

POPULATION EXPANSION AND HIGH GENE FLOW IN Aedes fluviatilis (DIPTERA: CULICIDAE) IN URBAN AREAS

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Abstract *Aedes fluviatilis* is an anthropophilic mosquito abundantly found in urban environments, however, its biology, epidemiological role and genetic characteristics are poorly known. Urbanization processes that resulted in environmental modifications together with climate change benefit certain anthropophilic mosquitoes' species such as *Ae. fluviatilis*, increasing its abundance in urban areas. Aiming for better understand whether urbanization processes may modulate the genetic structure of this species in the city of São Paulo, Brazil, there were utilized eight microsatellite loci to genetically characterize *Ae. fluviatilis* populations collected in nine urban parks located in the city of São Paulo. The results indicated that this species has high gene flow among their populations, low genetic structure, high genetic variation within populations, as a result of a population demographic expansion. Also, our findings suggest that *Ae. fluviatilis* populations are segregated in two main groups, the first composed by population collected in more conserved areas and the second composed by two populations collected in highly urbanized areas. This result led us to hypothesize that the population dynamics found in this study is probably correlated with the urbanization of the city of São Paulo, which consists mainly in the transformation of rural areas into urbanized areas, to accommodate the increasingly concentration of people living within the city.

Key words Populations genetics, Culicidae, Urbanization, Microsatellites.

INTRODUCTION

Aedes fluviatilis (Lutz, 1904) is a neotropical mosquito species abundantly found in urban areas (Forattini, 2002; WRBU, 2015). This species can thrive in urban environments and it is found throughout the city of São Paulo, where it represented around 10% of all mosquito specimens collected in previous studies (Ceretti-Júnior et al., 2015; Medeiros-Sousa et al., 2013). *Ae. fluviatilis* is highly anthropophilic and it is a potential vector of yellow fever virus (Davis and Shannon, 1931; de Carvalho et al., 2014), *Plasmodium gallinaceum* and *Dirofilaria immitis* (Camargo et al., 1983; Kasai, 1979; Vezzani et al., 2006).

The success of some species of mosquitoes in inhabiting urban environments depends on three main factors: the availability of breeding sites, blood-meal sources and climate variations. These factors can modulate the abundance of mosquito populations in urban areas (Brown et al., 2011; Chaves and Koenraad, 2010; Descloux et al., 2012; Edman, 1988). Therefore, a better knowledge of the genetic structure of urban mosquitoes can lead to a better understanding of how *Ae. fluviatilis* populations are modulated by selective pressures in the urban environment. Microsatellites have been used in genetic population studies since they are not subjected to selective pressures, indeed it can be a very useful tool for population genetic studies of mosquitoes on a macrogeographic (Brown et al., 2011; Fonseca et al., 2006; Wilke et al., 2014) and microgeographic (Olanratmanee et al., 2013; Piccinali and Gürtler, 2015) scale.

Considering the above facts, this study used microsatellite markers to investigate how *Ae. fluviatilis* populations are genetically structured in the city of São Paulo, Brazil and whether urbanization processes can modulate the genetic structure of this culicid.

MATERIAL AND METHODS

Ae. fluviatilis mosquitoes were collected from nine urban parks (Burle Marx, Ibirapuera, Piqueri, Previdência, Santo Dias, Shangrilá, Alfredo Volpi, Chico Mendes and Carmo) in the city of São Paulo, Brazil from 2011 to 2013. Mosquitoes were collected with portable, battery-powered aspirators and CDC CO₂-baited light traps. DNAs were extracted using the DNEasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. PCR reactions, visualization of fragments, dilution of PCR products, sequencing and fragment analysis were carried out as in Multini et al. (2015). The modified dendrogram displaying the Cavalli-Sforza and Edwards chord distance was constructed using Statistica 7.0 (StatSoft, 2004) by Multini et al. (2016). AMOVA was calculated using the software Arlequin (v3.5) (Excoffier et al., 2005). The amplified alleles were subjected to Bayesian model-based clustering analysis using Structure (v2.3.3) (Pritchard et al., 2007).

RESULTS AND DISCUSSION

The dendrogram displaying the Cavalli-Sforza and Edwards genetic distance in Figure 1, clearly showed the studied populations separated in 2 main clusters, one comprising the populations Burle Marx, Chico Mendes, Previdência, Shangrilá, Carmo, Santo Dias and Alfredo Volpi (Group 1) and the other comprising the populations Ibirapuera and Piqueri (Group 2), indicating some degree of differentiation between groups.

