Genetic Diversity in Dengue Mosquito, *Aedes aegypti* (Diptera: Culicidae) from Amazon Region: Comparative Analysis with Isozymes and RAPD Loci

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Abstract: Four populations of *Aedes aegypti* from Manaus were studied, using allozymes and RAPD loci, to determine intra- and interpopulation genetic variability and differentiation and to compare genetic structure parameters assessed with both markers. Five RAPD primers produced 52 polymorphic fragments, whereas only seven of 18 isozyme loci were polymorphic. The population from Praça 14 was the most polymorphic (P= 94.23% and P= 55.6%); while those from Coroado (P= 82.69% and P= 44.40%) and from Cidade Nova (P= 84.61% and P= 44.40%) were the least polymorphic, for both RAPD and isozymes respectively. The observed heterozygosity was higher between populations (Ho= 0.33 – 0.38) as assessed by RAPD. Wright's F statistics showed an F_{is} value higher than F_{st} ($F_{is} = 0.164 > F_{st} = 0.048$). AMOVA indicated that 95.12% of the genetic variability is intrapopulational. Even so, both of the genetic markers evaluated showed a relatively high gene flow ((N_m= 15.15), and possibly are still random couplings, although the F_{is} value was not low. The genetic distance between populations was similarly low for both markers: RAPD (0.012 – 0.016) and Isozymes (0.003 – 0.016). These results show that as assessed by both markers, the populations are genetically similar, and that isozymes (codominant) are the most efficient to detect the population genetic structure. Although isozymes revealed less genetic diversity than RAPDs, the estimated levels of genetic distance were identical.

Keywords: Population genetics, isozymes, RAPD, dengue vector, Amazonas.

INTRODUCTION

Aedes (Stegomyia) aegypti Linnaeus is the main vector of yellow-fever virus and of four serotypes of dengue virus and dengue hemorrhagic fever. This mosquito originated in the Old World, probably from the Ethiopian region, and was originally described in Egypt [1]. The species currently has an almost cosmotropical distribution.

Aedes aegypti was reintroduced into Brazil in 1967 and has now infested the entire country [2]. In recent years, dengue cases in Brazil have increased, and dengue fever has become one of the major public-health problems; the number of cases reported in 2007 (559,954) represents an increase of more than 100% since 2005 (248,189 cases) [3]. In Amazonas, *Ae. aegypti* was detected in 1996 in the city of Manaus, and today high population rates of this mosquito have been recorded, indicating the magnitude of the problem in the region [4, 5]. Unfortunately, the situation has worsened in recent years, especially due to the increasing number of cases of dengue hemorrhagic fever.

The epidemiological importance and the ease of laboratory manipulation of *Ae. aegypti* have facilitated studies on

its population genetics and molecular biology [5-13]. Many markers have been used in studies on genetic variability and differentiation, in an attempt to quantify and explain this variability, in terms of its origin, maintenance, and importance to evolution. In addition, different factors may contribute to the gene action that leads to the development of differentiated characteristics with respect to vector competence, resistance to insecticides, and ecological adaptation. Knowledge of the genetic structure of these populations may contribute to the development of control programs for this vector.

The isozyme loci segregate as codominant alleles and can therefore be used for the Hardy-Weinberg equilibrium test. So, through the analysis of the estimated intra- and interpopulation genetic variability and differentiation, valuable information is obtained to determine strategies for mosquito control. RAPD-PCR (Random Amplified Polymorphic DNA – Polymerase Chain Reaction) markers, which use only an arbitrary primer and amplify unknown regions of the DNA molecule, are also useful to determine genetic variability and to identify cryptic species in Culicidae. Nonetheless, they are not applicable to the Hardy-Weinberg equilibrium test [14].

In the present investigation, four populations of *Ae. aegypti* from Manaus were studied with the aim of estimating the genetic variability and differentiation, by comparing the genetic structure parameters between RAPD (dominant) and Isozyme (codominant) loci.

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MATERIAL AND METHODS

Sample Origin and Collection

Larvae and pupae of *Ae. aegypti* were collected in four districts of the municipality of Manaus: Praça 14 (South), Cidade Nova (North), Coroado (East), and Compensa (West), 10 to 20 km distant from each other, during the year 1999. The mosquitoes were transported to the insectarium and kept until the adults emerged, which were identified based on Consoli & Lourenço-de-Oliveira 1994 [1]. Males were fed with a 10% saccharose solution, and females with hamster blood (*Mesocricetus auratus* Waterhouse). After mating, females were isolated to obtain individual oviposition. Hatched larvae were maintained until the 4th instar and a small part until the adult stage, according to Santos *et al.* (1981) [15], and frozen at –70ºC.

