Movement of *Aedes aegypti* following a sugar meal and its implication in the development of control strategies in Durán, Ecuador

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ABSTRACT: We evaluated how the presence of sugar sources impacted the distribution of *Aedes aegypti* in different habitats in **Durán**, Ecuador. Land cover and normalized difference vegetation index maps were used to guide a random point sampling routine to select study grids (30 m x 30 m) in low vegetation (LV) and high vegetation (HV). Five individual plants, at one home in the LV and HV grid, were treated with a different colored, non-attractive, 60% sucrose solution to determine mosquito feeding and movement. Sugar alone is not attractive to mosquitoes, so spraying vegetation with a dyed sugar solution can be used for visual determination of sugar feeding. Outdoor collections using BG sentinel traps and indoor collections using aspirators were conducted at the treatment home and with collection points at 20, 40, and 60 m surrounding the treatment home for three consecutive days. A total of 3,245 mosquitoes in two genera, *Aedes* and *Culex*, was collected. The proportion of stained *Ae. aegypti* females was 56.8% (510/898) and 0% for males. For *Culex*, 63.9% (248/388) females and 36.1% (140/388) males were collected stained. *Aedes aegypti* and *Culex* spp. were found up to 60 m stained in both LV and HV grids. Significantly more stained females *Ae. aegypti* were found inside homes compared to females and males of *Culex spp*. in both habitats. This study identifies that outdoor sugar feeding is a common behavior of *Ae. aegypti* and can be targeted as a control strategy in urban habitats in Latin America. *Journal of Vector Ecology* 41 (2): 224-231. 2016.

Keyword Index: Resting behavior, mosquitoes, collections, Culex, sugar feeding.

INTRODUCTION

The tremendous public health problem of dengue (DENV) and the re-emerging chikungunya (CHIK) and Zika viruses (ZIKV) in Latin America are related to the ecology and behavior of the highly competent vectors Aedes aegypti and Aedes albopictus (Townson et al. 2005). Both species feed predominantly on humans, rest mainly inside houses, and can survive as adults for long periods increasing the potential for acquiring and transmitting multiple pathogens. These species are known to develop in inadequately managed containers (Grech et al. 2010), driving the focus of abatement programs to control the larval stage of these vectors (Luz et al. 2011). However, due to the number and types of containers producing Ae. aegypti larvae, targeted control is difficult, resulting in the lack of effectiveness and accounting for few success stories of reduction in DENV transmission in Latin America (Gratz and Jany 1994, Troyo et al. 2008a, Chadee et al. 2016). Additionally, Chadee et al. (2016) noted that Ae. aegypti are rapidly adapting their larval habitats to underground drains and septic tanks, exacerbating the already difficult problem of dengue and chikungunya control in Latin American countries.

The change in Aedes behavior identifies the need to

develop area-wide and targeted vector control approaches to effectively reduce the burden of DENV and other Aedesvectored arboviruses. One relatively unstudied aspect of Aedes ecology that has implications for control is sugar feeding. In nature, little is known about mosquito preferences for sugar sources and their attraction and orientation to plant odors (Grimstad and DeFoliart 1974, Foster 2008), but it has been demonstrated that mosquitoes are highly selective in their choice of flowering plants (Müller et al. 2010a,b). Laboratory studies have identified that plant sugar meals are a fundamental source of energy for female mosquitoes and the only food source for males (Yuval 1992, Foster 1995). Some blood-feeding studies suggested that sugar feeding was unimportant for Ae. aegypti (Scott et al. 2000, Braks et al. 2006). However, a field-based study conducted in Mexico investigated the sugar feeding behavior of Ae. aegypti and identified the importance of sugar-feeding in its life history (Martinez-Ibarra et al. 1997). Further investigations are needed to better understand the impact of sugar sources on the behavior of Ae. aegypti.

The current study examines the relationship between sugar feeding and mosquito movement following a sugar meal in different habitats in an urban environment in Ecuador. The study aims to better understand the sugar-feeding behavior of *Ae. aegypti* for developing and implementing new control strategies.

