Chapter 15
Characteristics of the Spatial Pattern of the Dengue Vector, *Aedes aegypti*, in Iquitos, Peru

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Abstract We determine the spatial pattern of *Aedes aegypti* and the containers in which they develop in two neighborhoods of the Amazonian city of Iquitos, Peru. Four variables were examined: adult *Ae. aegypti*, pupae, containers positive for larvae or pupae, and all water-holding containers. Adults clustered strongly within houses and weakly to a distance of 30 m beyond the household; clustering was not detected beyond 10 m for positive containers or pupae. Over short periods of time restricted flight range and frequent blood-feeding behavior of *Ae. aegypti* appear to be underlying factors in the clustering patterns of human dengue infections. Permanent, consistently infested containers (key premises) were not major producers of *Ae. aegypti*, indicating that larvaciding strategies by themselves may be less effective than reduction of mosquito development sites by source reduction and education campaigns. We conclude that entomologic risk of human dengue infection should be assessed at the household level at frequent time intervals.

15.1 Introduction

Patterns of dengue transmission are influenced by the abundance, survival, and behavior of the principal mosquito vector, *Aedes aegypti* (L.); the level of immunity to the circulating virus serotype in the local human population; density, distribution and movement of humans; and time required for development of virus in *Ae.*

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aegypti (Halstead, 1990). The relative influence of these factors on dynamics of virus transmission is poorly understood, including how they vary through space and time. Although the apparent clustering of human cases of dengue within households has been reported previously (Halstead et al., 1969; Waterman et al., 1985) there has been little formal spatial research on the distribution pattern of *Ae. aegypti* and dengue cases. An exception was the spatial statistics study of a dengue epidemic in Florida, Puerto Rico by Morrison and others (1998). They found that dengue cases clustered within individual households over short periods of time and that a large proportion of the entire municipality of 9,000 people was affected within seven weeks of the first reported case. Presumably the same, or very few, infected adult mosquitoes were causing the household case clusters while infected humans traveling within the town may have facilitated the rapid spread of infections. The most effective dengue control programs rely on entomologic, viral, serologic, and clinical surveillance (Gubler, 1993). Early detection of virus activity allows for more streamlined application of vector control measures. Because there is no vaccine or clinical cure for dengue, mosquito control is the only method of reducing virus transmission. Effective serologic and viral surveillance is often beyond the resources of the majority of affected, developing countries. Consequently, they rely on entomologic surveillance to estimate potential risk for virus transmission and disease.

Traditional *Ae. aegypti* control measures include elimination (source reduction) or treatment of larval habitats to prevent production of adults and insecticidal space spraying to reduce adult population densities (Gubler, 1993; Reiter and Gubler, 1997). Contemporary programs emphasize reducing *Ae. aegypti* populations to levels that prevent or slow virus transmission with the ultimate objective of decreasing the incidence of disease, especially severe, life-threatening illness. However, traditional entomologic surveillance techniques are based on a series of indices that were designed to detect the presence or absence of *Ae. aegypti* larvae. Those methods assume a strong positive correlation between the presence of larvae and adult females in a household: only adult females transmit virus to humans. There are, however, three important reasons to question the strength of the larvae-adult association. First, because larval mortality can be high, adults may not emerge from a container holding immature mosquitoes. Alternative entomologic surveillance methods, especially pupal surveys, were developed to circumvent this shortcoming (Focks and Chadee, 1997). Second, because adults are capable of flight, they can move away and become spatially disassociated from their development sites. Third, independent of the surveillance technique (larvae, pupae, or adult collections) city-wide surveys are often carried out in such a way that the number and location of households selected are derived from standard parametric sample size calculations. The assumption that there is no spatial structure among infested houses must be validated.

The purpose of this study was to characterize the spatial distribution of *Ae. aegypti* populations in two representative neighborhoods in the Amazonian city of Iquitos, Peru over two time periods. Specifically, from complete samples of households in two areas of Iquitos we examined the (1) underlying spatial structure of *Ae. aegypti* infestations (larvae, pupae, and adult), (2) temporal stability of that structure, and (3) correlation between clusters at different life stages of the mosquito. We conclude by discussing the implications of our findings on estimation of entomologic risk to epidemiologic studies of dengue and routine dengue surveillance.

15.2 Materials and Methods

Study Area

The area chosen for this study consists of two neighborhoods in Iquitos (73.2°W, 3.7°S, and 120 m above sea level), a city that is surrounded on three sides by the Amazon, Nanay, and Itaya Rivers. Because Iquitos is accessible only by air or river, it is a geographically isolated city of approximately 345,000 people in the Amazon forest (Watts et al., 1999) (Fig. 15.1). The major industries in Iquitos are small commercial enterprises, fishing, oil, lumber, and to some extent agriculture.

The two neighborhoods where we carried out entomologic surveys were Maynas, located in the north central part of the city, and Tupac Amaru, situated in the southwestern-most part of the city (Fig. 15.1). We selected these two neighborhoods because they were characterized as areas of high (Maynas) and low (Tupac Amaru) prevalence of human dengue infection in previous informal studies (Morrison, A.C. and Scott, T.W., unpublished data). Although Maynas could be characterized as the wealthier and older of the two neighborhoods, households within both areas vary greatly in socioeconomic status so that well constructed households with piped water and poorly constructed households with no water or sewer services exist in both neighborhoods in a patchwork. Nevertheless, there are some distinct differences between the two neighborhoods. Maynas has a higher proportion than Tupac Amaru of permanent houses constructed with bricks and concrete. Conversely, Tupac Amaru is a community in transition from predominantly temporary wood houses with palm roofs to houses constructed with brick and concrete. Even though Maynas has a better-developed sewer system than Tupac Amaru, the Maynas water supply is inconsistent. Consequently, Maynas residents are more likely than those in Tupac Amaru to store water in containers that are potential development sites for immature *Ae. aegypti*. In contrast, Tupac Amaru has many open sewers but because of close proximity to the city water plant most houses have a stable water supply and are less likely to store water than in Maynas.