Isozymes

Eighteen enzyme loci (*EST3, EST4, EST5, EST6, LAP1, LAP2, LAP4, LAP5, LAP6, HK1, HK2, ME, MDH, IDH, 6-PGDH, α-GPDH, PGI,* and *PGM*) were analyzed in 4th-instar larvae, individually homogenized, three per egg clutch, except for *α-GPDH*, where adults were used, for a total of 90 individuals per enzyme system/population. Horizontal electrophoresis was used in a gel-starch solution of 12.5% and a starch-agarose of 2% and 1%, respectively. Buffer systems and stain solutions were based on Steiner & Joslyn 1979 [16] and on Lima & Contel 1990 [17].

RAPD

One larva from each egg clutch, for a total of approximately 30 individuals per population, was used for the detection of DNA polymorphic fragments. DNA extraction and amplification reactions were carried out according to Williams *et al.* 1990 [18], using the following oligonucleotides (OPERON): OPA04 (5'–AATCGGGCTG–3'), OPA07 (5'–GAAACGGGTG –3'), OPA08 (5'–GTGACGTAGG–3'), OPA18 (5'–AGGTGACCGT–3'), and OPA20 (5'–GTTGCGATCC–3'). The amplification reaction was carried out with a total volume of 25 µl, containing 1 µl of DNA (10 ng/µl), 2.5 U Platinum *Taq* DNA polymerase (Invitrogen), 2.5 µl buffer reaction 10X, 1.5 µl MgCl$_2$ (50 mM), 2.5 µl dNTP (5 µM), 14.7 µl Milli-Q water, and 2.5 µl of a single initiator (Operon Technologies Inc., Alameda, CA/5µM). Amplification proceeded through 45 cycles at 94ºC for 1 min, 36ºC for 1 min, and 72ºC for 2 min, followed by one final extension step at 72ºC for 7 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel in ethidium bromide (5 ng/µL), and visualized on an ultraviolet transilluminator.

Statistical Analysis

For the analysis of population genetic variability with RAPD, the Tools for Population Genetics Analyses Program (TFPGA) was used in the option – Dominant Markers for Diploid Organisms [14]. In this case, each band is assumed to be a locus. The presence of the band represents the dominant genotype, which contains the two genotypes (homozygote and heterozygote); the absence of the band represents the recessive homozygote genotype. In the case of two heterozygote alleles, they are not distinguishable, as an estimate of the allelic frequency is obtained from the square root of the frequency of the observed absent bands. In this case it is assumed that the estimated allelic frequencies are in Hardy-Weinberg equilibrium. This essentially means that the inbreeding coefficients f and F are equal to 0 and θ, respectively. The inbreeding coefficients f and F correspond to Wright's F_{is} and F_{it}, respectively, while theta corresponds to F_{st} estimates [19, 20]. For the isozyme analysis, the BIOSYS Program was used [21]. The POPGENE program, version 1.32 [22] was employed for both markers. Effective migration rates (N_m) were estimated based on F_{st} for isozymes ($N_m=0.25(1-F_{st})/F_{st}$) and G_{st} for RAPD ($N_m= 0.5(1-G_{st})/G_{st}$) inbreeding rates, following McDermott & McDonald 1993 [23]. The hierarchical analyses of molecular variance (AMOVA) [24] were calculated using the Arlequin program, version 2000 [25], for both the isozyme and the RAPD data. The dendrogram was constructed using the UPGMA method [26]. F_{is}, F_{it} and F_{st} data for isozymes of the POPGENE Program were identical to those of BIOSYS, because they use the Wright's F statistics.

RESULTS

Levels of Genetic Variability

For the isozyme analyses, the observed and expected genotype frequencies and chi-square values are shown in Table **1**. Of the 18 loci studied, only seven were polymorphic in the four populations: *EST4, EST5, LAP2, LAP5, IDH, MDH,* and *PGM*. The chi-square values for the majority of the loci were significant in the four populations, indicating an imbalance according to the Hardy-Weinberg model. An exception was found in the population from Compensa, where most of the loci showed non-significant chi-square values (*EST3* - χ^2= 0.000, *EST4* - χ^2= 0.006, *LAP2* - χ^2= 0.582, *LAP5* - χ^2= 1.680, and *IDH* - χ^2= 0.624; degrees of freedom = 1). Based on gene-frequency analysis, only 43% of polymorphic loci were in Hardy-Weinberg equilibrium. Deviation from the Hardy-Weinberg equilibrium was due especially to the excess of homozygotes for the loci EST4, EST5, and LAP2.

