

Spatial Stability of Adult *Aedes aegypti* Populations

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Abstract. Vector control programs could be more efficient by identifying the location of highly productive sites of *Aedes aegypti*. This study explored if the number of female adults of *Ae. aegypti* in BG-Sentinel traps was clustered and if their spatial distribution changed in time in two neighborhoods in San Juan, Puerto Rico. Traps were uniformly distributed across each neighborhood (130 m from each other), and samples were taken every 3 weeks. Global and local spatial autocorrelations were explored. Spatial stability existed if the rank order of trap captures was kept in time. There was lack of global autocorrelation in both neighborhoods, precluding their stratification for control purposes. Hot and cold spots were identified, revealing the highly focal nature of *Ae. aegypti*. There was significant spatial stability throughout the study in both locations. The consistency in trap productivity in time could be used to increase the effectiveness of vector and dengue control programs.

INTRODUCTION

Vector control programs could be more efficient if the spatial locations of highly productive areas of *Aedes aegypti* were predictable. Several studies have reported that dengue vector abundance is highly heterogeneous, with some neighborhoods showing significantly higher infestation levels.^{1–3} Likewise, dengue incidence has been found to be highly variable, with a few areas having the largest values.^{4–8} For example, most dengue cases (70%) were reported from a relatively small fraction (35%) of all neighborhoods in a dengue hyperendemic city during 6 years of observations.⁴ It was observed throughout the period of study that the rank order of dengue incidence per neighborhood was kept practically unaltered between years. Also, neighborhoods having the largest dengue incidence were those neighborhoods with the highest average density of *Ae. aegypti* females per house, but vector abundance was also relatively large in neighborhoods with intermediate dengue incidence and correspondingly low in places with low dengue incidence.⁹ A distinct feature of the neighborhoods with the largest dengue incidence was that they also had the longest periods of uninterrupted dengue transmission (dengue persistence or endemicity). Thus, it is important to study the spatial and temporal dynamics of dengue transmission. This knowledge allows for the stratification of cities so that vector control programs can allocate their resources more effectively.^{4,10,11}

From the point of view of operational vector control, it would also be useful to understand the spatial and temporal patterns of mosquito abundance at finer geographical scales, such as city block or household levels. Getis and others¹² studied the spatial distribution of *Ae. aegypti* at the household level in two neighborhoods in Iquitos, Peru. They reported that *Ae. aegypti* adults clustered mostly at 10 m, with some degree of clustering up to 30 m. Chansang and Kittayapong¹³ found clusters of immature *Ae. aegypti* up to 20 m, and Getis and others¹² found clusters up to 10 m (households). Similarly, it has been reported that dengue cases cluster within households.^{14,15} Studies of space–time clustering of dengue cases showed clusters within and around households (< 10–15 m) and clusters that were close in time (3–6 days).^{16,17} Thus, both *Ae. aegypti*

and dengue cases seem to cluster at rather short distance and time. An important consequence of this highly clustered, local spatial pattern is that missing some houses during vector control operations can leave intact mosquito clusters that could repopulate the area. The primary question is whether the location of clusters can be determined in advance for operational vector control purposes. The household-level study of Getis and others¹² reported that most clusters of adult *Ae. aegypti* did not appear in the same places in the two surveys that they conducted 3 weeks apart. Strickman and Kittayapong¹⁸ reported that clusters of *Ae. aegypti* larvae in three villages in Thailand changed locations with the seasons. Pupal surveys conducted at two times of the year in a southern town in Puerto Rico showed that a significant number of households changed their status from producers (with pupae) to non-producers (without pupae) and *vice versa* between surveys.¹⁹ Thus, it would seem that the temporal instability of the spatial distribution of *Ae. aegypti* at very fine scales precludes the localization of highly productive premises that could be targeted for vector control.

