Acta Tropica 128 (2013) 598–605

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Vector control measures failed to affect genetic structure of *Aedes aegypti* in a sentinel metropolitan area of Brazil

Kathleen R. Souza[a], Gilmar Ribeiro Jr.[a], Carlos Gustavo Silva dos Santos[a], Eliaci Couto de Lima[b], Paulo R.S. Melo[c], Mitermayer G. Reis[a], Ronald E. Blanton[d], Luciano K. Silva[a,*]

[a] *Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Salvador, BA, Brazil*
[b] *Centro de Controle de Zoonoses (CCZ), Secretaria Municipal de Saúde, Salvador, BA, Brazil*
[c] *Universidade Estadual de Santa Cruz (UESC), BR-415 km 16, Ilhéus, BA, Brazil*
[d] *Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, USA*

ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form 22 August 2013
Accepted 2 September 2013
Available online 9 September 2013

Keywords:
Aedes aegypti
Microsatellites
Entomological surveillance
Population genetics
Genetic epidemiology
Georeferencing

ABSTRACT

In order to evaluate subpopulation differentiation, effective population size (N_e) and evidence for population bottlenecks at various geographic levels, *Aedes aegypti* larvae were collected longitudinally from 2007 to 2009 from four areas in the city of Salvador, Brazil. The DNA from each larva was isolated and genotyped with five independent microsatellite markers. F_{ST} and Jost's D revealed significant population structuring ($P < 0.05$) at the municipal and regional levels, while only R_{ST} was able to detect genetic differentiation at the level of strata within these areas. N_e analysis from longitudinal data did not show any evidence of significant change in population structure. The census population measured by the house index, however, showed a significant trend toward decrease in these areas. Active vector control measures did contribute to vector reduction, but this was not enough to decrease *A. aegypti* population genetic diversity in Salvador. The understanding of *A. aegypti* population dynamics may be helpful for planning and evaluation of control measures to make them more effective.

1. Introduction

Dengue is currently the most important flaviviral infection worldwide and presently there is no licensed effective vaccine. However, even if a vaccine were available, vector control would remain an important part of the management of this disease and other related arboviruses. In Brazil, the primary vector is the mosquito *Aedes aegypti* L. 1762. Since 1996 there have been nationally coordinated control efforts starting with the Program for the Eradication of *A. aegypti* which was reorganized in 2002 to be the National Program for the Control of Dengue. The latest program emphasizes regular house-to-house surveillance and quarterly survey of household infestation rates or house index (HI) in sentinel metropolitan areas. It uses a Rapid Index of *A. aegypti* Survey (Portuguese acronym LIRAa) designed to determine HI based on the presence of larvae at defined and formally randomized areas ("strata") at the neighborhood level across each city (BRASIL, 2005). The definition of a stratum is primarily geographic and not social or ecological. Infestation rates above a threshold of 4% are used to direct vector control units to institute various control measures. In addition to efforts to mobilize the community to eliminate breeding sites, both programs were based on the domiciliary or peridomiciliary use of chemical adulticides and larvicides. The measurement of success is usually based on results of subsequent LIRAas.

Population genetics can provide an additional way to view the effect of a control measure by revealing to what extent the structure of the *A. aegypti* population has or has not been changed and suggest how well a population can recover. *A. aegypti* is dependent on human habitation, which combined with a short flight distance (100 m, rarely 1 km) (Maciel-de-Freitas et al., 2006) suggests there may be significant population structure over this range. The typical urban landscape may present significant barriers to local gene flow due to parks, lakes, deserted properties and even roadways (Hemme et al., 2010). This low dispersal ability may be overcome by wind and human transport of the desiccation-resistant *Aedes* eggs or as females seek a blood meal and engage in "skip oviposition" (Reiter, 2007).

* Corresponding author at: Pathology and Molecular Biology lab at Gonçalo Moniz Research Centre, FIOCRUZ-BA, R. Waldemar Falcão, 121, Candeal, Salvador, BA, 40296-710, Brazil. Tel.: +55 71 3176 2301/71 3176 2265; fax: +55 71 3176 2289.
E-mail addresses: kathleen_ribeiro@yahoo.com.br (K.R. Souza), gilmar@bahia.fiocruz.br (G. Ribeiro Jr.), csilva@bahia.fiocruz.br (C.G. Silva dos Santos), eliaci.couto@gmail.com (E.C. de Lima), pmelo11@yahoo.com.br (P.R.S. Melo), miter@bahia.fiocruz.br (M.G. Reis), reb6@case.edu (R.E. Blanton), kalabric@bahia.fiocruz.br (L.K. Silva).

