Co-occurrence Patterns of the Dengue Vector *Aedes aegypti* and *Aedes mediovitattus*, a Dengue Competent Mosquito in Puerto Rico

Eliza Little[1], **Roberto Barrera**[2], **Karen C. Seto**[3], and **Maria Diuk-Wasser**[1]

[1]Yale School of Public Health and Yale School of Forestry and Environmental Studies, New Haven, CT

[2]Dengue Branch, Division of Vector-borne Infectious Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico

[3]Yale School of Forestry and Environmental Studies, New Haven, CT

Abstract

Aedes aegypti is implicated in dengue transmission in tropical and subtropical urban areas around the world. *Ae. aegypti* populations are controlled through integrative vector management. However, the efficacy of vector control may be undermined by the presence of alternative, competent species. In Puerto Rico, a native mosquito, *Ae. mediovittatus,* is a competent dengue vector in laboratory settings and spatially overlaps with *Ae. aegypti*. It has been proposed that *Ae. mediovittatus* may act as a dengue reservoir during inter-epidemic periods, perpetuating endemic dengue transmission in rural Puerto Rico. Dengue transmission dynamics may therefore be influenced by the spatial overlap of *Ae. mediovittatus*, *Ae. aegypti*, dengue viruses, and humans. We take a landscape epidemiology approach to examine the association between landscape composition and configuration and the distribution of each of these *Aedes* species and their co-occurrence. We used remotely sensed imagery from a newly launched satellite to map landscape features at very high spatial resolution. We found that the distribution of *Ae. aegypti* is positively predicted by urban density and by the number of tree patches, *Ae. mediovittatus* is positively predicted by the number of tree patches, but negatively predicted by large contiguous urban areas, and both species are predicted by urban density and the number of tree patches. This analysis provides evidence that landscape composition and configuration is a surrogate for mosquito community composition, and suggests that mapping landscape structure can be used to inform vector control efforts as well as to inform urban planning.

Keywords

Correspondence to: Eliza Little, eliza@elizalittle.com.

Introduction

Dengue is a vector-borne virus transmitted by the bites of infected mosquitoes, mainly *Aedes aegypti* (Hotez et al. 2008; Kyle and Harris 2008; WHO 2009). It is an acute febrile disease causing 50–100 million cases per year and for which more than 2.5 billion people are at risk (WHO 2009). Dengue incidence increased 30-fold in the last 50 years (CDC 2009), coinciding with rapid urbanization. Given the close link of the vector to urban areas (WHO 2009), dengue incidence is expected to rise with increasing urban population, especially in developing countries where urban growth outpaces government's capacity to provide infrastructure (Barrera et al. 1993, 1995; Troyo et al. 2009).

Individuals who acquire dengue develop immunity to the infecting serotype, but subsequent infection with a different serotype can lead to the more severe forms of dengue, including dengue hemorrhagic fever and dengue shock syndrome (Kendall et al. 2009). Due to this complication of multiple viral serotypes and immunological responses to sequential coinfection, there is currently no vaccine for dengue (CDC 2009); however, a tetravalent vaccine is currently in clinical trials (Morrison et al. 2010). With or without a vaccine, best practices for dengue prevention are integrated vector control methods including reduction of vector-producing containers and community education and participation (Gubler 2006). Sustaining community participation strategies in inter-epidemic years is difficult, though integral to the success of these programs (Lloyd 2003). By identifying areas of high dengue transmission risk, resources can be focused to the areas of greatest need (Wilson 2002).

Aedes aegypti lives in close association with humans and preferentially bites human hosts (Harrington et al. 2001; Kyle and Harris 2008; CDC 2009). Given its key role on the emergence of epidemic dengue in urban areas, vector control methods have focused on controlling *Ae. aegypti* by elimination of domestic and peridomestic water filled artificial containers that produce mosquitoes. However, the efficacy of vector control methods may be limited by the presence of alternate invasive or native container breeding species. In Puerto Rico, *Ae. mediovittatus* may be implicated in the transmission of dengue based on the laboratory observations that it feeds on humans, has greater vector competence and rates of vertical transmission of dengue viruses than *Ae. aegypti* (Gubler et al. 1985; Freier and Rosen 1988). *Aedes mediovittatus* is a native tree hole mosquito and is closely associated with arboreal vegetation (Cox et al. 2007). It has been proposed that *Ae. mediovittatus* can act as a reservoir in the maintenance of dengue viruses during inter-epidemic periods in rural areas with low human population density (Gubler et al. 1985). Areas where *Ae. aegypti* and *Ae. mediovittatus* co-occur may be at higher risk for dengue persistence between epidemics. The rationale is that: (1) during epidemics, high virus amplification by *Ae. aegypti* would increase the likelihood of *Ae. mediovittatus* becoming infected; (2) dengue viruses would persist in these areas during inter-epidemic periods due to the high rate of vertical transmission observed in *Ae. mediovittatus* in the laboratory; and (3) dengue transmission by *Ae. mediovittatus* to humans and *Ae. aegypti* areas of overlap could catalyze another epidemic. While targeting areas of high *Ae. aegypti* production is important during epidemic periods, habitats of high *Ae. mediovittatus* production may be better targets during inter-epidemic periods. Our aim in this study is to develop a predictive model of areas where both *Aedes* species overlap to conduct intensive sampling of *Ae. mediovittatus* and assess

