0.025	0.000	0.125	0.200	0.000	0.000	0.675	0.175	0.250
100	1.000	0.950	0.950	1.000	0.975	1.000	0.875	0.800	0.950	1.000	0.325	0.825	0.750
86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
Pgm													
168	0.250	0.107	0.000	0.000	0.105	0.125	0.000	0.150	0.091	0.200	0.025	0.175	0.450
153	0.250	0.393	0.000	0.133	0.158	0.050	0.175	0.275	0.409	0.400	0.100	0.250	0.050
100	0.500	0.500	1.000	0.867	0.737	0.825	0.825	0.575	0.500	0.400	0.874	0.575	0.500

Table 3. F statistics *per locus* in samples of *Aedes aegypti.*[1]

Loci	F_{IS}	F_{ST} (P)	F_{ST} (S)	F_{IT}
Est	0.410	0.177	0.153	0.500
Idh	0.101	−0.073	0.142	0.228
Mdh	0.106	0.074	0.077	0.175
Pgd	0.052	0.179	0.185	0.227
Pgi	0.271	0.280	0.319	0.503
Pgm	0.221	0.118	0.116	0.312
95% CI	0.103–0.277	−0.004–0.208	0.098–0.227	0.211–0.428
Total	0.172	0.088	0.144	0.292

[1] P, populations; S, subpopulation.

from the observation of higher levels of population subdivision in larger islands than in smaller ones, both in French Polynesia (Failloux et al. 1995) and Caribbean populations (Tabachnick and Wallis 1985).

Levels of heterozygosity estimated for the Brazilian *Ae. aegypti* populations ($0.05 < H < 0.28$) were similar to those observed in populations of this species from other parts of the world. For example, mean heterozygosity (H) was 0.152 ± 0.016 in populations from Puerto Rico (Wallis et al. 1984). In various locations throughout the world that were evaluated using allozymes, it was 0.129 ± 0.045 (Tabachnick and Powell 1979) and it varied from 0.090 to 0.161 in populations from Argentina (De Souza et al. 2000). Recently, Fraga et al. (2003) reported heterozygosity values ranging from 0.109 ± 0.037 to 0.152 ± 0.052 and polymorphism levels varying from 44.4 to 55.6% in *Ae. aegypti* populations from Manaus, Amazonas, similar to those observed by us (H = 0.112; P = 50%) on the same area.

Heterozygosity values based on RAPD analyses of *Ae. aegypti* populations are usually twice as high as isozyme estimates, i.e., in Brazil (H = 0.39; Ayres et al. 2003), Mexico (H = 0.34; Gorrochotegui-Escalante et al. 2000), and Puerto Rico (H = 0.35; Apostol et al. 1996). This probably reflects the higher mutation rates at primer hybridization sites in RAPD systems.

Lerdthusnee and Chareonviriyaphap (1999) observed a reduction in the heterozygosity values in *Ae. aegypti* populations collected in Thailand, following treatment with *Bacillus thuringiensis* serovar *israelensis.* The H value was 0.147 ± 0.035 before treatment and fell to 0.069 ± 0.031 after treatment, rising gradually up to 0.254 ± 0.045 after 5 months. This demonstrates that heterozygosity can rapidly re-establish after population depletion. In Brazil, an organophosphate insecticide, Temephos, has been applied as larvicide every 3 or 2 months. According to Melo-Santos (personal communication), approximately 2 generations of *Ae. aegypti* are produced every month under the ecological conditions observed in the northeast of Brazil. As a consequence, during the interval between insecticide applications, up to 4 generations of mosquitoes could have taken place. The heterozygosity levels, however, could not recover through normal mutation/drift effects during this period. For populations undergoing a bottleneck of very few individuals, and depending on the duration of the bottleneck, at least 10^5 generations are necessary before the population can reach its original heterozygosity values (Nei et al. 1975). Therefore, for *Ae. aegypti,* this would require a period of ca. 4,000

Table 4. Pairwise values of genetic distances (below the diagonal) and F_{ST} (above the diagonal) between samples of *Aedes aegypti.* Significant F_{ST} values (500 permutations; $P > 0.05$) are underlined.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Várzea	****	0.005	0.174	0.125	0.000	0.232	0.090	0.000	0.000	0.000	0.053	0.282	0.082
2 Casa Forte	0.016	****	0.124	0.279	0.059	0.413	0.122	0.052	0.000	0.017	0.106	0.202	0.160
3 Maceió	0.033	0.029	****	0.306	0.168	0.428	0.151	0.137	0.158	0.195	0.126	0.245	0.227
4 Arapiraca	0.026	0.064	0.051	****	0.104	0.071	0.101	0.116	0.153	0.147	0.109	0.379	0.239
5 Fortaleza	0.025	0.039	0.032	0.027	****	0.164	0.020	0.010	0.004	0.026	0.074	0.170	0.056
6 Natal	0.044	0.102	0.081	0.011	0.046	****	0.142	0.207	0.272	0.251	0.193	0.467	0.234
7 Aracaju	0.033	0.046	0.041	0.027	0.019	0.035	****	0.079	0.077	0.095	0.034	0.194	0.090
8 Baurú	0.006	0.019	0.030	0.025	0.026	0.044	0.029	****	0.008	0.012	0.074	0.182	0.067
9 Araçatuba	0.005	0.008	0.036	0.034	0.030	0.058	0.029	0.009	****	0.000	0.078	0.238	0.124
10 Campinas	0.003	0.015	0.044	0.033	0.023	0.055	0.037	0.009	0.005	****	0.053	0.276	0.097
11 B. Horizonte	0.087	0.065	0.060	0.110	0.093	0.150	0.071	0.056	0.078	0.097	****	0.250	0.103
12 Manaus	0.010	0.024	0.043	0.026	0.030	0.038	0.015	0.008	0.009	0.015	0.059	****	0.237
13 Macapá	0.028	0.055	0.053	0.057	0.043	0.049	0.041	0.024	0.043	0.037	0.088	0.026	****

years to return to the original heterozygosity value due solely to mutation (Tabachnick and Powell 1979). However, a large number of *Ae. aegypti* eggs remain in the environment because the insecticide-based treatments target only the larvae and the adults. Those eggs may permit enough genes to pass on from one generation to the other, precluding the effects of insecticide-based population bottlenecks, therefore maintaining high levels of heterozygosity. In addition, sites that did not receive treatment, which represent cryptic breeding sites, might favor the recolonization and recomposition of the genetic variability of the population. The high F_{ST} values indicate that levels of gene flow between areas are small, so this recolonization, if present, must be forcibly taking place from neighboring sites within each area.

Dinardo-Miranda and Contel (1996) analyzed samples of *Ae. aegypti* collected from the state of São Paulo in 1994, before the implementation of the PEAa, and recorded H values varying between 0.480 and 0.530, approximately twice as high as the maximum value we observed in samples from the same state (Campinas, Baurú, and Araçatuba). However, those authors did not analyze the populations after the implementation of the PEAa and the analyzed populations did not belong to the same municipalities. It is probable that control measures are effective in reducing vector population size, but, unfortunately, only for a short period. This means that those control measures have to be taken continuously, preferably with a rotation of other control agents, to minimize selection for resistance, and that new control strategies are needed, taking into account important biological aspects of the vector.

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