The spatial dispersal of *Ae. aegypti* has been studied at the level of city blocks. For example, Fernandes and others² found clusters of immature *Ae. aegypti* (Breteau Index) comprising one to three blocks in Rio de Janeiro, Brazil, and they concluded that analyses at the neighborhood level did not allow for the detection of such aggregation. The size of city blocks varies within a city and between countries, but generally, they are around 100 m or more. Given that spatial autocorrelation seems to fade beyond 30 m for adult *Ae. aegypti* and at even shorter distances for immatures,¹² clusters of mosquitoes per block should reflect the contributions of highly productive households within blocks. Unfortunately, the temporal stability or predictability of block-level clusters has not been investigated, and investigation could inform if these clusters are useful to guide vector control operations. Investigating vector processes at the scale of hundreds of meters may prove useful. Vazquez-Prokopec and others²⁰ found that 95% of dengue cases reported within the first week of onset of symptoms of an index case occurred at less than 125 m from it during an outbreak in Cairns, Australia.

We recently investigated the temporal dynamics of female adults of *Ae. aegypti* in two neighborhoods with a history of dengue in San Juan, Puerto Rico (CDC, unpublished). In this study, BG-Sentinel traps (Biogents, Regensburg, Germany) were spaced slightly over 100 m from each other to minimize trap interaction that could interfere with independent

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estimations of vector density. Traps were operated every 3 weeks to minimize sampling the same mosquito cohorts. We took advantage of this setup to investigate if *Ae. aegypti* adults showed spatial clustering and determine if the spatial pattern of adult abundance within each neighborhood changed in time. A static spatial pattern of *Ae. aegypti* could be valuable for preemptive vector control measures. This study reports significant concordance in the rank orders of trap catches throughout the study, showing high temporal consistency or stability in the spatial pattern of *Ae. aegypti* females in both neighborhoods that may be useful for vector and dengue control operations.

MATERIALS AND METHODS

Study sites. The study was carried out in two neighborhoods of the Metro Area of San Juan, Puerto Rico: El Comandante (EC, 6,951 persons and 1,979 buildings; US Census 2000) and Villa Carolina (VC, 9,240 persons and 1,996 buildings). These two neighborhoods are 3 km apart and belong to the adjacent municipalities of San Juan (EC, 18°24'02" N, 65°59'30" W) and Carolina (VC, 18°23'52" N, 65°57'26" W). Rainfall in the San Juan area occurs year round, with a relatively short dry season (< 100 mm/month) between January and March and two rainy peaks around May and November. Total annual rainfall at the nearby Muñoz–Marín International Airport (5–7 km) during 2008 was 1,388 mm, and mean annual temperature was 27.0°C. The population dynamics of *Ae. aegypti* were investigated in these neighborhoods to eventually compare the impact of vector control measures (control versus intervention).

Carolina Municipality had a spatial insecticide spraying program (truck-mounted Ultra Low Volume [ULV] equipment) that was active throughout the study, whereas the San Juan Municipality used a similar insecticide spraying technique but only around notified cases of dengue. Thus, we believe that VC was subjected to a more frequent application of ULV insecticide spraying. However, we could not establish the frequency or coverage of insecticide spraying in either neighborhood because of insufficient data.

Adult *Ae. aegypti* mosquitoes. The study consisted of capturing *Ae. aegypti* adults using 40 BG-Sentinel mosquito traps baited with BG-Lure (lactic acid, ammonia, and caproic acid; Biogents, Regensburg, Germany) in each neighborhood from November 2007 to December 2008 (20 samples). Each trap was operated for 4 consecutive days every 3 weeks to avoid collecting female *Ae. aegypti* from the same adult cohort given that they are not expected to live beyond that time in the field.²¹ Collection bags were replaced every day, and batteries were replaced after 2 days of operation. Traps were uniformly distributed across each neighborhood, resulting in intertrap average distances of 132 m in EC and 137 m in VC. We calculated the average number of female *Ae. aegypti* captured per trap per day for each sample as a measure of relative abundance.

A Geographical Information System (GIS; ArcView 9.2; Esri, Redlands, CA) was developed for each neighborhood with the following geo-spatial layers: polygons showing house boundaries (Tax Revenue Agency, San Juan, Puerto Rico), points showing house centroids, lines representing streets, and house centroids representing trap locations. Thematic maps showing the number of *Ae. aegypti* females captured per trap

per day for each of the 20 samples were made to visually examine the spatial patterns over time.