infection with DENV. Moreover, identifying the areas where these two species overlap is critical to focus interventions to curb future dengue epidemics.

The spatial overlap of adult *Ae. mediovittatus*, *Ae. aegypti,* dengue viruses, and humans in Puerto Rico has not been determined (Cox et al. 2007). In Puerto Rico, immature *Ae. aegypti* have been shown to occur close to houses (99%) while *Ae. mediovittatus* were more likely to occur far from houses (Moore 1983). In San Juan, Puerto Rico, immature *Ae. aegypti* and *Ae. mediovittatus* were found to segregate across an urban gradient (Cox et al. 2007) with *Ae. aegypti* found in areas with higher housing density (Smith et al. 2009). However, *Ae. mediovittatus* was found in containers within San Juan, particularly in areas of lower density housing and forested areas (Cox et al. 2007; Smith et al. 2009) and at the peak of the rainy season when most dengue viral disease transmission occurs (Smith et al. 2009). *Ae. mediovittatus* requires high humidity to survive as an adult (Cox et al. 2007) which may explain why it prefers vegetated areas.

Research utilizing remote sensing for the study of dengue has been limited (Eisen and Lozano-Fuentes 2009). There are four proposed explanations for the limited use of mapping, including remote sensing, for the study of dengue risk: (1) traps for adult *Ae. aegypti* had not been efficacious and emphasis has been placed on sampling immatures (Eisen and Lozano-Fuentes 2009); (2) dengue researchers have focused on the importance of small containers to determine the risk of dengue, precluding the utility of remote sensing which has not been shown to be able to discern containers at the fine scale needed (Moloney et al. 1998; Eisen and Lozano-Fuentes 2009); (3) environmental characteristics such as the normalized difference vegetation index (NDVI) may only be relevant in locations where containers are rain filled and trees prevent evapotranspiration rather than filled and maintained by humans (Eisen and Lozano-Fuentes 2009); and (4) the risk of dengue outbreaks is influenced not only by the abundance of mosquitoes but also the serotype specific herd immunity of the population (Eisen and Lozano-Fuentes 2009).

These concerns have resulted in a focus on epidemiological approaches rather than entomological or ecological. There are, however, exceptions. Remote sensing has been used to map landscape features such as tidiness and shade levels of backyards as predictors of suitable *Ae. aegypti* breeding habitats (Tun-Lin et al. 1995; Moloney et al. 1998) and to map environmental conditions, temperature, and precipitation, using NDVI as a proxy for precipitation (Estallo et al. 2008). Remote sensing has also been used to map dengue transmission risk by mapping land use change in Thailand (Vanwambeke et al. 2007) and landscape structure in Hawaii (Vanwambeke et al. 2011). Still there are few studies using remote sensing at high spatial resolution to assess *Ae. aegypti* habitat in urban areas (Troyo et al. 2009).

Here we used high resolution remote sensing to map elements of urban structure, tree cover and built-up areas, to predict *Ae. aegypti* within urban areas as has been done in previous studies (Troyo et al. 2008; Fuller et al. 2010). The goal of this research is to determine environmental features associated with high abundance of *Ae. aegypti* and *Ae. mediovittatus* at different spatial scales and identify and map the environmental conditions that result in high levels of co-occurrence. Since immature mosquitoes have been shown to segregate

along an urbanization gradient (Cox et al. 2007), we hypothesize that *Ae. aegypti* will be more closely associated with urban areas while *Ae. mediovittatus* will be more likely to be found in forested areas. We used novel BG-sentinel traps previously shown to be efficacious at collecting adult *Aedes* spp. mosquitoes (Kroeckel et al. 2006; Maciel de Freitas et al. 2006; Williams et al. 2006) and data from a newly launched satellite of high spatial and spectral resolution to characterize features of the urban environment predictive of habitat segregation of the two *Aedes* species. Mapping areas of co-occurrence will identify areas that will be used to refine a trapping scheme aimed at determining the role of *Ae. mediovittatus* in the transmission dynamics of dengue in Puerto Rico.

