Enzymatic variability in *Aedes aegypti* (Diptera: Culicidae) populations from Manaus-AM, Brazil

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Abstract

Eighteen enzymatic loci were analysed in *Aedes aegypti* populations from four neighbourhoods in the city of Manaus. The analyses showed that the Downtown population was the most polymorphic (p = 55.6%) with higher observed and expected mean heterozygosities (Hₒ = 0.152 ± 0.052; Hₑ = 0.174 ± 0.052, respectively). The least variability was detected in the Coroado and Cidade Nova populations, both with polymorphism of 44.4%. The latter population presented the least observed heterozygosity (Hₒ = 0.109 ± 0.037). Wright’s F statistics showed that the mean value of F is was higher than that of F st (Fᵢₛ > Fₛ = 0.048), and from analysis of molecular variance (AMOVA) it was found that 95.12% of the variability is found within populations indicating a certain intra-population differentiation possibly of the microgeographic structure resulting from some barrier in the random coupling. Although the four populations were similar genetically (D = 0.003 to 0.016), the 4.88% differentiation was significant.

Key words: *Aedes aegypti*, electrophoreses, isozymes, populations genetics.

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Introduction

*Aedes (Stegomyia) aegypti* is a widely geographically distributed species as well as of great epidemiological importance on account of being the major vector involved in the transmission of the yellow fever virus and four serotypes of dengue and its hemorrhagic fever viruses throughout most of the world’s tropical and subtropical areas (Chow et al., 1998).

The reintroduction of this vector insect to Brazil in 1967, which is presently infesting up to 3,592 municipalities nationwide (Honório and Lourenço-de-Oliveira, 2001), has consolidated dengue infection as a major public health problem. Nearly 1.8 million cases have been reported in the past few years. These cases account for about 80% of the Americas total (Schatzmayr, 2000). High population sizes of this mosquito have been recorded in the city of Manaus, highlighting the magnitude of this problem within the region.

Several studies have addressed the population genetics of this species (Tabachnick, 1982; Wallis et al., 1984; Harrington et al., 1984; Dinardo-Miranda and Contel, 1996; Failloux et al., 1995; de Sousa et al., 2000, 2001; Ravel et al., 2002). These studies dealt with the population genetical structure as an essential requirement to the understanding of population dynamics as well as factors that may interact with them, such as vectorial ability, insecticide resistance and ecological adaptation.

In the present study, four populations of *A. aegypti* were analysed using enzymatic variation of 18 loci to characterize the genetic structure of this species in the sampled regions.

Material and Methods

The mosquitoes were collected in Compensa, Cidade Nova, Coroado and Downtown, neighbourhoods of the city of Manaus. Larvae and pupae collected in artificial breeding sites outdoors were kept in an insectarium until the emergence of the adults. The adults were then transferred to a cage, where the males were fed on a 10% sucrose solution and the females were fed in a hamster (*Mesocricetus auratus*). After the couplings, females were isolated for individual oviposition and later identified by means of the Consoli and Lourenço-de-Oliveira (1994) key. Hatched larvae were kept until the electrophoretic analyses specific stages, according to Santos et al. (1981), and frozen in at -70 °C.

Eighteen enzymatic loci were analysed (*EST3, EST4, EST5, EST6, LAP1, LAP2, LAP4, LAP5, LAP6, PGI, HEX1, HEX2, MDH, IDH, ME, 6-PGD, PGM and α-GPDH*). Fourth instar larvae were used for most of the enzymes with the exception of *α-GPDH*, for which adults
were used. Three individuals from each progeny were used. Electrophoretic techniques and enzyme recipes were those described in Steiner and Joslyn (1979). The gels were prepared as described by Santos et al. (1996).

Allelic frequencies were estimated directly from the data. Polymorphic loci ratio (P), found (H₀) and expected (Hₑ) heterozygosities and Wright’s coefficients were estimated in each population by using BIOSYS (Swofford and Selander, 1981) program. The dendrogram was constructed employing the UPGMA method (Nei, 1978). Values of Fst (Weir and Cockerham, 1984), and hierarchical analysis of molecular variance (AMOVA) (Michalakis and Excoffier, 1996) were calculated using the ARLEQUIN program, version 2000 (Schneider et al. 2000), in which significance levels for the overall values were determined after 1023 permutations.

