Spatial and Temporal Patterns in Pupal and Adult Production of the Dengue Vector
*Aedes aegypti* in Kamphaeng Phet, Thailand

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Abstract. We investigated how temporal and spatial effects confound the functional relationship between pupal and adult populations of *Aedes aegypti* and thus the value of pupal numbers as predictors of dengue transmission risk in Kamphaeng Phet, Thailand. We found considerable seasonal shifts in productivity of key containers. Tires contained much less pupae in the dry season than in the wet season. Earthenware jars and cement tanks for washing purposes were consistent producers over the entire study period. Houses in the two villages, with approximately twice as many houses per unit area, were significantly more likely to have adults and pupae. No significant annual, seasonal, or spatial effects on the strength of correlations between pupal and adult populations were found. Except for 2 (of 16) occasions, pupal and adult populations were correlated strongly in time and space. Our results are consistent with application of the pupal survey technique for assessing dengue transmission risk.

INTRODUCTION

The increase in and rapid recurrence of major outbreaks of dengue and dengue hemorrhagic fever in many tropical and subtropical regions have made control of this disease a top-priority for many public health authorities.1,2 In the absence of an effective vaccine, efforts have focused on control of the mosquito vector *Aedes aegypti* L. through reduction of larval development sites (i.e., source reduction) and/or application of insecticides to kill immature forms or adults. Addition of the larvicide temephos to water-holding containers, use of biologic of insecticides to kill immature forms or adults. Introduction of the pupal/demographic survey technique is able to identify important insights into the ecology of *Ae. aegypti*. However, there is a need to better understand how temporal and spatial effects confound the functional relationship between pupal and adult populations and thus the value of pupal numbers as predictors of dengue transmission risk. Some previous studies focused on either immature8–11 or adult populations,12,13 whereas others investigated correlations between both life stages.14–17 In this study, we examined temporal and spatial patterns in pupal productivity and adult abundance in a dengue endemic area in north central Thailand. Temporal variations were seasonal and annual, whereas spatial variation was the result of varying house density and different geography. We determined how productivity of pupae and adults is correlated and if there is a spatial and temporal pattern in these relationships. Specifically, we asked whether *Ae. aegypti* life stages are structured differently among seasons, years, and geographic locations? And, does house density play a role in those associations?

MATERIALS AND METHODS

Study area. The study was conducted in Kamphaeng Phet Province, Thailand (Figure 1). All four dengue virus serotypes circulate in the study area and can cause mild to severe manifestations of dengue fever and dengue hemorrhagic fever.18,19 All studied households were part of a larger epidemiologic study that encompassed > 4,000 households in 19 villages in four subdistricts of Kamphaeng Phet.20 Houses were mapped at the onset of the study and demographic information (number of adults and children living in each house) was recorded.

Study design. House density, defined as the number of neighboring houses within a 100-meter radius from a house, was calculated for each house of the 19 villages and then averaged for all houses within a village with MapInfo professional version 6.0 software (MapInfo, Troy, NY). We then selected two of the four subdistricts for a comparative study: Kon Tee
Kon Tee is located along the Ping River, which runs from northwest to southeast. Na Bo Kham is situated 20 km west of the river (Figure 1) and is surrounded by gently rolling hills that are covered with open deciduous forest. Irrigated rice fields and sugarcane plantations form the main type of land use in both subdistricts.

We selected the two subdistricts so that we could capture what we thought a priori would be region wide the spatial variation in distribution of houses. Within each subdistrict, two villages were selected of which one had a high density of houses and the other a low density of houses. Kon Tee, village 2 and Kon Tee village 13 had mean ± SD house densities of 27.5 ± 12.0 (n = 152) and 14.0 ± 6.8 (n = 123), respectively. Na Bo Kham village 5 and Na Bo Kham village 15 had mean ± SD house densities of 25.6 ± 8.0 (n = 175) and 16.6 ± 9.0 (n = 132), respectively. In 2004 and 2005, entomologic surveys were carried out twice a year in these four selected villages daily for approximately one month: once at the end of the dry season (from March through April; beginning of the dengue transmission season) and once at the end of the wet season (from September through October; end of the dengue transmission season). In this way, we could investigate the temporal effects of year and season and the spatial effects of subdistrict (geographic location) and house density. In total, 604 households were included over the entire period, of which 482 (79.8%) were visited on all four survey occasions.