Study Design

A unique-house code was painted on the front of each of the 550 houses located on 20 blocks in Maynas and the 510 houses located on 14 blocks in Tupac Amaru. Almost all houses have at least one wall in common with a neighboring house. Beginning in mid-November 1998, five two-person entomology collection teams

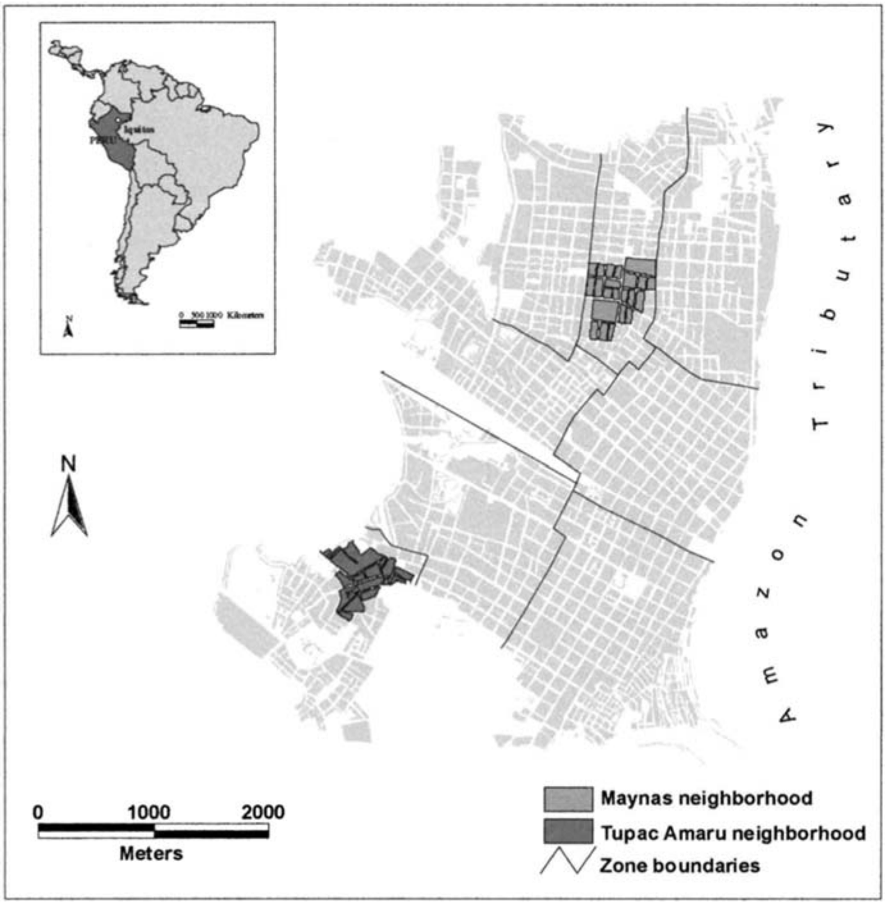

Fig. 15.1 Map of Iquitos, Peru and location of the Maynas and Tupac Amaru study areas

were provided a map of a block to be surveyed with a designated start house. Households were surveyed in sequence daily along the block from the start house between 7:00 a.m. and 1:00 p.m. Unoccupied or closed houses and houses where residents did not provide permission for the survey, businesses, offices, and schools were not sampled. Thus, we were able to survey 95% of the houses in both surveys: 528 in Maynas and 481 in Tupac Amaru. Collecting teams were rotated among blocks each day in an attempt to limit temporal and collector biases. Each day, prior to continuing surveys of unsampled households, an attempt was made to inspect houses that were previously closed or where access had been refused. Access to houses was attempted a minimum of three times. Maynas and Tupac Amaru were surveyed on alternating days. This process was carried out until all the houses in each neighborhood had been surveyed or repeated attempts to gain access failed. In mid-December 1998, immediately after termination of the first survey, the sampling procedure was repeated. The second survey was completed on January 18, 1999. To differentiate data associated with the four different collections, the two surveys will be referred to as a (November–December) and b (December–January).

Entomologic Surveys

Our survey methodology was based on techniques suggested by Focks and others (1993). Briefly, after asking permission to survey the household, one member of the team administered a demographic survey designed to determine the number of occupants, dimensions of the property, house construction materials, method of cooking, water use patterns, type of sewage disposal, and insecticide use. Simultaneously, the other team member began collecting adult mosquitoes using a backpack aspirator (John W. Hock Company, Gainesville, FL) (Scott et al., 2000a). Aspiration collections were attempted in all rooms of the house (when permitted) including walls, under furniture, and inside closets and other likely adult mosquito resting sites. Aspiration collections were similarly attempted outside the house from outside walls, under eaves, vegetation, and in and around outdoor stored materials.

In our field laboratory, larvae were identified as *Ae. aegypti* by the relative size of the siphon and their movement compared with the other most commonly found Culex species (Consoli and de Oliveira, 1994). *Limatus* larvae were differentiated by the characteristics on the eighth tergite (Consoli and de Oliveira, 1994). All larval samples were cross-checked with the entomology collection sheets provided by the field team. Pupae were counted and placed in plastic emergence vials, ≤ 30 per vial and labeled with the house, container code, and date. Each subsequent day, emerged adults were collected and placed in a $-20°\mathrm{C}$ freezer. After 30 min to 1 h, their species was identified, counted by sex, and data were recorded on the entomology collection sheet.

Data Management

A geographic information system (GIS), using ARC/INFO and ArcView software (Environmental Systems Research Institute, Inc., Redlands, CA), was developed for the city of Iquitos. A base map of city blocks in the form of AutoCAD files was obtained from the Peruvian Navy, which they created by digitizing ortho-corrected 1995 aerial photographs. The coordinate system and datum used were Universal Transverse Mercator and WGS-84, respectively. The AutoCAD files were converted to ARC/INFO export files and all polygons (city blocks) were closed using standard ARCEdit procedures. Files were then imported into ArcView and converted to shape files.

We then divided city blocks into individual housing lots that were identified by painted codes. The front end of each house lot was measured and recorded along with the house code and street address on a rough sketch of each block. Based on maps constructed in the field, each digital block in the GIS was split into lots of appropriate width using the measuring tool in ArcView. Lot length was estimated. Lot geometric centroids were then added to each individual lot and assigned a unique project code that was included on all subsequent survey forms. Construction of maps with resolution to the level of household lots allowed all entomologic data from the four surveys to be joined to geographic coordinates via house codes. Centroids allowed for spatial analysis to be performed from the level of the individual household upwards.

Analysis of the Data

Spatial patterns of four variables were examined (adult *Ae. aegypti*, pupae, all water-holding containers, and water-holding containers positive for larvae and pupae). Variables were explored by identifying the spatial distribution of each of the variables for each of the two time periods. Our study focused on (1) each of the two neighborhoods as a whole, (2) the magnitude of each variable in each household for each neighborhood, and (3) the presence or absence of a variable in a household for each neighborhood. Global K-functions, point and weighted, were used to identify clustering for (1) and the local statistic, G_i^*, was used for (2). These statistics are some of the suite of spatial statistical programs available as part of the Point Pattern Analysis (PPA) program. The program was developed by Arthur Getis with assistance from Laura Hungerford, Dong-Mei Chen, and Jared Aldstadt. An online version is available at http://zappa.nku.edu/~longa/cgi-bin/cgi-tcl-examples/generic/ppa/ppa.cgi. For (3), we used chi-square tests to compare similarities and differences among the various patterns.

K-functions

Pattern models are based on the K-function work of Ripley (1981) and Getis (1984). The K-function describes the number of pairs of observations between a point, which is the center of a disk and other points that are distance d away. For a stationary, isotropic process, $\lambda(d)$ is the expected number of points within distance d of an arbitrary point. The estimator of λ is N/A where N is the number of points in the study area A.

