HABITAT SEGREGATION OF DENGUE VECTORS ALONG AN URBAN ENVIRONMENTAL GRADIENT

JONATHAN COX, MARIA E. GRILLET, OLGA M. RAMOS, MANUEL AMADOR, AND ROBERTO BARRERA*

Abstract. Differential distributions of Aedes aegypti and Ae. mediovittatus (potential inter-epidemic dengue vector) and other mosquitoes colonizing bamboo pots in San Juan, Puerto Rico were studied along an urban-rural gradient. City regions (urban, suburban, and rural) and landscape elements within regions (forest [F], low-density housing [LDH], and high-density housing [HDH]) were identified using satellite imagery. Aedes species extensively overlapped in HDH of urban, suburban, and rural areas. Mosquito species showed their high specificity for landscape elements (96.6% correct classification by discriminant analysis); absence of Ae. mediovittatus in HDH or absence of Ae. aegypti in forests were the main indicator variables. The gradient was explained using a canonical correspondence analysis, which showed the association of Ae. aegypti with HDH in urban areas, Culex quinquefasciatus with LDH in suburbs, and Ae. mediovittatus and other native mosquitoes (Cx. antillum magnorum, Toxorhynchites portoricencis) with less disturbed habitats (forests, LDH).

INTRODUCTION

Aedes (Stegomyia) aegypti (L.) is the main dengue vector worldwide because of its close association with humans in tropical and sub-tropical urbanized areas. This mosquito encounters other invasive or native container mosquitoes (mostly treehole mosquitoes) with similar requirements of aquatic habitats for its immature development (natural and artificial containers) in some parts of the world. Examples of other container mosquitoes that overlap in aquatic habitat requirements are Ae. (Protomacleaya) triseriatus (Say) in eastern North America, Ae. (S.) albopictus (Skuse) in Asia, Africa and the Americas, Ae. (Stegomyia) polynesiensis in the South Pacific, and Aedes (Gymnometopa) mediovittatus (Coquillett) in the Caribbean.

Mosquitoes are insects with complex life cycles that undergo abrupt ontogenetic changes in morphology, physiology, and behavior (metamorphosis) associated with a change from an aquatic habitat in the immature stage to a terrestrial habitat in the adult stage. The immature stage is dedicated to growth whereas the adult stage to reproduction and dispersal. Thus, to understand the spatial distribution and co-occurrence between mosquito species, it is useful to compare their preadult and adult niche and habitat requirements. For example, Ae. albopictus and Ae. aegypti overlap in their use of artificial and natural containers, and when resources are limited the former species dominates, partly explaining the well-documented displacement of the latter species from much of its previous territory in North America. Thus, interactions between these species in the larval stage are important to understand their spatial distribution.

The degree of segregation of urban mosquito species in their terrestrial habitats may also be related to dispersal and blood-feeding behavior of adult mosquitoes. For example, Ae. albopictus seems to be restricted to wooded areas next to humans, and areas without tall vegetation seem to exclude Ae. albopictus. Conversely, Ae. aegypti can be found in a variety of urban habitats including the highly urbanized areas without wooded vegetation. Additionally, Ae. aegypti depends highly on human blood and tends to bite and rest indoors, whereas Ae. albopictus feeds on a variety of vertebrates outdoors. Studies on the spatial distribution of these species along urban gradients have shown that Ae. aegypti predominates in highly urbanized areas, Ae. albopictus in rural areas, and both species co-occur in suburban tropical areas. Environmental factors other than the presence or absence of trees may also be important determinants of the spatial segregation of container Aedes. For example, it has been shown that eggs of Ae. aegypti are more resistant to desiccation and elevated air temperatures than Ae. albopictus, which contributes evidence to understand why Ae. aegypti has not been totally displaced by Ae. albopictus from southern Florida.