Both the Isozyme (Fig. **1**) and RAPD (Fig. **2**) patterns indicated high genetic diversity. The genetic estimates in the four populations are shown in Tables **2** and **3**. The population from Praça 14 was the most polymorphic (P= 55.6%, P= 94.23%) with the highest level of heterozygosity (Ho= 0.152, H= 0.387) on the basis of both isozymes and RAPD loci, respectively. P and H values with RAPDs are higher than those obtained with isozymes, because hypervariable loci are frequently revealed by the RAPD procedure.

Based on the RAPD analysis, 52 markers were distinguished in the four populations, ranging in size from 300 to 2072 bp. The percentage of polymorphic loci varied from 82.69 to 94.23. The population from Praça 14 was the most polymorphic, and that from Coroado was the least. Heterozygosity ranged from 0.339 in Coroado to 0.387 in Praça 14. The expected mean heterozygosities within each

Table 1. Observed and Expected Genotype Frequency at each Polymorphic Locus, and Chi-Square Value for the Determination of the Hardy-Weinberg Equilibrium in *Aedes aegypti* Populations from Manaus, Based on Isozyme Analyses

Locus	Genotype	Compensa		Cidade Nova		Praça 14		Coroado	
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
EST3	100/100	89	89.000	Monomorphic		77	75.587	76	76.508
	100/96	1	1.000			2	3.687	8	7.419
	100/93	0	0.000			9	10.140	6	5.564
	96/96	0	0.000			0	0.034	0	0.156
	96/93	0	0.000			2	0.246	0	0.268
	93/93	0	0.000			0	0.307	0	0.084
$\chi^2(3)$		0.000 [ns]				13.786[*]		0.591 [ns]	
EST4	100/100	88	88.006	89	88.006	84	78.369	86	82.156
	100/98	2	1.989	0	1.989	0	11.263	0	7.687
	98/98	0	0.006	1	0.006	6	0.369	4	0.156
$\chi^2(1)$		0.006 [ns]		179.006[*]		97.672[*]		102.309[*]	
EST5	100/100	76	72.855	42	21.877	22	14.682	32	19.011
	100/98	5	11.765	3	39.279	16	34.665	8	31.067
	100/94	5	4.525	2	5.966	13	8.972	11	13.911
	98/98	4	0.436	33	17.212	30	19.944	24	12.352
	98/94	0	0.363	10	5.296	9	10.447	11	11.229
	94/94	0	0.056	0	0.369	0	1.291	4	2.430
$\chi^2(3)$		33.649[*]		73.682[*]		22.067[*]		38.613[*]	
LAP2	100/100	27	28.777	37	27.101	46	35.352	25	18.101
	100/98	48	44.447	25	44.799	21	42.296	31	44.799
	98/98	15	16.777	28	18.101	23	12.352	34	27.101
$\chi^2(1)$		0.582 [ns]		17.780[*]		23.109[*]		8.637[*]	
LAP5	100/100	55	52.810	60	59.134	49	49.782	49	52.810
	100/98	28	32.380	26	27.732	36	34.436	40	32.380
	98/98	7	4.810	4	3.134	5	5.782	1	4.810
$\chi^2(1)$		1.680 [ns]		0.360 [ns]		0.189 [ns]		5.086[**]	
LAP6	100/100	89	88.006	Monomorphic		89	88.006	monomorphic	
	100/98	0	1.989			0	1.989		
	98/98	1	0.006			1	0.006		
$\chi^2(1)$		179.006[*]				179.006[*]			
PGI	105/105			15	3.721	1	0.084		
	105/100			7	29.559	4	5.832		

(Table 1) Contd…..

