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

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Abstract

Aedes aegypti is the primary vector in the Western Hemisphere for the transmission of dengue fever viruses from viremic individuals to susceptibles. The purpose of this paper is to determine the spatial pattern of adult *Aedes aegypti* mosquitoes and the containers in which they breed in two neighborhoods of the Amazonian city of Iquitos, Peru. The study was carried out over two time periods. Specifically the spatial patterns of four variables are examined: adult *Aedes aegypti* mosquitoes, pupae, all water-holding containers, and containers positive for larvae or pupae. Associations between the spatial and temporal patterns of the four variables are described. Spatial referencing of our adult mosquito survey data and application of statistical tools, like K-function and *Gi**, provided insights into adult dispersal behavior that can help explain patterns of human dengue infections. The adult mosquitoes are observed to cluster strongly within houses and weakly to a distance of 30 meters beyond the household. Specific houses are identified as being members of statistically significant clusters of adult mosquitoes. We conclude that over short periods of time the flight range and blood feeding behavior of *Ae. aegypti* are underlying factors in the clustering patterns of human dengue infections. Results indicate that any source reduction campaign, such as decreasing the number of water-holding containers, needs to be undertaken at the scale of the individual household. *Key Words: Dengue, Aedes aegypti, K-function, local statistics, Iquitos*

Introduction

Dengue is a disease caused by any of four closely related but distinct viruses (Dengue-1, 2, 3, 4) that are transmitted to people by the bites of infected mosquitoes (Gubler, 1989). All 4 viruses infect humans and cause a range of responses including inapparent infections; mild illness; acute febrile illness with headache, body aches, and rash (classic dengue fever); and potentially lethal dengue hemorrhagic fever (DHF) with shock (dengue shock syndrome) (Waterman and Gubler, 1989). Worldwide an estimated 2-3 billion people are at risk for dengue infection each year. Reported cases range from 50-100 million annually, 500,000 of which are DHF. Dengue, which occurs in tropical locations around the world, causes more human morbidity and mortality than any other arthropod transmitted virus (Gubler 1997).

The mosquito *Aedes aegypti* (L.) is the principal vector of dengue. Like other mosquito species, it develops through four life stages: egg, larva, pupa, and adult. After taking a blood meal, which most often comes from a human (Scott et al. 2000b), adult females develop and lay eggs on the side of water filled containers just above the water line. After an obligatory drying period (~24 hr), eggs hatch when they are flooded by the addition of water to the container. Aquatic larvae and pupae develop in a variety of artificial and natural containers; e.g., 55-gallon drums, discarded appliances, used tires buckets, small plastic containers, flower-pots, and less frequently, bromeliads, coconut shells, and tree holes. Immature mosquitoes pass through a series of 4 larval stages, followed by a pupal stage when they do not feed but metamorphose into flying, terrestrial adults. The duration of larval development is temperature dependent and generally ranges from 5-8 days. Mortality is characteristically high for larvae and low for pupae (Focks et al. 1993a).

Patterns of dengue transmission are influenced by the abundance, survival, and behavior of *Ae. aegypti*; 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 the mosquito (Halstead 1997). The relative influence of these factors on the dynamics of virus transmission is poorly understood, including how they vary through space and time. Until recently there has been little research on the spatial distribution pattern of *Ae. aegypti* and dengue cases. An exception was the study of a dengue epidemic by Morrison et al. (1998) in Florida, Puerto Rico. 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 infected adult mosquitoes were causing the household case clusters while infected humans traveling within the town may have facilitated the rapid spread. Waterman et al. (1985) previously reported clustering of dengue infections within households in Puerto Rico.

The most effective dengue control programs rely on entomological, viral, serological, and clinical surveillance. Early detection of virus activity allows for more streamlined application of vector control measures. Because there is no vaccine or cure for dengue, mosquito control is the only method of reducing virus transmission. Effective serological and viral surveillance is often beyond the resources of the majority of affected, developing countries. Consequently, they rely on entomological 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 entomological 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 absence of larvae in a household and the presence of adult females—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. Second, because adults are capable of flight they can move away and become spatially disassociated from their larval development sites. Third, independent of the surveillance technique (larvae, pupae, or adult collections) citywide 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. Alternative entomological surveillance methods, especially pupal surveys, were developed to circumvent the first two shortcomings (Focks et al. 1997). For the third, the assumption that there is no spatial structure among infested houses must be validated.