Aedes albopictus has not been able to invade or become established in Puerto Rico despite its presence in nearby islands and the active exchange of goods and people with the United States. Another container mosquito seems to be more common in suburban and rural Puerto Rico. Aedes mediovittatus, the Caribbean treehole mosquito, is a native species of Jamaica, the Cayman Islands, and the Greater Antilles (Cuba, Hispaniola, Puerto Rico, Virgin Islands). It undergoes immature development in a variety of natural (treeholes, bamboo, rockholes) and artificial (tires, miscellaneous) containers. Moore found that Ae. aegypti, Ae. mediovittatus, and Culex quinquefasciatus Say co-occurred in containers close to houses, but most Ae. mediovittatus were found away from houses in Puerto Rico. Conversely, 99% of all Ae. aegypti collections were made from containers that were at least 85 meters from the nearest house and no Ae. aegypti was found more than 100 meters from houses. Aedes mediovittatus was reported around houses in Cuba, co-occurring with Ae. aegypti and Cx. quinquefasciatus in artificial containers. It has been suggested that Ae. mediovittatus increased in prevalence in artificial containers as a result of the eradication campaign against Ae. aegypti in Cuba. However, lack of comparative studies before 1981 makes it difficult to infer whether the induced reduction of Ae. aegypti populations has had a positive impact on the distribution and prevalence of Ae. mediovittatus on that island.

* Address correspondence to Roberto Barrera, Dengue Branch, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, PR 00920. E-mail: rbarrera@cdc.gov

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Aedes mediovittatus is a competent vector of dengue viruses, with a high rate of vertical transmission for all dengue serotypes. Also, it has been proposed that this mosquito can act as a reservoir in the maintenance of dengue viruses in Puerto Rico during inter-epidemic periods in rural areas with low human population densities. The extent to which *Ae. mediovittatus* overlaps with *Ae. aegypti*, dengue viruses, and humans in urban areas has not been determined. In this study, we investigated the differential terrestrial habitat distribution of *Ae. aegypti* and *Ae. mediovittatus* adults in San Juan, Puerto Rico along environmental gradients and major terrestrial habitat types to document species overlap, and to detect the main ecologic variables explaining their distribution and abundance. Special emphasis was placed on detecting arboreal vegetation within the urban area because of its importance for treehole mosquito species. Areas of overlap among container mosquito species, humans, and dengue viruses could be critical to generating evidence about the possible role of *Ae. mediovittatus* as an inter-epidemic dengue virus reservoir.

**MATERIALS AND METHODS**

**Study area.** The study site encompassed approximately 125 km² of the San Juan metropolitan area (Figure 1). Field collection sites extended north from Hato Rey to the southern portion of the San Juan municipality. A 1999 Landsat 7 Enhanced Thematic Mapper vegetation and land cover classified

**FIGURE 1.** Map (north at the top) of Puerto Rico highlighting the Metropolitan Area of San Juan city (upper map, dark area), and an inset showing a classified satellite image (Landsat 7) of the study area (urban, suburban, rural) and its land use/vegetation types. Elevation increases southwards as shown in Figure 2 (towards the bottom of the classified image).
image of San Juan\textsuperscript{26} was used to identify and divide the study area into three regions: urban (64.9% of study area), suburban (15.1% of study area), and rural (20% of study area) (Figure 1). The urban region was defined as an area with a few patches of forests surrounded by high-density housing (HDH). The suburban region was identified by many patches of low-density housing (LDH), few patches of HDH, and several forest patches. The rural region was defined by many larger forest patches and very little HDH.

Image classification, originally aimed at identifying urban forests,\textsuperscript{26} was used to derive landscape classes within each urban region: forests (F), LDH, and HDH. Although the classification map identified many more landscape classes (natural and artificial barren, wetlands, non-forest vegetation, water, clouds/shadows), preliminary surveys did not yield any container {	extit{Aedes}} mosquitoes in those areas. High-density housing was characterized by more than 80% concrete or built-up surface cover and small distances between houses. Typically vegetation in this class was sparse and was frequently mixed with small business or by gated residential communities with houses separated by lawns and ornamental vegetation. Low-density housing was characterized by having more than 20% vegetation cover, greater distances between houses, and large gardens or natural vegetation fragments. Forest areas were differentiated by patches of thick vegetation and large trees; no distinction was made with respect to forest age.