MATERIALS AND METHODS

Study area

The study area was located in the city of Durán, Guayas Province, Ecuador, where Ae. aegypti is the primary vector of DENV, CHIK, and ZIKV, and the study was conducted during the dry season. Within Durán, there are varying land cover classes. Variations in land-cover classes may affect the distribution of Ae. aegypti. For this study, sample grids were selected using geographic information system mapping tools based on both land cover and normalized difference vegetation index (NDVI) satellite imagery (Landsat 8 scene acquired on 16 September 2013). NDVI was calculated in each pixel of the image of Durán. Since the pixel resolution is 30 m, mixtures of land cover and NDVI are likely to exist in each pixel; however, classification was done to map each pixel to a dominant land cover class. Using ArcGIS software, the NDVI values that ranged from -0.125 to 0.559 from bands 5 (near infrared) and 4 (red) and the visible and near infrared bands (bands 1-7) were entered into a back-propagation neural network algorithm. Such values were stratified into three different levels guided through Jenks natural breaks. For open water, low vegetation, medium, and highly vegetated areas, the corresponding values were -0.125 to 0.038, 0.039 to 0.148, 0.149 to 0.346, and 0.347 to 0.559, respectively. Finally, land-cover classes we assigned as: 1. Low vegetation (LV); 2. medium vegetation; 3 high vegetation (HV); and 4. open water. This algorithm reduces the variance within classes and maximizes the variance among classes. We have used this GIS-based method earlier to eliminate the bias in selecting study sites for entomological field studies (Troyo et al. 2008b, Samson et al. 2015). Two randomly selected grids (30 m x 30 m) were chosen for the current evaluation: one in LV and one in HV. In one grid in LV and HV, one house was randomly selected until one with at least five different species of vegetation was identified. The vegetation was characterized for evaluation to identify *Ae. aegypti* post-sugar-feeding movement and any vegetation feeding preferences.

The six different species of vegetation per LV and HV grid were each treated with a specific color food-dye, nonattractive 60% sucrose solution (Figure 1). Both flowering and non-flowering vegetation was selected. One of each of the plants that represented that particular species found at the selected homes was sprayed. The ten plant species that were treated with a non-attractive 60% sucrose solution were: Allamanda cathartica L. (common name golden trumpet; Family Apocynaceae), Impatiens walleriana Hook.f. (common name busy Lizzie; Family Balsaminaceae), Ixora coccinea L. (common name jungle flame; Family Rubiaceae), Manihot esculenta Crantz (common name cassava; Family Euphorbiaceae), Turnera ulmifolia L. (common name yellow alder; Family Passifloraceae), Aloe vera (L.) (common name aloe; Family Asphodelaceae), Pilea sp. Lindl. (Family Urticaceae), Solanum sp. L. (Family name Solanaceae), Philodendron sp. Schott (Family Araceae), and Hibiscus rosa-sinensis L. (China rose; Family Malvaceae). Both A. cathartica and A. vera were found at the LV and HV grids. The addition of a plant-specific colored dye allows for visual determination of mosquito feeding on the solution (Müller and Schlein 2006, Qualls et al. 2012). As sucrose is a gustatory rather than an olfactory stimulant (Dethier and Chadwick 1948, Jorgensen et al. 2007, Isono and Morita 2010), spraying plants with a sucrose solution does not affect their attractiveness for mosquitoes. The application was applied by a 3 liter hand-held pump-up sprayer. All parts of the vegetation (flowers, leaves, stems) were sprayed until runoff, with the application covering ~40% of the plant. Prior to spraying, a plant sample was collected for identification.

Mosquito sampling

Mosquitoes were sampled by placing BG sentinel traps with a BG lure (BioQuip[®], Rancho Dominquez, CA) at the treatment site and at 20, 40, and 60 m increments in a



Figure 1. Left: 3-liter pump-up sprayers containing the different non-attractive, colordyed, 60% sucrose solutions. Center: Blue droplets of the non-attractive sucrose solution applied to *Allamanda cathartica*. Right: Green droplets of the non-attractive sucrose solution applied to *Aloe vera*.

