

## Low Gene Flow of *Aedes aegypti* between Dengue-Endemic and Dengue-Free Areas in Southeastern and Southern Brazil

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**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.<sup>1,2</sup> 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<sup>2–4</sup> and approximately 80% of all dengue cases registered in the Americas are reported in Brazil,<sup>5</sup> most of them acquired in the southeastern and northeastern regions.<sup>6</sup> 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.<sup>2,5</sup> 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),<sup>7,8</sup> passive migration through human transportation have been described and could explain mosquito dispersal and gene flow over long distances.<sup>9</sup> 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.<sup>10</sup> 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,<sup>11</sup>

A high degree of genetic differentiation of *Ae. aegypti* has been demonstrated in Rio de Janeiro.<sup>2</sup> 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 Espírito Santo (Consoação 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<sup>12</sup> per locality during two consecutive weeks to avoid collections of descendants 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 ( $25 \pm 1^\circ\text{C}$ , relative humidity of  $80 \pm 10\%$ , and a 12-hour light/dark cycle) and subsequently stored at  $-80^\circ\text{C}$  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  $\mu\text{L}$  of distilled water and centrifuged ( $12,000 \times g$  for 3 minutes at  $4^\circ\text{C}$ ). 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.<sup>13</sup> 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<sup>14</sup> and

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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 <sup>2</sup> | Total no. of dengue cases |       |
|---------------------|---------------------------------------|-------------------------------|---------------------------|-------|
|                     |                                       |                               | 2002                      | 2003  |
| Southeastern region |                                       |                               |                           |       |
| BARM                | Barra Mansa, Rio de Janeiro state     | 311.9                         | 3,198                     | 262   |
| BELH                | Belo Horizonte, 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                | Paraíba 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.<sup>15</sup> 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.<sup>16,17</sup>

**Genetic analysis.** Hardy-Weinberg proportions were compared using the GENEPOP software (version 3.4).<sup>18</sup> Deviations were based on an alternative hypothesis ( $H_1$  = deficits or excess) using an exact test procedure.<sup>19</sup> Linkage disequi-

librium was tested between pairs of loci for each sample using Fisher's exact test on rank  $\times$  column contingency tables.  $F_{IS}$ , the inbreeding coefficient, and  $F_{ST}$ , the fixation index, were estimated as described by Weir and Cockerham.<sup>20</sup> Genetic differentiation between populations or groups of populations was tested using Fisher's exact test on  $R \times C$  contingency tables for each locus. An unbiased estimate of the exact prob-

