Genetic Diversity in Brazilian Populations of *Aedes albopictus*

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Random amplified polymorphic DNA (RAPD) analysis technique was undertaken in Aedes albopictus *populations from three states in Brazil, Rio de Janeiro (RJ), Minas Gerais (MG) and Pernambuco (PE), to estimate the level of genetic variability and levels of genetic exchange between populations. Allele and genotype frequencies were measured on 47 RAPD loci. Average observed heterozigosity (H_o) ranged from 0.282 in MG to 0.355 in Casa Forte (PE) population. Genetic distances estimates indicated that RJ and MG were more genetically similar than populations from PE. Genetic variation observed in local Brazilian populations was attributed to genetic drift associated with restricted gene flow in recently established populations.*

Key words: *Aedes albopictus* - random amplified polymorphic DNA - genetic structure

Aedes albopictus, known as the Asian "tiger", is a mosquito that originates from the tropical forest of Southeast Asia. The area is also considered to be the origin of the dengue virus. *Ae. albopictus*, along with *Ae. aegypti*, is considered to be one of the most important dengue vectors. After a long time confined within the limits of its range in certain islands of the Indian Ocean and many countries in the oriental region of Asia and the Hawaiian islands in the Pacific Ocean (Huang 1972), an alarming and sudden change in the geographical picture occurred during the 1980s. In August 1985, large populations of *Ae. albopictus* were discovered throughout Texas. The species spread so rapidly that by 1989 it was widely distributed within 18 states in the United States (Rai 1991). The species is now known to occur in all continents except Australia and Antarctica.

Ae. albopictus was first observed in Brazil in 1986, in the states of Espírito Santo (ES), Minas Gerais (MG), Rio de Janeiro (RJ) and São Paulo (SP) (Forattini 1986). Currently, this species is found in several states of the country. In some MG municipalities, that reported *Ae. aegypti* in previous years, health workers have observed that *Ae. albopictus* is now the principal mosquito species. *Ae. albopictus* dissemination is due mainly to the international shipping trade of used tires, providing an ideal mechanism of immature stages passive dispersion (Reiter & Sprenger 1987, Craven et al. 1988).

Ae. albopictus presence poses a serious public health problem. Firstly, it has an important role in the transmission of several arboviruses and usually its susceptibility for the viruses is even greater than that of *Ae. aegypti* (Mitchell 1995). Secondly, the species has the possibility of naturally transmitting the serotypes 2 and 3 of the dengue virus vertically (Ibanez-Bernal et al. 1997). Additionally, the mosquito shows aggressive antropophilic behaviour and a great adaptability to different habitats (Miller & Ballinger 1988, Rodhain 1995). This success is perhaps due to its extreme variation in adaptative traits such as diapause (Hawley et al. 1989) and cold hardiness (Hanso et al. 1994).

The introduction and establishment of *Ae. albopictus* in Brazil has serious implications. Besides being a potential vector of dengue (Miller & Ballinger 1988, Mitchel & Miller 1990), *Ae. albopictus* may also transmit other diseases of public health concern, such as yellow fever and other arboviruses (Rai 1991). The species has the ability to colonize different environments, and could also sustain urban cycles of the yellow fever by connecting the sylvatic and urban environments (Consoli & Oliveira 1994).

Previous works on the genetics of *Ae. albopictus* indicated that Brazilian populations had probably originated from Japan, as demonstrated by Kambhampati et al. (1991), who studied 57 populations from different areas.

In a Control Program it is important to understand the genetic structure and the molecular basis of the genomic flexibility of the vector species and hence the mechanisms ensuring the diversity of its populations.

The population genetics of colonization is an important component of many models of speciation (Mayr 1963, Wright 1978). Because *Ae. albopictus* was detected in Brazil shortly after its introduction, it may provide a rare opportunity to study several aspects of the population genetics of the species and the genetic events which initially accompany colonization. In this study we analyzed for the first time the genetic variability of *Ae. albopictus* Brazilian populations from three different states using random amplified polymorphic DNA (RAPD).

