Rev. Inst. Med. trop. S. Paulo
45(2):99-101, March-April, 2003

BRIEF COMMUNICATION

ANALYSIS OF GENETIC RELATEDNESS BETWEEN POPULATIONS OF *Aedes aegypti* FROM DIFFERENT GEOGRAPHIC REGIONS OF SÃO PAULO STATE, BRAZIL

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SUMMARY

RAPD markers have been used for the analysis of genetic differentiation of *Aedes aegypti*, because they allow the study of genetic relationships among populations. The aim of this study was to identify populations in different geographic regions of the São Paulo State in order to understand the infestation pattern of *A. aegypti*. The dendrogram constructed with the combined data set of the RAPD patterns showed that the mosquitoes were segregated into two major clusters. Mosquitoes from the Western region of the São Paulo State constituted one cluster and the other was composed of mosquitoes from a laboratory strain and from a coastal city, where the largest Latin American port is located. These data are in agreement with the report on the infestation in the São Paulo State. The genetic proximity was greater between mosquitoes whose geographic origin was closer. However, mosquitoes from the coastal city were genetically closer to laboratory-reared mosquitoes than to field-collected mosquitoes from the São Paulo State. The origin of the infestation in this place remains unclear, but certainly it is related to mosquitoes of origins different from those that infested the West and North region of the State in the 80´s.

KEYWORDS: *Aedes aegypti*; RAPD-PCR; Mosquito; Random PCR; Dengue vectors; Population genetics.

INTRODUCTION

Aedes aegypti is the main vector of important diseases, such as yellow fever and dengue. In Brazil, 370,000 dengue cases were registered in 2001, with 50,000 cases notified in the São Paulo State. It is important to note that the number of infections registered in this State showed an increase of 14 times in relation to the total detected in 2000 (www.funasa.gov.br).

The geographical structure of *A. aegypti* populations is very important for the ecology and evolution of these mosquitoes. Demographic and genetic processes can help to understand several events, such as gene flow, migration, selection, and extinction of populations, among others. RAPD-PCR (Random Amplified Polymorphic DNA) is a technique that generates markers by the amplification of random DNA segments with single primers of arbitrary nucleotide sequence[14]. These markers have been used in the analysis of genetic differentiation of *A. aegypti* since they allow the study of genetic relationships among populations[1,10]. The RAPD-PCR methodology has many advantages. It avoids the use of radioactive materials, uses a minimum amount of DNA, is accomplished without the knowledge of a target sequence and is cost efficient[11,13]. The existence of random genetic differentiation on local scale and larger genetic homogeneity on wider geographical scales has been demonstrated in the *A. aegypti* analysis from Puerto Rico using this technique[3]. Thus, the aim of this study was to identify *A. aegypti* populations from different geographic regions of the São Paulo State in order to understand the infestation pattern of this mosquito in the State.

MATERIAL AND METHODS

Obtaining the mosquitoes. Eggs of *A. aegypti* were collected in ovitraps[5] in five cities of the São Paulo State (Bauru, Santos, Araçatuba, Marília and Presidente Prudente) (Fig. 1). The Rockefeller strain, which is considered a reference for insecticide susceptibility, was used as control. Eggs from mosquitoes of this strain were kindly provided by Centers for Disease Control and Prevention (CDC) and Dr. Paulo de Tarso Ribeiro Vilarinhos from the "Institut de Recherche pour le Développement" (IRD), in Brazil. Mosquitoes originated from the second source were originally brought to Brazil from the laboratory of the United States Department of Agriculture and here were named Florida. In the SUCEN laboratory in Marília, mosquitoes of the F1 generation were obtained from the field-collected eggs, and three females of each city were frozen