Methods

Mosquito Collection

The study was conducted from May to August 2010 in the municipality of Patillas in South-Eastern Puerto Rico. The selection of Patillas as a study site is based on the availability of preliminary data indicating the presence of both *Ae. aegypti* and *Ae. mediovittatus*. Dengue is endemic in Puerto Rico, all four serotypes have been involved with epidemics occurring every 2–3 years since before 1985 (Gubler et al. 1985) and surveillance in Patillas has shown that dengue is endemic to this area (CDC, unpublished).

Sampling was conducted in nine study sites, representing the highest density of urban development at three different altitude levels in Patillas, however, because of the presence of cloud cover in the satellite image over two of the sites, we report our findings only for seven of the study areas (Fig. 1). 90 BG-Sentinel traps were used to capture adult mosquitoes in the rainy season, between May and August 2010. Mosquito collection ran for 3 days: the traps were set on Monday with collection Tuesday through Thursday. A second sampling round was completed for each BG location at each study site 1 month later. All traps were baited with BG-Lure®, which contains non-toxic substances specifically found on human skin including ammonia, lactic acid, and fatty acids (Bhalala and Arias 2009). BG-Sentinel traps are highly effective at collecting host seeking adult mosquitoes especially *Ae. aegypti* (Kroeckel et al. 2006; Williams et al. 2006; Williams et al. 2007). A study in Brazil comparing BG-Sentinel traps to CDC backpack aspirators found that BG-Sentinel traps collected significantly more *Ae. aegypti* adult male and female mosquitoes than CDC aspirators (Maciel de Freitas et al. 2006). Trap sites were selected by locating one house per 100 m^2 sampling grid. The distance between sampling sites is based only on the flight range of *Ae. aegypti* (Reiter et al. 1995; Honorio et al. 2003) because the flight range of *Ae. mediovittatus* remains unknown. Trap placement was restricted to areas in close proximity to buildings to only include areas that were inhabited and where dengue transmission was likely to occur.

Land Cover/Land Use Classification and Characterization of Landscape Patterns

We used data acquired by the WorldView2 satellite sensor, launched on October 8, 2009. A WorldView2 scene was acquired on 25 March 2010. WorldView2's features include high spatial resolution of 1.82 m (2 m output pixels) and 8-band spectral resolution. The high spatial resolution provided by WorldView2 is important for analyzing the fine-scale

environmental drivers of these mosquito species, 8 bands provide higher ability to discriminate among features than the 4 bands available for other high resolution sensors.

We collected ground-based site-specific information on land cover types around BG-Sentinel traps by annotating printed satellite images. 'Regions of interest' composed of individual pixels were manually entered into ENVI, Environment for Visualizing Images (ITT 2011) for supervised classification training and testing. Seventy-five percent of pixels were used for training and 25% for testing the classification. Of the nine original study sites, two, Jagual and Pueblo, were occluded by clouds and excluded from the study. The remaining seven study sites were classified individually using an iterative approach. The first step was to mask out shadows, defined as those pixels with the lower 10% value for the WorldView2's blue band digital number (DN). Secondly, an urban mask was defined by an upper bound of NDVI equal to 0.4, to include the diverse urban structures in Patillas. Finally, a maximum-likelihood algorithm was used to perform a supervised classification of vegetation into four categories: bare soil, grass, scrub, and trees. Bare soil was defined as exposed soil in urban areas and very sparsely vegetated areas. The grass category was low vegetation. The scrub category was primarily deforested areas that have thick regenerated cover but lack tall vegetation (trees). The tree category included single trees as well as forested areas.

Landscape metrics were derived from the above classification using a combination of programs: ArcMap (ESRI 2011), Geospatial Modeling Environment (GME) (Beyer 2011), and FRAGSTATS (McGarigal et al. 2002). GME was used to clip the classified image into the areas around each trapping location (the 'landscape patch') at three spatial scales (25, 50, and 100 m) around each BG trap and then FRAGSTATS was used to calculate landscape metrics at those scales. The 25 m landscape patches represent the house-specific environmental factors. The larger landscape patches characterize environmental factors operating at larger spatial scales, such as the presence of a larger forested area. For each landscape patch, the total number of patches of each class, the class area distribution statistics, and a cohesion metric were calculated using FRAGSTATS for the two focal classes (forest and urban). The number of patches is a simple measure of the extent of fragmentation of the class-patch type; the class-patch area distribution metrics quantify the level of fragmentation for each focal class. Finally, the cohesion metric for the two main focal classes, forest and urban, characterize the configuration of the class-patches.

$$COHESION = \left[1 - \frac{\sum\limits_{j=1}^{n} Pij}{\sum\limits_{j=1}^{n} Pij \sqrt{aij}} \right] \cdot \left[1 - \frac{1}{\sqrt{A}} \right]^{-1} \cdot (100)$$

where P is the class-patch perimeter, a is the class-patch area, and A is the total number of cells in the landscape patch (McGarigal et al. 2002). Cohesion is a percentage ranging in values from 0 to 100. As the value approaches zero the proportion of the landscape patch comprised with the focal class-patch decreases and becomes increasingly subdivided and less connected (McGarigal et al. 2002).