Entomologic surveys. Seven two-person teams inspected the households between 8:00 AM and 12:30 PM. Two teams were responsible for collection of adults and the remaining five teams for collection of immature forms. Each team consisted of one staff member of the United States Armed Forces Research Institute of Medical Sciences Entomology Laboratory in Kamphaeng Phet and one collector was recruited from the village being sampled.

All water-holding containers on the plot of a household were inspected for larvae or pupae and described on entomologic survey forms. If pupae were encountered, all were collected. If only larvae were encountered, a sample was taken, depending on their density (all if density ≤ 10, 10 if density between 11 and 100, and 20 if density > 100). Recorded variables included container dimensions, water depth, location (indoor/outdoor), filling method (rain/manual), cover status (with/without), temephos status (with/without), and fish status (with/without). In the laboratory, larvae and pupae were cross-checked with the survey forms and reared to adults. Emerged mosquitoes were killed by freezing and identified as *Ae. aegypti*, *Ae. albopictus*, *Culex* spp., or *Toxorhynchites* spp. Their numbers were recorded by the individual container they were collected from, sex, and species. At least one larva or pupa was identified from 95% of the samples that were positive for immature mosquitoes. Larvae or pupae died or escaped before identification of adults in the remainder of the samples.

Adult mosquitoes were collected with battery-powered backpack aspirators (John W. Hock Company, Gainesville, FL). Collections were made from all indoor rooms, if permitted. Outdoor collections were made in separate collection cups from walls and objects located within three meters of the edge of the roof, but not from vegetation. For houses elevated on poles (approximately half of the houses), the outdoor area included the area underneath the house. Collections were also made from the toilet area. After collection of adult mosquitoes, we asked homeowners about the date of
last visit of Public Health authorities and the purpose of their visit (application of temephos or spraying of adulticides). This information was recorded on forms that also included information on house structure and water use practices.

In the laboratory, adult mosquitoes were killed by freezing and identified as *Ae. aegypti*, *Ae. albopictus*, *Culex* spp., *A. nigerris* spp. or *Anopheles* spp. Their numbers were recorded on entomologic survey forms by individual household, location (indoor/outdoor/toilet) and mosquito sex. All entomologic data were entered twice in Microsoft Office Access 2003 (Microsoft Corporation, Redmond, WA). The two databases were then synchronized and cleaned by checking empty cells, extreme values, and variables with inconsistent data when compared with other variables.

**Data analyses.** To overcome the non-normal distribution of pupal and adult populations across houses (Figure 2), we investigated spatial and temporal effects with multinomial regression, whereby the 2,123 samples that were obtained from houses over the entire study were assigned to one of three pupal and adult density categories: 1) no pupae or adults (negative), 2) less than the median (low), and 3) more than the median of positive houses (high). For pupae, the median value of positive houses was 2.25 pupae per person per house, whereas for adults the median was 0.5 females per person per house. A total of 1,167 (53.3%) were assigned to the negative pupal density category, 488 (22.3%) to the low pupal density category, and 477 (21.8%) to the high pupal density category. For adults, 1,334 (60.9%) were assigned to the negative category, 453 (20.7%) to the low category, and 345 (15.7%) to the high category (Figure 2).

**RESULTS**

During four entomologic surveys, 37,663 water-holding containers were characterized. Of those, 3,914 (10.4%) were positive with *Ae. aegypti* immature forms and 1,866 (5.0%) were positive for pupae. Most water-holding containers were located outdoors (66.1%), did not have a lid (72.1%), and were manually filled (77.9%) by the occupants of the household. In contrast, 56.8% of the positive containers were located outdoors, 83.7% did not have a lid, and 83.7% were manually filled. *Aedes albopictus* immature forms were found in 864 (2.3%) of inspected containers and 222 (25.7%) of these were shared with *Ae. aegypti*.