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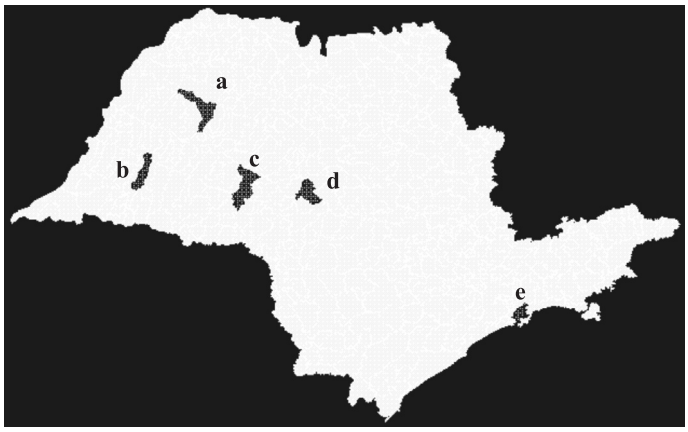

Fig. 1 - Map showing *Aedes aegypti* collection sites in the São Paulo State: Araçatuba (a); Presidente Prudente (b); Marília (c); Bauru (d) and Santos (e).

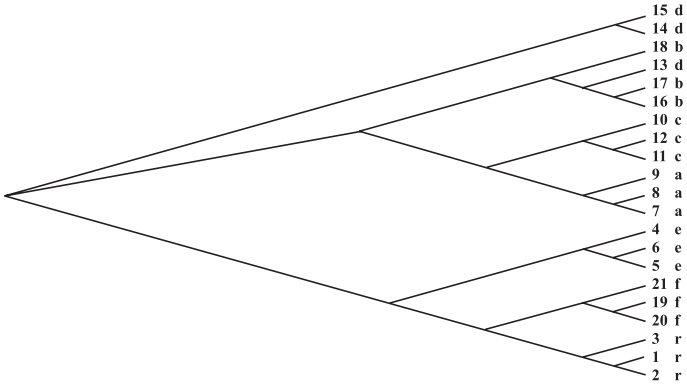

Fig. 2 - Dendrogram of genetic distance of *Aedes aegypti* based in Neighbor-joining analysis of RAPD data. The letters indicate the origin of the mosquitoes as illustrated in Fig. 1. Mosquitoes 1 to 3 (r) and 19 to 21 (f) are laboratory strains (Rockefeller and Florida, respectively).

in liquid nitrogen before any blood meal. The same procedure was performed with mosquitoes from the reference strain.

Extraction of the genomic DNA and RAPD-PCR. Twenty-one mosquitoes were ground separately, with disposable pestles in lysis buffer and the genomic DNA was obtained by digestion with proteinase K[7]. For primer selection and standardization of PCR protocols, nine oligonucleotides described in the literature (B1, B3, A2, B13, C4, C9, C13, C16 and C19)[2,4] and other six oligonucleotides (627 - GGATTCACAG, 641 - TGGAACCATG, 662 - GGCTACGTCT, 669 - GTTACACCAC, 683 - TATTACCGCC and 686 - CGTGACAGGA) were tested. The oligonucleotides B1, B3, B13, C4, C16 and 683 were chosen for their capacity to detect differences among the populations. RAPD-PCR reactions were accomplished with all the samples including the controls, essentially as described by BALLINGER-CRABTREE *et al.*[4]. Amplified products were separated electrophoretically on 1.2% agarose gel stained with ethidium bromide or on 8% polyacrylamide gel stained with silver. The gels were photographed with UV-light or scanned for subsequent analysis.

Data Analysis. The band pattern was analyzed using the RAPDistance program, version 1.03, which constructed a discrete character matrix to assess the relationships among individuals through band sharing. With this analysis it is possible to set up dendrograms that determine the proximity among the populations. Consistently amplified DNA fragments were used to produce a pair-wise matrix with Jaccard similarity coefficients, which was used to construct dendrograms based on the Neighbor-joining method, using a program from the PHYLIP 3.5 package[6].

RESULTS

A total of 58 reproducible gel bands, which were amplified by RAPD-PCR with the six selected primers, were number-coded and scored as present or absent for each mosquito. To verify the relationship between the populations, a dendrogram was constructed using the combined data set of the RAPD patterns. The mosquitoes were segregated into two major clusters (Fig. 2). One cluster consisted of 10 mosquitoes, all of them from the Western region of the São Paulo State. The other cluster was composed of six mosquitoes from a laboratory strain and three from Santos. The two clusters were subdivided into sub clusters containing all the mosquito triplicates, except mosquito 13.