The purpose of this paper is to characterize the spatial distribution of *Ae. aegypti* populations in two representative neighborhoods in the Amazonian city of Iquitos, Peru. Specifically, from complete samples of households in two areas of Iquitos we examine 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 to epidemiological studies of dengue and routine dengue surveillance of our findings on estimation of entomological risk.

This study is part of a much larger effort investigating dengue activity in Iquitos. The primary goal of the larger study is to quantify the relationship between vector population abundance and transmission of dengue viruses, using a large data set, so that the assumed minimum vector density required for sustained virus transmission—the entomological threshold—can be determined. Identifying and quantifying the characteristics that increase the risk of contracting dengue are crucial components of any control program. This is especially important for dengue because most of the affected people live in poor countries, where resources available to devote to control programs are limited. Focusing available resources on identified risk factors and developing specific control goals can be an efficient means for eliminating or reducing dengue. Iquitos was chosen as the site for testing these fundamental elements of dengue control because of its geographic isolation and well-documented history of dengue (Hayes et al. 1996). Although conclusions regarding the assumptions of dengue control that we tested will require confirmation in other parts of the world, they will be the basis for comparison with dengue in other locations as well as other mosquito-borne diseases in general.

Data and Methods

Study Area

The area chosen for this study consists of two neighborhoods in Iquitos (73.2°W, 3.7°S, altitude 120 m above sea level); a city that is surrounded on three sides by the Amazon, Nanay, and Itaya Rivers. Iquitos is geographically isolated, so that it is accessible only by air or river. It is in essence an ecological island of approximately 345,000 people in the Amazon forest (Watts et al. 1999) (Figure 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 entomological surveys were Maynas, located in the north central part of the city, and Tupac Amaru, situated in the southwestern-most part of the city. These two neighborhoods were selected for study because they were characterized as areas of high (Maynas) and low (Tupac Amaru) prevalence of human dengue infection in previous informal studies. Although Maynas could be characterized as the wealthier and older of the two neighborhoods, households within both areas vary greatly in socio-economic 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 in the 20 blocks of the Maynas neighborhood and the 510 houses in the 14 blocks of the Tupac Amaru neighborhood. All houses have at least one wall in common with a neighboring house. Entomological surveys were carried out using the following strategy. Beginning in Mid-November 1998, five two-person entomology collection teams 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 07:00-13:00 h. 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; 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 an attempt was made to inspect houses that were closed or where access was refused. This was done prior to continuing surveys of unsampled households. Access to houses was attempted a minimum of three times. On alternating days each neighborhood was surveyed. This process was carried out until the houses in each neighborhood were 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 18 January 1999. In order to differentiate data associated with four different collections, the surveys will be referred to as Maynas *a* and *b* and Tupac Amaru *a* and *b*.

Entomological Surveys

Survey methodology employed in this study was based on techniques suggested by Focks et al. (1993b). 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, 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.

After recording demographic information, team members examined all potential *Ae. aegypti* development sites for water, larvae and pupae. All containers were measured (diameter, length, width and height), scored for solar exposure (proportion of day with direct sunlight, 0-1), evaluated for fill method (manually-filled/frequency, rain-filled and rain-filled with aid of rain gutter or roof) and examined for whether or not they had a lid. For containers that held immature *Ae. aegypti*, the number of larvae was estimated (1-10, 11-100 and > 100) and all pupae were collected and counted. Pupae and a sample of larvae were placed in a twist-top plastic bag and labeled with a house and container code. Larvae, pupae, and adults were transported the same day to the field laboratory in Iquitos for processing. Total collection time for a house (larvae, pupae and adult collections) varied with the size and complexity of the property (average 7 min inside and 5 min outside; range 2 - 45 total minutes).

In our field laboratory larvae were identified as *Ae. aegypti* by the relative size of the sifon and their movement compared with the other most commonly found *Culex* species. *Limatus* larvae were differentiated by the characteristics on the 8th tergite. 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, $\leq$ 30 per vial and labeled with the house and container code. Each subsequent day, emerged adults were aspirated with a mechanical aspirator, transferred to small plastic cups and placed in a 20°C freezer. After 30 minutes to 1 hour, adults were removed from the freezer and their species identified, counted by sex, and data were recorded on the entomology collection sheet.