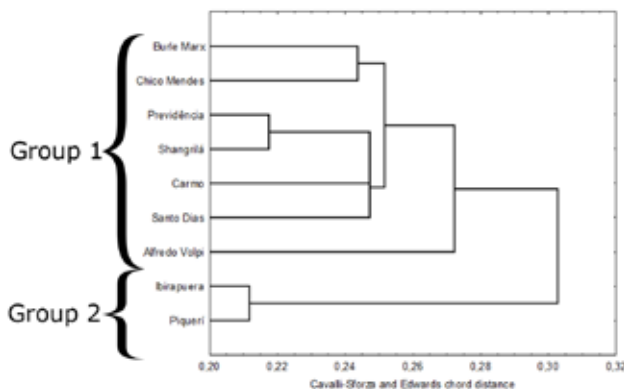


Figure 1. Genetic-distance dendrogram for *Aedes fluviatilis*, modified from Multini et al. (2016).

The AMOVA results showed that differences within population account for 98% of the genetic variation, meaning that variations within population were higher than between population. The Bayesian analysis displayed in Figure 2 showed that although the populations were partially segregated, the differences between them are subtle, indicating the presence of high levels of gene flow, which is probably responsible for the low genetic structure found in *Ae. fluviatilis* populations.

The urbanization processes present in Brazil are characterized by rapid and unplanned growth of the cities, resulting in the lack of basic sanitation, unsanitary houses, polluted rivers and untreated sewage. These factors have been resulting in the increasing in abundance of mosquitoes' species that are well adapted to man-made alteration. These features are acting as an obstacle in vector control strategies (Taípe-Lagos and Natal, 2003; Li et al., 2014).

The main findings of this study suggest that *Ae. fluviatilis* populations are segregated in two main groups. Since Group 2 is composed by the two populations collected in the most pressed by urbanization areas, it may indicate how man-made alteration in the environments might be modulating

Ae. fluviatilis populations.

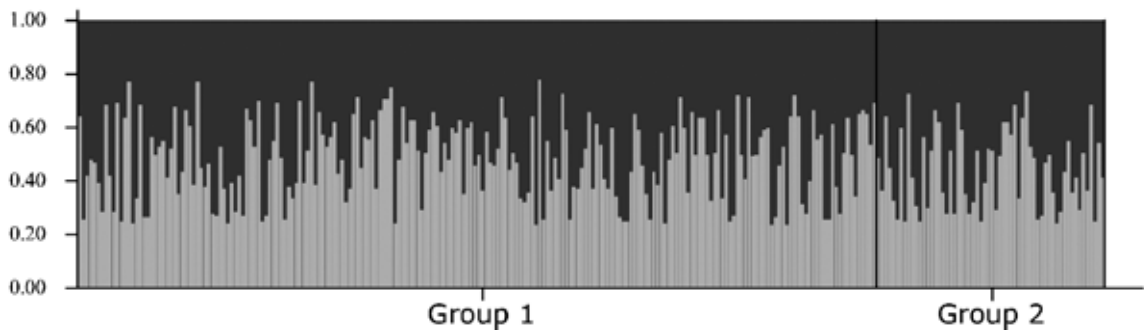


Figure 2. Bayesian analysis of structure for the two groups of *Aedes fluviatilis* populations showing the subdivision of individuals $k = 2$. Each of the 270 individuals is represented by a vertical line divided into different colored segments. The length of each segment represents the probability of the individual belonging to the genetic cluster represented by that color.

It was previously found by Multini et al. (2016) that this culicid undergone a population expansion, a common trend among mosquito species that can thrive in urbanized environments (Michel, 2006; Mirabello and Conn, 2006). The population expansion hypothesis can be also corroborated by the low genetic structure and high gene flow found in the Bayesian analysis. Moreover, the AMOVA results indicate that the low genetic variation between population can be a result of a genetic homogenization of these populations (LaDeau et al., 2015).

CONCLUSION

The structure patterns found for *Ae. fluviatilis* populations are probably correlated with the urbanization of the city of São Paulo, fitting the hypothesis that the transformation of rural areas into urbanized areas might be modulating *Ae. fluviatilis* population dynamics.

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