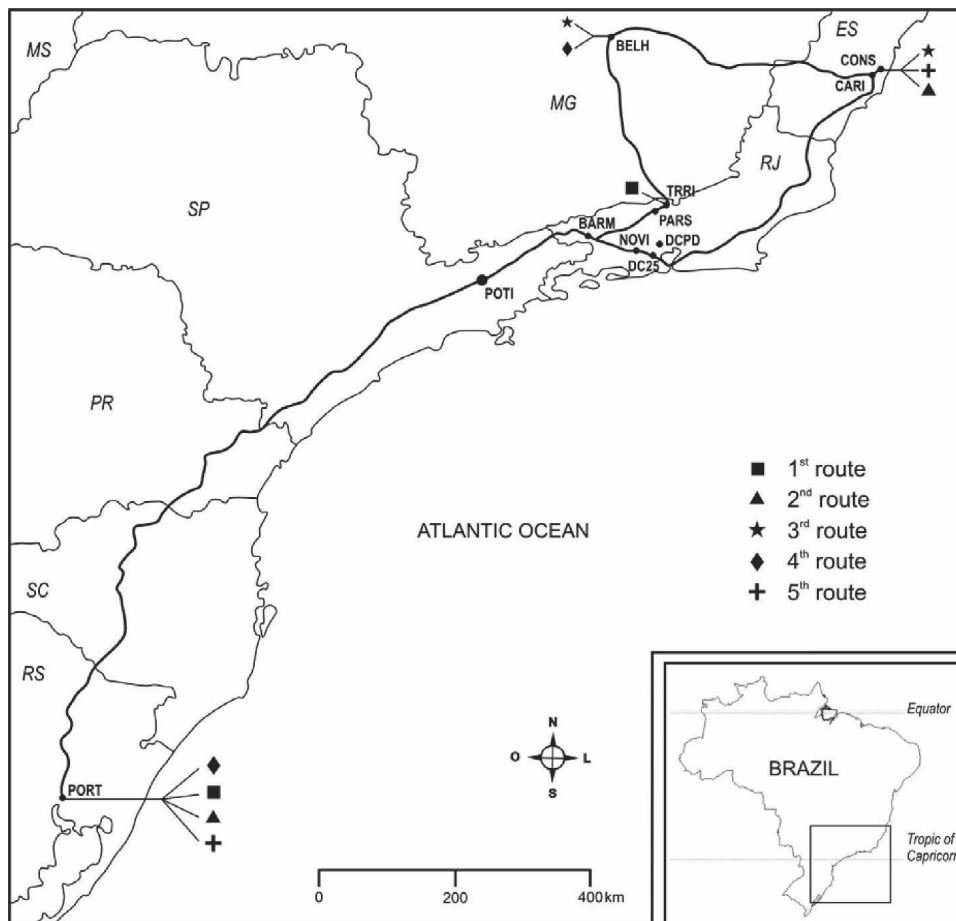


FIGURE 1. *Aedes aegypti* samples collected in southern and southeastern Brazil in 2003. District abbreviations are shown in Table 1. Routes correspond to roads or railways connecting cities. MS = Mato Grosso do Sul; MG = Minas Gerais; ES = Espírito Santo; RJ = Rio de Janeiro; SP = São Paulo; PR = Parana;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

ability was obtained with a Markov chain method.<sup>18</sup> Significance levels for multiple testing were corrected using sequential Bonferroni's procedures.<sup>21</sup> 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.<sup>22</sup>

## 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<sup>23</sup> 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.<sup>24</sup> 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).<sup>25</sup> 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<sup>2,26–29</sup> and other countries,<sup>30,31</sup> 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   |       |          |                 |     |
|---------|-------|-------|-------|-------|-------|----------|--------|-----|-------|-------|----------|-----------------|-----|
|         | 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   | <b>0.0024</b> † | 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 |          |                  |
|---------|-------|-------|-------|----------|------------------|-----|-------|-------|----------|--------|----------|----------|------------------|
|         | 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   | <b>0.00001</b> † | 47  | 0.188 | 0.812 | -0.2208  | 0.1812 | 48       | 0.3357   | <b>0.00001</b> † |
| 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  | <b>0.0043</b> ‡  | 45  | 0.115 | 0.885 | 0.0864   | 0.4753 | 48       | -0.0577  | <b>0.0151</b> ‡  |
| DCPD    | 0.245 | 0.745 | 0.011 | -0.3151  | 0.0552           | 47  | 0.156 | 0.844 | -0.0166  | 1      | 48       | -0.1834  | <b>0.0097</b> ‡  |
| 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  | <b>0.0003</b> ‡  | 46  | 0.083 | 0.917 | -0.0805  | 1      | 48       | -0.1877  | <b>0.0117</b> ‡  |
| TRRI    | 0.000 | 1.000 | 0.000 | -        | -                | 48  | 0.219 | 0.781 | 0.2177   | 0.1952 | 48       | 0.0046   | 0.6017           |

| Samples | Pgm   |       |       |       |          |                 |     |       | Mdh   |          |                  |     |  |
|---------|-------|-------|-------|-------|----------|-----------------|-----|-------|-------|----------|------------------|-----|--|
|         | 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   | <b>0.0148</b> † | 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   | <b>0.0017</b> † | 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  | <b>0.0013</b> ‡  | 47  |  |
| TRRI    | 0.906 | 0.000 | 0.094 | 0.000 | 0.1521   | 0.3377          | 48  | 0.531 | 0.469 | -0.7098  | <b>0.00001</b> ‡ | 48  |  |