Results

Only 7 out of the 18 loci analysed presented polymorphism in the four populations: EST4, EST5, LAP2, LAP5, IDH, MDH and PGM. The EST3 and LAP6 loci were polymorphic in the Compensa and Downtown populations. The EST3 locus was also polymorphic in the Coroado population. The PGI locus was polymorphic in the Cidade Nova and Downtown populations. The Compensa and Coroado populations were monomorphic (Table 1). Chi-square values for most Compensa population polymorphic loci were

Table 1 - Allele frequency at each polymorphic locus and chi-square value for the determination of Hardy-Weinberg equilibrium in Aedes aegypti populations from Manaus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Compensa</th>
<th>Cidade Nova</th>
<th>Downtown</th>
<th>Coroado</th>
</tr>
</thead>
<tbody>
<tr>
<td>EST3</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.994</td>
<td>1.000</td>
<td>0.917</td>
<td>0.922</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>0.006</td>
<td>0.000</td>
<td>0.022</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>93</td>
<td>0.000</td>
<td>0.000</td>
<td>0.061</td>
<td>0.033</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>0.000</td>
<td>(df = 1)</td>
<td>13.786 (df = 3)*</td>
<td>0.591 (df = 3)**</td>
<td></td>
</tr>
<tr>
<td>EST4</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.989</td>
<td>0.989</td>
<td>0.933</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>0.011</td>
<td>0.011</td>
<td>0.067</td>
<td>0.044</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>0.006</td>
<td>(df = 1)</td>
<td>179.006 (df = 1)*</td>
<td>97.672 (df = 1)*</td>
<td>102.309 (df = 1)*</td>
</tr>
<tr>
<td>EST5</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.900</td>
<td>0.494</td>
<td>0.406</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>0.072</td>
<td>0.439</td>
<td>0.472</td>
<td>0.372</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>0.028</td>
<td>0.067</td>
<td>0.122</td>
<td>0.167</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>33.649</td>
<td>(df = 3)*</td>
<td>73.682 (df = 3)*</td>
<td>22.067 (df = 3)*</td>
<td>38.613 (df = 3)*</td>
</tr>
<tr>
<td>LAP2</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.567</td>
<td>0.550</td>
<td>0.628</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>0.433</td>
<td>0.450</td>
<td>0.372</td>
<td>0.550</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>0.582</td>
<td>(df = 1)</td>
<td>17.780 (df = 1)*</td>
<td>23.109 (df = 1)*</td>
<td>8.637 (df = 1)*</td>
</tr>
<tr>
<td>LAP5</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.767</td>
<td>0.811</td>
<td>0.744</td>
<td>0.767</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>0.233</td>
<td>0.189</td>
<td>0.256</td>
<td>0.233</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>1.680</td>
<td>(df = 1)</td>
<td>0.360 (df = 1)**</td>
<td>0.189 (df = 1)**</td>
<td>5.086 (df = 1)**</td>
</tr>
<tr>
<td>LAP6</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.989</td>
<td>1.000</td>
<td>0.989</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>0.011</td>
<td>0.000</td>
<td>0.011</td>
<td>0.000</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>179.006</td>
<td>(df = 1)*</td>
<td>179.006 (df = 1)*</td>
<td>179.006 (df = 1)*</td>
<td>179.006 (df = 1)*</td>
</tr>
<tr>
<td>PGI</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>0.000</td>
<td>0.206</td>
<td>0.033</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.000</td>
<td>0.794</td>
<td>0.967</td>
<td>1.000</td>
</tr>
<tr>
<td>χ²H-W</td>
<td>53.653</td>
<td>(df = 1)*</td>
<td>10.603 (df = 1)*</td>
<td>10.603 (df = 1)*</td>
<td>10.603 (df = 1)*</td>
</tr>
</tbody>
</table>
not significant, indicating equilibrium according to the Hardy-Weinberg equilibrium. Exceptions were found in the loci EST5, LAP6, MDH and PGM which presented significant deviations. In the Cidade Nova population, four out of the eight polymorphic loci were not in equilibrium (EST5, LAP6, MDH and PGI). However, a higher number of polymorphic loci that showed significant deviations were found in the Downtown (EST3, EST4, EST5, LAP2, LAP6, PGI and PGM), and in the Coroado (EST5, EST4, LAP2, LAP5 and IDH) populations as well. According to the four populations genotype frequency analyses, only 45% of the polymorphic loci were in Hardy-Weinberg equilibrium.