Housing density was derived from a digitized point shapefile of all structures in Patillas, Puerto Rico, derived from the Internal Revenue Agency of Puerto Rico. These points were subjected to kernel density estimation using the spatial analyst toolbox of ArcMap. To calculate the mean density at each spatial scale, ArcMap zonal statistics tool was used.

Weather Data

To account for temporal changes in weather parameters between samplings and to examine the response of each mosquito species to these variables, we operated three weather stations at three elevations (Jagual, Recio, and Quebrada Arriba) (Hobo Micro Station; Onset Computer, Bourne, MA). Rainfall is an important factor because its accumulation is necessary for larval development of mosquitoes. At each mosquito collection observation, the accumulated rainfall during weeks 3 and 2 before sampling was recorded. The week prior to sampling is omitted because immature development lasts 7 days and so this rainfall does not contribute to the number of adult mosquitoes. Seasonal patterns related to temperature and rainfall are reflected in the breeding patterns of the vectors and dispersal of dengue (Moore et al. 1978; Honorio et al. 2009; Kyle and Harris 2008).

Statistical Analyses

Population-averaged panel-data models (xtgee procedure) were run in STATA (StataCorp 2007) to model the association of mosquito counts or presence/absence and environmental parameters. Generalized estimating equations (GEE) are widely used in the analysis of longitudinal data in epidemiologic studies (Cui 2007) and spatially explicit data in ecological studies (Kraan et al. 2010). The xtgee procedure is suited to fit generalized linear models when there is within-group correlation structure for the panels (StataCorp 2011). To account for the possibility of temporal and/or spatial within-group correlation we specified both the location of the BG-Sentinel traps as well as the date of trapping. GEE are based on quasi-likelihood theory, therefore traditionally used Akaike's information criterion (AIC) cannot be used to compare models (Pan 2001). Pan (2001) proposed an alternative method called the Quasi likelihood under Independence model Criterion (QIC), which can be used to determine the best set of covariates to explain an outcome (Pan 2001; Cui 2007).

We used a GEE model with binomial family, logit link, two panel variables location and date, and an independent correlation structure to assess the landscape features predictive of the distribution of *Ae. aegypti* and *Ae. mediovittatus* and those that are predictive of co-occurrence. We calculated two-category variables for *Ae. aegypti* and *Ae. mediovittatus* splitting the distribution of *Ae. aegypti* at the mean to create a high/low variable and splitting the distribution of *Ae. mediovittatus* to presence/absence. We assessed the predictive value of individual variables through a univariate analysis using QIC values. To avoid including collinear variables in the model, correlated variables ($P < 0.01$) were compared in the univariate analysis and the variable with higher QIC was excluded from the final model. To determine the influence of scale, we ran models with the best set of covariates at each scale as well as a mixed scale model using the best set of covariates across all three scales. We used QIC to determine the models with the best set of covariates to explain the outcomes of interest: presence of high numbers of *Ae. aegypti* compared to low numbers; presence of *Ae. mediovittatus* compared to absence; and co-occurrence of high numbers of *Ae. aegypti*; and

presence of *Ae. mediovittatus*. We calculated Moran's I spatial autocorrelation statistics based on the regression residuals to determine if the covariates explained all of the model's variation.

Predictive Map

The final predictive model was then mapped using ArcMap to determine risk of co-occurrence across the study area. We generated raster grids for the predictive variables. Using the spatial analyst tool raster calculator we then ran the regression equation for each pixel resulting in a risk map of co-occurrence throughout the study area with resolution of 2 m.

Results

We obtained 1,260 mosquito collections from 235 trapping sites, with an average of 5.4 collections per trap site (range 2–6). Over the entire sampling period we collected 7,057 males and 8,787 females (0.8♂:1♀) of *Ae. aegypti* and 92 males and 1,090 females (0.08♂: 1♀) of *Ae. mediovittatus*. Average numbers of *Ae. aegypti* adults per trap per day were higher than those of *Ae. mediovittatus* in most study sites, with the exception of Mulas (Table 1). The two localities with the highest densities of *Ae. aegypti* per trap, such as Lamboglia and Quebrada Arriba also showed the highest densities of *Ae. mediovittatus* (Table 1). The percentages of traps with both species (co-occurrence) also were the highest in Lamboglia and Quebrada Arriba (Table 1). Co-occurrence was minimal in the two localities with the lowest densities (Mamey and Mulas). These areas received the lowest levels of rainfall that were observed during the field study (Table 1). Other mosquito species collected in BG traps during the study were *Aedes taeniorhynchus*, *Ae. tortilis*, *Culex bahamensis*, *Cx. quinquefasciatus*, *Cx.antil-lummagnorum*, and *Toxorhynchites portoricensis*.

