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Research Article

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 (H_o = 0.152 ± 0.052; H_e = 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 (H_o = 0.109 ± 0.037). Wright's F statistics showed that the mean value of F_{is} was higher than that of F_{st} (F_{is} = 0.164 > F_{st} = 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

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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 (H_o) and expected (H_e) 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 F_{st} (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.

		Population			
Locus	Allele	Compensa	Cidade Nova	Downtown	Coroado
EST3 (**n**)		90	90	90	90
	100	0.994	1.000	0.917	0.922
	96	0.006	0.000	0.022	0.044
	93	0.000	0.000	0.061	0.033
χ^2H-W		0.000 (df = 1) ^{ns}		13.786 (df = 3)*	0.591 (df = 3) ^{ns}
EST4 (**n**)		90	90	90	90
	100	0.989	0.989	0.933	0,956
	98	0.011	0.011	0.067	0.044
$\chi^2_{H\text{-}W}$		0.006 (df = 1) ^{ns}	179.006 (df = 1)*	97.672 (df = 1)*	102.309 (df = 1)*
EST5 (**n**)		90	90	90	90
	100	0.900	0.494	0.406	0.461
	98	0.072	0.439	0.472	0.372
	94	0.028	0.067	0.122	0.167
$\chi^2_{H\text{-}W}$		33.649 (df = 3)*	73.682 (df = 3)*	22.067 (df = 3)*	38.613 (df = 3)*
LAP2 (**n**)		90	90	90	90
	100	0.567	0.550	0.628	0.450
	98	0.433	0.450	0.372	0.550
$\chi^2_{H\text{-}W}$		0.582 (df = 1) ^{ns}	17.780 (df = 1)*	23.109 (df = 1)*	8.637 (df = 1)*
LAP5 (**n**)		90	90	90	90
	100	0.767	0.811	0.744	0.767
	98	0.233	0.189	0.256	0.233
$\chi^2_{H\text{-}W}$		1.680 (df = 1) ^{ns}	0.360 (df = 1) ^{ns}	0.189 (df = 1) ^{ns}	5.086 (df = 1)**
LAP6 (**n**)		90	90	90	90
	100	0.989	1.000	0.989	1.000
	98	0.011	0.000	0.011	0.000
$\chi^2_{H\text{-}W}$		179.006 (df = 1)*		179.006 (df = 1)*	
PGI (**n**)		90	90	90	90
	105	0.000	0.206	0.033	0.000
	100	1.000	0.794	0.967	1.000
$\chi^2_{H\text{-}W}$			53.653 (df = 1)*	10.603 (df = 1)*	

Table 1 (cont.)

Locus	Allele	Population			
		Compensa	Cidade Nova	Downtown	Coroado
IDH (**n**)		90	90	90	90
	110	0.311	0.183	0.200	0.128
	100	0.689	0.817	0.800	0.872
$\chi^2_{H\text{-}W}$		0.624 (df = 1) ^{ns}	1.913 (df = 1) ^{ns}	1.015 (df = 1) ^{ns}	6.158 (df = 1)**
MDH (**n**)		90	90	90	90
	110	0.350	0.217	0.300	0.422
	100	0.650	0.783	0.700	0.578
$\chi^2_{H\text{-}W}$		5.573 (df = 1)**	0.008 (df = 1) ^{ns}	0.000 (df = 1) ^{ns}	0.159 (df = 1)^{ns}
PGM (**n**)		90	90	90	90
	115	0.183	0.167	0.211	0.183
	105	0.050	0.139	0.183	0.167
	100	0.767	0.694	0.606	0.650
$\chi^2_{H\text{-}W}$		8.556 (df = 3)**	4.845 (df = 3) ^{ns}	16.160 (df = 3)*	5.893 (df = 3)^{ns}

n - sample size.
χ^2 H-W - chi-square/Hardy-Weinberg equilibrium.
df - degrees of freedom.
*p < 0.01; ** p < 0.05; ns - not significant.

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*, *EST4*, *LAP2* 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 F_{is} mean value relative to F_{st} (0.164 > 0.048). Higher F_{is} values as compared with F_{st} were found in loci *LAP6*, *EST4* and *PGI* with 1.000, 0.912 and 0.687 respectively, suggesting a certain intra-populational differentiation (Table 3). Population structure was also tested at different hierarchical levels using F_{st} 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 2 - Estimate of measures of genetic variability in *A. aegypti* populations.

Population	Mean sample size/locus	Mean nº of alleles/locus	% Polymorphic loci*	Mean heterozygosity	
				Observed	Expected**
Compensa	90.0 ± 0.0	1.6 ± 0.2	50.0	0.117 ± 0.043	0.131 ± 0.046
Cidade Nova	90.0 ± 0.0	1.6 ± 0.2	44.4	0.109 ± 0.037	0.157 ± 0.050
Downtown	90.0 ± 0.0	1.7 ± 0.2	55.6	0.152 ± 0.052	0.174 ± 0.052
Coroado	90.0 ± 0.0	1.6 ± 0.2	44.4	0.143 ± 0.049	0.164 ± 0.053

* A locus was considered polymorphic if more than one allele was detected.
** Expected heterozygosity of Hardy-Weinberg, Nei's unbiased estimate (Nei, 1978).