The urban to rural gradient was associated with changes in land use and vegetation extents (human intervention), and with an elevation gradient from the coastal lowlands (Atlantic Ocean north) up to nearly 300 meters in altitude (humid northern foothills, south) (Figure 2). Elevation was obtained from a raster image with a resolution of 30 meter (National Elevation Dataset, United States Geological Service, Reston, VA). Thus, urban San Juan can be described as a mosaic of landscape elements (F, LDH, HDH) scattered along a north-south gradient in elevation and disturbance (urban, suburban, rural).

**Sampling container mosquitoes.** The combination of regions (urban, suburban, rural) and landscape classes per region (HDH, LDH, F) was incomplete (e.g., HDH was nearly absent in rural areas). As expected, HDH predominated in the urban region, F and LDH in the suburban region, and F in the rural region (Table 1). Thus, we sampled in three landscape elements in the urban area (F, LDH, HDH), three in the suburban area (F, LDH, HDH), and two in the rural area (F, LDH). Three to six locations fitting each of the landscape classes within each region were sampled as replicates (urban: 6 HDH, 3 LDH, 3 F; suburban: 3 HDH, 3 LDH, 3 F; rural: 3 LDH, 3 F), and within each replicate site we placed five bamboo pots spread over each location (20–30 meters from each other) to minimize false-negative results (135 pots). The presence and relative abundance of container mosquitoes were assessed by means of larvae collected weekly in bamboo (\textit{Bambusa vulgaris}) pots with water.\textsuperscript{27} Bamboo internodes were sectioned from fresh green bamboo canes. Each bamboo pot was hung with a nylon cord tied to holes drilled approximately an inch from the top, and placed in partially or totally shaded locations 1–1.5 meters off the ground. Bamboo pots varied in diameter (4–8 cm) and volume (130–900 mL). Samples were collected weekly for an eight-week period in July and August 2003. Each bamboo pot was emptied and rinsed into an enamel pan. Larvae were pipetted into Whirl-Paks (Nasco, Fort Atkins, WI) containing de-chlorinated fresh water and sealed for transport to the laboratory in an ice chest for rearing and species identification.\textsuperscript{10–12} Mosquito species identification was made on advanced instars reared in the laboratory. After removing specimens, water and debris were returned to the bamboo pot, and additional water was then added to fill the container, preventing the bamboo pot from drying up. During each weekly collection, we measured pH, temperature, and conductivity of the water between 10:00 AM and 2:00 PM (model HI 98129; Hanna Instruments, Woonsocket, RI). We also recorded the diameter and capacity of the bamboo pots. Geographic coordinates were recorded using global positioning system units (model Rino 120; Garmin, Olathe, KS).

**Statistical analyses.** We averaged the number of larvae in the five pots every week from each landscape replicate; we then averaged those values for the eight weeks of observations. Data were log\textsubscript{10} (bamboo pot diameter, water temperature, conductivity) or angular (percentage of landscape type) transformed prior to statistical analyses. A discriminant function analysis (DFA) was performed in SPSS software (SPSS Inc., Chicago, IL) to examine whether the composition and abundance of mosquito species per landscape class (forest, LDH, HDH) could be used to discriminate (classify) landscape classes. This analysis provides a measure of mosquito community similarity within landscape classes and the divergence of the mosquito community among landscape classes. A cross-validation procedure was used to estimate classification error. The standardized coefficients for discriminant functions were used to determine the contribution of each mosquito species to habitat separation. By plotting DFA scores for the first two discriminant functions, we examined...
the spread of samples among habitats and the results of the classification.