The genetic variability estimates in the four populations are shown in Table 2. The Downtown population was the most polymorphic (P = 55.6%), with the largest number of alleles per locus (1.7) and highest level of heterozygosity (H_o = 0.152). The least variability was found in the Coroado and Cidade Nova (P = 44.4%) populations with the smallest number of alleles per locus (1.6), and the latter presented the least observed heterozygosity (H_o = 0.109).

Genetic structure of the populations analysed through Wright’s F statistics showed higher Fis mean value relative to Fst (0.164 > 0.048). Higher Fis values as compared with Fst were found in loci LAP6, EST4 and PGI with 1.000, 0.912 and 0.687 respectively, suggesting a certain intrapopulational differentiation (Table 3). Population structure was also tested at different hierarchical levels using Fst by AMOVA analysis (Table 4). Most of the variation was found within populations (95%), indicating large differentiation within population differentiation. There was little variation among populations (5%).

However, the genetic distance values shown in Table 5 (D = 0.003 - 0.016) indicate that these populations are very similar genetically, grouping the Cidade Nova, Downtown and Coroado populations in one single “cluster”, while the Compensa population was separated in another “cluster” (Figure 1).

### Table 1 (cont.)

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Compensa</th>
<th>Cidade Nova</th>
<th>Downtown</th>
<th>Coroado</th>
<th>χ²/H-W</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH (n)</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>6.158 (df = 1)**</td>
<td></td>
</tr>
<tr>
<td>MDH (n)</td>
<td>110</td>
<td>0.311</td>
<td>0.183</td>
<td>0.200</td>
<td>0.128</td>
<td>1.913 (df = 1)**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.889</td>
<td>0.817</td>
<td>0.800</td>
<td>0.872</td>
<td></td>
</tr>
<tr>
<td>PGM (n)</td>
<td>110</td>
<td>0.350</td>
<td>0.217</td>
<td>0.300</td>
<td>0.422</td>
<td>0.000 (df = 1)**</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.650</td>
<td>0.783</td>
<td>0.700</td>
<td>0.578</td>
<td></td>
</tr>
<tr>
<td>χ²/H-W</td>
<td>5.573 (df = 1)**</td>
<td>0.008 (df = 1)**</td>
<td>0.159 (df = 1)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>0.183</td>
<td>0.167</td>
<td>0.211</td>
<td>0.183</td>
<td></td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>0.050</td>
<td>0.139</td>
<td>0.183</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.767</td>
<td>0.694</td>
<td>0.606</td>
<td>0.650</td>
<td></td>
</tr>
<tr>
<td>χ²/H-W</td>
<td>8.556 (df = 3)**</td>
<td>4.845 (df = 3)**</td>
<td>5.893 (df = 3)**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n - sample size.  
χ² H-W - chi-square/Hardy-Weinberg equilibrium.  
df - degrees of freedom.  
*p < 0.01; ** p < 0.05; ns - not significant.

### Table 2 - Estimate of measures of genetic variability in A. aegypti populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean sample size/locus</th>
<th>Mean n° of alleles/locus</th>
<th>% Polymorphic loci*</th>
<th>Mean heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compensa</td>
<td>90.0 ± 0.0</td>
<td>1.6 ± 0.2</td>
<td>50.0</td>
<td>0.117 ± 0.043</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.131 ± 0.046</td>
</tr>
<tr>
<td>Cidade Nova</td>
<td>90.0 ± 0.0</td>
<td>1.6 ± 0.2</td>
<td>44.4</td>
<td>0.109 ± 0.037</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.157 ± 0.050</td>
</tr>
<tr>
<td>Downtown</td>
<td>90.0 ± 0.0</td>
<td>1.7 ± 0.2</td>
<td>55.6</td>
<td>0.152 ± 0.052</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.174 ± 0.052</td>
</tr>
<tr>
<td>Coroado</td>
<td>90.0 ± 0.0</td>
<td>1.6 ± 0.2</td>
<td>44.4</td>
<td>0.143 ± 0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.164 ± 0.053</td>
</tr>
</tbody>
</table>