| Samples | Hk2   |       |       |          |                 |     |       | Got1  |          |        | All loci |          |                  |
|---------|-------|-------|-------|----------|-----------------|-----|-------|-------|----------|--------|----------|----------|------------------|
|         | 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  | <b>0.0099</b> ‡  |
| 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  | <b>0.0001</b> ‡ | 47  | 0.188 | 0.812 | -0.2208  | 0.1812 | 48       | -0.1408  | <b>0.0005</b> ‡  |
| DC25    | 0.000 | 1.000 | 0.000 | -        | -               | 48  | 0.209 | 0.791 | -0.2537  | 0.1657 | 43       | -0.0839  | <b>0.0023</b> ‡  |
| 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  | <b>0.0012</b> ‡  |
| TRRI    | 0.000 | 1.000 | 0.000 | -        | -               | 48  | 0.281 | 0.719 | -0.0717  | 0.7299 | 48       | -0.3313  | <b>0.00001</b> ‡ |

| Samples | Pgm   |       |       |       |          |        |     |       | Mdh   |       |          |                 |     |
|---------|-------|-------|-------|-------|----------|--------|-----|-------|-------|-------|----------|-----------------|-----|
|         | 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   | <b>0.0168</b> † | 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 |                  |
|-----------|-------|-------|-------|----------|-----------------|----|-------|-------|----------|-----------------|-----------------|----------|------------------|
|           | 80    | 100   | 110   | $F_{IS}$ | $P$             | N  | 60    | 100   | $F_{IS}$ | $P$             | N               | $F_{IS}$ | $P$              |
| September |       |       |       |          |                 |    |       |       |          |                 |                 |          |                  |
| 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           |
| December  |       |       |       |          |                 |    |       |       |          |                 |                 |          |                  |
| Samples   | Pgm   |       |       |          |                 |    | Mdh   |       |          |                 |                 |          |                  |
|           | 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   | <b>0.0021</b> † | 46 | 0.000 | 0.468 | 0.532    | 0.2411          | 0.1421          | 47       |                  |
| CONS      | 0.967 | 0.000 | 0.033 | 0.6617   | <b>0.0330</b> † | 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   | <b>0.0283</b> † | 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          | <b>0.0000</b> † | 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          | <b>0.0394</b> † | 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   | <b>0.0030</b> † | 42 | 0.000 | 0.793 | 0.207    | 0.4900          | <b>0.0052</b> † | 41       |                  |
| December  |       |       |       |          |                 |    |       |       |          |                 |                 |          |                  |
| Samples   | Hk2   |       |       |          |                 |    | Got1  |       |          |                 |                 | All loci |                  |
|           | 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   | <b>0.0101</b> †  |
| 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   | <b>0.0002</b> † | 39 | 0.250 | 0.750 | 0.0255   | 1               | 46              | 0.2954   | <b>0.0007</b> †  |
| NOVI      | 0.000 | 1.000 | 0.000 | –        | –               | 48 | 0.104 | 0.896 | 0.7809   | <b>0.0001</b> † | 48              | 0.4780   | <b>0.00001</b> † |
| 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   | <b>0.0223</b> † | 48 | 0.085 | 0.915 | 0.1906   | 0.2768          | 47              | 0.2698   | <b>0.0330</b> †  |
| TRRI      | 0.000 | 1.000 | 0.000 | –        | –               | 42 | 0.110 | 0.890 | 0.6330   | <b>0.0025</b> † | 41              | 0.5468   | <b>0.00001</b> † |

\*  $F_{IS}$  = inbreeding coefficient; N = sample size;  $P$  = probability for rejecting Hardy-Weinberg equilibrium; significant  $P$  values (< 0.05) are in **bold**; Pgm = phosphoglucotomutase; 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,<sup>32</sup> with a peak occurring usually between January and March.<sup>3,33</sup>

TABLE 3

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

| Comparison     | $N_s$ | $N_i$ | $F_{ST}$ † |        |        |        |        |
|----------------|-------|-------|------------|--------|--------|--------|--------|
|                |       |       | 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.  
 $P \leq 10^{-6}$ .

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 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.<sup>34</sup> 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,<sup>28</sup> 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.<sup>35</sup>

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,<sup>36</sup> 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<sup>37,38</sup> (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).<sup>38</sup> 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).<sup>37</sup> This tends to reduce genetic differentiation. The flight range of *A. aegypti* in urban areas is oviposition-driven, making the dis-

persal and feeding frequency as functions of the availability of oviposition sites.<sup>39</sup> 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<sup>2</sup> and insecticide resistant<sup>40,41</sup> is a threat for dengue control.<sup>42</sup> 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<sup>28</sup> and in Mexico.<sup>30</sup> 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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