Locus	Genotype	Population							
		Compensa		Cidade Nova		Praça 14		Coroado	
		Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
	100/100	monomorphic		68	56.721	85	84.084	monomorphic	
$\chi^2(1)$				53.653*		10.603*			
IDH	110/110	7	8.603	1	2.950	2	3.520	4	1.413
	110/100	42	38.793	31	27.101	32	28.961	15	20.173
	100/100	41	42.603	58	59.950	56	57.520	71	68.413
$\chi^2(1)$		0.624 ns		1.913 ns		1.015 ns		6.158**	
MDH1	110/110	16	10.911	4	4.140	8	7.994	15	15.922
	110/100	31	41.179	31	30.721	38	38.011	46	44.156
	100/100	43	37.911	55	55.140	44	43.994	29	29.922
$\chi^2(1)$		5.573**		0.008 ns		0.000 ns		0.159 ns	
PGM	115/115	7	2.950	4	2.430	1	3.927	0	2.950
	115/105	1	1.659	6	4.190	4	7.006	6	5.531
	115/100	18	25.441	16	20.950	32	23.140	27	21.570
	105/105	0	0.201	0	1.676	0	2.950	1	2.430
	105/100	8	6.939	19	17.458	29	20.095	22	19.609
	100/100	56	52.810	45	43.296	24	32.883	34	37.911
$\chi^2(3)$		8.556**		4.845 ns		16.160*		5.893 ns	

χ^2 – chi-square/Hardy-Weinberg equilibrium
( ) = degrees of freedom
*= $P < 0.01$; **= $P < 0.05$; ns= not significant
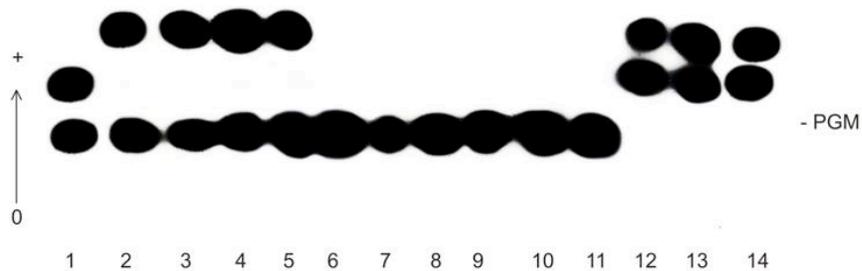
Fig. (1). Starch gel electrophoresis Phosphoglycomutase isoenzymatic profiles for 4th-instar larvae of *Aedes aegypti* populations from Manaus. Samples: 1 to 3 (Praça 14); 4 to 7 (Coroado); 8 to 10 (Compensa); and 11 to 14 (Cidade Nova). TEEM buffer system, pH 7.4.

population and in the total population were also higher (Hs= 0.3600; Ht= 0.3719, respectively), reflecting high genetic diversity among the populations (h*= 0.162[i]; h*= 0.372[r]) (Table **3**).

Population Genetic Structure

The analysis of population structure with RAPD and isozyme loci is presented in Table **4**. For isozymes, the Wright's F statistics showed a mean value relative to F_{is} higher than for F_{st} (0.164 > 0.041). Higher F_{is} values compared with F_{st} were found in loci LAP6, EST 4, and PGI with 1.000, 0.912, and 0.687 respectively, suggesting intrapopulation differentiation. However, for RAPD, the coefficient of genetic differentiation based on G_{st} was lower (0.032). A still lower value was observed when we analyzed the genetic structure based on the θ method to verify the inbreeding coefficients among populations (θ = 0.025), with the "bootstrapping" rate of consistency for 1000 replications
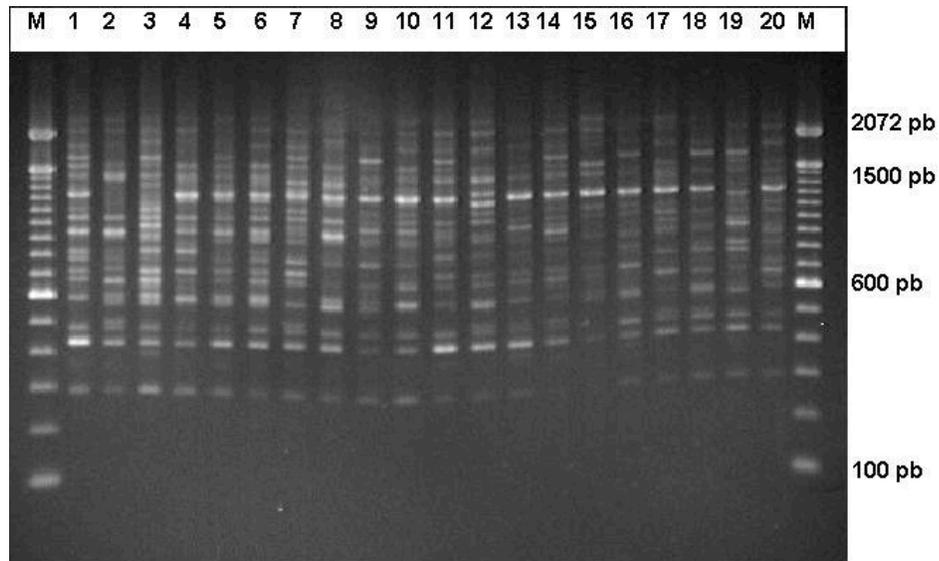
Fig. (2). RAPD profiles for 4th-instar larvae of *Aedes aegypti* populations from Manaus. Primer OPA 08. Samples: 1 to 5 (Coroado), 6 to 10 (Compensa), 11 to 15 (Cidade Nova), and 16 to 20 (Praça 14). M= 100 bp DNA Ladder.