The estimator of $K(d)$ is

$$\hat{K}(d) = A/N^2 \Sigma_i \Sigma_j u_{ij}^{-1} I_d(d_{ij} \leq d), \quad i \neq j, \tag{15.1}$$

where d_{ij} is the distance between the ith and jth observed points and $I_d(d_{ij} \leq d)$ is an indicator function that is 1 if d_{ij} is less than or equal to d and 0 otherwise. For a circle centered on i passing through point j, u_{ij} is the proportion of the circumference of the circle that lies within A. When d_{ij} is less than the distance from i to one or more borders of the study area, u_{ij} is 1. The "border correction" makes $\hat{K}(d)$ an approximately unbiased estimator of $K(d)$ provided that d is less than the circumference of A. A square-root scale makes the function linear and stabilizes the variance. Thus, we have

$$\hat{L}(d) \equiv \sqrt{\hat{K}(d)/\pi} \tag{15.2}$$

which is the estimator of $L(d) \equiv \sqrt{K(d)/\pi}$. The mean of $L(d)$ is d and the approximate variance is $\frac{1}{2}(\pi N^2)$ (Ripley, 1979a). The expectation of $L(d)$ given the hypothesis of complete spatial randomness (CSR) is d. CSR is a homogenous planar Poisson process where all points are independent of all other points and all locations are equally likely to contain a point. For CSR, a plot of $\hat{L}(d)$ against d on similarly scaled axes yields a 45° line beginning at the natural origin. A clustered pattern occurs when $\hat{L}(d)$ is greater than d and a dispersed pattern can be identified when $\hat{L}(d)$ is less than d. In the spirit of a exploratory diagnostic tool, statistical significance at the $P \leq 0.05$ level is assumed to exist when the observed $\hat{L}(d)$ function falls outside of an envelope containing 19 permutations of the location of the N objects where each permutation is based on CSR. $\hat{L}(d)$ is usually calculated for a series of distances d.

Instead of considering each point as a nominal scale variable, points can be weighted according to some measure of size or intensity (Getis, 1984),

$$\hat{L}_w(d) = [\{A\Sigma_i\Sigma_j u_{ij}^{-1} I_d(d_{ij} \leq d)x_i x_j\}/\{\pi[(\Sigma_i x_i)^2 - \Sigma_i x_i^2]\}]^{1/2}, \quad i \neq j, \tag{15.3}$$

where X is a random variable having values x for adult mosquitoes in houses at sites i. Equation (15.3) is the estimator for $L_w(d)$, which is equal to $E[\hat{L}_w(d)]$. In the cases discussed in this paper, the weights are in turn numbers of adult mosquitoes, pupae, water-holding containers, and positive containers. For each x_i, there are $(N-1)$ values x_j. In this case, the numerator of $\hat{L}_w(d)$ represents the product of the pairs of values x_i x_j within distance d of each x. The denominator is scaled such that if all x are of equal value, then $\hat{L}(d)$ will be approximately equal to $\hat{L}_w(d)$. Thus, (15.3) represents a measure of clustering or dispersion identified in (15.2). If the number of adult mosquitoes, for example, is independently distributed within the plots of houses, $\hat{L}(d)$ will be approximately equal to $\hat{L}_w(d)$. Upper and lower significance boundaries for $\hat{L}_w(d)$ can be determined by a permutation procedure in which the various observed values for number of adult mosquitoes, x_i, are permuted among the house locations a specified number of times.

We also explored the increments to $\hat{L}(d)$ and $\hat{L}_w(d)$ observed for each equal increase of distance. In a CSR pattern of adult mosquitoes, these successive values will be the same for each equal increase of d. The focus is on the noncumulative properties of these pattern indicators. When the change in $\hat{L}(d)$ is greater or less than the change in $\hat{L}_w(d)$ for a given distance band, the adult mosquitoes are less concentrated or more concentrated, respectively, than that expected in the observed pattern, no matter how clustered the pattern of houses. That is, the number of adult mosquitoes is not randomly distributed among the houses. In essence, we compare $\Delta\hat{L}(d)$ with $\Delta\hat{L}_w(d)$ for a given small change in d.

Table 15.1 Summary of clustering statistics

Test	Purpose	Scale	Cut-off for statistic
$\hat{L}(d)$	To identify the existence of clustering for a 1/0 variable in a neighborhood	d	19 simulations of random occurrence within neighborhood (0.05 level)
$\hat{L}_w(d)$	To identify clustering of a weighted variable in a neighborhood	d	99 simulations of random occurrence within eligible locations of variable (0.01 level)
$G_i^*(d)$	To identify individual observations of a variable who are members of clusters	Z	$> +2.575$ (0.01 level)

$G_i^*(d)$ Statistic

In addition to $L(d)$, we used the local statistic, G_i^* (Ord and Getis, 1995), to identify individual members of clusters. For G_i^* we take each house as a center, one at a time, and search the nearby area for occurrences of more or fewer adult mosquitoes than expected. In this way, specific houses are identified as members or non-members of clusters. This statistic is written as

$$G_i^*(d) = [\Sigma_j w_{ij}(d)x_j - W_i^*\bar{x}]/[s\{[NS_{1i}^* - W_i^{*2}]/(N-1)\}^{1/2}], \quad \text{all } j, \quad (15.4)$$

where $w_{ij}(d)$ is the i, jth element of a one/zero spatial weights matrix with ones if the jth house is within d of a given ith house; all other elements are zero; $W_i^* = \Sigma w_{ij}(d)$, where w_{ii} is included, and $S_{1i}^* = \Sigma w_{ij}^2$ (all j). The mean of the adult mosquitoes in houses is $\bar{x}$ and s is the standard deviation. The value of $G_i^*(d)$ is given in normal standard deviates. Note that this statistic has as its expectation, $W_i\bar{x}$, which controls for the number of houses within d of each house. Note, too, that $G_i^*(d)$ is 0 in a pattern where adult mosquitoes are randomly distributed within d of house i. For this study, we arbitrarily define values greater than 2.575 (the 0.01 level of confidence) as representing houses which are members of clusters of adult mosquitoes. The statistics used in the analysis and the test criteria are summarized in Table 15.1.

15.3 Results

We begin the explanation of results from our study by focusing on one neighborhood, Maynas, using data from the initial survey a. We first consider the general, neighborhood (global) spatial pattern of adult mosquitoes and then focus on the pattern of the numbers of *Ae. aegypti* in individual houses (local) followed by an analysis of the presence or absence of adult mosquitoes in households. Next we examine the same processes for immature mosquitoes. Finally, we compare the four entomologic variables in the two neighborhoods and two time periods.