Canonical correspondence analysis (CCA) was used (CANOCO 4)\textsuperscript{28} to determine the amount of variation in the mosquito data that could be explained by various environmental variables, including elevation above sea level as a covariate. Statistical validity of resulting environmental axes, the model, and the selected environmental variables explaining the variation of mosquito species abundance were evaluated by means of unrestricted Monte Carlo permutation tests ($n = 999$).

**RESULTS**

A total 18,146 larvae of the following species was collected in the bamboo pots: 8,440 *Ae. aegypti* (L.), 3,104 *Ae. mediocivittatus* (Coquillett), 5,045 *Cx. quinquefasciatus* Say, 1,465 *Cx. antillummagnorum* Dyar, and 92 *Toxorhynchites portoricensis* (Roeder). More mosquitoes were captured in the bamboo pots located in LDH (55.7\%) than in HDH (27.0\%) or in forests (17.3\%). *Aedes aegypti* predominated in HDH and LDH areas of urban settings, whereas *Cx. quinquefasciatus* did so in corresponding suburban areas (Figure 3). *Aedes mediocivittatus* was the most prevalent mosquito species in rural forests, but it was also present in LDH areas of urban, suburban and rural areas (Figure 3). *Culex antillummagnorum* and *Tx. portoricensis* occurred at low densities and were more common in LDH and forests. It would appear that *Ae. aegypti* and *Cx. quinquefasciatus* were negatively correlated in bamboo pots (Figure 3), but the correlation analysis between the abundance of the two species in the bamboo pots per sampling site was not significant (Spearman $r_s = 0.29$; $n = 27$; $P > 0.05$).

Discriminant function analysis showed that most samples (96.6\%) could be correctly assigned to each landscape category (HDH, LDH, F) based on their mosquito species composition. The first discriminant function was highly correlated with *Ae. mediocivittatus*, whereas the second function was correlated with *Ae. aegypti*. The smaller values of Wilks’ lambda statistic indicated greater discriminatory ability of the first function (Table 2). Significant chi-square values indicated that the discriminant function did better than chance at separating the landscape classes ($P < 0.001$). The DFA plot showed that the first function separated HDH areas from both, LDH and forests, where *Ae. mediocivittatus* predominated (towards the right of Figure 4). The second function almost completely separated urbanized areas (toward the top of Figure 4) from forests (bottom of Figure 4) based on mainly the prevalence of *Ae. aegypti*. These results underscored the uncommon occurrence of *Ae. mediocivittatus* in HDH areas and the uncommon occurrence of *Ae. aegypti* in forested areas.

Canonical correspondence analysis of the relationship between mosquito species abundance and habitat variables showed significant results ($F = 2.08$; $P < 0.05$; Figure 5), with the first two axes accounting for 87% of the explained species-environment relationship. The first CCA axis reflected a gradient in major environmental variables from urban areas with HDH associated with higher water temperature in the bamboo pots and abundance of *Ae. aegypti* (left of Figure 5) to rural, forested areas at higher elevations, lower water temperaure, and prevalence of *Ae. mediocivittatus*, *Tx. portoricensis*, and *Cx. antillummagnorum* (right of Figure 5). Suburban areas fell in between those extremes with their typical LDH developments and prevalence of non-forest vegetation, which was also associated with elevation and *Cx. quinquefasciatus* abundance (bottom of Figure 5). The second CCA axis was mostly related to the aquatic variables of the bamboo pots, which showed that *Cx. quinquefasciatus* immature forms were more abundant in bamboo pots with larger water volume and diameter and higher pH and conductivity (Figure 5).