The global Moran's *I* correlation coefficient²² was used to determine if the number of *Ae. aegypti* females per trap per day was spatially autocorrelated in each neighborhood. Spatial autocorrelation occurs when the numbers of mosquitoes per trap per day in nearby traps are more similar than in traps that are farther away. The Moran's *I* correlation coefficient is said to be a global measure of spatial dependence, because it uses a single summary statistics to describe the overall spatial autocorrelation in the neighborhood. The test was applied to each sample to determine if autocorrelation changed in time. To detect local spatial patterns of *Ae. aegypti* abundance within each neighborhood, we calculated the Getis–Ord *G*_i^{*} spatial statistics.²³ This test detects hot/cold spots or traps with unusually larger or smaller captures. Calculations were performed using ArcView's Spatial Analyst tool. Thematic maps showing summaries of average trap captures and hot/cold spots were produced for visual analyses.

Spatial stability of mosquito abundance occurs when the observed spatial pattern repeats in time. In this case, there is spatial stability if the order of mosquito captures per trap is kept from one sample to the next. To test for spatial stability, a Spearman rank correlation coefficient of trap captures between consecutive samples (forward lag = 1) was calculated. A significant and positive correlation coefficient indicates that the rank order of trap yields was kept between consecutive observations. Spearman's rank correlations were also calculated for all other forward time lags (2–19 lags of 3 weeks each) to determine if the correlations faded between samples at different future times. For example, a significant positive correlation between samples with a forward time lag of two means similarity in ranks of trap captures that were spaced in time by 6 weeks. To test for overall spatial stability in each neighborhood, we calculated a Kendall's *W* coefficient of concordance for each neighborhood using all 20 samples. This statistic measures the overall concordance among the rank order of trap yields for all samples and varies between zero and one. Significant values of Kendall's *W* imply that there was overall consistency in trap ranks throughout the study. Accumulated rainfall during the second and third weeks before a given sampling date was calculated to determine whether changes in rainfall and average mosquito population were associated with periods of spatial stability or instability. Rainfall during the week of sampling does not contribute many new adult *Ae. aegypti*, because its immature development lasts about 1 week.

RESULTS

Spatial patterns. The average number of *Ae. aegypti* females per trap per day was 4.76 ± 0.22 ($\pm 95\%$ confidence interval [CI], $N = 3,059$ trap days) in EC. There was significant spatial autocorrelation at $\alpha = 0.05$ in only 1 of 20 samples during the first week of July of 2008 ($I = 0.32$, $Z = 2.803$). In general, average *Ae. aegypti* captures in EC were spread throughout the neighborhood, without appreciable global clustering (Figure 1A). This figure depicts how traps were spaced throughout the neighborhood, although the location of houses, blocks, or streets is not shown. Global clustering would typically show areas with one or more traps with large numbers of mosquitoes surrounded by traps with numbers that