Data Management

A geographic information system (GIS), using ARC/INFO and ARC/VIEW software was developed for the city of Iquitos. Base maps of city-blocks, obtained courtesy of the Peruvian Navy, were digitized from 1995 aerial photographs and ortho-corrected. The coordinate system and datum used was Universal Transverse Mercator and WGS-84, respectively. They were converted to ARC/INFO export files and all polygons (city blocks) were closed using standard Arc-Edit procedures. Files were then imported into ARC/VIEW.

To obtain more detailed maps of household lots use was made of the painted house 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 block in the GIS was split into lots of appropriate width using the measuring tool in ARC/VIEW. Lot length was estimated. Lot centroids were extracted 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 entomological data from the four surveys to be joined to geographic coordinates via house codes.

Analysis of the Data

In the work reported on for this study, spatial patterns of four variables were examined: adult *Ae. aegypti*, pupae, all water-holding containers, and water-holding containers positive for larvae or pupae (positive containers). These were explored by identifying the level of clustering for 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.[1] For (3), we use chi-square tests to compare similarities and differences among the various patterns.

The 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, ë(*d*) is the expected number of points within distance *d* of an arbitrary point. The estimator of *ë* is *N/A* where *N* is the number of points in the study area *A*.

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[1] The program was developed by Getis with assistance from Laura Hungerford, Dong-Mei Chen, and Jared Aldstadt. An online version is available at http://xerxes.sph.umich.edu:2000/cgi-bin/cgi-tcl-examples/generic/ppa/ppa.cgi.

The estimator of *K(d)* is

$$K(d) = A/N^2\, S_i S_j\, u_{ij}^{-1} I_d(d_{ij}\ \pounds\ d) \quad , \qquad i\ {}^{1}\ j \qquad (1)$$

where d_{ij} is the distance between the *i*th and *j*th 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 *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:

$$L(d) \equiv \surd\ [K(d)/ð] \qquad (2)$$

which is the estimator of $L(d) \equiv \surd\ [K(d)/ð]$. The mean of $L(d)$ is *d* and the approximate variance is $½(ð\ N^2)$ (Ripley 1979). 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 $L(d)$ against *d* on similarly scaled axes yields a 45-deg line beginning at the natural origin. A clustered pattern occurs when $L\ (d)$ is greater than *d* and a dispersed pattern can be identified when $L\ (d)$ is less than *d*. In the spirit of a exploratory diagnostic tool, statistical significance at the p=<0.05 level is assumed to exist when the observed $L\ (d)$ function falls outside of an envelope containing 19 permutations of the location of the *N* objects where each permutation is based on CSR. $L\ (d)$ 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),

$$L_w(d)=[\{A\, S_i S_j\, u_{ij}^{-1} I_d(d_{ij}\ \pounds\ d) x_i x_j\} / \{p[(S_i x_i)^2 - S_i x_i{}^2]\}]^{1/2} \quad , \quad i\ {}^{1}\ j \qquad (3)$$

where *X* is a random variable having values *x* for adult mosquitoes in houses at sites *i*. Equation (3) is the estimator for $L_w(d)$, which is equal to $E[L_{\mathrm{w}}(\mathrm{d})]$. 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 $(N\text{-}1)$ values x_j. In this case the numerator of $L_{\mathrm{w}}(d)$ 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 $L\,(d)$ will be approximately equal to $L_{\mathrm{w}}(d)$. Thus, Equation (3) represents a measure of clustering or dispersion identified in Equation (2). If the number of adult mosquitoes, for example, is independently distributed within the plots of houses, $L\ (d)$ will be approximately equal to $L_{\mathrm{w}}(d)$. Upper and lower significance boundaries for $L_{\mathrm{w}}(d)$ 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 explore the increments to L (d) and 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 non-cumulative properties of these pattern indicators. When the change in L (d) is greater or less than the change in 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 Ä L (d) with ÄL_{w}(d) for a given small change in d.

The G_i*(d) *Statistic*. In addition to $L(d)$, we use 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:

$$G_i*(d)=[S_j\, w_{ij}\,(d)x_j\, -\, W_i\, *x] \;\;/\;\; [s\{[N\, S_{1i}*\, -\, W_i\, *^2\,]/(N-1)\}^{1/2}] \;\; , \quad \text{all } j \tag{4}$$

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 * = Ów_{ij}(d), where w_{ii} is included, and S_{1i}* = Ów^2_{ij} (all j). The mean of the adult mosquitoes in houses is 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, $W_i\, x$, 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. Table 1 summarizes the statistics used in the analysis and the test criteria.