Table 15.2 $L(d)$ values for distances 10–100 m for houses and adult mosquitoes in Maynas a*

Distance (m)	Houses	Adult mosquitoes	House increment	Adult increment
10	16.33	22.86	16.33	22.86
20	27.13	36.79	10.80	13.93
30	38.70	50.58	11.57	13.79
40	52.85	61.13	14.15	10.55
50	65.67	74.24	12.82	13.11
60	76.70	83.94	11.03	9.70
70	88.03	93.71	11.33	9.77
80	100.98	104.12	12.95	10.41
90	111.77	113.10	10.79	8.98
100	122.19	120.57	10.42	7.47

* i does not equal j

Neighborhood Pattern Analysis

The results of the K-function analysis for adult *Ae. aegypti* in Maynas in time period a are shown in Table 15.2. Adult mosquito clustering occurs if values of $\hat{L}(d)$ are higher not only than adult mosquitoes distributed at random in the Maynas neighborhood for a given distance (i.e., d), but also higher than the $\hat{L}(d)$ value for the pattern of houses at that same distance. Clearly, it is not enough that adult mosquitoes were spatially concentrated at the same rate as the spatial concentration of houses. Note that in column 3 in Table 15.2, the $\hat{L}_w(d)$ value for adult mosquitoes at 10 m is 22.86, which is quite a bit higher than the 10.00 (random expectation) shown in column 1. However, houses were much more clustered than random (16.33 vs. 10.00 at 10 m). Even so, adult mosquitoes were more clustered than houses. In addition, using 19 permutations to identify the range of possible values for adult mosquitoes among houses (at the 0.05 level), we find that adult mosquitoes at 22.86 fall outside of that range (low of 11.88 to high of 19.10) at 10 m. This gives strong statistical evidence that adult mosquitoes were clustered in the Maynas neighborhood during time period a. Clustering is at the 10-m level; thus, we can conclude that there is clustering around houses to at least 10 m distant.

Notice that in column 2 of Table 15.2, as distance increases to 20, 30 m, and so on, the $\hat{L}(d)$ values for houses increase at a rate that is not dissimilar from random expectation. This means that although houses are closely spaced at short distances, there is little or no increase in clustering as distance increases. The $\hat{L}_w(d)$ value for adult mosquitoes shown in column 3 at 20 and 30 m, however, increases at a slightly higher rate than houses (column 5 vs. column 4), indicating a continuing of the clustering identified at 10 m to at least 30 m. This pattern of increase changes by 40 m (the increment is 10.55, less than the house increment of 14.15) indicating an end to the increase in clustering. That is, beyond 30 m, any further clustering of adult mosquitoes corresponds to clustering of houses. We conclude that adult mosquitoes cluster heavily at nearest house distances and moderately to approximately 30 m. In

Table 15.3 $L(d)$ values for distances 10–100 m for houses and adult mosquitoes in Maynas a*

Distance (m)	Houses	Adult mosquitoes	House increment	Adult increment
10	21.44	39.30	21.44	39.30
20	30.46	48.65	9.03	9.35
30	41.08	59.67	10.62	11.02
40	54.60	68.75	13.51	9.08
50	67.06	80.52	12.47	11.77
60	77.88	89.46	10.81	8.94
70	89.04	98.60	11.16	9.14
80	101.83	108.44	12.79	9.84
90	112.52	117.00	10.69	8.56
100	122.87	124.17	10.34	7.17

* i may equal j

Maynas, the mean house width was 7 ± 3 m; thus, adult clusters could extend to about two households on each side.

We altered (15.1) and (15.3) to include houses themselves; that is, we allowed i to equal j (Table 15.3; see Getis (1984) for an explanation of the methodology). Our focus now is on houses and their neighbors rather than neighboring houses only. In this circumstance, the clustering of houses (column 2) is inflated to include not only near neighbors at 10 m, but also the houses themselves. The original value of 16.33 at 10 m now increases to 21.44 for houses indicating that in this view, houses are more clustered than was indicated previously (an increase of 31%). More importantly, however, are the results when adult mosquitoes within houses are taken into account. Here the value at 10 m increases to 39.30 from 22.86, an increase of 72%. The implication is that adult mosquitoes are heavily clustered within houses. Note also that as distance increases, the increment to houses and adult mosquitoes is approximately 10, indicating that there is a cessation of clustering beyond 10 m. Again there is additional, albeit weak clustering up to 30 m because the increase in the mosquito value is higher than that for the houses at 20 and 30 m. These results taken together with the earlier ones unequivocally indicate that adult mosquitoes cluster heavily within or among nearest neighboring houses. In addition, there is evidence of further, albeit minor, clustering as far as 30 m. The clustering within houses in the Maynas neighborhood quantitatively overwhelms this further clustering.

Household Pattern Analysis by Numbers of Adult Mosquitoes

After it was evident that there was short distance clustering of adult mosquitoes in Maynas a, we identified the exact houses that could be considered as members of clusters. First, we considered the actual numbers of adult mosquitoes in each house in Maynas a (Fig. 15.2). If clustering was within households, the G_i^* statistic will be above $+2.575$ at short distances, say 1 m at the 0.01 level of statistical significance. If clustering continues to near neighbors within 10 m of a house, the value of G_i^*

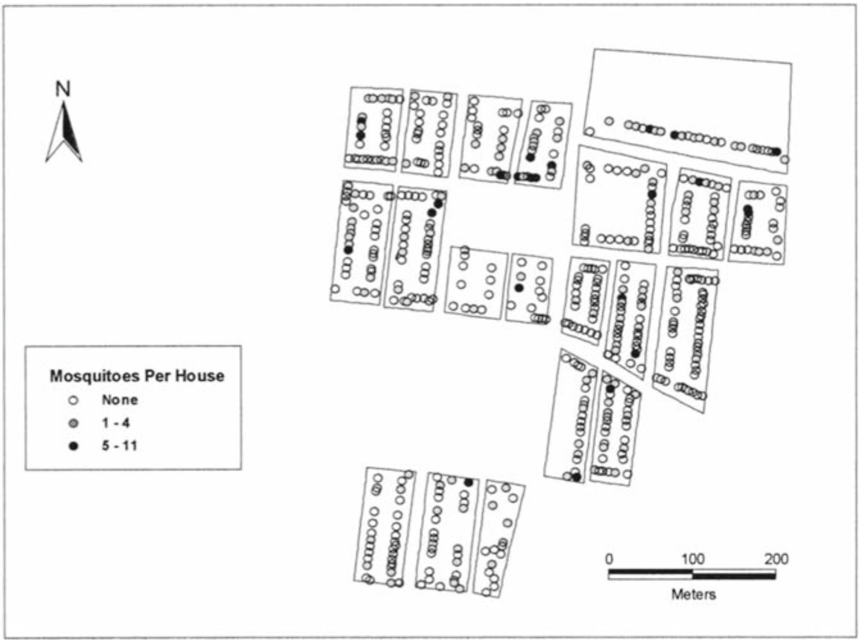

Fig. 15.2 Mosquitoes per house in the Maynas a study

will be higher at 10 m than at 1 m. If values of G_i^* do not increase with increases in distance, then whatever clustering existed at the shorter distance ceases to exist at longer distances. The houses that are members of significant clusters at 1, 10, 20, and 30 m are shown in Fig. 15.3. Note that of the 528 houses in Maynas during time period a, 35 (6.6%) are members of statistically significant clusters of adult mosquitoes. Of the 35, 10 exhibit clustering with near neighbors beyond the house itself. Of these 10, seven show clustering to 10 m, two to 20 m, and one to 30 m. This result reinforces the notion that adult mosquitoes tend to cluster in single households with a modest spread to as far as 30 m.