0001-706X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2013.09.002

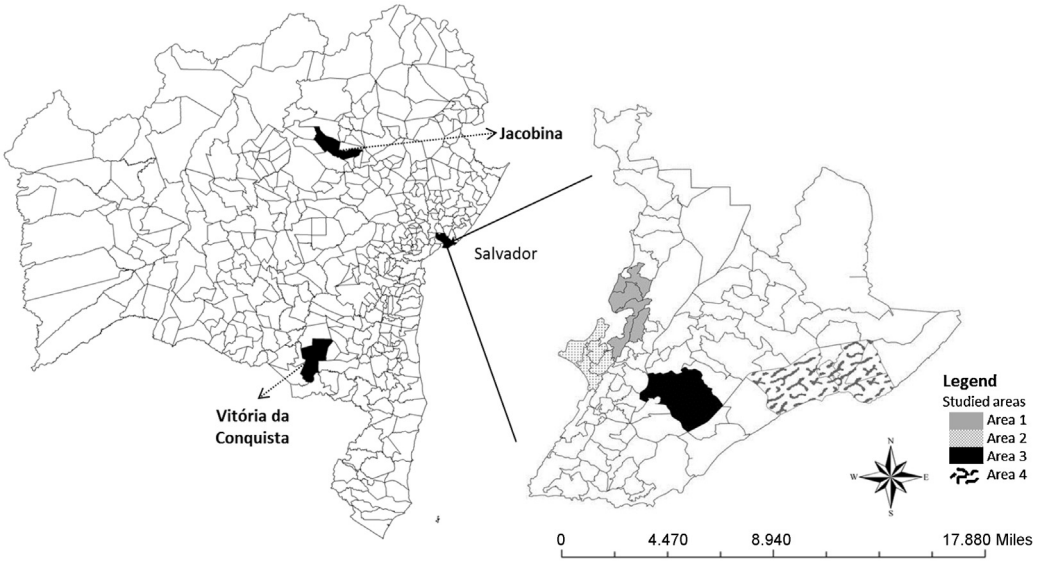

Fig. 1. Location of *A. aegypti*-collecting sites in the State of Bahia and in Salvador.

A large number of genetic markers have been used for studies of population genetics in mosquitoes: isozymes, polymorphisms in mitochondrial DNA, random amplified polymorphic DNA (RAPD) (Apostol et al., 1996) and microsatellites. Microsatellites, in particular, have become some of the most important markers due to their ease of use, scorability and high information content (da Costa-Ribeiro et al., 2006). Microsatellites are genomic sequences characterized by a variable number of short nucleotide repeats (2–6 nucleotides each), in tandem, which are highly polymorphic and widely distributed in the genome. In contrast to many other organisms, the genome of *A. aegypti* is microsatellite-poor (Fagerberg et al., 2001). One of the significant benefits of the *A. aegypti* genome project has been the ability to identify a larger number of these markers than was previously possible (Lovin et al., 2009). In Brazil, several studies have used microsatellite markers to investigate *A. aegypti* population differentiation in the cities of Rio de Janeiro and Recife, in the Brazilian Amazon and in Paraná state (da Costa-Ribeiro et al., 2006, 2007; Fantinatti, 2009; Lima, 2007, 2010). Only one study in the city of São Paulo has examined longitudinal patterns of change in these populations (Campos et al., 2012).

The use of spatial analysis of entomological indicators as well as genetic characteristics can be an aid for the monitoring and control of *A. aegypti* (Hemme et al., 2010; Lagrotta et al., 2008). Understanding of mosquito dispersal or gene flow may be helpful to redefine strata in a more ecological way, guide targeted release of genetically modified mosquitoes or increase the effectiveness of other vector control strategies. Based on microsatellite data, the current study evaluated vector genetic parameters such as differentiation, effective population size (N_e) and evidence for population bottlenecks at various geographic levels in the city of Salvador from 2007 to 2009.

2. Materials and methods

2.1. Study sites and samples

The quarterly *A. aegypti* survey (LIRAa) organized by the National Dengue Control Program is distributed based on "strata". "Strata" are defined as 8100 to 12,000 adjacent residential and commercial buildings that are grouped independent of neighborhood boundaries. After the conclusion of each LIRAa, localities with high risk for dengue transmission were targeted for treatment with insecticides and reservoir elimination. For analysis in this study 4–6 adjacent strata were combined to form "areas". Five hundred and twelve larvae of *A. aegypti* from infested houses or buildings from four areas of Salvador-BA, Brazil, were collected by local agents from the Zoonosis Control Center (CCZ) during four cycles of the LIRAa performed between 2007 and 2009 (designated here SSA Aug 2007, Oct 2008, Aug 2009 and Oct 2009). All four cycles were collected in the Southern Hemisphere's spring. Areas 1, 2 and 4 are at sea level. Area 3 is at approximately 75 m above sea level and separated from the coast by the steep uplift that divides the city into "upper" and "lower" sections. Areas 1 and 2 are contiguous while area 3 and 4 are 8 km apart (Fig. 1). For comparison, sixty-eight larvae were also collected in from two separate municipalities of Bahia state, i.e. Jacobina (JAC) and Vitória da Conquista (VC), 340 km and 520 km from Salvador, respectively (Fig. 1), as well as larvae representing the Rockefeller (ROCK) laboratory strain. JAC ($n = 36$) and VC ($n = 32$) samples were collected in 2009 and ROCK ($n = 44$) samples in 2010. According to the type of breeding site, a maximum of ten larvae were collected and transported in 70% alcohol to the lab for species identification. The study sample included only one confirmed *A. aegypti* larva from each breeding site. Each larva was frozen individually at −70 °C for subsequent DNA analysis.

2.2. House index (HI) and incidence of dengue, larvae georeferencing

Aggregated larval house index (HI; Infested houses/Houses Inspected × 100) for each cycle and stratum of the LIRAa and the annual incidence of dengue from 2003 to 2012 were obtained from the CCZ and Bahia State Secretary of Health (SESAB), respectively. Individual larva from the study sample was georeferenced using Arcview 9.1 (ESRI, 1999) for stratification of the larvae into various geographic levels (municipality, area and stratum).

2.3. Microsatellite identification

The GenBank database from 2002 to 2004 was searched for *A. aegypti* short tandem repeat sequences using BLAST and primer pairs were designed for the sequences flanking these regions. Forty-five potential markers were validated by amplification of DNA obtained from individual mosquitoes of the Salvador strain kept