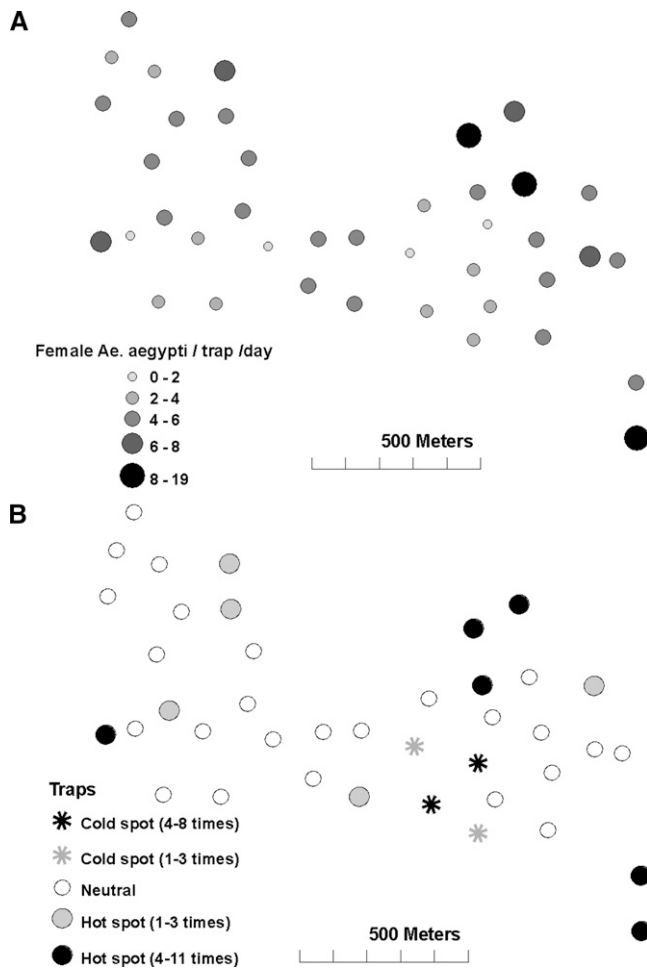


FIGURE 1. Spatial pattern of (A) the overall average number of *Ae. aegypti* females per trap per day in 40 traps scattered throughout EC neighborhood (sampled every 3 weeks; total of 20 samples) between November 2007 and December 2008, San Juan, Puerto Rico, and (B) a summary of traps classified as hot or cold spots based on the Getis-Ord's (G_i^*) statistics.

gradually decrease with the distance. Getis-Ord's local spatial statistics were calculated for every sample to detect traps with unusually large (hot spots) or small (cold spots) captures. To summarize and map hot spots throughout the study period, we assigned a value of one to each trap that was identified as a hot spot ($P < 0.05$) in any sampling date and added the values to represent the number of times that a trap was a hot spot (Figure 1B). Likewise, traps that were classified as cold spots ($P < 0.05$) within a sampling date were assigned a value of -1 and added up. Cold spots are traps with low values that are surrounded by other traps with low values. Cold spots in EC were localized close to each other, whereas hot spots were observed throughout the neighborhood (Figure 1B). Despite the lack of global spatial autocorrelation, local clustering was common, and some traps were frequent hot spots (4–11 of 20 samples) (Figure 1B). It can be observed that traps with large average captures of *Ae. aegypti* were usually classified as hot spots.

The average number of *Ae. aegypti* females per trap per day was 3.80 ± 0.14 ($N = 3,048$) in VC. None of the Moran's I correlation coefficients were significant at $\alpha = 0.05$ for any sample, indicating lack of global spatial autocorrelation

throughout the study. Similar to EC, average numbers of *Ae. aegypti* females per trap in VC were spread throughout the neighborhood, which is the main reason why the global autocorrelation analysis did not detect significant clustering (Figure 2A). Getis-Ord's local spatial statistics revealed the presence of hot and cold spots in VC (Figure 2B). The spatial dispersal of hot spots corresponded well with the location of traps with large mosquito yields. Cold spots were scattered, particularly around the periphery of the neighborhood in VC (Figure 2B).

Spatial stability. The rank order of mosquito captures per trap (every 3 weeks) was compared using the Spearman correlation coefficient to determine the similarity of trap yields between sampling dates. For example, the correlation between rank orders of *Ae. aegypti* females per trap between samples two and one was 0.514 ($P < 0.05$) in EC. Most correlation coefficients between consecutive samples (future time lag = 1) were highly ($P < 0.01$) significant in EC, with slight reductions in significance ($P < 0.05$) on occasions that seemed to be associated with marked increases in rainfall and numbers of *Ae. aegypti* females per trap (Figure 3A). Those reductions can be observed between samples 4 and 5, 10 and 11, and 14 and 15. There were significant and negative associations between Spearman's rank correlations and both the number of *Ae. aegypti* females per trap ($r = -0.534$, $P < 0.05$) and rainfall ($r = -632$, $P < 0.01$) per sampling date. That is, spatial stability decreased at times when *Ae. aegypti* populations increased or expanded because of rainfall. Average Spearman's correlation coefficients were largest at forward time lags one and two, but correlations did not fade out and stayed above significant levels ($\alpha = 0.05$) for most time lags (Figure 4). The Kendall's W coefficient of concordance of mosquito yields per trap throughout the study was significant ($W = 0.305$, $P < 0.01$) in EC, showing overall consistency in the rank order of trap yields.