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Figure 2. *Aedes* sampling scheme depicted at the HV site. At each location marked on the map, a BG Sentinel trap was operated for one trap night. Additionally, indoor aspirations were conducted at each marked location. The sampling scheme was the same at the LV site (not shown here).

circular radius surrounding the treatment site (Figure 2). This accounted for 13 houses in the LV and HV grid for a total of 13 BG traps per grid operated for 24 h for three consecutive days (39 trap nights). In addition, at the LV and HV grid, at the 13 houses where the BG sentinel traps were placed, indoor aspirations were conducted using a backpack aspirator (InsectaZooka, Bioquip*, Rancho Dominquez, CA) on three separate occasions. BG traps and the indoor aspirations were used to compare the proportion of dye-marked mosquitoes and determine the *in situ* order of preference of the different vegetation types. Mosquito movement following the sugar

Table 1. Mean number of females and males collected by the two sampling methods.

	Collecti	on Methods
	Aspiration	BG Sentinel Traps
Females		
Aedes aegypti	851	707
Culex sp	348	592 [*]
Males		
Aedes aegypti	54	118*
Culex sp	207	368 [*]

*Significant differences between rows P <0.05 (generalized linear model). meal was determined relative to the proportion of stained mosquitoes collected at the varying distances. The trap and aspirator mosquito collections were removed in the field and stored on ice until the specimens could be brought back to the laboratory and stored in a -20° C freezer until they were processed. The mosquitoes were sexed, counted, determined whether or not their abdomens were color-stained, and identified to species (Rueda 2004).

Statistical analysis

The data were counts of stained mosquitos and had a Poisson distribution that was overdispersed. Therefore, we used a generalized linear model for a dependent variable with a negative binomial distribution. The outcome variable was stained (yes/no) and the predictors were flower type, species, and the interaction of flower type and species. Covariates for habitat type, collection method, and distance from the bait station were included to control for their possible confounding effects. An offset of the number of mosquitos in the catch was also included in the model to produce proportions of the catch that were stained. The data are presented as mean, percents, and standard error for each species-flower type combination. Planned comparisons were made between species. Separate analyses were run for male and female mosquitos. The twotailed alpha level was set at 0.05. SAS 9.3 (SAS Institute, Cary, NC) was used for all analyses.

Table 2. Effect of covariates on sugar-stained males and females.

Effect.		Males			Females	
Effect	DF	F Value	Pr > F	DF	F Value	$\Pr > F$
Habitat	1	6.28	0.013	1	5.24	0.022
species	1	21.66	< 0.001	1	26.44	< 0.001
Habitat*species	1	0	0.987	1	2.17	0.141
collection	1	59.32	< 0.001	1	16.41	< 0.001
Habitat*collection	1	0.08	0.784	1	0.86	0.353
species*collection	1	6.76	0.01	1	19.86	< 0.001
Habitat*species*collection	1	5.40	0.021	1	0.67	0.414

by nower species, species, and gender.	_				_							_	
- - -		Aed	es aegypt	i				Culex	species				
Flower species (arid located in HV or 1V)	-E10		Femá	ale*	071-5		Ma	lle	010-2		Fem	ale	
(BITH IDEALER III II V OF LV)	016=11	ME	SD	LCL-UCL	n=140	ME	SD	LCL-UCL	n=248	ME	SD	LCL-UCL	
Dazzler merlot LV	4	4.00		•	0				7	2.38	1.36	1.46-3.31	
Musa sp. LV	76	6.82	3.99	6.45-7.19	18	3.67	2.38	2.98-4.35	12	1.80	0.54	1.53-2.07	
Manihot esculenta HV	42	7.85	8.48	6.68-9.02	9	1.00	0.00	•	27	5.90	2.89	5.41-6.39	
Turnera ulmifolia HV	66	6.52	4.41	6.06-6.99	15	3.00	1.60	2.45-3.55	22	6.68	4.53	5.79-7.57	
Philodendron sp. HV	0			•	0			•	6	3.26	1.95	2.32-4.2	
Pilea sp. HV	39	3.84	2.02	3.49-4.2	14	2.47	1.09	2.94-2.9	21	2.60	1.62	2.09-3.11	
Solanum sp. LV	2	2.00	0.00	•	1	1.00		•	12	1.91	0.41	1.72-2.1	
Ixora coccinea LV	20	3.61	1.53	3.21-4.02	23	5.34	2.58	4.83-5.85	11	1.71	0.59	1.38-2.03	
Allamanda cathartica HV and LV	137	6.61	5.44	6.22-7.01	55	7.23	4.98	6.67-7.77	102	8.73	8.82	8.87-9.4	
Aloe vera HV and LV	124	6.48	5.25	6.07-6.88	8	3.40	2.03	2.33-4.47	25	1.98	0.88	1.7-2.26	