Satellite image classification accuracy ranged from 80 to 99% with associated kappa coefficients of 0.73 to 0.9 (Fig. 1). For all three outcomes (presence of high numbers of *Ae. aegypti* compared to low numbers, presence of *Ae. mediovittatus* compared to absence, and co-occurrence of high numbers of *Ae. aegypti* and presence of *Ae. mediovittatus*), we found that mixed scale models had the best predictive values, indicated by lowest QIC scores (Table 2). For *Ae. aegypti*, the model with the best fit included the covariates number of tree patches within 50 m, housing density within 25 m, rainfall, and temperature and was statistically significant ($\chi^2 = 273$, df = 5, $P < 0.001$). For *Ae. mediovittatus*, the final model included number of tree patches within 100 m, urban patch area within 50 m, rainfall, and temperature and was statistically significant ($\chi^2 = 62$, df = 5, $P < 0.001$), The overall model predicting co-occurrence (statistically significant ($\chi^2 = 137$, df = 5, $P < 0.001$)) included the covariates housing density within 25 m, the number of tree patches within 100 m, and rainfall.

We used Receiver Operating Characteristic (ROC) to plot the true positive rate (sensitivity) as a function of one minus the false positive rate (specificity) (STATA SE 10 2007). The model predicting *Ae. aegypti* results in an AUC value of 0.83, suggesting that a random sample of positive observations has a test value larger than that of negative observations

83% of the time (mediovittatus 64% of the time; co-occurrence 75% of the time). Moran's I statistic based on the regression residuals was not significant in five of the seven study sites, and significant for two, indicating some residual spatial structure not explained by the covariates specified.

To create an ecological risk map of co-occurrence across the study area we used the logistic regression model predicting co-occurrence. To create the raster grid for house density we calculated the kernel density at 25 m throughout the entire study area. To extract values of the number of tree patches over the entire study area we overlaid a 50 m × 50 m grid and calculated the number of tree patches in each grid cell. The map depicting risk of cooccurrence illustrates the heterogeneity across the landscape within the inhabited areas of Patillas, Puerto Rico (Fig. 2). The communities Lamboglia and Providencia show the greatest risk for co-occurrence of *Ae. aegypti* and *Ae. mediovittatus*. These are areas of higher urban density but also have a high number of tree patches.

Discussion

In this study, we identified density of urban and vegetation structure as important habitat features for *Ae. aegypti* and *Ae. mediovittatus* abundance and for their co-occurrence. The analysis of high resolution remotely sensed data enabled us to look at the scale at which the landscape metrics had the most influence on predicting mosquito distribution. *Ae. aegypti* is positively predicted by urban density but also by the number of tree patches. The scale at which these covariates predict *Ae. aegypti* distribution are localized to the immediate (25 m) urban environment and to an intermediate scale (50 m) for the influence of trees. *Ae. mediovittatus* is negatively predicted by the large urban areas at an intermediate scale (50 m) and positively predicted by the number of tree patches at a larger spatial scale of 100 m. The models suggest that *Ae. aegypti* may be more influenced by local environmental features, while *Ae. mediovittatus* may be more influenced by landscape structure at a coarser scale. The use of a model considering multiple scales simultaneously indicates the importance of landscape structure and composition at different spatial scales. However, the three scales analyzed are all relatively local. Further research should incorporate the influence of landscape structure and composition at a larger geographical scale.

The models showed significant positive associations of each mosquito species with accumulated rainfall (2–3 weeks before sampling) that must have resulted from an increased number of water-filled containers. Temperature was positively associated with high *Ae. aegypti* and negatively associated with presence of *Ae. mediovittatus*. This difference is likely associated with the main types of terrestrial habitats occupied by these species. For example, *Ae. mediovittatus* is more prevalent in areas with tall vegetation where air temperature should be lower than in built-up areas where *Ae. aegypti* is more abundant. Rainfall and temperature are important drivers of the population dynamics of *Ae. aegypti* (Moore et al. 1978) and, arguably more so, for *Ae. mediovittatus* (Gubler et al. 2001; Smith et al. 2009) as well as dengue in Puerto Rico (Johansson et al. 2009).