Pattern of Houses Infested with Adult *Ae. aegypti* ($<$1 Mosquito)

Figure 15.4 is a map of the presence of one or more mosquitoes in households. One hundred sixty-four (31.1%) of the houses had one or more adult mosquitoes present; however, only 35 of them (21.3%) were members of statistically significant clusters. This indicates that clusters were made up mainly of household concentrations, and that 79.7% of the households with mosquitoes were spread about in a random pattern among all households.

Fig. 15.3 Clusters of *Aedes aegypti* adults in the Maynas *a* study based on the number of mosquitoes in houses

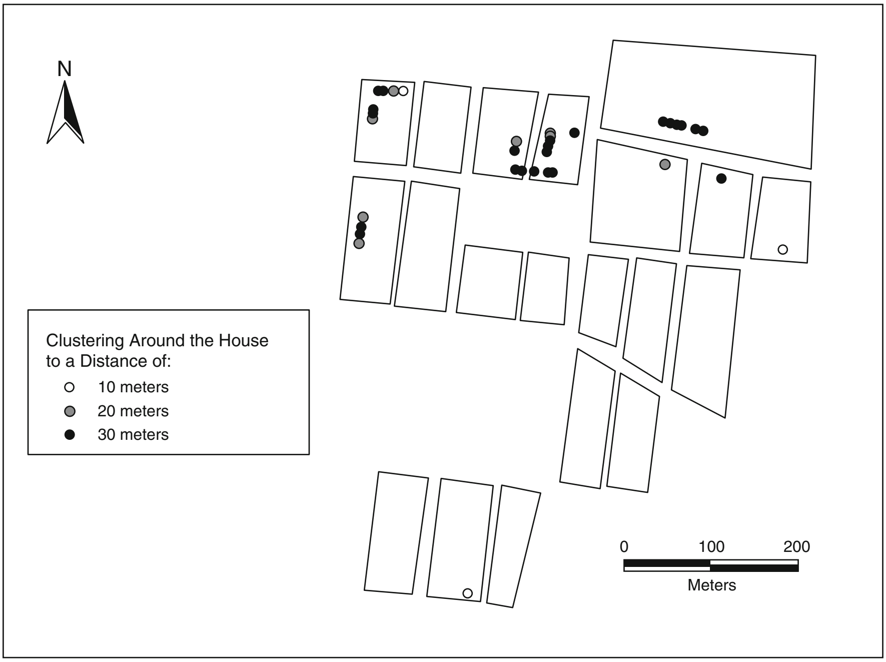

Fig. 15.4 Clusters of *Aedes aegypti* adults in the Maynas *a* study based on presence or absence of mosquitoes

Neighborhood Pattern Analysis of Immature Mosquitoes

Results in Tables 15.4 and 15.5 allow for the comparison of K-function values for water-holding containers, positive containers, and pupae with house and adult mosquito patterns in the Maynas neighborhood [(15.1) and (15.3)]. The $d = 10\,\mathrm{m}$ row in Table 15.4 shows, as before, that adult mosquitoes cluster more so than houses (22.86–16.33), but the pattern of water-holding containers and positive containers is more nearly like the pattern of houses (16.25–16.33 and 15.40–16.33). Thus, there is evidence of no clustering for these variables. In the case of pupae, however, there is a significantly lower value (12.03), indicating that pupae do not cluster beyond the household and, in fact, are dispersed rather evenly throughout the neighborhood. However, when we allow i to equal j (Table 15.5), pupae increase from 12.03 to 56.13, an extremely high and statistically significant value.

Table 15.4 $\hat{L}(d)$ values for distances 10–100 for houses, adult mosquitoes, pupae, water-holding containers, positive water-holding containers in Maynas a*

Distance (m)	Houses	Adult mosquitoes	Pupae	Containers	Positive containers
10	16.33	22.86	12.03	16.25	15.40
20	27.13	36.79	22.73	27.43	27.03
30	38.70	50.58	36.82	40.03	37.66
40	52.85	61.13	46.40	54.16	51.88
50	65.67	74.24	56.15	66.86	64.55
60	76.70	83.94	70.50	78.42	76.20
70	88.03	93.71	80.66	90.19	86.40
80	100.98	104.12	92.23	102.57	99.59
90	111.77	113.10	102.49	113.17	110.28
100	122.19	120.57	110.86	123.36	119.91

* i does not equal j

Table 15.5 $\hat{L}(d)$ values for distances 10–100 m for houses, adult mosquitoes, pupae, water-holding containers, positive water-holding containers in Maynas a*

Distance (m)	Houses	Adult mosquitoes	Pupae	Containers	Positive containers
10	21.44	39.30	56.13	23.44	29.05
20	30.46	48.65	59.26	32.18	36.53
30	41.08	59.67	65.77	43.36	44.93
40	54.60	68.75	71.41	56.61	57.3
50	67.06	80.52	77.91	68.74	68.92
60	77.88	89.46	88.51	79.93	79.88
70	89.04	98.60	96.56	91.49	69.60
80	101.83	108.44	106.14	103.62	102.31
90	112.52	117.00	114.91	114.12	112.68
100	122.87	124.17	122.22	124.26	122.07

* i may equal j

This indicates that pupae cluster strongly within houses, but households infested with pupae are dispersed rather evenly throughout the neighborhood (Table 15.4).

Because water-holding container spatial data are similar to the house location data (Tables 15.4 and 15.5), we conclude that water-holding containers are ubiquitous in Maynas. That is, nearly all houses have water-holding containers. Conversely, containers positive for pupae and/or larvae are more concentrated in some houses than others and infested houses are dispersed evenly throughout the neighborhood.

Continuing on to 20, 30 m, and further (Tables 15.4 and 15.5), only pupae act differently than containers and positive containers. For both of the container variables, increases mirror those of houses, reinforcing our earlier results that show ubiquitous occurrences of these variables. Pupae values (Table 15.5), however, increase at a much slower rate than houses after 10 m, indicating that households infested with pupae are less common than households with water-holding containers or positive containers, and that the spatial pattern of pupae is characterized by strong clustering within households.

Household Pattern Analysis of Non-adult Mosquitoes

Our G_i^* statistic results show that there is a lack of statistically significant clustering beyond households for container and immature mosquito variables. In the case of pupae, there were 18 households exhibiting clustering with no clustering beyond the household. Of the 24 houses with clusters of containers, only two were clustered to a neighboring distance of 10 m. For positive containers, 23 houses exhibit clustering, but only three of those were clustered beyond the household, 2–10 m, and 1–20 m.

Patterns of Pupae: Presence or Absence in Houses

In this analysis, the concern is less with numbers of pupae in houses and more with their spatial occurrence in houses. Data in Fig. 15.5 were derived from a G_i^* analysis that assigned a 1 to houses with one or more pupae present and 0 for the absence of pupae. We found that 18 (3.4%) of the 528 houses can be considered as members of clusters at the 99% level of confidence. There are two distinct clusters: one in the middle block in the south and a smaller cluster in the north. These concentrations raise the question of the relationship of the location of pupae to adult mosquitoes.

