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Indoor Resting Behavior of *Aedes aegypti* (Diptera: Culicidae) in Acapulco, Mexico

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Abstract

The markedly anthropophilic and endophilic behaviors of *Aedes aegypti* (L.) make it a very efficient vector of dengue, chikungunya, and Zika viruses. Although a large body of research has investigated the immature habitats and conditions for adult emergence, relatively few studies have focused on the indoor resting behavior and distribution of vectors within houses. We investigated the resting behavior of *Ae. aegypti* indoors in 979 houses of the city of Acapulco, Mexico, by performing exhaustive indoor mosquito collections to describe the rooms and height at which mosquitoes were found resting. In total, 1,403 adult and 747 female *Ae. aegypti* were collected, primarily indoors (98% adults and 99% females). Primary resting locations included bedrooms (44%), living rooms (25%), and bathrooms (20%), followed by kitchens (9%). *Aedes aegypti* significantly rested below 1.5 m of height (82% adults, 83% females, and 87% bloodfed females); the odds of finding adult *Ae. aegypti* mosquitoes below 1.5 m was 17 times higher than above 1.5 m. Our findings provide relevant information for the design of insecticide-based interventions selectively targeting the adult resting population, such as indoor residual spraying.

Key words: mosquito abundance, dengue, indoor residual spraying, vector behavior

Aedes aegypti (L.) is one of the world's most widely distributed mosquito species and, as a vector of dengue, yellow fever, chikungunya, and Zika viruses, a major contributor to the global burden of mosquito-borne illness (Bhatt et al. 2013, Brady et al. 2014). Remarkable behavioral and ecological attributes make this mosquito an efficient vector. *Aedes aegypti* is well-adapted to completing its entire life cycle within urban areas in and around houses, primarily feeding on humans at a very high frequency (<2 d), a trait that leads to very high human–mosquito contacts and dengue virus attack rates (Kuno 1995, De Benedictis et al. 2003, Stoddard et al. 2013, Liebman et al. 2014). Given that *Ae. aegypti* is primarily found indoors (Perich et al. 2000, Chadee 2013) and that both males and females seldom disperse beyond 100 m (Harrington et al. 2005, Russell et al. 2005), identifying the environmental and behavioral conditions that influence its resting behavior is crucial for devising innovative targets for vector control.

Early studies on the resting behavior of *Ae. aegypti* in Thailand have shown that the mosquito predominantly rests indoors, primarily on hanging objects (over 90% of all collected specimens; Pant and Yasuno 1971). In contrast, studies performed in Panama (where houses are built with concrete and are more enclosed than the wooden and often elevated houses found in Thailand) have shown that *Ae. aegypti* rests both in walls and objects and that 57–64% of adults rest below 1 m of height and predominantly inside bedrooms and on surfaces made of cement, wood, and cloth (Perich et al.

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2000). Data from Iquitos, Peru, provided further evidence of mosquitoes resting at low heights (Vazquez-Prokopec et al. 2009), a pattern also supported by observational studies in experimental huts performed in Thailand (Tainchum et al. 2013).

During the yellow fever eradication campaign in the Americas, the perifocal residual spraying of DDT or malathion applied directly to actual breeding habitats and adjacent resting places was very effective at controlling *Ae. aegypti* (Soper 1965). More recently, in Australia, selective indoor residual spraying (IRS) applications in known *Ae. aegypti* resting locations (e.g., under furniture, closets, lower walls, dark areas) not only reduced intervention costs but also provided an unparalleled effectiveness against dengue (Ritchie et al. 2002; Vazquez-Prokopec et al. 2010a,b). Before such results could be translated to existing *Ae. aegypti* programs in endemic areas (which may differ from Australia in building construction and availability of potential resting sites), more research is needed about the applicability and acceptability of selective IRS. In this study, we quantified *Ae. aegypti* indoor resting behavior in the city of Acapulco (Mexico) and discuss the relevance of our findings for the adoption of selective IRS, as an alternative to "traditional" IRS for *Anopheles* spp. mosquitoes, for the control of *Aedes* vectors in urban areas within the Americas.

Material and Methods

Study Area

The study was performed in the city of Acapulco (32° 43′−14° 32′ N, 86° 42′−118° 22′ W), in the State of Guerrero, located in the Pacific coast of southern Mexico. Acapulco has a population of approximately 789,971 living in ~360,000 households (INEGI, 2014). From the total households in the city, 10% lacks domestic piped water supply, and 60% receives water sporadically within a scheduled plan. The lack of a reliable water supply promotes the need for water storage, leading to the permanent presence of large tanks and drums. Consequently, *Ae. aegypti* abundance and productivity are very high (Che-Mendoza et al. 2015, Manrique-Saide et al. 2015).