The Spatial Pattern of Adult *Aedes aegypti* in Maynas *a*

In order to efficiently explain the various results of the study, we begin by focusing on one neighborhood, Maynas, using data from the first 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) in the neighborhood, followed by a study of the presence or absence of adult mosquitoes in households.

Neighborhood Pattern Analysis

Table 2 and Figure 2 give the results of the K-Function analysis for adult *Ae. aegypti* in Maynas in time period *a*. Adult mosquito clustering obtains if values of $L(d)$ 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 $L(d)$ value for the pattern of houses at that same distance. Clearly, it is not enough that adult mosquitoes are spatially concentrated at the same rate as the spatial concentration of houses. Note that in Table 2, column (3), the $L_w(d)$ value for adult mosquitoes at 10 meters is 22.86, quite a bit higher than the 10.00 (random expectation) shown in column (1). But, houses are much more clustered than random (16.33 versus 10.00 at 10 meters). Even so, adult mosquitoes are more clustered than houses. In addition, using 19 permutations to identify the range of possible values for adult mosquitoes among houses (at the .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 meters. *This gives strong statistical evidence that adult mosquitoes were clustered in the Maynas neighborhood during time period "a".* The clustering is at the 10 meter level, thus, we can conclude that there is clustering around houses to at least 10 meters distant. Notice that in column (2), as distance increases to 20, 30 meters and so on, the $L(d)$ values for houses increase at a rate 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 $L_w(d)$ value for adult mosquitoes shown in column (3) at 20 and 30 meters, however, increases at a slightly higher rate than houses [column (5) versus column (4)], indicating a continuing of the clustering identified at 10 meters to at least 30 meters. This pattern of increase changes by 40 meters (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 meters any further clustering of adult mosquitoes corresponds to clustering of houses, therefore, *we conclude that adult mosquitoes cluster heavily at nearest house distances and moderately to about 30 meters.* In Maynas, the average house width was 7 m (± 3 m) and thus adult clusters could extend to about 2 households on each side.

As a special feature of our analysis, we altered equations (1) and (3) to include houses themselves, that is, we allowed i to equal j. This means that our focus now is on *houses and their neighbors* rather than neighboring houses only. Table 3 and Figure 3 presents the results of this special case (see Getis 1984 for an explanation of this methodology). In this circumstance, the clustering of houses (column 2) is inflated to include not only near neighbors at 10 meters, but also the houses themselves. The original value of 16.33 at 10 meters now rises to 21.44 for houses indicating that, in this view, houses are more clustered than was indicated previously (an increase of 31 per cent). More importantly, however, are the results when adult mosquitoes within houses are taken into account. Here the value at 10 meters rises to 39.30 from 22.86, a rise of 72 per cent. The implication is that adult mosquitoes are heavily clustered within houses. Note, too, that as distance increases, the increment to houses and adult mosquitoes is about 10, indicating that there is a cessation of clustering beyond 10 meters. *These results taken together with those above 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 meters. The clustering within houses in the Maynas neighborhood quantitatively overwhelms this further clustering.

Household Pattern Analysis by Numbers of Adult Mosquitoes

Now that it is evident that there is short distance clustering of adult mosquitoes in Maynas *a*, we now identify the exact houses that can be considered as members of clusters. First, we consider the actual numbers of adult mosquitoes in each house in

Maynas *a* (see Figure 4). If the clustering is within households, the G_i* statistic will be above +2.575 at short distances, say 1 meter at the 0.01 level of statistical significance. If clustering continues to near neighbors within 10 meters of a house, the value of G_i* will be higher at 10 meters than at 1 meter. 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 meter, 10 meters, 20, and 30 meters are shown in Figure 5. Note that of the 528 houses in Maynas during time period *a*, 35, or 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, 7 show clustering to 10 meters, 2 to 20 meters and 1 to thirty meters. *Again, this reinforces the notion that adult mosquitoes tend to cluster in single households with a modest spread to as far as 30 meters.*

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

One further aspect of the nature of the pattern of adult mosquitoes is presented in Figure 6. Here we see a map of the presence of one or more mosquitoes in households. One hundred sixty-four, or 31.1%, of the houses have one or more adult mosquitoes present. This is a large number when one considers, as shown above, that only 35 of them, or 21.3%, are members of statistically significant clusters. *The implication here is that although there is clustering present in Maynas in time period a, the clusters are made up mainly of household concentrations and that 79.7% of the households with mosquitoes are spread about in a random pattern among all households.*

Patterns of Pupae, Water-holding Containers, and Positive Containers

The analysis described above for adult *Ae. aegypti* was carried out for the other variables in the study. Following is a brief review of the results.