**DISCUSSION**

*Aedes aegypti* and *Cx. quinquefasciatus* were the most prevalent mosquitoes in urban and suburban areas of San Juan. *Aedes aegypti* was the dominant species in areas of HDH in urban areas, whereas *Cx. quinquefasciatus* was more prevalent in suburban areas (Figure 3). Average number of *Ae. aegypti* and *Cx. quinquefasciatus* larvae per bamboo pot had an inverse relationship in HDH and LDH of urban and suburban areas (Figure 3). A natural question is whether that...
pattern is due to interspecific larval competition in the bamboo pots. Because we removed all larvae from the bamboo pots every week, there probably was little opportunity for competitive interactions to operate between samplings. Also, a correlation analysis between the abundance of these species per site was not significant, possibly indicating that factors foreign to the bamboo pots could have caused such a contrasting pattern. Candidate factors to explain this pattern could be the presence of sewage systems in urban areas leading to low Cx. quinquefasciatus populations, compared with suburban areas where septic tanks are common.

The native mosquitoes (Ae. mediovittatus, Cx. antillum-magnorum, and Tx. portoricensis) were more common in less urbanized areas, mainly in forests and places with LDH, especially in suburban and forested areas. These indigenous mosquitoes use natural containers as their immature aquatic habitats (bamboo internodes, treeholes, leaf axils of bromeliads), and it is natural that they would be found in less urbanized areas, but they have also been collected in artificial containers. Therefore, native mosquito species overlap with cosmopolitan urban mosquitoes, but each species is segregated along a gradient in urban disturbance. The discriminant analysis clearly showed that the composition of mosquito species separated the three main land use classes (Table 2 and Figure 4). Ae. mediovittatus was a rare mosquito at places with HDH in urban and suburban areas, and Ae. aegypti was a rare mosquito in forests of suburban and rural areas. Otherwise, these two species consistently overlapped in areas with LDH in urban, suburban and rural areas (Figure 3). Commonness of Ae. mediovittatus in LDH was most likely related to the presence of trees. Discriminant function analysis has been useful to characterize terrestrial habitats based on the composition of mosquito species and to identify key environmental variables associated with the presence or absence of mosquito species.

Variables involved in the segregation of mosquito species along an environmental gradient in urban San Juan were well illustrated by the results of CCA (Figure 5). The first CCA axis depicted the terrestrial habitat or land use gradient, from an extreme with HDH in urban areas and dominance of Ae. aegypti, passing through suburban areas with prevalence of Cx. quinquefasciatus, to the other end where forests, Ae. mediovittatus and the other native species prevailed. Ae. aegypti was positively associated with water temperature, which reflected the greater exposure to the sun of bamboo pots in highly urbanized areas, whereas Ae. mediovittatus showed a negative correlation with water temperature, which is to be expected in forested areas. The second CCA axis represented a micro-habitat gradient, where variables of the aquatic habitat in bamboo pots separated the mosquito species. Culex quinquefasciatus was separated from the other mosquito species because of its preference for colonizing bamboo pots with larger diameter and water volume and higher conductivity and pH values (Figure 5). Canonical correspondence analysis

<table>
<thead>
<tr>
<th>Discriminant variables</th>
<th>Correlation coefficients between variables and functions</th>
<th>Function 1</th>
<th>Function 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes mediovittatus</td>
<td>0.81*</td>
<td>−0.19</td>
<td></td>
</tr>
<tr>
<td>Culex antillum-magnorum</td>
<td>0.26*</td>
<td>0.05</td>
<td></td>
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<tr>
<td>Aedes aegypti</td>
<td>0.05</td>
<td>0.70*</td>
<td></td>
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<tr>
<td>Culex quinquefasciatus</td>
<td>0.17</td>
<td>0.26*</td>
<td></td>
</tr>
<tr>
<td>Toxorhynchites</td>
<td>0.22</td>
<td>−0.23*</td>
<td></td>
</tr>
</tbody>
</table>

Summary statistic

- Wilk’s lambda: 0.16 for Function 1, 0.47 for Function 2
- Chi square: 40.22 for Function 1, 16.51 for Function 2
- P: <0.001 for both functions
- Eigenvalues (%): 63.4 for Function 1, 36.6 for Function 2
- Canonical correlation: 0.81 for Function 1, 0.73 for Function 2