MATERIALS AND METHODS

Mosquito collection - A total of six *Ae. albopictus* populations were analyzed. Collections were made in three different states in Brazil, RJ city (RJ, 22.90S, 43.20W, 2 m) and Belo Horizonte municipality (MG, 19.92S, 43.94W, 850 m), both located in the Southeast region and PE in the

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Northeast region. In PE, samples were taken from three neighborhoods in the capital Recife (8.05S, 34.88W, 4 m): Parnamirim (PN), Várzea (VZ) and Casa Forte (CF) 1 to 7 km apart, and from the municipality of Jaboatão dos Guararapes (8.11S, 35.01W, 76 m) in the neighborhoods of Sucupira (SU), 15 km east of the town of Recife (Fig. 1). *Ae. albopictus* eggs were collected using ovitraps from January 1999 to January 2001 in the Southeast region and pupae and larvae were collected from natural breeding sites in PE in the same period. Eggs, pupae and larvae were reared to adults in the insectarium of the Department of Entomology at the Centro de Pesquisas Aggeu Magalhães, under standard conditions (temperature 27 to 30°C, relative humidity 65 to 95% and 12-h light/dark cycle). The number of individuals analyzed in each locality are shown in Table II. Adult females belonging to the same six populations were pooled and transferred to a -70°C freezer until DNA extraction.

DNA extraction - Individual female mosquitoes were homogenized in 500 µl of lysis buffer containing 0.4 M NaCl, 2 mM EDTA, and 10 mM Tris-HCl pH 8.0, Proteinase K (150 mg/µl) and 1.5% sodium dodecyl sulphate (SDS). The homogenates were incubated at 60°C overnight and 420 µl of 5M NaCl was added to the suspension. The mixture was gently vortexed for at least 30 sec followed by centrifugation at 14,000 rpm for 20 min. The DNA was precipitated from the supernatant by the addition of an equal volume of isopropanol, kept at -20°C for 1 h and then recovered by centrifugation at 14,000 rpm for 20 min. The pellet was washed with 70% ethanol, vacuum-dried and re-suspended in 300 µl of sterile TE buffer (10 mM Tris, 1 mM EDTA). The DNA concentration was estimated by comparison with known amounts of electrophoresis standards.

RAPD-PCR and analysis - Ten arbitrary primers (Gibco-BRL) were tested and four were selected on the basis of their reproducibility and efficiency in the PCR amplification. The reproducibility of the amplifications was tested using the same DNA sample in three different PCR reactions. The sequences of the oligonucleotide primers are given in Table I. Each PCR was carried out in a final volume of 30 µl of 10 mM Tris-HCl pH 9.0, containing 10 ng of mosquito genomic DNA, 50 mM of KCl, 1.5 mM of $MgCl_2$, 400 pmol of each primer, 2.0 units of Taq DNA polymerase and 0.2 mM of each dNTP. The PCR amplification was performed in a Techne thermocycler. The PCR

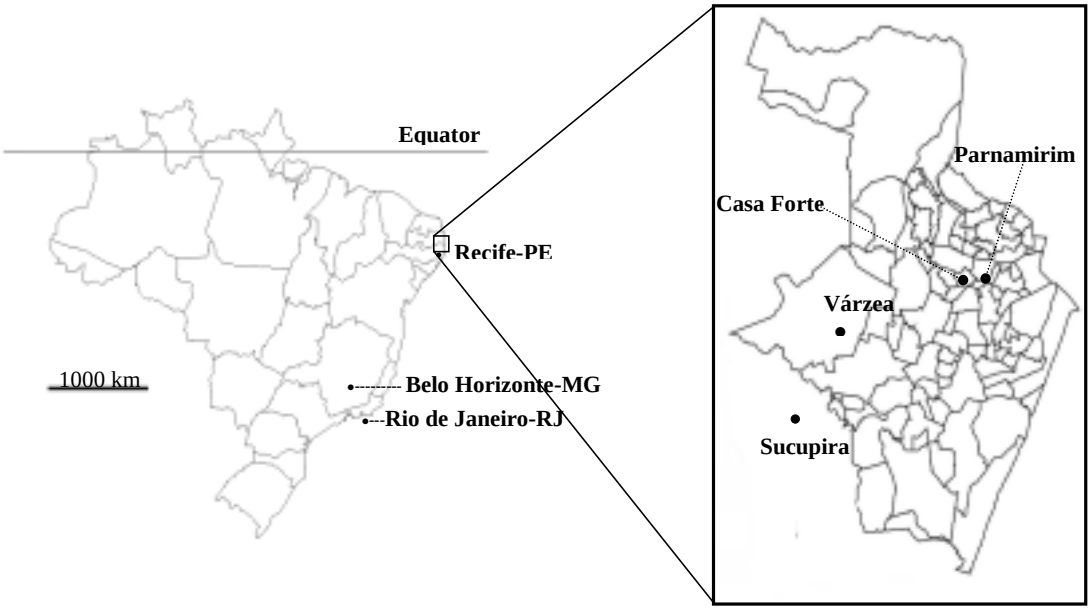

Fig. 1: map of sampling locations in Brazil and in the municipality of Recife, PE, Brazil.

