Swarming Behavior of *Aedes polynesiensis* (Diptera: Culicidae) and Characterization of Swarm Markers in American Samoa

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ABSTRACT We characterize the swarming behavior of male *Aedes polynesiensis* (Marks) in American Samoa. Instead of swarming around a blood host, males used the base of certain trees as a marker. Repeated sampling proved nondestructive and allowed us to investigate the impact of static (e.g., tree species) and dynamic (e.g., barometric pressure) characters on the likelihood of swarm presence and intensity. Tree circumference and oviposition activity (number of *Ae. polynesiensis* reared from oviposition cups) were significant positive predictors of the number of males in a swarm. Tree circumference and diameter were significantly positively associated, and canopy height was significantly negatively associated, with swarm occurrence. Comparisons between males swarming early and late during the swarming period allowed for insight into swarm composition in terms of male size and the amount of putative fluid (e.g., nectar) in the crop, indicators of energetic reserves. Males collected during the late period had significantly larger wings and less crop contents than did males of the early cohort. Because the ecology of male *Ae. polynesiensis* remains understudied, we consider how the current results could facilitate further studies related to applied autocidal strategies as well as the evolution of host-based mating behavior.

KEY WORDS *Aedes polynesiensis*, ecology, marker, mating, mosquito

An in-depth understanding of insect mating assemblies is crucial to studies of breeding systems, gene flow, population structuring, and evolution. The mating system of a species itself is also subject to selective pressures, and constrained by phylogeny and current ecological conditions, such as the spatial and temporal distribution of the sexes (Emlen and Oring 1977, Thornhill and Alcock 1983, Yuval 2006). Additionally, mating assemblies are the implicit focal point of applied insect control strategies that rely on the insemination of wild females by released males.

Mating in flight is typical for Culicidae. Short-range attraction is facilitated by auditory interactions between the sexes (Belton 1994, Gibson and Russell 2006). The swarm, a group of mostly male mosquitoes in sustained dance-like flight, near or over conspicuous elements of sharp contrast in the landscape (i.e., swarm markers), forms an assembly point at which short-range attraction and copulation can take place. The landscape elements used vary by species and can range from lakeshores to breaks in the forest canopy to the tip of a branch (Downes 1969). In a number of groups, the swarming habit has been modified to use the blood host as the swarm marker; most notably in the culicid genus *Mansonia* Blanchard, where attraction of males to mammal odors has been demonstrated (McIver et al. 1980), and within the culicid subgenus *Stegomyia* Theobald for species such as *Aedes aegypti* (L.) (Hartberg 1971) and *Aedes albopictus* (Skuse) (Gubler and Bhattacharva 1972). Host-based mating systems might be likely to evolve in response to temporally staggered emergence patterns (such as might be found when larvae develop in scattered small container habitats) or highly specific host utilization rates (Yuval 2006).

The mating system of *Aedes polynesiensis* (Marks), a member of the *Scutellaris* complex, has likewise been described as host based, but the literature suggests that within this species, this behavior may be less strongly developed or dependent on ecological conditions. For instance, the "following swarms" of *Ae. polynesiensis* in coconut groves in Polynesia were described by Ali and Rozeboom (1971) as being more diffuse than those of *Ae. albopictus.* A lower percentage of unmated nulliparous female *Ae. polynesiensis* collected at human baits, compared with the percentage of unmated *Ae. aegpyti*, led Russell et al. (2005) to conclude that mating in *Ae. polynesiensis* may take place earlier in life, closer to the larval habitat, and less often near the blood host. Two accounts state that males swarm and mate in flight with females on or around breeding containers, interrupting females when they are attempting to oviposit, and swarming around blood

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hosts when females attack humans in numbers, suggesting that any swarm marker used by this species could shift with population density (O'Connor 1923, Jachowski 1954). The current study focuses on the distinct use of another type of swarm marker, after the serendipitous discovery by one of the authors (H.C.T.) of stationary swarms of *Ae. polynesiensis* at the base of certain trees in American Samoa.

Ae. polynesiensis is the primary vector of *Wuchereria bancrofti* Cobbold in the South Pacific area between Fiji and French Polynesia. To date, vector control has proven most efficacious against filariasis transmission where *Anopheles* (bed nets and indoor residual spraying) and *Culex* (larval control using polystyrene beads) mosquitoes are the vectors. The behavior of *Ae. polynesiensis*, an opportunistic, outdoor, and daytime biting mosquito, and larval development in small ephemeral and cryptic habitats, makes it less amenable to these methods of vector control. Although source reduction has proven effective against *Ae. polynesiensis* in the past, breeding sites—ranging from crab holes of *Cardisoma carnifex* Herbst, tree holes, coconut shells, and various artificial containers—can be difficult to locate and can rapidly reappear after cessation of a breeding site-elimination campaign (Burkot and Ichimori 2002).