**Container productivity.** Container numbers and total production were divided by container class for three consecutive surveys and are shown in Table 1. We had not developed a consistent method of container classification for the first survey in March–April 2004. Therefore, those data are not included here. A standardized classification scheme was applied to the remaining three surveys. Total production of pupae from all containers ranged from 1.29 to 1.92 pupae per person. Ranking container classes by pupae productivity showed that earthenware jars and cement tanks, both used for general washing purposes, were consistently the two most productive container classes. Their relative contributions to the total pupae standing crop were 42.3%, 59.0%, and 42.6% for September–October 2004, March–April 2005, and September–October 2005, respectively. Comparing only the wet season surveys (September–October) showed that rubber tires (no use) and cement tanks used for flushing toilets were consistently productive in 2004 and 2005, whereas these container classes were much less productive during the dry season (March–April 2005, ranks 5 and 19). Relative production was consistent throughout the three surveys with 58.7%, 63.8%, and 59.4% in September–October 2004, March–April 2005, and September–October 2005, respectively, being produced by earthenware jars used for washing, cement tanks used for washing and flushing, and tires. In March–April 2005, the four most productive containers (ranks 1–4) accounted for 70.3% of pupae production.

**Temporal and spatial patterns in vector abundance.** Figure 3 shows the temporal dynamics of the number of positive containers per house, pupae per person, and adult female *Ae. aegypti* per person for each of the four villages. Numbers shown are pupal and adult female densities at the village level calculated as total pupae/adults per village divided by total
persons per village. Numbers of positive containers were higher than 2.0 (equivalent to a Breteau index of 200) on 7 of 8 occasions in Kon Tee subdistrict, whereas they were always less than 2.0 in Na Bo Kham. Pupal densities ranged from 1.7 to 3.6 pupae per person in Kon Tee and from 0.24 to 2.8 pupae per person in Na Bo Kham. Adult densities were more constant over the seasons and two years of study, especially in Kon Tee.

To overcome problems with analysis of the highly skewed distribution of pupae and adults at the household level (many

<table>
<thead>
<tr>
<th>Shape</th>
<th>Use</th>
<th>Material</th>
<th>Sep/Oct 2004</th>
<th>Mar/Apr 2005</th>
<th>Sep/Oct 2005</th>
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## Table 1

| Container classes (as defined by shape, use, and material) and their numbers per house (CT/HSE), numbers pupae positive per house (PPC/HSE), contribution to *Aedes aegypti* pupae per person (PU/PER), and their relative rank during three surveys in Kamphaeng Phet Province, Thailand |

*Figure 3.* Temporal dynamics in densities of positive *Aedes aegypti* containers, *Ae. aegypti* pupae, and adult female *Ae. aegypti* in Kon Tee (A and B) and Na Bo Kham (C and D), Thailand.
houses are devoid of pupae and few houses have many, Figure 2), we further investigated spatial and temporal effects with multinomial regression. In this case, pupal/adult densities were calculated as their numbers per house divided by the number of persons living in that house. Thus, the 2,123 house samples that were obtained over the entire study were assigned to one of three pupal and adult density categories; i.e., negative, low, and high, as described earlier.

First, we modeled the effects of year, season, subdistrict, and house density and their two-way interactions. That analysis showed strong interacting effects of subdistrict \( \times \) year and subdistrict \( \times \) season for the multinomial pupal density model. There was a strong interacting effect of subdistrict \( \times \) season for the multinomial adult density model (all \( P < 0.001 \), by likelihood ratio test).

Next, we modeled annual, seasonal, and house density effects separately for the two subdistricts. Distributions of the relative number of houses in each density category are shown in Figures 4 and 5 for pupae and adults, respectively. The pupal density model for Kon Tee showed no significant effects of year or season (\( \chi^2 = 2.511 \), degrees of freedom \([df] = 2 \), \( P = 0.285 \) and \( \chi^2 = 0.892 \), \( df = 2 \), \( P = 0.640 \), respectively; Figures 4A and B). In Na Bo Kham, the second year had significantly more houses without pupae at the expense of both low and high density category houses (\( \chi^2 = 22.812 \), \( df = 2 \), \( P < 0.001 \); Figure 4D). The seasonal effect in Na Bo Kham was strong and opposite of what was expected: the dry season had significantly more houses belonging to the low and high categories (\( \chi^2 = 83.759 \), \( df = 2 \), \( P < 0.001 \); Figure 4E).