Overall the results of the model support previous findings about the habitat preferences of immature *Ae. aegypti* and *Ae. mediovittatus*, with *Ae. aegypti* preferring containers located

in high density urban areas, *Ae. mediovittatus* preferring lower density urban areas and both *Aedes* species overlapping in urban areas with high canopy cover (Cox et al. 2007). We determined that the same variables are associated with co-occurrence of adults. Previous studies on the influence of landscape structure on *Aedes* spp. distribution and abundance, habitat preferences, and community structure are limited (Rey et al., 2006). Heterogeneities of urban/built up structure have been linked to *Ae. aegypti* distribution (Troyo et al. 2009). Recent research on *Ae. albopictus* in Hawaii specifically investigated vegetation and urban structure using landscape metrics (Vanwambeke et al. 2011). Vanwambeke et al. (2011) incorporated residential density data, recreational areas, mosquito abundance, and environmental factors to identify areas with abundant mosquitoes and where humans actively visit to pinpoint areas of high transmission risk. They found that the areas with highest abundance of *Ae. albopictus* were in a mixture of urban/built up and vegetated areas (Vanwambeke et al. 2011). This result is remarkably similar to our findings for *Ae. mediovittatus*. *Ae. mediovittatus* has been likened to *Ae. albopictus*, ''the albopictus of the Caribbean'', due to similar habitat requirements (Gubler et al. 1985). Understanding the habitat preferences of *Ae. aegypti* and *Ae. mediovittatus* should guide research of habitat preferences and community structure of *Ae. aegypti* and *Ae. albopictus*.

The biotic interactions between larvae of *Ae. aegypti* and *Ae. mediovittatus* have not yet been investigated. *Ae. aegypti* and *Ae. albopictus* overlap during immature development in containers and when natural (leaf litter) resources are limited *Ae. albopictus* outcompetes *Ae. aegypti* (Barrera 1996). Research on land use change in Thailand found *Ae. aegypti* was limited to artificial containers in settled areas while *Ae. albopictus* was in artificial and natural containers in both settled and agricultural areas (orchards) (Vanwambeke et al. 2007). *Ae. aegypti* adaptation to urban life and increased dengue transmission in densely populated areas are cited as the main reasons for the emergence of dengue as a public health problem in the last 50 years (WHO 2009). Rapid urbanization that outpaces governmental capacity to maintain housing standards and provide reliable access to drinking water, trash collection, and excreta disposal are main drivers for dengue fever emergence (Barrera et al. 1993; Barrera et al. 1995; Troyo et al. 2009). *Ae. aegypti* is well adapted to crowded tropical cities, but has also been found in other urban habitats (Cox et al. 2007). Troyo et al. (Troyo et al. 2009) developed a model including tree cover and built-up areas and found that moderately built up residential areas with moderate tree cover were likely to have high numbers of habitats suitable for *Ae. aegypti* larvae and that an important portion of habitats containing mosquito larvae were not near houses. A study in Brazil found that *Ae. aegypti* oviposition was related to the urbanization gradient, indicating preference for certain resources at the rural urban interface (Carbajo et al. 2006). And, in Australia an increase in shade in backyards was linked to increased risk of immature *Ae. aegypti* (Tun-Lin et al. 1995). *Ae. aegypti* exploits a wide range of containers as developmental habitats from discarded rain-filled containers to anthropogenic water storage containers (Barrera et al. 2006; Kendall et al. 2009). Previous observations in Puerto Rico suggest that most containers with water suitable for mosquito development are outdoors (Moore et al. 1978) and in unattended rain-filled containers (Barrera et al. 2006). Containers filled with water and organic matter or under trees were found to produce the largest adult *Ae. aegypti*, which has faster development and better survival rates (Tun-Lin et al. 2000). Tree canopies may

provide benefits to larvae by reducing evapotranspiration and adding nutritious leaf detritus in rain filled containers (Barrera et al. 2006). Urban areas with high canopy cover may therefore be important targets for *Aedes* spp. vector control efforts.

The metrics developed in this study to map areas of cooccurrence will be employed to refine a trapping schedule aimed at determining the role of *Ae. mediovittatus* in the transmission dynamics of dengue in Puerto Rico. We speculate that in areas of co-occurrence *Ae. mediovittatus* will be more likely to carry dengue then in areas where it does not overlap with *Ae. aegypti*. However, this study is limited by the exclusion of human movement and human interaction with the environment as mechanisms of dengue transmission. Human movement is thought to be an important driver of dengue transmission and targeting key individuals and areas frequented by large numbers of people may improve disease prevention (Stoddard et al. 2009). Because *Ae. mediovittatus* prefers more forested areas it is possible that human movement between urban and rural areas facilitates transmission of dengue. If *Ae. mediovittatus* is incriminated as a competent dengue vector in the wild, future research should investigate where humans and *Ae. mediovittatus* come into contact.