DISCUSSION

The infestation of the State of São Paulo by *A. aegypti* probably begun in the 80's due to the presence of this species in cities of the neighboring States of Mato Grosso and Paraná. In the State of São Paulo mosquitoes spread from the West to the East. In 1995, 66% of the cities of the State were infested. The occupation did not occur in a homogeneous way in all municipal districts, being much faster in areas with lower demographic density, such as Araçatuba, Marília, Presidente Prudente and Bauru, which were infested in 1985, 1989, 1985 and 1986, respectively. On the other hand, in areas of high demographic density like Santos, the infestation was slower, occurring in 1995[8].

The purpose of this work was to examine the usefulness of RAPD in defining the genetic relatedness within *A. aegypti* populations obtained from distinct geographic regions of the São Paulo State. Although individual genotypes cannot be discerned by the RAPD analysis, since the majority of the alleles (more than 90%) segregates as dominant markers, RAPD-PCR revealed large number of genetic polymorphism. It is important to emphasize that the data obtained with our molecular analysis coincide with the report of the infestation described above. The results obtained with RAPD showed that subpopulations of mosquitoes that form sub clusters exist in the different municipal districts. The sub clusters could be grouped in major clusters, and we have noticed that the genetic proximity was greater between mosquitoes, whose geographic origin was closer. This conformity between geographic origin and genetic relationship has been described for other organisms[9,12]. As expected, mosquitoes from the same lineage, but that have been maintained in different colonies for many years, remained genetically close. Surprisingly, mosquitoes from Santos were closer genetically to laboratory-reared mosquitoes than to the others collected in São Paulo State. The origin of the infestation of this place remains unclear. As Santos has the most important port in Brazil, the infestation could be

related to North American mosquitoes, but it was certainly caused by mosquitoes of origins different from those that infested the West and North region of the State by the 80´s. It will be necessary to examine mosquitoes from other geographic regions in order to clarify this finding. Additionally, male mosquitoes obtained at the same places could be used to confirm our results.

In view of the importance of dengue epidemics in the São Paulo State, studies to determine genetic differences among *A. aegypti* populations from different sites could help to plan dengue prevention activities. Finally, it would be interesting to demonstrate if genetic differences observed in different cities are associated to different abilities to transmit the dengue virus.

RESUMO

Análise de relacionamento genético entre populações de *Aedes aegypti* de diferentes regiões geográficas do Estado de São Paulo, Brasil

Marcadores de RAPD são utilizados para a análise de diferenciação genética de *Aedes aegypti*, pois permitem o estudo do relacionamento genético entre populações. Este estudo procurou identificar populações em diferentes regiões geográficas do Estado de São Paulo visando entender o padrão de infestação do *A. aegypti*. O dendrograma construído com os dados combinados dos padrões de RAPD mostrou que os mosquitos foram separados em dois grupos principais. Mosquitos da região oeste do Estado de São Paulo constituíram um grupo e o outro grupo foi composto de mosquitos de uma cepa de laboratório juntamente com mosquitos de uma cidade litorânea onde se localiza o maior porto da América Latina. Estes dados concordam com o relato de infestação do Estado de São Paulo. A proximidade genética foi maior entre mosquitos cuja origem geográfica foi mais próxima, entretanto, mosquitos da cidade litorânea foram geneticamente mais próximos aos mosquitos criados em laboratório que àqueles coletados no Estado de São Paulo. A origem da infestação deste local permanece obscura mas certamente está relacionada a mosquitos de origens diferentes daqueles que infestaram a região oeste e norte do Estado na década de 80.

ACKNOWLEDGMENTS

This work was supported by a grant obtained from OPAS (Organización Panamericana de la Salud).

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Received: 22 October 2002
Accepted: 18 February 2003