The effect of house density was consistent for both subdistricts. In villages where houses were more distantly spaced apart (i.e., lower density), houses were more likely to be negative (\( \chi^2 = 7.222 \), \( df = 2 \), \( P = 0.027 \) and \( \chi^2 = 9.138 \), \( df = 2 \), \( P = 0.010 \) for Kon Tee and Na Bo Kham, respectively; Figures 4C and F).

In Kon Tee, the adult density model showed no significant shifts between years, which was consistent with pupal distributions (\( \chi^2 = 4.181 \), \( df = 2 \), \( P = 0.124 \); Figure 5A). In Na Bo Kham, during the second year there was a significant increase in houses with low densities of adult female \textit{Ae. aegypti} compared with the first year (\( \chi^2 = 6.455 \), \( df = 2 \), \( P = 0.040 \); Figure 5D), opposite of what was seen for the pupal population (Figure 4D).

Although the relative proportion of negative houses remained the same between seasons, there was an increase in high-density houses at the expense of low-density houses in the wet season in Kon Tee (\( \chi^2 = 9.397 \), \( df = 2 \), \( P = 0.009 \); Figure 5B). Densities were lower in the wet season in Na Bo Kham (\( \chi^2 = 171.802 \), \( df = 2 \), \( P < 0.001 \); Figure 5E), which is consistent with the pupal distributions. The effects of house density were highly significant in both subdistricts: low-density villages had more negative houses and less houses in the low and high density categories (\( \chi^2 = 30.826 \), \( df = 2 \), \( P < 0.001 \) and \( \chi^2 = 14.147 \), \( df = 2 \), \( P = 0.001 \) for Kon Tee and Na Bo Kham, respectively; Figures 5C and F), which is consistent with the effect of house density in the population model.

**Correlations between pupal and adult populations.** Pearson correlation coefficients among numbers of \textit{Ae. aegypti} pupae per person and adult female \textit{Ae. aegypti} per person were highly significant, except on two occasions. The two wet season surveys in Na Bo Kham village 5 (high density) showed no correlation between pupae and adult numbers (Table 2). Because this village had many houses without pupae or adults during the wet season (Figures 4E and 5E), we confirmed our findings by calculating Kendall’s \( \tau \) between nominal pupal and adult categories for these two wet season surveys. Values of \( \tau \) remained insignificant (\( \tau = -0.013 \), \( P = 0.875 \), \( \tau = 0.14 \), \( P = 0.096 \)). The remaining Pearson correlation coefficients ranged between 0.246 and 0.460. We did not find any variable (year, season, subdistrict, or house density) that significantly explained variation in observed coefficients (\( P > 0.24 \), by Mann-Whitney test).

To investigate the role of ongoing vector control activities, we calculated the proportion of houses where Public Health authorities had visited during the survey and either applied adulticides or larvicides (Figure 6). Although there were marked differences between subdistricts and seasons, these differences could not explain the absence of correlation between pupal and adult populations in Na Bo Kham village 5 (high density) during the two wet season surveys.

**DISCUSSION**

The identification of epidemiologically important containers for \textit{Ae. aegypti} pupal productivity is a focus of evaluation for improving contemporary vector control programs. In our study area, there were four container classes that consistently produced approximately 60% of \textit{Ae. aegypti} pupae; i.e., jars and tanks used for general washing purposes, tanks used for flushing bathrooms, and tires. Although these were the top four producers near the end of the wet season (September–October of 2004 and 2005), two container classes (i.e., jars and tanks that were not in use), ranked 3 and 4 at the end of the dry season in 2005. There are two factors contributing to this finding. First, rain-filled tires were much less numerous in the dry season, and thus dropped out of the top of pupae producers. Simultaneously, jars and tanks not in use became more productive although they were not more abundant (Table 1). The reason for this finding is not entirely clear, but may be related to a more economical use of water during the dry season. Water is scarce when it cannot be collected from rain and thus may be stored and stand in containers for a longer time with less turnover. As a result, water storage jars during the dry season may accumulate more food and become more productive. Unfortunately, we were not able to confirm if the same trend was true in 2004 because of inconsistently classified containers.