After the successful introgression of a *Wolbachia* Hertig endosymbiont of a clade occurring in *Aedes riversi* Bohart & Ingram into *Ae. polynesiensis*, an autocidal vector control method based on *Wolbachia*-induced cytoplasmic incompatibility was proposed for the South Pacific islands that relies on repeated inundations of a natural *Ae. polynesiensis* population with incompatible males, similar to classic Sterile Insect Technique (SIT) (Brelsfoard et al. 2008). An essential component of a successful SIT campaign is the maintenance of mating competitiveness for wild-type females of the released males versus wild-type males, as a lack of competitiveness or evolution of assortative mating, or both, has been the downfall of several prior mosquito SIT programs (Benedict and Robinson 2003). A telling example warning that competitiveness has to be assured in unconstrained field settings is that colonized *Anopheles culicifacies* Giles were competitive with wild-type males under laboratory settings, but not in the field (Reisen 2004). One method by which this can occur is through assortative swarming behavior, which was observed in *Culex tarsalis* Coquillet when released males swarmed mostly in breaks in the vegetation near the ground, whereas wild-type males tended to swarm above the vegetation (Reisen 2004).

Understanding the mating ecology of a species (i.e., the occurrence and distribution of mating in relation to ecological conditions) pertains to the logistics of incompatible male releases. For instance, if mating occurs in clumped aggregations while released males are spread evenly over the environment, the release rate of incompatible males might need to be ≈4 times as high as when clumping does not occur (Barclay 1992). Knowledge of where mating occurs, and whether released males can locate these sites successfully, could therefore be an important prerequisite for successful SIT releases. The implications of male swarming behavior for the effective sampling of male mosquitoes, allowing investigations on aspects of modified males' mating competitiveness, dispersal, and survival, are considered in a companion article (Stone et al. 2013). The objectives of the current study were to describe the swarming behavior and the markers used by *Ae. polynesiensis* in American Samoa, to investigate the composition and temporal consistency of swarms, and understand the biotic and abiotic factors that influence swarm presence and size, in preparation for a proposed release of modified males.

Materials and Methods

While looking for larval mosquito development habitats at our field station in Tafuna, American Samoa (global positioning system: 14° 19′31.00″ S, 170° 44′02.05″ W), a serendipitous discovery of mosquito swarms was made on 24 April 2012. The swarm was noticed when walking out of a gully ≈2 h before sunset and probably was evident because the sun was shining behind the swarm. The swarm was located in proximity to the base of a mango tree (*Mangifera indica* L.). A sample was taken with a sweep net, and on examination using a dissection microscope (MZFLIII, Leica, Bannockburn, IL) consisted only of male *Ae. polynesiensis.*

Subsequently, all accessible trees on the property (an area of ≈11,460 m^2) with a diameter ≥10 cm were marked on a map. They were examined in the morning and evening, and if swarms were seen, samples were taken. All samples were composed of male *Ae. polynesiensis.* Over 8 d, sampling methods were refined to ensure adequate and consistent sampling among sites, determine times of swarm occurrences, and elucidate possible static (e.g., tree spp.) and dynamic (e.g., ambient temperature) characters influencing swarm presence and size.

Trees with any previous swarming activity were then sampled on 13 separate days over a period occurring from 25 May to 20 June, 2012. For ease of sampling, a circuit was established at the beginning of the sampling period, and each day the start site was picked at random. Sites were sampled in two cohorts—seven to eight sites during the early cohort (between 16:45 and 17:10) and the remaining seven to eight sites during the late cohort (between 17:25 and 17:50).

Static measurements of the trees were taken once, which consisted of tree circumference and diameter 0.5 m above ground, width of tree canopy along two 90° axes (estimated from the ground), visual estimation of canopy cover (>50% full, equal to 50% full, <50% full), and species of tree. Before sampling each day, at ≈16:40, we recorded dynamic variables as the amount of rain in the past 24 h (measured with a catchment basin), noted whether any rain had fallen in the last 12 h, recorded barometric pressure, relative humidity, and temperature (all measured with a Kestrel 3500 wind speed meter, Nielsen–Kellerman, Boothwyn, PA), and visually estimated cloud cover (blue sky, partial cover, or cloudy).

After taking dynamic variables each sample day, sites were then assessed for the presence of swarms. If

a swarm was present, a sample was taken using a handheld aspirator made from a handheld PVC tube fitted with a fan, connected to a 12V battery, to which was added a black PVC suction tube from a Modified CDC Backpack Aspirator (model 1412, J.W. Hock Co., Gainesville, FL). The sampling method consisted of placing the tip of the aspirator into the perceived middle of the swarm and moving the tip in side to side and up and down motions over the area of the swarm for 10 s, then sweeping the tip of the aspirator across the space above the ground and tree trunk immediately below the swarm. While sampling occurred, the time, cardinal location of the swarm, and presence of biting females were noted. Aspiration cups containing live mosquitoes were then placed in a caddy until the particular cohort (early or late) was returned to the lab, whereupon cups were placed in a −20°C freezer to kill specimens for later identification.