Most of the Spearman's correlation coefficients of mosquito captures between consecutive samples (lag = 1) in VC were highly significant ($P < 0.01$) (Figure 3B). Similar to EC, Spearman's correlation coefficients between rank orders of trap captures were largest at forward time lags one and two and stayed above significant levels for all time lags (Figure 4). The Kendall's W coefficient of concordance in VC was lower than in EC but nevertheless, significant ($W = 0.152$, $P < 0.01$). There did not seem to be any consistency in the changes of the correlation coefficients and rainfall or mosquitoes per trap (Figure 3B), which was observed in EC (Figure 3A). There was a lack of correlation between the Spearman's rank coefficients and the number of *Ae. aegypti* females per trap ($r = 0.271$, $P > 0.05$) or rainfall ($r = 212$, $P > 0.05$) in VC.

DISCUSSION

This investigation showed lack of global spatial dependence of the number of female adult *Ae. aegypti* captured in BG-Sentinel traps that were uniformly spaced (130 m) in each of two neighborhoods during 20 consecutive population samples every 3 weeks in San Juan, Puerto Rico. This finding means that adult females of *Ae. aegypti* were not clustered in particular areas of the neighborhoods, which is unfortunate; these neighborhoods could not be stratified into areas with varying mosquito densities that would simplify vector control operations. This finding is possibly because of the functional

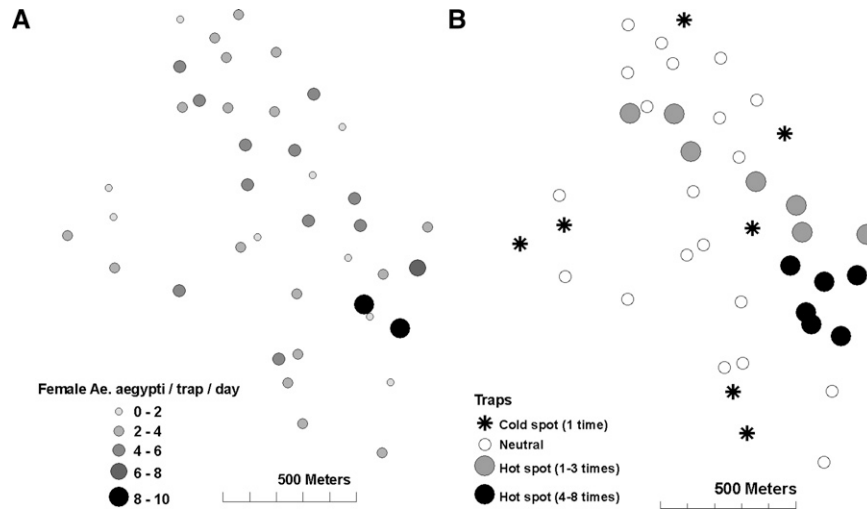


FIGURE 2. Spatial pattern of (A) the overall average number of *Ae. aegypti* females per trap per day in 40 traps scattered throughout VC neighborhood (sampled every 3 weeks; total of 20 samples) between November 2007 and December 2008, San Juan, Puerto Rico, and (B) a summary of traps classified as hot or cold spots based on the Getis-Ord's (G_i^*) statistics.

homogeneity in terms of housing type, basic public services, etc. of the residential neighborhoods investigated here.

The analysis of local spatial dependence did reveal local clustering or hot spots scattered throughout the neighborhoods,

and the temporal analyses showed a relatively high concordance in the rank order of trap productivity in time, which translates into a pattern of spatial stability of *Ae. aegypti* females in both neighborhoods. Spatial stability, expressed as the persistence of hot spots for periods of time at the same locations, has been reported for tsetse flies in Luke Community, Ethiopia.²⁴ A previous study using BG traps revealed significant spatial clustering of adult *Ae. aegypti* at the household scale but little temporal clustering in individual traps that were operated for 15 days in Cairns, Australia.²⁵ Other previous studies conducted at the household scale did not show spatial consistency in adult or immature density in time.^{12,18,19} This study differs from previous ones in that we sampled every 3 weeks for over 1 year, which allows for the observation of how spatial