Table 2.   Estimates of Measures of Genetic Variability in Populations of *Aedes aegypti* from Manaus, Based on Isozymes [i] and RAPD [r] Loci

Population	Mean number of samples per locus	Polymorphic locus percentage*	Mean heterozygosity	
			Observed	Expected**
Coroado	90.0[i]	44.40[i]	0.143 ± 0.05[i]	0.164 ± 0.05[i]
	29.04[r]	82.69[r]	0.339[r]	0.345[r]
Compensa	90.0[i]	50.00[i]	0.117 ± 0.04[i]	0.131 ± 0.05[i]
	28.83[r]	86.54[r]	0.364[r]	0.371[r]
Cidade Nova	90.0[i]	44.40[i]	0.109 ± 0.04[i]	0.157 ± 0.05[i]
	28.60[r]	84.61[r]	0.350[r]	0.356[r]
Praça 14	90.0[i]	55.60[i]	0.152 ± 0.05[i]	0.174 ± 0.05[i]
	28.07[r]	94.23[r]	0.387[r]	0.394[r]

*Frequency of most common allele less than or equal to 0.95.
**Hardy-Weinberg expected heterozygosity; unbiased estimate (Nei 1978).

Table 3. Genetic Variation for all Loci in Populations of *Aedes aegypti* from Manaus, Estimated Using Isozymes and RAPD Markers

Population	Sample Size	ne*	h*	I*	P*
Coroado	90[i]	1.324 ± 0.474[i]	0.163 ± 0.225[i]	0.280 ± 0.344[i]	44.44[i]
	29[r]	1.612 ± 0.373[r]	0.339 ± 0.183[r]	0.492 ± 0.251[r]	82.69[r]
Compensa	90[i]	1.222 ± 0.346[i]	0.130 ± 0.192[i]	0.206 ± 0.287[i]	50.00[i]
	29[r]	1.664 ± 0.353[r]	0.364 ± 0.172[r]	0.525 ± 0.234[r]	86.54[r]
Cidade Nova	90[i]	1.279 ± 0.408[i]	0.157 ± 0.209[i]	0.248 ± 0.329[i]	44.44[i]
	29[r]	1.624 ± 0.343[r]	0.350 ± 0.169[r]	0.509 ± 0.232[r]	86.54[r]
Praça 14	90[i]	1.324 ± 0.474[i]	0.173 ± 0.219[i]	0.280 ± 0.344[i]	55.56[i]
	28[r]	1.690 ± 0.283[r]	0.387 ± 0.131[r]	0.563 ± 0.172[r]	94.23[r]
Total	360[i]	1.293 ± 0.423[i]	0.162 ± 0.212[i]	0.263 ± 0.329[i]	55.56[i]
	115[r]	1.664 ± 0.324[r]	0.372 ± 0.145[r]	0.545 ± 0.183[r]	96.15[r]
$Hs = 0.3600 ± 0.0212$ $Ht = 0.3719 ± 0.0210$					

* ne = Effective number of alleles [40]; * h = gene diversity [41];* I = Shannon's Information index [42]; * P = Percentage of polymorphic loci; Hs = expected within-population heterozygosity; Ht = expected total heterozygosity; i = Isozymes; r = RAPD.