Comparison of Entomologic Spatial Patterns in Maynas a

Does the pattern of adult mosquito clusters correspond to the patterns of the other variables? We answer this question in three ways. First, we consider the overlap of clusters among the four variables. Second, we note the presence (one or more) of each variable occurring simultaneously in individual houses. Third, we focus on the number of water-holding containers, positive containers, pupae, and adult mosquitoes in households

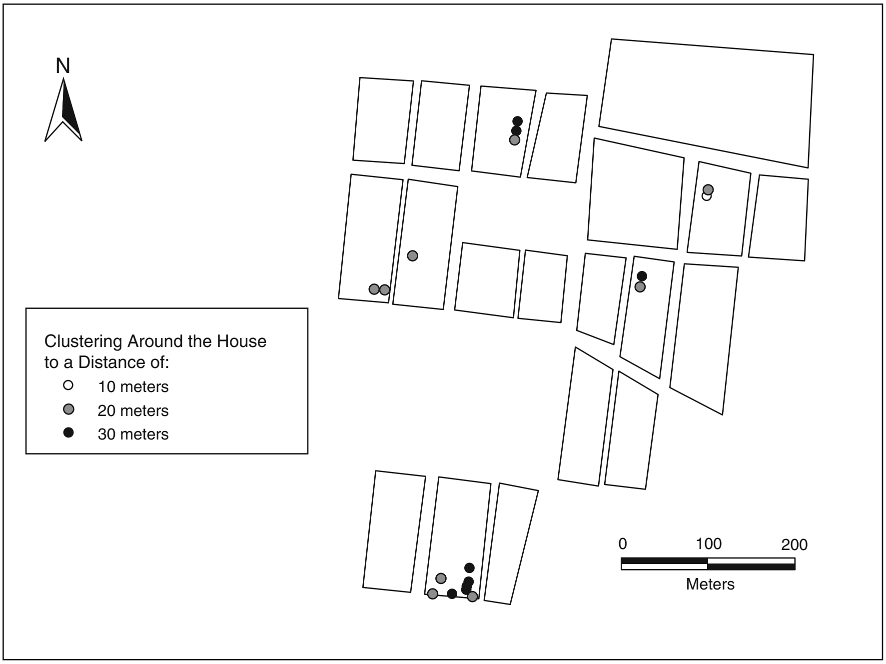

Fig. 15.5 Clusters of *Aedes aegypti* pupae in the Maynas a study based on presence or absence of pupae

Table 15.6 Number of members of clusters in Maynas and Tupac Amaru in time periods a and b

	Maynas	Tupac Amaru
Houses	528	481
Adults in time period a	35	40
Adults in time period b	27	32
Pupae in time period a	18	18
Pupae in time period b	4	24
Adults in a and b	7[a]	2
Pupae in a and b	0	6[a]
Adults in a and pupae in b	0	1
Pupae in a and adults in b	2	3
Adults in a and pupae in a	3	4[b]
Adults in b and pupae in b	0	0

[a] Significant at the 0.01 level
[b] Significant at the 0.05 level

Association Among Clusters

In Table 15.6 we see, as before, that of the 528 houses in Maynas, 35 were members of clusters of adult mosquitoes and 18 were members of clusters of pupae in time period a. Only three houses were constituents of both clusters, a non-statistically significant result at the 0.05 level ($\chi^2 = 1.60$, degrees of freedom = 1, Yates'

Table 15.7 One or more adult mosquitoes and/or pupae present in houses in Maynas and Tupac Amaru in time periods a and b

	Maynas	Percent	Tupac Amaru	Percent
Houses	528		481	
Adults in time period a	164	31.06	87	18.09
Adults in time period b	151	28.60	92	19.13
Pupae in time period a	155	29.36	86	17.88
Pupae in time period b	134	25.38	65	13.51
	Maynas		Tupac Amaru	
	Observed	Expected	Observed	Expected
Adults in a and b	67	47[a]	20	15
Pupae in a and b	70	39[a]	25	11[a]
Adults in a and pupae in b	53	42[b]	14	11
Pupae in a and adults in b	50	44	20	15
Adults in a and pupae in a	66	48[a]	25	14[a]
Adults in b and pupae in b	50	38[a]	15	11

[a] Significant at the 0.01 level
[b] Significant at the 0.05 level

correction for small expectations). There was not a significant correlation between pupal and adult abundance within household or neighborhood clusters detected during the same survey.

Association Among Households Having One or More of Each Variable Present

The analysis summarized in Table 15.7 reveals the overlap of households that have as few as one mosquito or one pupae present. Note that of the 528 houses in Maynas, 164 had at least one mosquito present and 155 had at least one pupae present in time period a. Expectation from a chi-square two-by-two contingency test indicate that the two types of occurrence come together in households 48 times. A total of 66 households were infested with both pupae and adults, demonstrating that the presence of these two variables are not independent ($P < 0.01$).

Association of Water-Holding Containers and Adult Mosquitoes and Pupae

Because there are water-holding containers in every household in Maynas, we compared the relative abundance of positive containers, pupae and adult mosquitoes. Table 15.8 shows the results of Spearman's rank correlation test where the number of water-holding containers per household were ranked from 1 to 14. Ranks 15 and 16 were made up of 15–19 and 20–35 containers, respectively. The final two ranks were grouped because of the few numbers of observations at these high levels. The

Table 15.8 Spearman's rank correlations of the number of containers per house with the number of mosquitoes and pupae per house

Location of containers	Mosquitoes	Pupae
Maynas *a*	+0.615[a]	+0.487
Maynas *b*	+0.682[b]	+0.594[a]
Tupac Amaru *a*	+0.284	+0.486
Tupac Amaru *b*	−0.199	+0.481

[a] Significant at the 0.05 level
[b] Significant at the 0.01 level

Table 15.9 $\hat{L}(d)$ values for 10 m for Maynas and Tupac Amaru for time periods *a* and *b**

	Maynas *a*	Maynas *b*	Tupac Amaru *a*	Tupac Amaru *b*
Houses	21.44	21.44	25.00	25.00
Mosquitoes	39.30	51.06	76.64	51.08
Pupae	56.13	71.42	80.34	76.14
Containers	23.44	23.43	27.68	27.87
Positive containers	29.05	31.00	38.56	44.30

* i may equal j

mean number of adult mosquitoes per house was ranked for each container level. The result was a moderately high positive correlation for adults (+0.615, $P < 0.05$), and a modest correlation for pupae (+0.487, not significant). Our analysis indicates that elevated numbers of water-holding containers in houses increase the likelihood for elevated numbers of adult mosquitoes and/or pupae to be present.