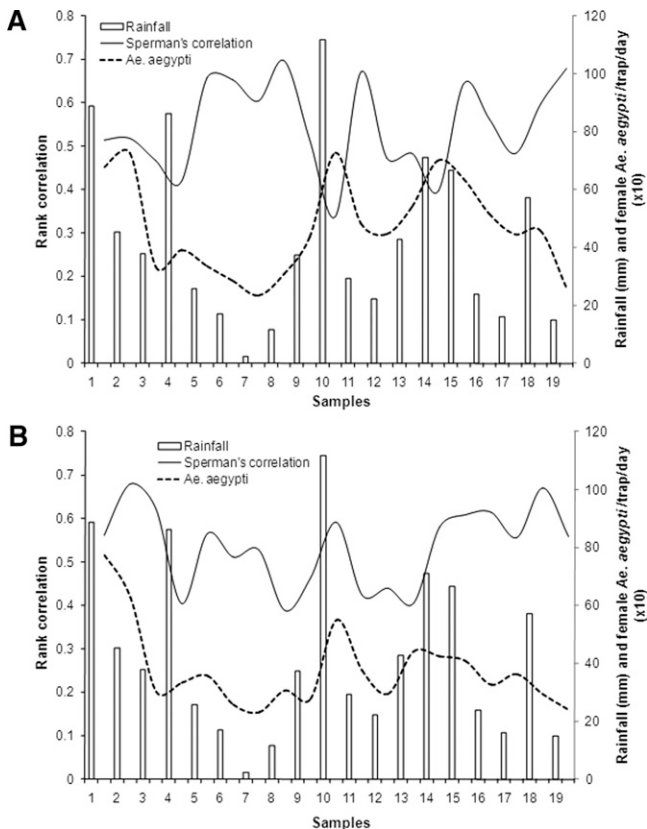


FIGURE 3. Changes in Spearman's correlation coefficients between consecutive sampling dates (every 3 weeks), accumulated rainfall during the second and third weeks before mosquito sampling, and number of female *Ae. aegypti* per BG-Sentinel trap per day ($\times 10$) from November 2007 to December 2008 in (A) EC and (B) VC, San Juan, Puerto Rico. Lines were smoothed using the cubic spline function of Excel.

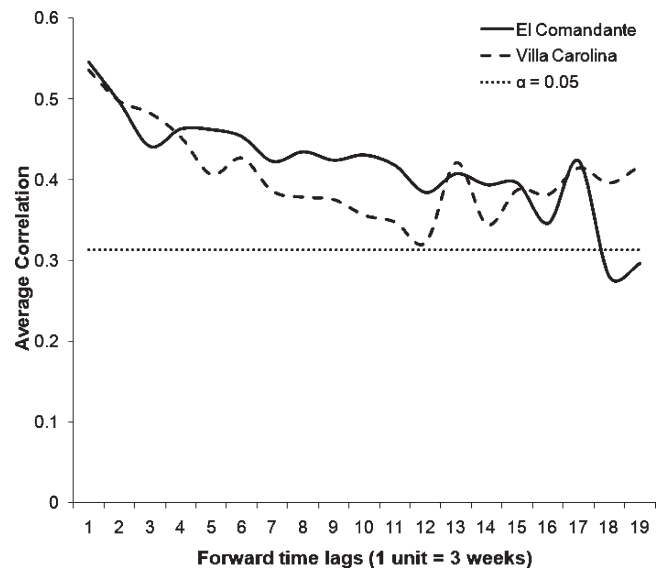


FIGURE 4. Average Spearman's correlation coefficients of the number of female *Ae. aegypti* per BG-Sentinel trap per day between samples in each neighborhood at various forward time lags (each lag = 3 weeks). Significant correlation coefficients are greater than 0.313 (two-sided test, $N = 40$, $\alpha = 0.05$). Lines were smoothed using the cubic spline function of Excel.

patterns change in time in greater detail, and our sampling was done at the scale of city blocks (130 m). The scale at which observations are made seems to be an important component that merits additional investigations. For example, Getis and others¹² showed that the spatial dependence of *Ae. aegypti* disappeared beyond 30 m in Iquitos, Peru. Exploring scale effects can help optimize entomological surveillance and vector control.²⁴