Neighborhood Pattern Analysis

Tables 4 and 5 allow for the comparison of K-function values for the three non-adult mosquito variables with house and adult mosquito patterns in the Maynas neighborhood (Equations 1 and 3). The *d=10 meters* row in Table 4 shows, as before, that adult mosquitoes cluster more so than houses (22.86 to 16.33), but the pattern of water-holding containers and positive containers is more nearly like the pattern of houses (16.25 to 16.33 and 15.40 to 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. When we allow *i* to equal *j*, however, as seen in Table 5, pupae jump from 12.03 to 56.13, an extremely high and statistically significant value. *This means that pupae are extremely heavily clustered within houses, even more so than adult mosquitoes, while the clusters themselves are dispersed rather evenly throughout the neighborhood* (Table 4).

Since the water-holding container spatial data are similar to the house location data in both Tables 4 and 5, we conclude that water-holding containers are ubquitous in Maynas.

That is, nearly all houses have water-holding containers. On the other hand, containers positive for pupae and/or larvae are more concentrated in some houses than in others, but not at the level of concentration of adult mosquitoes or pupae.

Continuing on to 20, 30 meters, and further distances in Tables 4 and 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 items. Pupae values, in Table 5, however, increase at a much slower rate than houses after 10 meters, *indicating that as before the dispersed pattern of pupae is characterized by extreme clustering within households*.

Household Pattern Analysis

The *Gi** statistic allows us to identify the particular houses with clusters of the three variables. *Our results show that there is a lack of statistically significant clustering beyond households for all three variables*. In the case of pupae, there are 18 households exhibiting clustering with no clustering beyond the household. Of the 24 houses with clusters of containers, only 2 are clustered to a neighboring distance of 10 meters. For positive containers, 23 houses exhibit clustering, but only 3 of those are clustered beyond the household, two to 10 meters and one to 20 meters.

Patterns of Pupae: Presence or Absence in Houses

Here the concern is less with numbers of pupae in houses and more with their spatial occurrence in houses. Figure 7 shows the location of statistically significant clusters of houses having one or more pupae present in the survey. This was 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 of the 528 (3.4%) houses can be considered as members of clusters at the 99 percent 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, which we address in the next section.

Comparison of Entomological Spatial Patterns in Maynas *a*

Before we treat results from all four surveys, we briefly focus on a comparison of patterns in Maynas *a*. The degree of similarity between the patterns of adult mosquitoes and pupae allow us to identify any relationship that might exist between them. 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

Association Among Clusters

In Table 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-square = 1.60, 1 df, Yates correction for small expectations). *We conclude that there is 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

Table 7 reveals a somewhat different pattern of association. Here we are looking for the overlap of households that have as few as one mosquito or one pupa present. Note that of the 528 houses in Maynas 164 had at least one mosquito present and 155 had at least one pupa present in time period *a*. In terms of traditional *Ae. aegypti* indices this represents a House (Premise) Index of 31. Results from a chi-square two-by-two contingency test indicate that the two types of occurrence come together in households 48 times. In fact, on 66 occasions this was the case; a statistically significant result at the 0.01 level. *Thus, although elevated numbers of the two variables do not correspond, the mere presence of pupae in a household indicates that one or more adult mosquitoes are present.*

Association of Water-Holding Containers and Adult Mosquitoes and Pupae

Because there are water-holding containers in every household in Maynas , we will focus on the number of containers, adult mosquitoes and pupae. Table 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 and ranks 15 and 16 were made up of 15 to 19 and 20 to 35 containers, respectively. The final two ranks were grouped because of the few numbers of observations at these high levels. The mean number of adult mosquitoes per house was ranked for each container level. The result is that there is a moderately high positive correlation for mosquitoes (+0.615, significantly different than 0 at the 0.05 level), and a modest correlation for pupae (+0.487, not significant at the 0.05 level). *We conclude from this that elevated numbers of water-holding containers in houses increase the likelihood for elevated numbers of mosquitoes and/or pupae to be present.* This result for Maynas *a*, although important, may not represent results from the other surveys. We now consider result from the other three surveys

The Degree of Consistency Between Entomological Spatial Patterns in Maynas *a* and *b* and Tupac Amaru *a* and *b*

In this section we consider the similarities and differences among the four variables in the two surveyed neighborhoods and between the two time periods. As in the previous section, we test for statistically significant relationships among the variables.