Entomologic Surveys

A cross-sectional entomologic study was performed between February-March and August-September 2013 to collect resting *Ae. aegypti* adult mosquitoes within 26 neighborhoods of Acapulco. In each neighborhood, city blocks were randomly selected, aiming to obtain a minimum of 10 blocks per neighborhood. Once city blocks were identified, one house per side of the block was randomly selected for exhaustive entomological surveys. Collections were carried out by four teams composed of three entomologists each from 0800 to 1500 hours using Prokopack aspirators (Vazquez-Prokopec et al. 2009). Given that our goal was to compare the abundance of resting *Ae. aegypti* within houses, we standardized our aspiration time to not last more than 10 min per house (Getis et al. 2003, LaCon et al. 2014), with two technicians collecting indoors and a third one outdoors. Indoors, collections were stratified by height by having one technician collecting mosquitoes from the bottom (<1.5 m) walls and resting sites and another technician collecting from the top wall (and any objects or spaces that may serve as resting areas) and ceiling (>1.5 m) using a telescopic aspirator handle, as previously described (Vazquez-Prokopec et al. 2009). Prior to the study, technicians measured the angle of their arm on a wall with a horizontal line marking the 1.5 m threshold. Both technicians collecting indoors worked simultaneously on each room, with the technician collecting at <1.5 m starting from the wall located on the right side of the room's door and the technician collecting >1.5 m starting from the wall located on the left (this procedure made collection time comparable above and below 1.5 m). The same procedure was followed on each room by using different collection cups per room (i.e., living-dining room, bedrooms, kitchen, bathroom). Backyard (patio) collections were also stored on a separate cup, but not stratified by height. All cups containing at least one mosquito were transported to the State Laboratory for further processing, which involved species identification, sex identification, and determination of level of engorgement (as bloodfed or non-bloodfed) following the methods described by Vazquez-Prokopec et al. (2009). We considered the indoor entomological collections (<1.5 and >1.5 m) within each house as matched pairs and used conditional logistic regression models to evaluate differences in collections by height using the function *clogit* in the R package *Survival* (Therneau and Grambsch 2013).

Results and Discussion

From 979 premises accessed and examined, 593 (60.6%) were infested with *Ae. aegypti* adult mosquitoes and 452 (46.2%) with female *Ae. aegypti*. The total number of adult *Ae. aegypti* collected was 1,403 (of which 747 were females). More than half of the females (473, 63.3%) showed evidence of a recent bloodmeal. The average (SD) number of *Ae. aegypti* collected per house was 0.72 (1.23), and the average per positive house was 1.87 (1.34, range = 1–12 mosquitoes). Most of the collections were obtained indoors (98% positive for adult and 99% for female *Ae. aegypti*) in comparison with patios. *Aedes aegypti* adults predominantly rested in bedrooms (44%), followed by living rooms (25%), bathrooms (20%), and kitchens (9%). When stratified by height, 82% (1,151/1,403) of all adults, 83% (626/747) of all females, and 87% (410/473) of all bloodfed females were found resting below 1.5 m. Plotting the household abundance also shows that the predominance of *Ae. aegypti* below 1.5 m occurred across all indoor abundance values (Fig. 1). A very low fraction of infested houses (3.9–7.1%) had *Ae. aegypti* mosquitoes (adults, females, or bloodfeds) resting only in the upper wall and ceiling (Table 1). Thus, the sensitivity of collections (i.e., the percentage of infested houses detected by aspiration) varied dramatically if collections were performed only in the lower wall (93–96%) or only in the upper wall and ceiling (17–30%; Table 1). The odds of finding adult *Ae. aegypti* mosquitoes resting below 1.5 m was 17 times higher than the odds of finding mosquitoes resting above 1.5 m (conditional logistic regression model, odds ratio, OR = 17.96; 95% CI = 11.80–27.33; Wald test = 181.7; df = 1; $P < 0.001$). Similar OR values were calculated for female *Ae. aegypti* (OR = 11.0; 95% CI = 7.66–15.80; Wald test = 168.7; df = 1; $P < 0.001$) and bloodfed female *Ae. aegypti* (OR = 11.87, 95% CI = 7.76–18.17; Wald test = 129.8; df = 1; $P < 0.001$).

There is strong consensus in the scientific and public health communities that current vector control tools against *Ae. aegypti* have limited entomological efficacy and unknown epidemiological impact (Bowman et al. 2016). Although several novel methods (*Wolbachia*, genetically modified mosquitoes) show a promising outlook (Achee et al. 2015), more evidence of their epidemiological impact is required before they are available for widespread implementation (Reiner et al. 2016). Thus, insecticide-based interventions directed to the adult resting population represent a relevant approach for *Ae. aegypti* control and disease prevention. In Acapulco, where houses are made of brick and mortar, bedrooms are the key *Ae. aegypti*