Table 4.   Analysis of Genetic Structure in Populations of *Aedes aegypti* from Manaus, using Wright's F Statistics

Marker	POPGENE				TFPGA		
	F_{is}	F_{it}	F_{st}/G_{st}	N_m	f	F	θ
Isozymes	0.164	0.198	0.041	5.827*	0.169	0.209	0.048
RAPD			0.032	15.15⁺	0.000	0.025	0.025

F_{is}= Inbreeding coefficient among individuals in subpopulations; F_{it} = Level of genetic differentiation in the total population; F_{st} = Level of genetic differentiation among the subpopulations. G_{st} = coefficient of genetic differentiation. The inbreeding coefficients f and F correspond to Wright's F_{is} and F_{it}, respectively, while theta corresponds to F_{st} estimates [19, 20]. * = Gene flow estimated from F_{st}= 0.25(1 - F_{st})/F_{st}; ⁺N_m = 0.5(1 - G_{st})/G_{st} [23].

of 95%. The population structure was also tested at different hierarchical levels using F_{st} by AMOVA analysis for both markers, Isozymes and RAPD. Most of the variation was found within populations (95.12% and 99.90%, respectively), indicating a high within-population differentiation (Table **5**). The variation among populations was very low (4.88% and 0.10%, respectively). The results suggest that despite the low level of genetic structuring observed in these populations, the gene flow among them is high (N_m= 15.15). Significant differences among the populations were observed only in the isoenzyme analyses.

Table 5.   Hierarchical Analysis of Molecular Variance of Four *Aedes aegypti* Populations from Manaus, Considering Isozymes[i] and RAPD-PCR[r]

Source of Variation	d.f	% Variation F_{st}	P F_{st}
Among populations	3[i] 3[r]	4.88[i] 0.10[r]	<0.001[i] >0.05[r]
Within populations	716[i] 116[r]	95.12[i] 99.90[r]	<0.001[i] >0.05[r]

d.f = degrees of freedom; %= molecular variance percentage explained by hierarchical level; P= significance level for hierarchical distribution to be non-random.

The genetic distance values indicate that these populations are genetically very similar (Table **6**), from both the

Table 6.   Matrix of Genetic Distance and Similarity among the Four *Aedes aegypti* Populations from Manaus, Based on Isozymes[i] and RAPD-PCR[r]

	Population	1	2	3	4
1.	Coroado	*****	0.987[i] 0.987[r]	0.993[i] 0.988[r]	0.997[i] 0.985[r]
2.	Compensa	0.013[i] 0.013[r]	*****	0.986[i] 0.984[r]	0.984[i] 0.984[r]
3.	Cidade Nova	0.007[i] 0.012[r]	0.014[i] 0.016[r]	*****	0.997[i] 0.985[r]
4.	Praça 14	0.003[i] 0.015[r]	0.016[i] 0.016[r]	0.003[i] 0.015[r]	*****

The values above the diagonal correspond to unbiased genetic identity, and those below the diagonal correspond to unbiased genetic distance [26].

data with isozymes and those with RAPD (D = 0.003 – 0.016 and D= 0.012 - 0.016, respectively). Despite the high genetic similarity observed among the populations, it was possible to separate them into three clusters, considering both markers: isozymes (Fig. **3**) - Praça 14 and Coroado, and another that separated Cidade Nova from Compensa; RAPD (Fig. **4**) - Coroado and Cidade Nova which are distinct from Compensa, and another consisting of the population from Praça 14. Likewise, the greatest genetic distance (0.016) for both markers was between Praça 14 and Compensa.
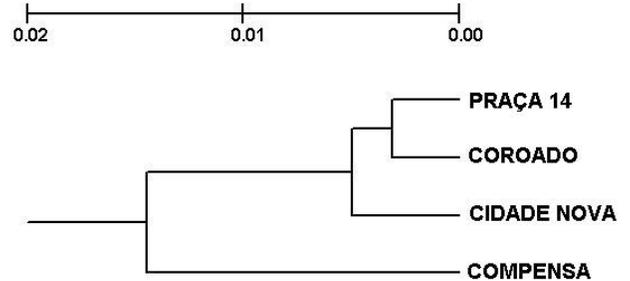
Fig. (3). Dendrogram resulting from grouping among the four Manaus populations of *Aedes aegypti*, using Isozymes, on the basis of genetic distance [26] by the unweighted pair group method with arithmetic mean - UPGMA, using the TFPGA program [14].
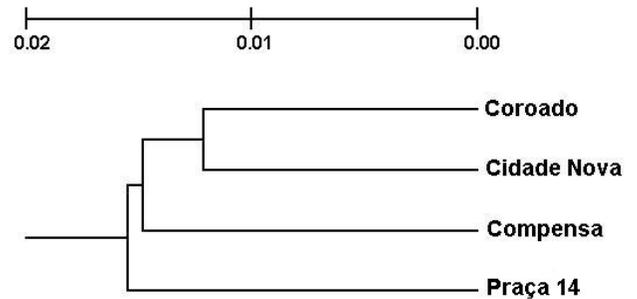
Fig. (4). Dendrogram resulting from grouping among the four Manaus populations of *Aedes aegypti*, using RAPD, on the basis of genetic distance [26] by the unweighted pair group method with arithmetic mean - UPGMA, based on the TFPGA program [14].