Aedes aegypti males, n=288; stained=0.

RESULTS

A total of 3,245 mosquitoes in two genera, *Aedes* and *Culex*, was collected using the two methods of BG sentinel traps and indoor aspirations. *Aedes aegypti* represented 53.3% (1,730/3,245) of the total collection with 90.1% (1,558/1,730) females and 9.9.% (172/1,730) males. *Culex* represented 46.68% (1,515/3,245) of the total collection with 62.1% (940/1,515) females and 38% (575/1,515) males.

Table 1 shows the mean collections by method, sex, and species. There were significant interactions on total number of mosquitoes collected by species, habitat, and by collection method. There were no significant differences observed by collection method or habitat for female Ae. aegypti. For Ae. aegypti, an average of 6.33 (SE=0.96) were collected outside and 6.54 (SE=1.00) were collected inside at the LV grid. In the HV grid, 4.10 (SE=0.64) Ae. aegypti were collected outside and 4.34 (SE=0.66) were collected inside. In the HV grid, signifcantly more Ae. aegypti females were collected with both methods (P < 0.001) compared to Culex females. For Ae. aegypti males, there were significant differences observed with significanlty more males collected with BG sentinel traps in the LV grid (1.29,SE=0.33) than the HV grid (0.51, SE=0.15) (P=0.017). However, there were no significant differences in aspiration collections between habitats for Ae. aegypti males. Significantly more males of the Culex sp. were collected with both collection types and in both habitats compared to the collection of male Ae. aegypti (P < 0.05).

From the total collection of mosquitoes, 27.7% were collected stained (898/3,245). Of those collected stained, 56.8% (510/898) were *Ae. aegypti* and 43.2% (388/898) were *Culex* spp. The proportion of stained *Ae. aegypti*, 56.8% (510/898) were females. There were no male *Ae. aegypti* stained (0/510); The proportion of *Culex* females that were stained was 63.9% (248/388) and 36.1% (140/388) for males.

In both the LV and HV grids, there was a significant difference in the staining rate at the different distances, with *Culex* males found to be stained at 0 and 20 m from the house where the application was made (p <0.001). When looking at the distance, there is a significant species interaction for females (F=2.5, df_{1,2}= 3,561, P=0.058) with *Ae. aegypti* found stained at the application site and at 20 m from the application site. *Aedes* males were not stained at any distance in any habitat. The findings that contrast the percent stained *Aedes* and *Culex spp.* per habitat are portrayed in Figure 3.

Table 2 demonstrates the effects of the covariates associated with the staining rate of female and male mosquitoes. Signficantly more stained *Ae. aegypti* were found aspirated inside homes compared to *Ae. aegypti* males, and females and males of *Culex spp.* in both LV and HV (F=19.8, $df_{1,2}$ = 3,561, P < 0.001). There were significant differences observed in flower type and species for males (P< 0.05) but not for females (P=0.58). Table 3 summarizes the mean number of mosquitoes caught attributed to be feeding in different flower types by species and sex.



Figure 3. Percent stained A) *Aedes aegypti* and B) *Culex spp.* female and male mosquitoes aspirated indoors at the application site (0 m) and at 20, 40, and 60 m from the application site.