Table 3 - Genetic structure analysis of *Aedes aegypti* populations from Manaus, using Wright's F statistics.

Locus	F_{is}	F_{it}	F_{st}
EST3	0.006	0.036	0.035***
EST4	0.912	0.914	0.017
EST5	0.473	0.536	0.147***
LAP2	0.287	0.299	0.016*
LAP5	-0.030	-0.026	-0.001
LAP6	1.000	1.000	0.001
PGI	0.687	0.728	0.160***
IDH	-0.049	-0.021	0.030***
MDH1	0.047	0.072	0.028***
PGM	-0.089	-0.075	0.012***
Mean	0.164	0.198	0.048***

F_{is} = coefficient of inbreeding among individuals in the subpopulations; F_{it} = degree of genetic differentiation among the total populations; F_{st} = degree of genetic differentiation among the subpopulations; levels of significance for $F_{st} > 0$ are: *p < 0.005; **p < 0.01; ***p > 0.001.

Table 4 - Hierarchical analysis of molecular variance of four *Aedes aegypti* populations from Manaus.

Source of variation	d.f	% Variation F_{st}	P F_{st}
Among populations	3	4.88	< 0.001
Within populations	716	95.12	< 0.001

%, percentage molecular variation explained by the hierarchical level; P, level of significance for the distribution for that hierarchical level being different from random.

Table 5 - Matrix of genetic distance and similarity among the four *Aedes aegypti* populations.

Population	Compensa	Cidade Nova	Downtown	Coroado
Compensa	*****	0.986	0.984	0.987
Cidade Nova	0.014	*****	0.997	0.993
Downtown	0.016	0.003	*****	0.997
Coroado	0.013	0.007	0.003	*****

The values below the diagonal correspond to unbiased genetic distance and those above the diagonal correspond to unbiased genetic identity (Nei, 1978).

Discussion

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.

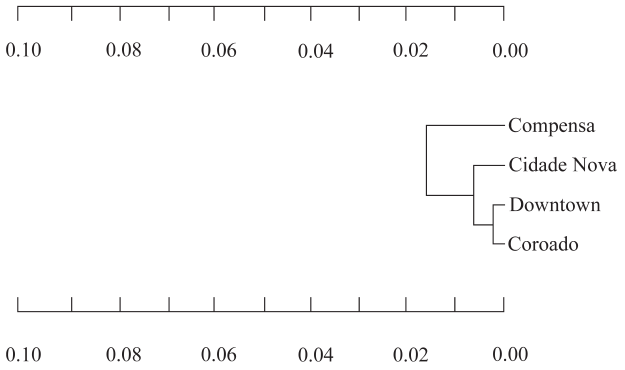

Figure 1 - Dendrogram resulting from grouping populations of *Aedes aegypti* on the basis genetic distance by the unweighted pairing group method which arithmetic mean - UPGMA (Nei, 1978).

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 resemble 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 Argentina. 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 heterozygosity values for this species ($H_e = 0.118 \pm 0.009$) were detected by Tabachnick (1982) in the Caribbean, and by Harrington *et al.* (1984) ($H_e = 0.097 \pm 0.055$) in Houston populations. Nevertheless, Dinardo-Miranda and Contel (1996) obtained expected mean heterozygosity value divergent 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 estimates ($H_e = 0.350$) 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* populations here analysed would not be less polymorphic than the others analysed around the world. Therefore, heterozygosity indexes found in the Manaus *A. aegypti* populations, 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 undergoes an initial decrease whose recovery will come about slowly, and only following nearly 10^5 generations will be established back to the initial population levels.

Therefore, these data suggest that the in loci with distribution of their genotypic frequencies according to the expected 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, detected 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 vector, 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 deficiency 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 populations 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 differentiation between populations, making it possible to assert that the allelic frequency variability origin is intrapopulacional ($F_{is} = 0.057$).

According to Eanes and Koehn (1978) population genetic structures is a consequence from the coupling patterns, and the genetic flow magnitude between populations and this is expressed by the Hardy-Weinberg equilibrium deviation and by the amount of differentiation or allelic frequency 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_{is} value was relatively high. Tabachnick and Wallis (1985) and Failloux *et al*. (1995), reported similar findings on this mosquito populations genetic structure to those obtained in Manaus, where control measures of both immature and winged forms with insecticide systematic applications led to a decrease in population size within a determined period of time. However, a population increase occurs again whenever there is a relaxation of these measures.

The genetic distance values found among the four Manaus populations were small, indicating that these populations 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 genetic 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 present study showed to be very useful since it helped to understand the polymorphism levels and indexes which

determine the genetic structure of the *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 *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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