Depending on assumptions regarding initial seroprevalence of dengue antibody in the human population and number of viral introductions, our values of pupal densities per person (ranging from 1.7 to 3.6 in Kon Tee and from 0.2 to 2.8 in Na Bo Kham) are either above or below transmission thresholds predicted from dengue transmission threshold models (Container Inhabiting Mosquito Simulation Model and Dengue Transmission Simulation Model). For example, at 28°C, the threshold with 67% seroprevalence and monthly introductions is approximately 1.3 pupae per person, whereas the same threshold with 33% prevalence is approximately 0.6. In theory, both threshold values would lead to control efforts in Kon Tee, but not necessarily in Na Bo Kham. This finding further stresses the importance of the collection of seropreva-
FIGURE 4. Annual, seasonal, and house density effects on the distribution of *Aedes aegypti* pupal densities in Kon Tee (A, B, and C) and Na Bo Kham (D, E, and F), Thailand. *P* values indicate significance resulting from multinomial logistic regression.
FIGURE 5. Annual, seasonal, and house density effects on the distribution of *Aedes aegypti* adult female densities in Kon Tee (A, B, and C) and Na Bo Kham (D, E, and F), Thailand. *P* values indicate significance resulting from multinomial logistic regression.
producing houses from the high group becoming low productivity from low houses becoming negative or from extreme positive or negative. For example, a decrease in density could be detected by mial logistic regression in which houses are classified as either decreases in density that would not be possible with logistic regression. The latter study included multiple locations (8 geographic zones) and sample events (12 sampling circuits over 4 years) to determine spatial and temporal patterns in mosquito productivity.

Our analyses showed that house density had the strongest and most consistent effect on pupal and adult densities. Houses in the two villages with approximately twice as many houses per surface area were significantly more likely to have increased housing density may provide increased opportunities for \textit{Ae. aegypti} larval development and adult survival. Second, for adult \textit{Ae. aegypti}, documented patterns of dispersal may be an important factor. In Iquitos, Peru (a crowded urban environment where most houses are close to one another or are adjoining) a spatial analysis demonstrated that adults clustered within a 30-meter radius. Mark-release-recapture studies in rural Thailand villages and Puerto Rico showed little dispersal of \textit{Ae. aegypti} beyond neighboring houses. In terms of distances between houses, we calculated that in our high-density villages 5.2% and 6.9% of houses were spaced > 30 meters apart, whereas in the low-density villages 15.5% and 16.5% of houses were spaced > 30 meters apart. Because of the closer distance between houses in high-density villages, adult mosquitoes that emerge in one house may be more likely to disperse to a neighboring house. Thus the proportion of houses with adult \textit{Ae. aegypti} may be relatively higher in these high-density villages. Confirmation of this proposition requires additional field research.

We hypothesized that the strength of correlations between pupal and adult populations depends on season, year, or geographic location. We also hypothesized that houses that were more closely spaced would show less correlation because of a more pronounced smoothing effect as a result of adult dispersal that is more likely if houses are spaced closer. However, no significant main effects on the strength of the correlations were found. Pupal and adult populations correlated strongly, except for two occasions. These occasions were from the two wet season surveys in Na Bo Kham village 5 (high density). Because the study was designed in such a way that it did not interfere with ongoing vector control activities by public health authorities, which mostly consisted of the application of temephos, we suspected that vector control activities may be the cause of the absence of correlation between pupal and adult populations during the wet, high transmission seasons in Na Bo Kham village 5. Although there was more vector control activity during the wet season of year 1 in Na Bo Kham village 5 (Figure 6), the other village in Na Bo Kham had what appeared to be a similar amount of vector control and showed a significant correlation between pupal and adult populations. An earlier study in the same villages found that households in
Na Bo Kham were more likely to protect containers (but this included temephos use provided by public health authorities) and use preventive measures against adult mosquitoes. This finding may partially explain the absence of correlation in current study.

Our results are consistent with the concept of adaptive Ae. aegypti interventions. Across different villages and sampling time periods, we detected considerable shifts in productivity of key containers. For example, tires were main producers in the wet season, whereas they did not contribute to pupal production in the dry season. However, two types of containers (earthenware jars and cement tanks for washing purposes) were consistent producers over the entire study period. The strong correlation between pupal and adult populations is consistent with application of the pupal survey technique for assessing dengue transmission risk. However, development of a precise Ae. aegypti control package for a given location will require tailoring control activities to site-specific conditions, patterns of mosquito vector production, and susceptibility of the human population to dengue infection.

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