DISCUSSION

Studies of genetic variability at the molecular level have demonstrated that different regions in the genome have different rates of change. Single-copy genes have relatively low substitution rates because some alteration in a coding sequence may cause a severe effect on the codified protein. Nonetheless, changes in repetitive DNA apparently do not have the same effects, and thus the variability in this genome region is greater than single-copy genes [27]. Although the RAPD technique has limitations, especially to assess genetic relationships beyond the species level, it offers some advantages when applied to studies within the intraspecific level [8]. When RAPD markers were analyzed to estimate the population genetic variability of one species, generally high levels of polymorphism were found, because they offer random polymorphic regions in the genome. Although isozymes are restricted to genes coding for specific proteins,

they are also useful molecular markers for the study of gene variation.

The analysis of allele frequencies showed that 39% of the loci were polymorphic in the four populations, using isozymes. Similar results were found in three populations of Guariba, state of São Paulo for *Ae. Aegypti* [6]. Our results using RAPDs revealed 52 markers, with the percentage of polymorphic loci varying from 82.69 to 94.23. Heterozygosity ranged from 0.338 in Coroado to 0.387 in Praça 14. The genetic diversity was significantly higher than the calculated diversity based on isozyme markers (P= 44.40 – 55.60; Ho= 0.109 – 0.152) for the four populations. The estimated He was similar to those found in *Ae. aegypti* populations from Puerto Rico (He= 0.354) [28], Trinidad (He= 0.390) [29], Mexico (He= 0.339) [30], Argentina (He= 0.350) [8], and some populations from Brazil (He= 0.238) [31]. H values with RAPDs are greater than those obtained with allozymes, because hyper-variable loci are frequently revealed by the RAPD procedure [28].

In this context, RAPD markers have shown genetic differentiation of *Ae. aegypti* populations all over the world [30, 32] and in Brazil [12, 31, 33]. Gorrochotegui-Escalante *et al.* (2000) [30] analyzed populations of *Ae. aegypti* from ten cities of the Mexican northeast coast, using 60 RAPD polymorphic loci. These authors observed that those populations were isolated by distance and that gene flow only occurred among populations separated by 90 to 250 km. Garcia-Franco *et al.* (2002) [32], also using these markers, analyzed the genetic variation in 131 loci of 20 *Ae. aegypti* populations from Mexico, where they also observed isolation by distance.

For Brazilian *Ae. aegypti*, Ayres *et al.* (2003) [31] analyzed 47 RAPD polymorphic loci in 15 populations from five states, and detected a high level of genetic differentiation, suggesting population structuring. Santos *et al.* (2003) [33] analyzed populations from different regions of São Paulo, using these markers, and observed that genetic similarity was higher among mosquitoes whose geographic origins were closer. Paduan *et al.* (2006) [12], studying *Ae. aegypti* populations from six Brazilian states, also using RAPD markers, detected 21 polymorphic loci with high polymorphism levels, revealing two main groups.

Population genetic structure is a consequence of the mating patterns and the magnitude of gene flow among them, and is expressed mainly by the quantity of differentiation or variation in allelic frequency among the populations [34]. In this context, the F_{st} values detected in this study from isozyme markers may indicate the beginning of a gene-flow reduction process as well as the occurrence of nonrandom couplings, since the F_{is} value was relatively high. Fraga *et al.* (2003) [5], Dinardo-Miranda & Contel (1996) [6], Tabachnick & Wallis (1985) [35], and Failloux *et al.* (1995) [36], using this marker, reported similar results for the population genetic structure of this mosquito. However, in populations from São Paulo, Dinardo-Miranda & Contel (1996) [6] found lower values of $F_{is} > F_{st}$ (0.057 > 0.018). In that study, although the F_{st} value was low, it was significant and indicated slight differentiation among the populations, showing that the variability of allelic frequencies has an intrapopulation origin.