Maynas Vs. Tupac Amaru

Although non-spatial measures of *Ae. aegypti* population densities decreased in both sites in the second surveys, they were higher in both surveys in Maynas than in Tupac Amaru. For example, the house index (percentage of surveyed houses with ≥ 1 positive container) was 45% in Maynas *a*, 38% in Maynas *b*, 29% in Tupac Amaru *a*, and 23% in Tupac Amaru *b*.

Clustering patterns of adult mosquitoes and pupae were consistent among the four surveys, but the level of clustering was greatest during the first Tupac Amaru survey. Table 15.9 shows the $\hat{L}(d)$ values (i may equal j) for each of the four surveys for 10 m. Houses in Tupac Amaru were slightly more clustered than in Maynas (25.00–21.44). Note also that in both neighborhoods water-holding containers are distributed much the same as were houses, but positive containers tend to cluster. Maynas with 29.05 and 31.00 in the two time periods are approximately 8–10 L units higher than the pattern of houses. Tupac Amaru with 38.56 and 44.30 are about 13–19 units higher than the pattern of houses. This implies that positive containers were more clustered in Tupac Amaru than Maynas, which may be a reflection of lower infestation rates in Tupac Amaru. Nevertheless, in both sites the level of clustering was relatively low.

Time Period *a* Vs. *b*

The objective of carrying out back-to-back surveys in two sites was to account for variability in collector aptitude; a commonly cited limitation of entomologic surveys (Reiter and Gubler, 1997). Despite only three weeks separating surveys, the number of water-holding containers and immature mosquito indices decreased between the two sampling periods. Reasons for this are not known, but the possibility that our survey methodology affected immature populations must be considered. During the first survey, small containers not used for water storage were tipped over and homeowners may have cleaned or drained larger containers that our field team identified as being infested with larvae or pupae. Following a reduction in immature mosquitoes, we would expect a decrease in emergence of adults and in turn a measurable reduction in adult population density. Curiously, a reduction in adult density was only detected in Tupac Amaru, where the number of adults per household decreased from 0.4 to 0.3. In Maynas, the number of adult *Ae. aegypti* per household was 0.7 in both surveys. In the second surveys the number of water-holding containers decreased by 13% in Tupac Amaru compared with only 3% in Maynas.

Overall Patterns of Adult Mosquito and Pupae Household Clustering

Table 15.6 shows the number of houses that were members of statistically significant clusters of pupae and adult *Ae. aegypti*. The number of houses included in clusters for pupae in Maynas decreased from 18 to 4 from time period *a* to *b*. Interestingly, the location of adult clusters changed between the two surveys. Twenty-eight households were members of adult clusters in the first Maynas survey that were not members of clusters in the second, a statistically significant finding that was not the case in Tupac Amaru. Only seven households were members of adult clusters in both Maynas surveys. Twenty Maynas households were members of clusters in the second but not first survey. The same type of result, changing cluster locations, was evident with member houses of pupae clusters. In Maynas none of the houses were members of pupae clusters in both surveys, whereas six households were part of pupae clusters during both time periods in Tupac Amaru (Table 15.6). This result indicates that the spatial distribution of entomologic data varies greatly within short periods of time.

Association Among Households Having One or More of Each Variable Present in Each Neighborhood over Time

Although clusters of positive containers, pupae, and adult mosquitoes identified by G_i^* were not consistent with time, *Ae. aegypti* infestations of individual households were clearly a risk factor for future infestation. That is, there is evidence of repeat offenders. Table 15.7 shows the number of houses observed to be infested with either pupae or adults in survey a, survey b, or both. Pupae in a are again found in the same houses in b in both neighborhoods between 29% and 45% of the time, a statistically significant result. The implication is that for unknown reasons mosquitoes are more likely to lay eggs in containers on some house lots than others. Another risk factor for infestation is the number of water-holding containers in a household. Results in Table 15.8 indicate that there is a tendency for houses in both neighborhoods and both time periods to contain more pupae when more water-holding containers are present.

15.4 Discussion

Historically, entomologic surveillance for dengue was dominated by the use of larval surveys, in large part because *Ae. aegypti* control grew out of an eradication paradigm that promoted complete, thorough and repeated coverage of infested areas (Reiter and Gubler, 1997). In 1994, however, the Pan American Health Organization declared *Ae. aegypti* eradication an unattainable goal and promoted *Ae. aegypti* control, which they defined as the "cost effective utilization of limited resources to reduce vector populations to levels at which they are no longer of significant public health importance" (PAHO, 1994). Although this recommendation intuitively makes sense, it is not specific enough for public health officials to use as a guideline to control dengue. For example, experience with yellow fever and recent computer simulation estimates indicate that entomologic thresholds below which dengue transmission will cease are low (Reiter and Gubler, 1997; Focks and Chadee, 1997; Focks et al., 1995), but threshold values have not been systematically derived or tested (Reiter and Gubler, 1997). Empirically defined thresholds will require prospective, longitudinal studies in which investigators simultaneous monitor relationship between dengue virus transmission in a human cohort and *Ae. aegypti* population densities. Interpretation of data from those kinds of studies will require careful consideration of (1) spatial auto-correlation and scale in statistical analyses; (2) the most appropriate measure of entomologic risk-should absolute numbers or indices be measured and what life stage of the mosquito provides the best estimate for risk of human dengue virus infection; and (3) survey design, including the extent of data collection. Our study contributed to an improved understanding for each of these issues.

The lack of spatial structure for immature forms of *Ae. aegypti* supports recommended vector surveillance strategies where standard sample size calculations and resource limitations are used to determine in a systematic way the number of houses to be sampled, typically every ith house. Our K-function analysis indicates that individual households are the appropriate spatial unit for entomologic surveys. From a temporal perspective because water-holding containers were ubiquitous in Iquitos, all households are at risk of infestation over any considerable period of time. Our results, however, imply that as the number of containers on a premise increases so does the risk of *Ae. aegypti* pupae and adult infestations. In other words, positive containers and pupae cluster within individual households, but the location of clusters changes through time. Biologically this makes sense. Infestation of a household is largely a function of container management practices by the occupants of the property and the ecology of *Ae. aegypti* egg-laying behavior. We did not detect larger scale structure that might have been affected by other factors (data not presented or discussed in this paper) such as the availability of piped water, local temperature, rainfall patterns, or garbage disposal.

Identification of "key premises" or households that are superproducers of *Ae. aegypti* has been proposed as a way to streamline surveys (Tun-Lin et al., 1995). The idea is that the presence of pupae or adults during an initial survey is a significant risk factor for observing the same life stage at the same location during subsequent surveys. If we adopt the notion of controlling key premises as a way of reducing but not eliminating *Ae. aegypti* populations, the fundamental need to refine our understanding of entomologic thresholds is reinforced. Until we quantitatively define the relationship between mosquito density and risk of virus transmission, we cannot predict the effect that eliminating key premises will have on the risk of human infection and disease. For example, eliminating key premises may not reduce the adult mosquito population below the threshold density and, depending on the nature of the relationship between virus transmission and vector density, the pattern of human infections could continue unabated. Interestingly, the transient pattern of immature mosquito cluster locations observed in our study indicates that even if key premises can be identified and eliminated there may still be a sufficient number of *Ae. aegypti* to sustain dengue virus transmission. It should be noted, however, that because Iquitos has a relatively low percentage *Ae. aegypti* production in permanent water holding containers, our results may be site specific. The same kind of thorough examination may need to be carried out (large sample sizes and spatial analysis) at other locations.