* A locus was considered polymorphic if more than one allele was detected.  
** Expected heterozygosity of Hardy-Weinberg, Nei’s unbiased estimate (Nei, 1978).
The analysis of the polymorphism found in the four populations, through the allelic frequencies, showed that most loci in Hardy-Weinberg equilibrium were in the Compensa population. In the four population group, it was verified that only 45% of loci were in equilibrium. Similar results were found in the Guariba population (SP) for this vector by Dinardo-Miranda and Contel (1996). Considering the three populations from their study, it was found that only (35%) of the loci were in equilibrium. However, divergent findings from other countries’ A. aegypti populations have been reported, such as observed by Tabachnick and Powell (1978), where most analysed loci did not show significant deviations for the Hardy-Weinberg equilibrium. Likewise, Harrington et al. (1984) found similar results when analysing 20 populations of this vector. Significant deviations from the expected values of the Hardy-Weinberg equilibrium were found in some loci. These deviations are due to the occurrence of a single homozygote individual for one rare allele, such as the alleles LAP6*98 in the Compensa, EST4*98 in the Cidade Nova, LAP6*98 and PGI*105 in the Downtown populations. Similar findings were reported by Scarpassa et al. (1999) in Anopheles nuneztovari populations from Tucuruí (PA) for three nonspecific esterase loci.

One of the most important implications of Hardy-Weinberg equilibrium is that when an allele is rare, most of the individuals should be heterozygous (Hartl, 1981). For the loci with deviation from genetic equilibrium due to the heterozygotes deficiency, Crouau-Roy (1988) mentioned that usually these deficiencies are found in some enzymatic loci and/or in some populations presenting other loci whose genotype proportions are in equilibrium.

The Downtown population presented the highest polymorphism indexes. These findings are similar to those detected in populations of São Paulo State by Dinardo-Miranda and Contel (1996), who found a polymorphic loci rate ranging from 37.5% in the Guariba to 50% in the Ribeirão Preto populations. In populations of this species collected in Kenya, Tabachnick and Powell (1976) found variation in 59% of the loci. Harrington et al. (1984), found a polymorphic loci rate ranging from 30% to 40% in A. aegypti populations from Houston. Recently, de Sousa et al. (2000) detected polymorphic loci rates ranging from 27.3% to 63.6% in Argentinean populations.

Higher mean heterozygosity values detected in the Downtown population indicated that this population was
the most variable among the four studied. These values re-
semble those found by de Sousa et al. (2000) who detected
expected mean heterozygosities ranging from 0.090 to
0.161 for 11 allozymic loci in A. aegypti samples from Ar-
gentina. This also resembles the analyses reported by
Tabachnick et al. (1979), where the expected mean heterozygosity was of 0.141 for domestic, and of 0.163 for
East African wild populations. Lower expected mean heterozygosities for this species (H_e = 0.118 ± 0.009)
were detected by Tabachnick (1982) in the Caribbean, and
by Harrington et al. (1984) (H_e = 0.097 ± 0.055) in Houston
populations. Nevertheless, Dinardo-Miranda and Contel
(1996) obtained expected mean heterozygosity value diver-
gent findings, ranging from 0.48 to 0.53, in this mosquito’s
São Paulo populations.

Higher heterozygosity estimates have been detected
in this species with the use of other molecular markers. Apostol et al. (1996) detected mean heterozygosity values
equal to 0.354, twice the level found with the use of isozymes in Puerto Rico populations, using RAPD-PCR.
Yan et al. (1999) found mean heterozygosity values of 0.39
in this vector’s populations with the use of AFLP, and this
value was similar in all populations, whereas the values were from 0.44 to 0.58 with the use of RFLP. Similar esti-
mates (H_e = 0.350) by using RAPD-PCR in this species were reported by de Sousa et al. (2001).

On the basis of the data presented, it may be inferred
that the heterozygosity values found in this study by using
isoenzymes would have a very close correspondence to
those found with other markers, and that the A. aegypti pop-
ulations here analysed would not be less polymorphic than
the others analysed around the world. Therefore, hetero-
yzosity indexes found in the Manaus A. aegypti popula-
tions, point out that there is no “founder effect” occurrence.
Since according to Nei et al. (1975), for a new population
started by 2 to 10 founder individuals, heterozygosity un-
dergoes an initial decrease whose recovery will come about
slowly, and only following nearly 10^7 generations will be
established back to the initial population levels.