Maynas Neighborhood versus the Tupac Amaru Neighborhood

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 (proportion of surveyed houses with $\geq$ 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 4 surveys, but the level of clustering was greatest during the first Tupac Amaru survey. Table 9 gives L(d) values (*i* may equal *j*) for each of the four surveys for 10 meters. We can see that houses in Tupac Amaru are slightly more clustered than in Maynas (25.00 to 21.44). Note, too, that in both neighborhoods water-holding containers are distributed much the same as are houses, but positive containers tend to cluster. Maynas with 29.05 and 31.00 in the two time periods are about 8 to 10 L units higher than the pattern of houses. Tupac Amaru with 38.56 and 44.30 are about 13 to 19 units higher than the pattern of houses. This implies that positive containers are 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 is relatively low.*

Time Period *a* versus Time Period *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 entomological surveys (Reiter and Gubler 1997). Despite only 3 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, were 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 water-holding containers decreased by 13% in Tupac Amaru compared to only 3% in Maynas. This indicates that our survey methodology had an impact on measures of mosquito abundance at both sites, with the greatest effect being in Tupac Amaru.

Adult Mosquito and Pupae Household Clustering

Table 6 indicates the number of houses that were members of statistically significant clusters of pupae and adult *Ae. aegypti*. Interestingly, the number of houses included in clusters for pupae in Maynas decreased from 18 to 4 from time period *a* to *b*. Perhaps of greatest interest is that the location of adult clusters changed between the two surveys. Twenty-eight households were members of adult clusters in the first Mynas survey that were not members of clusters in the second, a statistically significant finding that was not the case in Tupac Amaru. Only 7 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. No Maynas houses infested with pupae were detected in survey *a* while an elevated number were found in both time periods in Tupac Amaru. *This result gives us a picture of an entomological surface that 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 Gi* are not consistent overtime, infestation of individual households by *Ae. aegypti* is clearly a risk factor for future infestation. That is, there is evidence of repeat offenders. Table 7 shows the number 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 29-45% of the time, a statistically significant result. The implication is that for unknown reasons *mosquitoes are more likely to lay eggs on some house lots than others*. Another risk factor for infestation is the number of water-holding containers in a household. Results in Table 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*.

Implications for Dengue Control

Historically, entomological 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 (PAHO) declared 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 a guideline to control dengue. For example, experience with yellow fever and recent computer simulation estimates indicate that entomological thresholds for dengue are low (Reiter and Gubler 1997, Focks et al. 1995, 1997), 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 the 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 autocorrelation and scale in statistical analyses; (2) the most appropriate measure of entomological risk—should absolute numbers or indices be measured and what life stage of the mosquito provides the best estimate for risk of dengue virus transmission; 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 i^{th} house. Our K-function analysis indicates that individual households are the appropriate spatial unit for entomological 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. However, results from our analysis 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, not discussed in this paper, like the availability of piped water, local temperature, rainfall patterns or garbage disposal.

Identification of "key premises" or households that are super producers of *Ae. aegypti* has been proposed as a way to streamline surveys (Tun-Lin et al. 1995). The idea here 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 entomological 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. 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 of permanent water holding containers (data not shown) our results may be site specific and require the same kind of thorough examination that we carried out—large sample sizes and spatial analysis—at other locations.

Although small, there was significant spatial structure of adult mosquito populations compared to pupae and positive containers. Adults cluster most to distances of about 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 entomological 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) because viremic people can have an inapparent infection or may not seek medical assistance, the homes and surrounding areas of most people infective to mosquitoes will not be sprayed.