DISCUSSION

The results show that both Ae. aegypti and Culex spp. are flying at least 60 m from where they are obtaining a sugar meal in both LV and HV urban environments. Importantly, the findings identify that Ae. aegypti is significantly more likely to move indoors at the site and up to 20 m away from where they are obtaining a sugar meal. One previous field study in Latin America, investigating the sugar-feeding behavior of Ae. aegypti, did not identify any sugar meal preference but did identify that the proportion of mosquitoes containing sugar varied from 8% to 21% in direct relation to blooming plant abundance (Martinez-Ibarra et al. 1997). In the current study, for Ae. aegypti females, the lack of specificity on the different vegetation types, as demonstrated by no significant differences in staining rate by plant type and impact of habitat, suggests that this species may be opportunistic in its sugarfeeding behavior. More in-depth field studies are needed to determine to what extent Ae. aegypti will feed on different types of flowering vegetation in urban environments.

Interestingly, no male Ae. aegypti stained from feeding on the non-attractive sugar solution were collected. Male collections of both species were low with proportions of roughly 38% Culex and 10% Aedes, regardless of collection method and when compared to female collections. However, most of the Ae. aegypti males collected were outside in BG sentinel traps. Possibly these males were collected following mating and had depleted their energy reserves prior to being collected. Thus, no color would be detected in the abdomen. One study demonstrated that male An. gambiae mosquitoes lose over 50% of the carbohydrate reserves during swarming (Maiga et al. 2012). Another possibility is that males may imbibe less sugar while feeding than do females, resulting in a more rapid depletion of their energy reserves and thus not detecting the color dye from the non-attractive sugar solution. Additionally, Ae. aegypti males may not have been attracted to any of the plants selected in the study and therefore were not obtaining a sugar meal from the selected vegetation. Yu et al. (2016) found that males were consistently sugar-positive, but when they were exposed to different sugar sources the proportion declined rapidly when provided with less preferred plants.

The high staining rates found in this study of female Ae. aegypti on a non-attractive sucrose solution indicates that sugar feeding may be a common behavior of Ae. aegypti females. In order to target the sugar-feeding behavior of mosquitoes and develop control strategies, knowledge of these behaviors is important. Based on the mosquito staining rates demonstrated in previous studies following the incorporation of an attractive sugar bait, our staining rates without an attractive sugar bait indicate that the sugarfeeding behavior could be a successful control method (Müller and Schlein 2006, Müller et al. 2010a, Beier et al. 2012, Qualls et al. 2012, Junnila et al. 2015). In most of the studies with a highly attractive sugar bait (attractant added), >50% staining rate was achieved with at least that percentage of control if the attractive sugar mixture was toxic (Müller and Schlein 2006, Müller et al. 2010a, Beier et al. 2012, Qualls et al. 2012, Junnila et al. 2015). Specifically for Aedes species, we have demonstrated a 95% staining rate for Ae. albopictus exiting storm drains and cisterns in a controlled study in Florida (Qualls et al. 2012) and a 17% staining rate following a dyed attractive sugar bait application to vegetation in Israel (Junnila et al. 2015). One modeling study demonstrated that a daily feeding rate of 33% would result in a 90% reduction in anopheline populations (Marshall et al. 2013). Based on our staining rate of 58.7% for female Ae. aegypti, similar population reductions should be expected. It should be noted that in these described studies attractive sugar bait solutions were applied to large areas of vegetation depending on the treatment sites, whereas in the current study only five plants per treatment grid were sprayed with a non-attractive sugar solution. This suggests that small-targeted applications with an attractive toxic sugar mixture could increase the feeding rate and control of *Ae. aegypti* because of the addition of both an attractant and a toxin.