* Coefficients with largest absolute correlation between each species and discriminant functions.
has been useful for ordering mosquito species along environmental gradients and to describe the relationships between mosquito species and sets of complex environmental variables.\textsuperscript{29,31} Observed spatial segregation between container \textit{Aedes} in this study resembles patterns reported for \textit{Ae. aegypti} and \textit{Ae. albopictus} in recent studies,\textsuperscript{14,15} which showed that \textit{Ae. aegypti} dominated in highly urbanized areas, \textit{Ae. albopictus} predominated in rural areas, and both species overlapped in suburban areas. Our study allowed us to more finely separate types of urban habitats, showing that the overlap between \textit{Ae. aegypti} and \textit{Ae. mediovittatus} was greatest in areas of LDH. This type of terrestrial habitat was characterized by having tall vegetation and LDH in urban, suburban and rural areas of San Juan.

Container mosquito species vary in their domesticity, and \textit{Ae. aegypti} seems to be well adapted to the environment of crowded tropical cities. Although \textit{Ae. aegypti} is the dominant container mosquito in highly urbanized areas, the species is not restricted to this type of habitat. In our study, \textit{Ae. aegypti} was rather abundant in other urban habitats of San Juan, with the exception of forested areas (Figure 3). In Buenos Aires, \textit{Ae. aegypti} oviposition was higher in areas of HDH but lower in areas with the highest human densities living in apartment buildings.\textsuperscript{52} Thus, success of \textit{Ae. aegypti} in highly modified, crowded urban areas is possibly related to its capacity to exploit a variety of terrestrial environments with or without a tree cover. Conversely, highly disturbed urban centers would not seem to attract forest mosquitoes such as \textit{Ae. mediovittatus} or \textit{Ae. albopictus} possibly because of a lack of vegetation, which may be an indicator for treeholes, high humidity, and vertebrate hosts. We have observed in the laboratory that \textit{Ae. mediovittatus} requires a high humidity environment to survive in the adult stage. Thus, the distribution of \textit{Ae. aegypti} conforms to that of a generalist species, which is adapted to exploiting a variety of environmental conditions associated with humans, whereas \textit{Ae. mediovittatus} appears as a specialist forest mosquito that is dependent upon the presence of vegetation and its associated resources, and not necessarily associated with humans.

The co-occurrence of the main dengue vector \textit{Ae. aegypti} with \textit{Ae. mediovittatus}, a potential inter-epidemic dengue virus reservoir,\textsuperscript{24} in LDH of urban, suburban, and rural areas may indicate that those are the sites with the greatest potential for dengue viruses passing from infected humans to biting \textit{Ae. mediovittatus} females. Given the high rates of vertical transmission for all four dengue serotypes in this mosquito species, areas of LDH might then be the most likely places where to investigate whether \textit{Ae. mediovittatus} acts as a virus reservoir or inter-epidemic vector. If dengue viruses are vertically maintained in the \textit{Ae. mediovittatus} populations, then eventual infections in humans could occur without further virus introductions. Thus, current results contributed to identifying the likely places where this process may be occurring.

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Authors’ addresses: Jonathan Cox, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520, E-mail: jonathan.cox@yale.edu. Maria E. Grillet, Laboratorio de Biología de Vectores, Instituto de Zoológica Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas 1041-A, Venezuela. E-mail: mgrillet@ciens.ucv.ve. Olga M. Ramos, International Institute of Tropical Forestry, United States Department of Agriculture Forest Service, Jardín Botánico Sur 1201 Calle Ceiba, Río Piedras, PR 00926-1119, E-mail: oramos@fs.fed.us. Manuel Amador and Roberto Barrera, Dengue Branch, Centers for Disease Control and Prevention, 1324 Calle Cañada, San Juan, PR 00920, Telephone: 787-706-2399, Fax: 787-706-2496, E-mails: mamador@cdc.gov and rbarrera@cdc.gov.

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