The results of the present study also showed significant correlations in the rank order of mosquito abundance per trap at most forward time lags throughout the study (Figure 4), which means high predictability in the spatial pattern of *Ae. aegypti* productivity. Captured mosquitoes were most likely produced nearby, because in most mark–release–recapture studies, *Ae. aegypti* adults are captured within 100 m a few days after release,^{21,26,27} with the exception of gravid females that can fly longer distances in search of containers with water.²⁸ The permanency of the rank orders of abundance of *Ae. aegypti* females in time must reflect the existence of persistent, local sources of mosquitoes near the traps.²⁹ The important consequence of the existence of relative stability in the spatial pattern of trap yields is that the hot spots could be targeted for a more efficient vector and dengue control. However, this strategy clearly points out that vector control organizations would need to conduct vector surveillance at similar scales. The advent of mosquito surveillance devices, such as the BG-Sentinel trap or similar devices that reflect the local abundance of adult *Ae. aegypti*, provides the opportunity to do this surveillance.

The spatial heterogeneity of *Ae. aegypti* females per trap was considerable (Figures 1 and 2). One trap captured 91 females and 153 males of *Ae. aegypti* in a single day in the porch of a house. It is conceivable that, if a dengue-infected person stays at one of such hot spots in the study areas, it could initiate the local transmission of dengue viruses. Furthermore, it is reasonable to propose the hypothesis that *Ae. aegypti*'s hot spots are the most likely places where dengue viruses get established and from which dengue viruses can be exported to other areas. It has been shown that dengue virus transmission is highly focal in nature and associated with the abundance of *Ae. aegypti*,³⁰ but it has not been shown if the elimination of local hot spots could prevent the establishment of dengue viruses. There is evidence showing that dengue infections tend to recur at or near the same places in time,^{31,32} which might be because of persistent *Ae. aegypti*'s hot spots.

Spatial stability faded during periods of significant increases in rainfall and high *Ae. aegypti* adult density in EC, which was revealed by the negative correlations between these variables and the Spearman's correlation coefficients. The negative correlations mean that the rank orders of trap captures drastically changed from one sampling date to the next. This transient change in the spatial pattern of trap captures may be indicative of the recruitment of many containers that were filled with water in the study area, but the spatial pattern in mosquito productivity soon returned to its previous order after reductions in population density (Figures 3 and 4). This observation seems to suggest that mosquito sampling after heavy rains would not necessarily reflect the prevalent spatial pattern of productivity. From a vector control perspective, it implies that vector surveillance should be conducted more frequently during periods in which the population of *Ae. aegypti* expands. Our results are strikingly similar to the results of Sciarretta and others,³³ who have recently described patterns of spatial

stability in tsetse flies that were transiently disrupted after significant increases in the size of the fly populations; this stability was followed by a quick return to the previous spatial pattern associated with lower fly densities.

The effect of rain and mosquito density on the dissimilarity of rank order trap captures was not observed in VC (Figure 4). This neighborhood was more intensely subjected to spatial spraying of insecticides than EC, and perhaps for that reason, *Ae. aegypti* adult abundance during the peak of the rainy season in VC was also smaller than in EC: (CDC, unpublished). There is evidence that effective vector control changes the spatial pattern of adult *Ae. aegypti*. For example, immature control measures targeting surface containers in a southern Puerto Rican town changed the spatial pattern of adult mosquitoes from one in which there was no clustering before control to one in which significant clusters appeared around untreated, underground aquatic habitats.³⁴ Thus, it is likely that the spatial pattern of *Ae. aegypti* is bound to change after the application of effective vector control measures. For this reason, it is recommended that vector control measures be monitored for their effectiveness in reducing adult mosquito abundance and the spatial distribution of mosquitoes.³⁴

Given that hot spots tend to be stationary for periods of time, it is likely that an approach based on adaptive population management could result in an efficient way to reduce the risk of local dengue transmission. Adaptive population management has been successfully applied to reduce stationary hot spots of tsetse flies.²⁴ This management approach relies on the dynamic interaction between entomological surveillance, aimed at identifying hot spots, and application of local vector control in and around hot spots. Clearly, adaptive management depends on efficient vector surveillance, prompt data analysis, and mapping capabilities. Future research on novel ways to control dengue could focus on developing inexpensive but efficient traps for adult *Ae. aegypti*, establishing proper scales for trap deployment, and testing the effectiveness of adaptive control.

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