Although small, there was significant spatial structure of adult mosquito populations compared with pupae and positive containers. Adults cluster most to distances of approximately 10 m and to a lesser extent out to 30 m, which could include neighboring houses. This finding is consistent with our conclusion to use the household as the basic unit of entomologic surveillance. It also superficially supports focal insecticide treatments for dengue control, a practice in which households are treated with insecticides within a 50–100 m radius of the residence of a detected dengue case (PAHO, 1994). There are, however, at least three shortcomings to focal treatments that extend beyond spatial patterns of adult *Ae. aegypti*. The approach does not take into account (1) the time delay between when a person is infective to mosquitoes and they are detected as being clinically ill with dengue, (2) that infected people can transport virus rapidly over greater distances than flying infected mosquitoes, and (3) that viremic people can have an inapparent infection or may not seek medical assistance, the homes and surrounding areas of many people infective to mosquitoes will not be sprayed.

Our statistical approach corroborates results from mark-release-recapture experiments on the dispersal of adult *Ae. aegypti*. Most researchers have concluded that the typical flight range of this species is short ($<$100 m). Rodhain and Rosen (1997) stated that spontaneous dispersal of adult *Ae. aegypti* averages from 30 to 50 m per day, so that females are rarely expected to visit more than two or three houses in their lifetime. The length of an *Ae. aegypti* lifetime is difficult to estimate, but is generally believed to range from 8 to 16 days (Focks et al., 1993). Ordonez and others (1997) reported minimum and maximum daily flight distance for *Ae. aegypti* of 8 and 120 m, respectively, with a mean of 30.5 m. In a Kenyan village, McDonald (1977) found that most adult *Ae. aegypti* dispersed to less than 20 m and the majority of those recaptured were collected in the same house where they were released. Edman and others (1998) similarly collected most of their recaptured *Ae. aegypti* in Puerto Rico from their release house. In Kenya, Trpis and Hausermann (1986) reported 57 m as the mean daily flight distance for females, with a maximum dispersal of 154 m. Sixty percent of their recaptured females were collected in 11 houses that were within 50 m from their release point. Our spatial analysis agrees with the preponderance of evidence that in a place such as Iquitos most adult *Ae. aegypti* do not fly far from the container where they developed as larvae and pupae.

Spatial referencing of our adult survey data and application of statistical tools, such as K-function and G_i^*, provided insights into adult dispersal behavior that help explain patterns of human dengue infections. We propose that over short periods of time the restricted flight range and frequent blood-feeding behavior of *Ae. aegypti* (Scott et al., 2000b) are underlying factors in the clustering patterns of human dengue infections. In addition to the studies cited above on *Ae. aegypti* dispersal, several researchers have reported spatial and temporal clusters of clinically ill dengue patients in the same household or adjacent houses (Halstead et al., 1969; Waterman et al., 1985; Chan, 1985; Gubler, 1997). In the first spatial statistics analysis of this phenomenon, Morrison and others (1998) found that dengue cases reported within a three-day interval during an epidemic in Florida, Puerto Rico clustered up to 10 m. With regard to blood-feeding behavior, *Ae. aegypti* is know to frequently and preferentially imbibe human blood meals (Scott et al., 2000b; Harrington et al., 2001) and infected females can transmit dengue virus to as many as 20 consecutive hosts, one after another (Putnam and Scott, 1995). It is conceivable that a single or very few infected *Ae. aegypti* that remain in the same general area could bite and transmit virus to several susceptible family members or their immediate neighbors within a period of a few days.

Upon further investigation, we may discover that the extent to which infected humans are clustered is influenced by house construction and distribution. For example, households in our study area were small and often located close together; most were row houses with common walls. Although features of housing in Iquitos might facilitate *Ae. aegypti* movement, we do not expect that the tendency for adult females to disperse will be dramatically different at other locations. In Iquitos, water-holding containers were found in all households surveyed, something that is expected to decrease the probability of female dispersal (Edman et al., 1998).

Abundance of adult female mosquitoes should be the most appropriate measure of entomologic risk because they are in the life stage from which viruses are transmitted. Interestingly, in at least one previous study adult *Ae. aegypti* abundance was correlated with diagnosed dengue cases (Rodriguez-Figueroa et al., 1995). The value of larval indices was recently challenged because their relationship with adult densities is questionable (Reiter and Gubler, 1997). Pupal indices are now being considered as alternatives to traditional larval indices (Focks and Chadee, 1997; Focks et al., 1993). Pupal indices are attractive for three reasons. First, it is theoretically possible to make absolute counts of their abundance, something that cannot be done for flying and difficult to capture adults. Second, pupal mortality is low. The magnitude of the pupal population should, therefore, be directly and relatively easily correlated with adult densities. Third, because the pupa is the life stage that directly precedes the virus-transmitting adult, pupae should be a more direct measure of transmission risk than larvae, which are a developmental step removed from adults.

Results from our spatial analyses, however, identified some limitations of pupal indices. The transient nature and high variability of containers positive for pupae can lead to misleading survey results, especially if the goal is to identify "key premises" and if only a single survey is carried out. Examination of spatial correlations among water-holding containers, larvae, pupae, and adults reveal significant correlations between life stages that are directly linked in their developmental sequence. For example, larval clusters correlated with pupal clusters and pupal with adults, but larval clusters were not correlated with adult clusters. This indicates that many containers exhibited a cohort effect. That is to say, cohorts of mosquitoes in a given container move in synchrony through the different stages of their life cycle without overlapping other cohorts. A noteworthy observation in that regard is that we did not consistently collect all stages of mosquitoes at the same time in the same household. This indicates that containers in Iquitos are not in equilibrium with the mosquito population. Instead houses are positive for a limited period of time as mosquitoes develop, disperse, and the household reverts to being negative. Other households subsequently become positive and the process repeats itself. In locations where positive containers are ubiquitous and permanent a different pattern of cluster spatial stability may emerge.

We conclude that pattern analysis can efficiently describe local *Ae. aegypti* populations and substantially aid in our understanding of dengue epidemiology and the development of dengue surveillance and control strategies. We argue that development of long-term entomologic risk assessment strategies requires thorough surveys of all mosquito life stages. Our results highlight the importance of scale when investigating the dynamics of dengue transmission. In Iquitos, the appropriate scale for assessing mosquito vector density is the household level at frequent time intervals.

This work is being extended with more extensive studies in additional areas of Iquitos, including an entire city study of the affinity that *Ae. aegypti* may have for particular types of water-holding containers and the relationship of various measures of mosquito density to human dengue infection. In addition, related work is underway in Thailand, which will allow comparison of concepts and processes described for Iquitos to results from an ecologically and epidemiologically distinct study area.

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