RAPD markers have also given results that indicate population genetic differentiation of this mosquito, as described by Ayres *et al.* (2003) [31], studying 15 populations of *Ae. aegypti* from five Brazilian states. They analyzed 47 RAPD polymorphic loci and reported high levels of genetic polymorphism (Hs = 0.274) among the different states (G_{st} = 0.317), as well as among cities (G_{st} = 0.085 − 0.265). Ayres *et al.* (2003) [31] suggested that this high polymorphism level results from the frequent use of insecticides in areas of treatment for these vectors, which could establish a cycle of extinction and recolonization resulting in genetic differentiation. Similarly, Paduan *et al.* (2006) [12] detected 21 polymorphic loci with high levels of polymorphism (Hs= 0.224) and high levels of genetic differentiation among populations of *Ae. aegypti* from those different states (G_{st} = 0.430), as well as among populations in cities (G_{st} = 0.410), indicating significant differences among populations from the six states. According to Ayres *et al.* (2003) [31], this high differentiation may reflect important differences in competence, susceptibility to the parasite, and resistance to insecticides, so that populations from different areas of Brazil must be treated as independent epidemiological units.

The differences observed in the estimated F_{st} with both types of markers could be the result of differences in the mutation rate of these loci, as suggested by Yan *et al.* (1999) [29] when they studied populations of *Ae. aegypti* based on RAPD, AFLP, RFLP, and isozyme markers. They argued that the F_{st} value can be seriously underestimated if the loci mutation rates are relatively high. RAPD loci tend to have higher mutation rates than isozyme loci, and therefore the F_{st} calculated from RAPD markers may be underestimated. On the other hand, the mutation rate of isozyme markers is generally lower than the DNA markers, so a higher F_{st} estimate can be expected. According to Slatkin & Barton (1989) [37], the statistic theta tends to overestimate mutation rates, as was observed with our RAPD data, which showed lower values than with Wright's $F_{st.}$

The analysis of molecular variation (Table **5**) demonstrated that the majority of the genetic variability is within the populations (95.12% for Isoenzymes and 99.90% for RAPD), although there is a significant difference between the populations when we consider only the isoenzyme analysis (4.88%; P < 0.0001). The data from RAPD were not significant, due largely to the analyses with dominant markers, which consider the allele frequencies of the populations in Hardy-Weinberg equilibrium. The low proportion of interpopulation in relation to intrapopulation variance lends support to the data in Table **4**, where the values of F_{st}/G_{st} and N_m show a small genetic differentiation, indicating a high migration rate and that the populations still have a metapopulation structure, i.e., there is gene flow among them.

The genetic distance obtained from the square root of gene frequencies varied from 0.0122 to 0.0166 (Table **6**), indicating homogeneity among the four populations from Manaus. These results indicate that levels of gene flow between *Ae. aegypti* populations can be high. Studies based on allozymes [38] and RAPD [28] demonstrated continuous gene flow among *Ae. aegypti* populations in Puerto Rico that were approximately 100 km apart. This observation is

congruent with our estimate using RAPD and Isozyme markers, with which the same genetic distance value was obtained among the populations, which showed great genetic similarity. The high observed genetic diversity as well as other factors may be related to the high dispersion rate of this mosquito, which is favored by dense human populations such as in Manaus (2,000,000 inhabitants) [4]. The consequence is a relatively high gene flow, despite systematic applications of insecticides in dengue control that lead to a reduction in the size of the population, followed by an increase when these measures are relaxed.

These data reflect a low level of genetic differentiation among the *Ae. aegypti* populations studied. Taking the direct relationship between genetic distances and evolving time into account, it may be inferred that the divergence between populations is fairly recent. They are possibly the result of a single introduction into Manaus, in November 1996 through its high dispersion and adaptive capacity, the species propagated in other districts of the city [4, 39]. The results not only suggest the existence of a single lineage of *Ae. aegypti* in Manaus, and possibly the same susceptibility for transmitting the dengue and urban yellow-fever viruses, but also their similar responses to control programs.

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