TABLE I

Nucleotide sequence of primers used in random amplified polymorphic DNA showing size and number of fragments obtained (No.) after amplification of DNA samples from specimens of six *Aedes albopictus* populations from Brazil

		States					
		Pernambuco		Rio de Janeiro		Minas Gerais	
Primers	Nucleotide sequence	Size (bp)	No.	Size (bp)	No.	Size (bp)	No.
G06	5'-GCG GAA ATA G-3'	210-1500	12	240-2000	12	240-2000	12
G08	5'-GTC AAC GAA G-3'	310-1650	11	390-1890	11	390-1890	11
G09	5'-GAG GAC AAA C-3'	120-1000	10	265-1000	8	265-1000	8
G10	5'-GGT ACT CCC A-3'	210-1300	10	375-1200	9	375-1200	9

Pernambuco includes Várzea, Parnamirim, Casa Forte and Sucupira.

TABLE II

Origin and sample size (N) of six Brazilian *Aedes albopictus* populations and genetic variability indices (polymorphism, and gene diversity) for each population based on random amplified polymorphic DNA analysis

Localities	N	Polymorphism (%)	Heterozigosity
Parnamirim-PE	8	74.5	0.294
Casa Forte-PE	20	87.2	0.355
Várzea-PE	20	85.1	0.339
Sucupira-PE	20	83.0	0.296
Rio de Janeiro-RJ	15	83.0	0.296
Belo Horizonte-MG	20	80.8	0.282

program consisted of 40 cycles at 94ºC for 1 min, 35ºC for 1 min, 72ºC for 2 min followed by one final extension step at 72ºC for 10 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel in Tris-borate-EDTA (TBE) buffer (0.089 M Tris; 0.0089 M boric acid, 0.002 M EDTA, pH 8.3) with ethidium bromide (5 mg/ml) according to Sambrook et al. (1989) and visualized on an UV transilluminator. The possibility of contamination was checked using negative controls on each set of reactions. The negative control samples included all reaction material except template DNA.

Statistical analyses - The RAPD markers were analyzed using the following assumptions: (1) RAPD alleles segregate in a Mendelian fashion; (2) bands that co-migrate are homologous; (3) different loci segregate independently and (4) populations are in Hardy-Weinberg equilibrium. Gene frequencies (calculated under the assumptions above) were used to estimate genetic distances (Nei 1978). A dendrogram, summarizing the genetic relationships among all populations was built, using the unweighted pair-group mean analysis (UPGMA) algorithm. Effective migration rates (Nm) were estimated based on inbreeding indices (G_{ST}) according to McDermott and McDonald (1993), where $Nm = 0{,}5\,(1 - G_{ST})/G_{ST}$. Calculations were performed with the help of the POPGENE (version 1.32) population genetics package.

RESULTS

Due to their reproducibility and polymorphism, the primers G06, G08, G09 and G10 were used to analyze the six *Ae. albopictus* populations. A total of 47 amplification products were generated by the four primers used, ranging from 120 to 2000 bp, with an average of 10 bands per primer. Table I shows the numbers and the size range of fragments amplified by each primer in each population. Two markers were exclusive for SU population and 5 for PN, VZ, CF and SU together. Two other markers were present only in the RJ and MG populations. No fragments differentiated the populations of RJ from MG. The gene diversity (H) ranged from 0.282 in MG to 0.355 in the CF population. The total average heterozigosity was 0.365 and the genetic distances varied between 0.049 and 0.133 (Table III). The overall differentiation among the six populations presented $G_{ST} = 0.149$ and $Nm = 2.86$. The G_{ST} value for the four populations within the state of PE (PA, VZ, CF, SU) was 0.121 ($Nm = 3.64$). Between the states of RJ and MG G_{ST} was 0.077, such that the number of migrants was 5.95 (Table IV). The UPGMA dendrogram constructed based on Nei's genetic distances (1978), demonstrated two distinct groups, one representing all the four populations from PE ($D \leq 0.111$) and the other containing the populations from MG and RJ ($D \leq 0.050$). The SU population was the most divergent of the populations from PE (Fig. 2).