Aloe vera was a common indoor and outdoor plant species of the residents in Durán because of their belief that this plant provides good luck. Most of the homes, if not all, have a number of these plants surrounding their homes. Our findings revealed that female Aedes and Culex mosquitoes will feed on this plant species, which has been used in the past for its mosquitocidal and repellent properties (Qualls and Xue 2009, Dinesh et al. 2015). This identifies an opportunity to capitalize on the local ecology to develop local vector control tools that can further attract and kill mosquitoes in an environmentally appropriate approach. In addition, of the plants commonly found in the yards of the residents in Durán, both natives and non-native plant species were identified. The planting of non-natives in urban settings could potentially create more favorable habitats for Aedes mosquitoes. A recent study found that an invasive species had significant impact on survivorship and maximum larval size of the West Nile virus vector, Culex pipiens, suggesting positive effects on certain life history traits. This study is one of the first to demonstrate the potential facilitative effect of an invasive plant species on an insect vector and suggests that plant invasion could have positive feedback on mosquito population dynamics and, ultimately, human disease (Shewhart et al. 2014). Another study evaluated the effects of native and non-native plants on the oviposition site selection, emergence rates, development time, and adult body size of Cx. pipiens. Leaf detritus from invasive plants yielded significantly higher adult emergence rates compared to detritus from the remaining leaf species and the invasive honeysuckle alleviated the negative effects of intraspecific competition on adult emergence. Conversely, leaves of native plants acted as an ecological trap, generating high oviposition but low emergence rates. The authors conclude that the displacement of native understory plant species by certain invasive shrubs may increase production of Cx. pipiens with potential negative repercussions for human and wildlife health (Gardner et al. 2015). These findings may be relevant to mosquito control and invasive plant management practices in the geographic range of Cx. pipiens. Native and non-native plant interactions in the context of resting and sugar feeding have not been extensively studied and may provide insight into Aedes control.

In previous studies, female populations of *Ae. aegypti* have been associated with indoor resting (Pant and Yasuno 1970, Nelson 1986, Perich et al. 2000, Chadee 2013). Interestingly, there were no significant differences in collection methods outdoors or indoors for female *Ae. aegypti*. However, significantly more *Ae. aegypti* females were collected stained indoors than outdoors. These findings are contrary to many studies that found *Ae. aegypti* rarely fed on plant sugars and derived all of their adult energy requirements from human blood alone (Foster and Eischen 1987, Edman et al. 1992, Scott et al. 1997, Costero et al. 1998). Our findings suggest that sugar-feeding behavior occurs before *Ae. aegypti* enter houses. This finding is supported by Chadee et al. (2014) that identified that both male and female *Ae. aegypti* exhibited diel sugar feeding periodicity with a significant evening peak for both sexes at 16:00-18:00 just prior to blood-feeding that generally takes places indoors for this species. Importantly, it has been demonstrated that *Ae. aegypti* rests indoors from 36-50 h post-blood-feeding before taking another blood-meal (Fuchs and Kang 1978, Bowen 1991, Klowden and Briegel 1994, Klowden 1994, Chadee 2012, Chadee 2013). However, these studies do not identify if *Ae. aegypti* would sugar feed during this resting period. Additonal information is needed to identify if *Ae. aegypti* mosquitoes prefer to sugar feed outdoors or indoors. As noted, the importance of *Aedes* resting behavior indoors has potential for developing targeted control measures, such as indoor residual spraying or attractive toxic sugar baits indoors.

There were differences among the species collected based on HV and LV sites. In the HV site, *Ae. aegypti* females were collected more frequently than *Cx. spp.* This suggests that, at least during the dry season when this study was conducted, the habitats characterized as HV may serve as a preferred resting or sugar-feeding habitat for *Ae. aegypti* populations. Shade cast by dense vegetation cover reduces sub-canopy evaporative demand and heat stress and also provides for wetter containers. Thus, these factors may combine to create ideal *Ae. aegypti* habitats. Targeting these areas specifically during the dry season could decrease the dispersal and population increase of *Ae. aegypti* in the rainy season.

The sugar-feeding behavior of *Ae. aegypti* deserves additional research. The findings of our study provide the first evidence that the DENV, CHIK, and ZIKV vector is likely to move indoors at the site in which the sugar meal was taken. Studies have demonstrated that certain overlooked behaviors (sugar feeding, outdoor resting) are common and important in the life history of vectors and establish the basis for developing and implementing novel control strategies. Determining that sugar feeding does occur for *Ae. aegypti* in tropical environments demonstrates a new target for control. Further studies are ongoing to evaluate the role of attractive toxic sugar baits for the prevention and control of DENV, CHIK, and ZIKV in urban environments in Ecuador.

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