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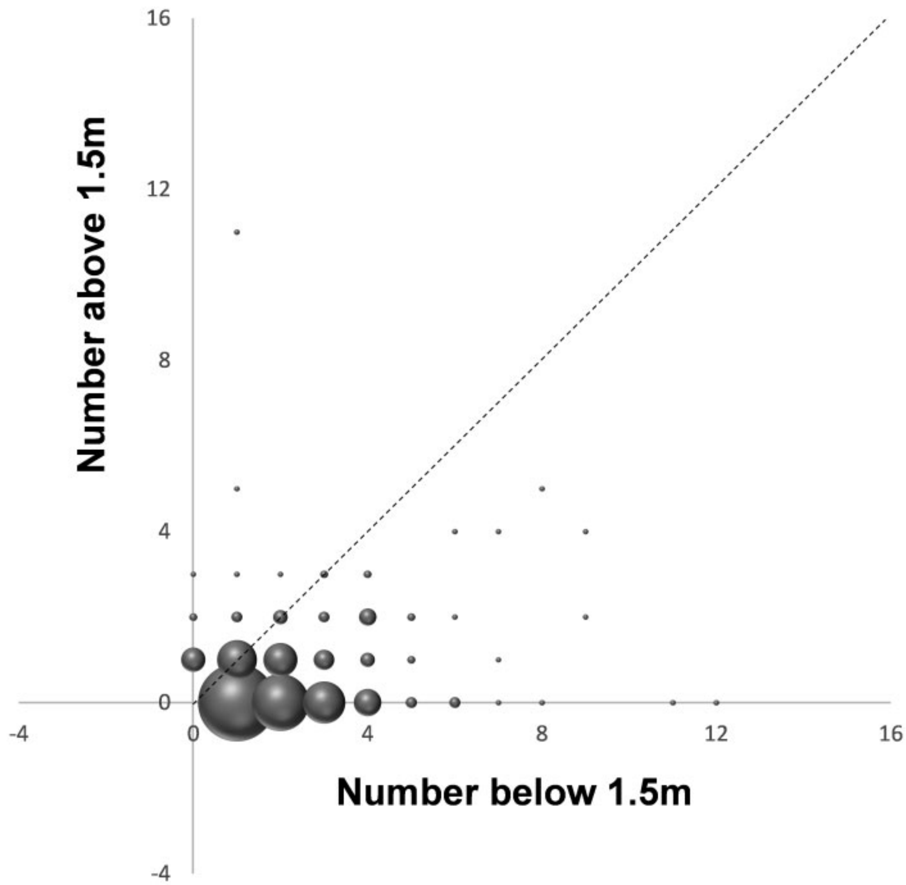

Fig. 1. Distribution of paired *Ae. aegypti* abundances inside houses stratified by collection height. Each symbol indicates an abundance occurrence (e.g., the point at [x = 12, y = 0] shows a house that had 12 *Ae. aegypti* below 1.5 m and none above 1.5 m). The size of the spheres is proportional to the number of houses with a given *Ae. aegypti* abundance above and below 1.5 m (e.g., the largest sphere indicates that 205 houses had the values [1,0], whereas the smallest spheres represent a single house). The dashed line indicates the point of indifference, where abundance is the same above and below 1.5 m.

Table 1. House positivity by different *Ae. aegypti* adult indices, stratified by height of collection indoors (low, <1.5 m; high, >1.5 m and ceiling)

Adult Index	Negative houses	*Ae. aegypti* presence, No. houses (% positive houses)			Sensitivity (%)	
		Low	High	Both	Low	High
Adults	386	413 (69.6)	23 (3.9)	157 (26.5)	96.1	30.4
Females	527	352 (77.9)	32 (7.1)	68 (15.0)	92.9	22.1
Bloodfeds	649	273 (82.7)	23 (7.0)	34 (10.3)	93.0	17.3

Sensitivity indicates the percentage of detection of infested houses when collections are done in the lower or only in the higher walls.

resting location, and the vast majority of adult mosquitoes rest below 1.5 m. Our observations confirm, with a much larger sample size, initial findings from Iquitos, Peru, showing that of 56 *Ae. aegypti* collected indoors, 82% were found resting below 1.5 m (Vazquez-Prokopec et al. 2009), and observations from Thailand (where houses were elevated on wooden poles and had wooden walls and unscreened windows) showing 57–64% of adults rest below 1 m (Perich et al. 2000).

The "traditional" approach for IRS (used for *Anopheles* spp. and other disease vectors) relies on impregnation of the entire wall with insecticides (World Health Organization 2006). Such spraying requires moving all furniture and removing picture frames or other objects from walls, which increases the time it takes to spray a house (and requires householders to prepare premises before spraying). This is a major barrier for community acceptance of traditional IRS in urban areas (Paz-Soldan et al. 2016). The finding of a strong preference by *Ae. aegypti* for resting below 1.5 m has important practical implications for the consideration of "selective" IRS (Ritchie et al. 2002) as a possible approach for controlling *Ae. aegypti* in the Americas. Our results also suggest that not spraying kitchens (a key area where contamination of food items with insecticides is very likely) may not lead to a dramatic loss of insecticidal coverage to control resting mosquitoes. In urban areas such as Acapulco, the selective application of residual insecticides to which *Ae. aegypti* is not resistant in resting areas located below 1.5 m can not only reach the majority of the vector population but also reduce operational time of spraying a house, the operators' exposure to insecticides, and the per-household intervention costs and increase community acceptance. Such increases in efficiency can favor the adoption of this expensive and labor-intensive vector control method under specific circumstances. Specifically, we

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feel that performing selective IRS can be suitable for the emergency control of *Ae. aegypti* in homes of pregnant woman (or other high-risk groups) in areas currently experiencing Zika virus transmission.

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