TABLE IV

Diversity (total heterozigosity, Ht), genetic differentiation (Gst) and gene flow (Nm) among *Aedes albopictus* Brazilian populations, pooled by state based on random amplified polymorphic DNA analysis

Populations	Gst	Nm	Ht
PE	0.121	3.645	0.365
RJ, MG	0.077	5.949	0.314
PE, RJ, MG	0.149	2.860	0.365

PE: Pernambuco; RJ: Rio de Janeiro; MG: Minas Gerais

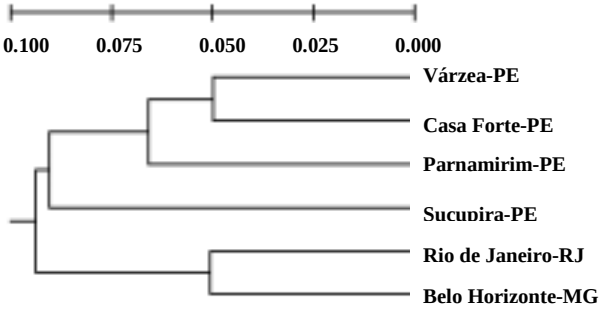

Fig. 2: dendrogram based on Nei's genetic distances among *Aedes albopictus* populations analyzed.

TABLE III

Pairwise genetic identities (diagonal above) and genetic distances (diagonal below) between six Brazilian *Aedes albopictus* populations based on random amplified polymorphic DNA analysis

	Parnamirim	Casa Forte	Várzea	Sucupira	Rio de Janeiro	B. Horizonte
Parnamirim-PE	-	0.927	0.945	0.914	0.907	0.875
Casa Forte-PE	0.075	-	0.951	0.895	0.913	0.914
Várzea-PE	0.056	0.049	-	0.932	0.940	0.926
Sucupira-PE	0.089	0.111	0.071	-	0.922	0.889
Rio de Janeiro-RJ	0.097	0.091	0.062	0.081	-	0.951
Belo Horizonte-MG	0.133	0.090	0.077	0.118	0.050	-

DISCUSSION

The results presented here are the first evaluation of the genetic variability of *Ae. albopictus* in Brazil. Heterozigosity and polymorphism values (H = 0.365 and P = 80%) were similar to those described for other populations of this species and other species of the genus *Aedes* (Black et al. 1988a, b).

Kambhampati and Rai (1991) analyzed the variation in the ribosomal DNA non-transcribed spacer within and among populations of *Ae. albopictus* in Texas over several years and observed that intrapopulational variation was 2-3 times higher than interpopulational variation. The differentiation pattern among populations observed in the present study (moderate differentiation at local level) has been also found in *Ae. albopictus* populations in the United States and Malaysia when analyzed by allozyme markers (Black et al. 1988a, b). Our data confirm that genetic drift accompanies the establishment of local populations. This occurs because only a few individuals found a new population and so, effective migration rates are not sufficiently high to homogenize the populations during colonization process.

The G_{ST} value observed among PE populations was 0.120, almost twice than that observed in the populations of Southeast states (RJ and MG). The Southeast region was the first area of Brazil to be colonized by *Ae. albopictus* in 1986 (Consoli & Oliveira 1994). The presence of this species in PE was first observed few years later (1994) in the city of Igarassu (7.83S, 34.90W, 20 m), 40 km away from Recife (Secretaria de Saúde do Estado de Pernambuco, unpublished data). This suggests that the presence of *Ae. albopictus* is very recent in PE. Indeed, many municipalities are still *Ae. albopictus* free in PE. The highest G_{ST} values observed in PE populations, compared to RJ and MG ones, could also imply that, in the Southeast region a significant gene flow among populations is leading to a certain homogenization. This is in the contrary to the statement made by Black et al. (1988a), that the amount of differentiation reported among local populations in the United States is a consequence of the natural breeding structure of the species.

Crossing experiments between different mosquito populations indicated that the susceptibility to dengue viruses in *Ae. albopictus* is genetically controlled and that the level of infection can vary according to the mosquito geographic origin (Gubler & Rosen 1976).

Brazilian populations of *Ae. albopictus* from Espírito Santo exhibited a relatively high degree of susceptibility to oral infection (52% for DEN-1, 38% for DEN-2, 19% for DEN-3 and 35% for DEN-4) under experimental conditions. Vertical transmission of the dengue virus is possible for this population (Miller & Ballinger 1988). These data indicate that *Ae. albopictus* has the potential to play an important role in the maintenance of the dengue virus in Brazil. Therefore, vector control programs could be directed not only to *Ae. aegypti* but also to *Ae. albopictus*, which breeds in different habitats from those of *Ae. aegypti*.

Miller and Ballinger (1988) demonstrated that the *Ae. albopictus* Brazilian population from Espírito Santo (Southeast region) was not an efficient vector of the yellow fever virus. However, other populations may have a different vector potential in Brazil.

The extensive *Ae. albopictus* intrapopulational differentiation with regard to RAPD markers and the findings of diagnostic fragments between different populations, emphasize the need for more detailed epidemiological and breeding structure studies.

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