Therefore, these data suggest that the in loci with dis-
tribution of their genotypic frequencies according to the ex-
pected by the Hardy-Weinberg equilibrium, the couplings
are random between the individuals. However, for those
where significant deviations between found and expected
frequencies were detected, it is possible that it is a result of
a higher number of found than expected homozygotes, de-
tected in the majority of loci in non-equilibrium in this
study’s populations. This hypothesis is forwarded by
Failloux et al. (1995) in studies on populations of this vec-
tor, as well as by Santos et al. (1999) on Anopheles darlingi
populations from the Amazonian region.

Genetic structure data from Wright’s F statistics
showed disequilibrium resulting from homozygote excess
and suggested a certain intrapopulational differentiation, in
which F_{is} values were higher than F_{st} values. Similar results
in French Polynesian A. aegypti populations were reported
by Failloux et al. (1995), who detected heterozygote defi-
ciency in loci EST1, EST3 and PGM (F_{is} = 0.26; 0.20 and
0.13, respectively). de Sousa et al. (2000) found F_{st} mean
value equal to 0.065, in this vector’s Argentinean popula-
tions indicating low levels of genetic differentiation among
populations from different localities. Nevertheless, Dinardo-Miranda and Contel (1996) found lower F_{st} values
(0.018) in São Paulo populations. In their study the author
considers that even though actual F_{st} values had been low
they nevertheless were significant, and indicate a differen-
tiation between populations, making it possible to assert
that the allelic frequency variability origin is intra-
populacional (F_{is} = 0.057).

According to Eanes and Koehn (1978) population ge-
netic structures is a consequence from the coupling pat-
terns, and the genetic flow magnitude between populations
and this is expressed by the Hardy-Weinberg equilibrium
deivation and by the amount of differentiation or allelic fre-
cuency between the populations. They further consider that
the high genetic flow rates among sub-populations and the
tendency for intrapopulational random coupling may lead
to a genetic structure decrease. Given this information, it is
possible to admit that the F_{st} values detected in this study
may indicate the onset of a genetic flow reduction process
as well as of the non random couplings occurrence, since
the F_{st} value was relatively high. Tabachnick and Wallis
(1985) and Failloux et al. (1995), reported similar findings
on this mosquito populations genetic structure to those ob-
tained in Manaus, where control measures of both immu-
ture and winged forms with insecticide systematic
applications led to a decrease in population size within a de-
termined period of time. However, a population increase
occurs again whenever there is a relaxation of these mea-
sures.

The genetic distance values found among the four
Manaus populations were small, indicating that these popu-
lations are very similar. These findings are similar to those
detected in São Paulo populations by Dinardo-Miranda and
Contel (1996) who found genetic distance values between
0.009 and 0.018. Tabachnick et al. (1979) and Wallis et al.
(1984) working with this vector’s populations who found
generic distance values from 0.002 to 0.082 and 0.002 to
0.084. These findings confirm a low genetic differentiation
level among all the populations around the world, including
those in Brazil. Taking the direct relation between genetic
distance and evolving time into account (Nei, 1972) it may
be inferred that, for A. aegypti, the divergence between
populations is fairly recent.

The dendrogram in Figure 1 showed that the Cidade
Nova, Downtown and Coroado populations were grouped
in a “cluster”, being closely related, whereas the Compensa
population was separated into another “cluster”. The pre-
ent study showed to be very useful since it helped to under-
stand the polymorphism levels and indexes which
determine the genetic structure of the \textit{A. aegypti} populations that infest the city of Manaus, and thus, subsidise these vectors’ control strategies. Currently, vector control is the only available method for reducing the incidence of dengue fever. Mosquito populations can be limited by insecticides used against larvae and/or adults. Extended use of insecticides for dengue control may enhance the resistance to insecticides in mosquito populations (Pasteur and Raymond, 1996). Furthermore, rebuilding from selected resistant individuals gives rise to a population genetically different from the original one. Therefore, knowledge about geographical genetic variation in \textit{A. aegypti} populations regarding dengue transmission would be informative. However, further molecular studies involving other Brazilian populations must be carried out in the attempt to provide more information on the genetic structure of this vector’s populations, its variability and possibly about its vectorial ability, insecticide resistance and ecological adaptation.

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References