Mapping areas of co-occurrence also has relevance to urban planning. The habitat of co-occurrence with a mix of urban and tree patches could be described as low-density development patterns that cut into forested areas. If *Ae. mediovittatus* is incriminated as a reservoir for dengue during inter-epidemic periods then areas of co-occurrence may be at higher risk for dengue emergence. If *Ae. mediovittatus* is involved in dengue transmission then it should be the object of vector control wherever it overlaps with humans.

The results also suggest that in rural areas like Patillas the intermixing of urban areas with tree patches positively predicts *Ae. aegypti* and reducing this type of development pattern may be important for reducing dengue transmission. The landscape of Puerto Rico is undergoing rapid transformation due to both urban expansion and forest recovery; Puerto Rico underwent conversion from an agricultural to an industrial economy 60 years ago (Martinuzzi et al. 2007). A total of 11% of the island is urban surfaces and a large portion of this development occurs in low-density patterns. Like other mountainous islands, urban areas are concentrated to the coasts and valleys, however, in Puerto Rico much of the low-density development occurs at the forest–urban interface (Martinuzzi et al. 2007). Land use change mediated by humans is linked to many vector-borne diseases (Patz et al. 2004) through the creation of new habitats, the expanding range of vectors, and changing mosquito community structure (Vanwambeke et al. 2007). Vanwambeke et al. (2007) challenge the theory that intact forest ecosystems regulate pathogens and disease, and suggest that some ecosystem services are actually ''disservices'' that create new nodes of disease transmission. In the case of Patillas, Puerto Rico the intermixing of urban with tree patches may result in such an ecosystem ''disservice.'' Puerto Rico is a ''window to the future'' for Caribbean islands because many Caribbean islands are only now making the transition from an agricultural economy (Martinuzzi et al. 2007). Informed urban planning that provides public infrastructure and minimizes sprawl through zoning initiatives such as urban growth boundaries may significantly reduce dengue transmission in Puerto Rico and provide a model for other Caribbean islands.

In conclusion, this research highlights the importance of landscape structure and composition for predicting *Aedes* mosquitoes and risk of dengue transmission. The use of high resolution remote sensing is key to determine the structure and composition of the landscape at a local scale. The use of high resolution or potentially medium-resolution remote sensing could be used to look at larger scale landscape patterns that influence the distribution of *Ae. aegypti* and *Ae. mediovittatus*. Further research should aim to clarify the occurrence and persistence of dengue in *Ae. mediovittatus* around areas of co-occurrence with *Ae. aegypti*. A landscape ecology approach contributes to our understanding of vector biology by adding the importance of landscape pattern to what has been previously elucidated about land cover types predictive of mosquitoes (e.g., NDVI). The importance of landscape pattern contributes to our understanding of vector control. Localized vector control efforts may fail if they do not consider the importance of urban development patterns. Future research should directly address how urban development patterns influence dengue epidemiology.

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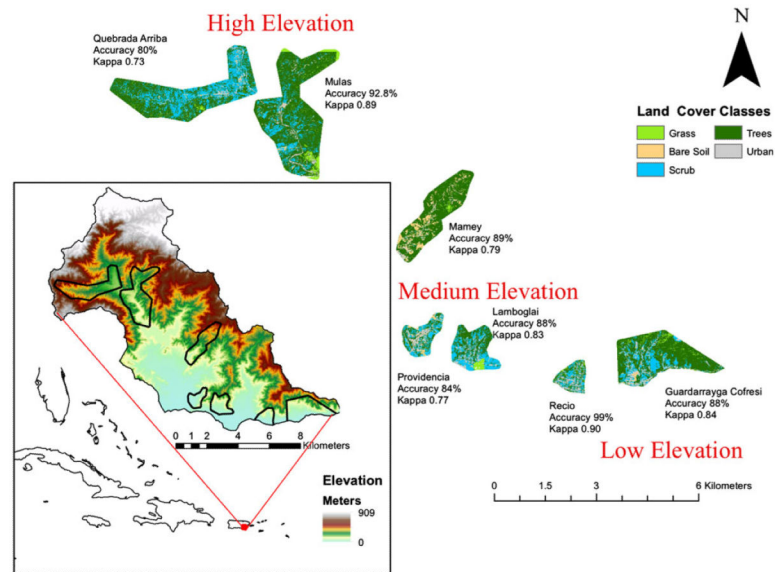
Figure 1.
Study area in Patillas municipality, southern Puerto Rico that shows the elevation above sea level (meters) and maximum likelihood classification of a WorlView2 satellite imageof the seven study sites broken down into elevation categories.
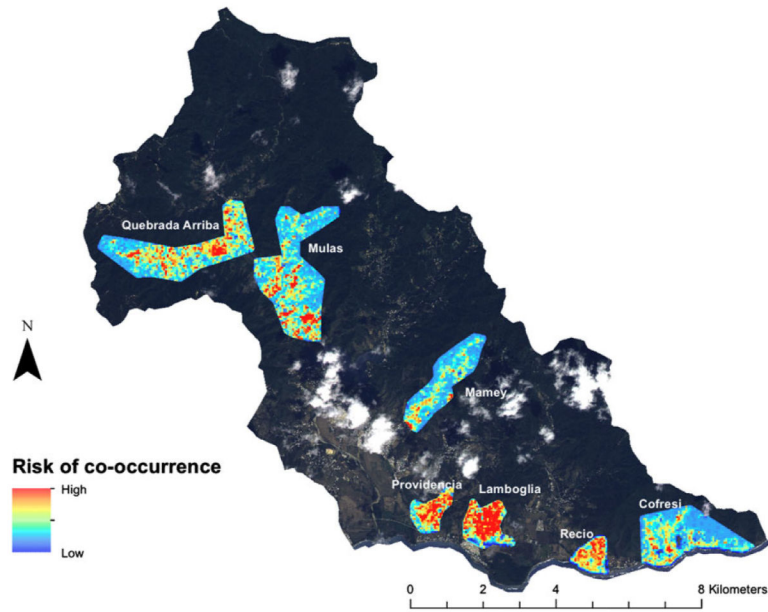
Figure 2.
Risk of co-occurrence for *Ae. aegypti* and *Ae. mediovittatus* predicted by urban density and the number of tree patches in Patillas, Puerto Rico May–August 2010.