Our statistical approach does corroborate 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. Ordonez et al. (1997) reported minimum and maximum daily flight distance for *Ae. aegypti* of 8 m 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 et al. (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 like 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, like K-function and *Gi**, provided insights into adult dispersal behavior that help explain patterns of human dengue infections. We propose that over short periods of time the flight range and blood feeding behavior of *Ae. aegypti* are underlying factors in the clustering patterns of human dengue infections. In addition to the studies cited above on *Ae. aegypti* dispersal, numerous researchers have reported spatial and temporal clusters of clinically ill dengue patients in the same household or adjacent houses (Halstead et al. 1969, Chan 1985, Waterman et al. 1985, Gubler 1989). In the first, spatial statistics analysis of this phenomenon, Morrison et al. (1998) found that dengue cases reported within a 3-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 closer together than in Puerto Rico. Houses in Iquitos were mostly row houses with common walls. Homes in Florida, Puerto Rico, were larger and often separated by at least 10 m. 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 the two locations. In Iquitos water-holding containers were found in all the households surveyed, something that is expected to decrease the probability of female dispersal. Edman et al. (1998) demonstrated that the availability of suitable ovipostion sites is positively correlated with the ability of the female *Ae. aegypti* to disperse her eggs.

Abundance of adult female mosquitoes should be the most appropriate measure of entomological risk because they are in the life stage in 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, Focks and Chadee 1997). Pupal indices are now being considered as alternatives to traditional larval indices (Focks et al. 1993b, 1997). 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 proceeds 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 identified 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 the 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.

Conclusions

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 entomological risk assessment strategies require thorough surveys of all mosquito life stages. Permanent containers—key premises—were not major producers of *Ae. aegypti* in Iquitos, indicating that larvaciding strategies by themselves may be less effective than reduction of mosquito development sites by source reduction and education campaigns.

For purposes of investigating the dynamics of dengue transmission, our results point out the need to assess risk of human infection at the household level at frequent time intervals. Our detailed spatial approach constitutes the framework for analysis of data from ongoing longitudinal studies in Iquitos in which we will assess entomological risk at the level of the household with human dengue infection, and ultimately severity of disease.

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Table 1. Summary of Clustering Statistics

Test	Purpose	Scale	Cut-off for Statistic
$Å(d)$	To identify the existence of clustering for a 1/0 variable in a neighborhood	*d*	19 simulations of random occurrence within neighborhood (.05 level)
$Å_w(d)$	To identify clustering of a weighted variable in a neighborhood	*d*	99 simulations of random occurrence within eligible locations of variable (.01 level)
$G_i^*(d)$	To identify individual observations of a variable who are members of clusters	Z	>+2.575 (.01 level)

Table 2. L(d) Values for Distances 10 to 100 Meters for Houses and Adult Mosquitoes in Maynas *a*: *i* Does Not Equal *j*

Distance	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

Table 3. L(d) Values for Distances 10 to 100 Meters for Houses and Adult Mosquitoes in Maynas *a*: *i* May Equal *j*

Distance	Houses	Adult Mosquitoes	House Increment	Adults 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

Table 4. L(d) Values for Distances 10 to 100 for Houses, Adult Mosquitoes, Pupae, Water-Holding Containers, Positive Water-Holding Containers in Maynas *a*: *i* Does Not Equal *j*

Diatance	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

Table 5. L(d) Values for Distances 10 to 100 Meters for Houses, Adult Mosquitoes, Pupae, Water-Holding Containers, Positive Water-Holding Containers in Maynas *a*: *i* May Equal *j*

Distance	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

Table 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**	2
Pupae in *a* and *b*	0	6**
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*
Adults in *b* and Pupae in *b*	0	0

* Significant at 0.05 level
** Significant at 0.01 level

Table 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**	20	15
Pupae in *a* and *b*	70	39**	25	11**
Adults in *a* and Pupae in *b*	53	42*	14	11
Pupae in *a* and Adults in *b*	50	44	20	15
Adults in *a* and Pupae in *a*	66	48**	25	14**
Adults in *b* and Pupae in *b*	50	38**	15	11

* Significant at 0.05 level
** Significant at 0.01 level

Table 8. Spearman's Rank Correlations of the Number of Containers Per House With the Number of Mosquitoes and Pupae Per House

Containers in	Mosquitoes	Pupae
Maynas *a*	+0.615*	+0.487
Maynas *b*	+0.682**	+0.594*
Tupac Amaru *a*	+0.284	+0.486
Tupac Amaru *b*	-0.199	+0.481

* Significant at 0.05 level
** Significant at 0.01 level

Table 9. L(d) Values for 10 Meters for Maynas and Tupac Amaru for Time periods *a* and *b*: *i* May Equal *j*

	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