Table 1

Abundance (± Standard Error) of *Aedes aegypti* and *Aedes mediovittatus* and Their Co-occurrence (Percentage of Traps with Both Species) in BG-Sentinel Trap Per Day, Average Air Temperature, and Accumulated Rainfall Per Study Site in Patillas Municipality, Puerto Rico Between May and August 2010

Study site/species	*Aedes aegypti*	*Aedes mediovittatus*	Co-occurrence (%)	Air temperature (°C)	Rainfall (mm)
Cofresi	14.15 ± 2.97	0.77 ± 0.10	31.7	28.36 ± 0.01	163.25 ± 4.43
Lamboglia	26.44 ± 4.60	1.71 ± 0.70	57.9	28.14 ± 0.001	121.27 ± 0.50
Mamey	0.52 ± 0.09	0.48 ± 0.07	10.2	27.34 ± 0.01	45.73 ± 0.01
Mulas	0.63 ± 0.10	0.74 ± 0.07	14.9	27.44 ± 0.02	43.80 ± 1.61
Providencia	11.25 ± 0.93	0.82 ± 0.11	42.2	28.14 ± 0.001	122.03 ± 0.60
Quebrada Arriba	15.71 ± 4.11	1.58 ± 0.19	59.5	26.61 ± 0.01	197.14 ± 7.52
Recio	14.96 ± 1.52	0.70 ± 0.09	33.2	28.36 ± 0.01	159.76 ± 4.84

Table 2

Mixed Scale Models Predicting Mosquito Distribution in Patillas, Puerto Rico May–August 2010

	Odds	Std. error	Z value	Pr (> z)	95% CI
Ae. aegypti					
(Intercept)	<0.001	3.214	−5.04	<0.001	(<0.001, <0.001)
Rain	1.004	0.001	5.56	<0.001	(1.003, 1.006)
Temp	1.618	0.115	4.20	<0.001	(1.292, 2.024)
TNP 50 m	1.017	0.004	4.03	<0.001	(1.009, 1.026)
Density 25 m	68.993	0.538	7.87	<0.001	(24.023, 197.947)
Ae. mediovittatus					
(Intercept)	48.231	2.114	1.83	0.06	(0.766, 3038.138)
Rain	1.001	0.001	2.24	0.025	(1.0002, 1.002)
Temp	0.837	0.077	−2.33	0.020	(0.720, 0.972)
TNP 100 m	1.004	0.001	4.21	<0.001	(1.002, 1.005)
UAM 50 m	0.130	0.446	−4.58	<0.001	(0.312, 0.974)
Co-occurrence					
(Intercept)	<0.001	3.655	−3.08	0.002	(<0.001, <0.001)
Rain	1.005	0.001	5.36	<0.001	(1.003, 1.007)
Temp	1.307	0.135	1.99	0.047	(1.004, 1.702)
Density 25 m	46.155	0.738	5.19	<0.001	(10.870, 195.980)
TNP 100 m	1.008	0.001	5.50	<0.001	(1.005, 1.011)

TNP tree number of patches, *UAM* urban area average weighted mean.