Within 24 h, specimens were sorted by sex and identified to species (Ramalingam 1976). During the period of protocol refinement, we noted whether male *Ae. polynesiensis* had rotated or unrotated terminalia. This character was not scored during the experimental sampling period. However, during experimental identifications, we did assess the putative amount of nectar in male *Ae. polynesiensis* crops through visual estimation. If all abdominal sternites were sunken into the tergites, males were scored as having an "empty crop"; if all abdominal sternites excepting the most proximal two segments (A1 and A2) were sunken into the tergites, they were scored as having a "partial crop"; and if less than A3-A8 were sunken, they were scored as having a "full crop." After identification, the wing size of males was measured by mounting one wing from each male at a given collection site on a slide, photographing at 10× magnification, and measuring from alular notch to distal edge, excluding the fringe, using ImageJ (Abramoff et al. 2004).

In addition to static and dynamic measurements, we estimated the number of larval mosquito development habitats near each tree and the amount of oviposition activity. To estimate the number of habitats, we performed a single survey 4 d before the beginning of the study period by sampling any observed artificial (e.g., plastic bottles) and natural (e.g., coconut shells) water-containing bodies previously described as *Ae. polynesiensis* habitats (Burkot et al. 2007) within a 5 m radius of the bases of swarm trees, using either a 21-ml turkey baster or 470-ml "pint" dipper and sieving through mesh net, then rinsing strained material into labeled cups. If a turkey baster was used, we made up to three draws, and if the pint dipper was used, we made up to three dips. Larvae were returned to the field station, reared to adults, and identified as adults (Ramalingam 1976).

To complement the larval survey and provide additional insight into localized breeding activity, oviposition activity was estimated by leaving out 354-ml plastic cups (Hallmark, Kansas City, MO), lined with seed germination paper and filled with distilled water, at the bases of swarm trees for 1-wk periods three times over the study month. Papers were retrieved and replaced at the end of each week; existing water was dumped and fresh water was added. After retrieval, the total numbers of hatched and unhatched eggs (visual examination for a burst operculum) on the papers were counted using a dissecting microscope, then papers were placed in hatching water, larvae reared, and adults identified using the aforementioned keys. Eggs were hatched, and larvae were reared in 21.5 by 21.5 by 7.5-cm clear plastic clamshell pans (Pactiv, Lake Forest, IL) containing 200 ml conditioned water and 300 ml distilled water. Larvae were fed a 6% liver powder (ICN Biomedicals, Aurora, OH) solution (60 g/liter) ad libitum. The conditioned water was brewed in 5-gallon plastic jugs and made by adding 6 g of liver powder to 10 liters of distilled water and topping off (at 0.6 g liver powder/1 liter of distilled water) every 4–7 d, as needed. A screen was placed over the top of the jug to allow aerobic exchange while preventing contamination with debris and insects.

In addition to characterizing swarms at the field site, we visually assessed off-site locations within 1.5 km of the original site for swarms but did not take samples. In addition, we inspected spermathecae of females that were sampled haphazardly by being found incidentally in male swarm samples, or caught between 1700 and 1900 hours with either handheld aspirators (human landing) or BG-Sentinel (BioGents, Regensburg, Germany) traps baited with BG-Lure Attractant. Collection sites for females were either at swarm trees ("near") or at least 15 m away from any known swarm tree ("far"). Females were identified to species and then stored in a −20°C freezer until spermathecae could be dissected and visually inspected with a compound microscope for presence of sperm.

Data were analyzed using the JMP 9 statistical package (SAS, Raleigh, NC). For the static site-based predictor variables, a univariate screen was performed with the total number of males caught at each site over the entire study period as the response, and for the dynamic day-based predictor variables, a univariate screen was performed with the total number of males caught each day over all sites as the response; in both instances, males were summed over time and space to increase sample sizes and eliminate pseudoreplication. Two sites, trees 4 and 8, were not included in analyses because swarms were never sighted at them during experimental sampling; one site, tree 6, was also excluded from analysis because it caused extreme nonnormality in residual plots while adding few data of biological relevance. Power analyses were conducted on data, and subsequent tests performed only when sample sizes were adequate to explain differences.

Results

Swarms were never observed >0.5 m above the ground, and although some male activity was noticed at swarm trees in the morning (0600–0800 hours), this activity was not consistent, and no characteristic swarming behavior was observed (i.e., we saw only individual males flying and landing, resting, and entering and exiting crevices formed by rock piles and roots at base of trees). Swarms were never seen at

Fig. 1. Photographic examples of trees by which male swarms were consistently found; left is site 2, middle is site 10, right is site 13. Trunk shot top row, roots middle, and canopy is bottom row.

vertical elements in the landscape (e.g., posts, light poles) other than trees. Additionally, destructive sampling did not occur although samples were taken from trees several days in succession on multiple occasions.

Based on anecdotal observations, we determined that static variables of potential importance for swarm presence were 1) amount of shade available at the base of the tree, which we attempted to quantify by measuring tree canopy size (i.e., area covering the ground), and estimating canopy density and height above the ground; 2) tree circumference and diameter; and 3) tree species. At the field station, we saw swarms regularly at "koa" (*Acacia* Mill sp.), mango (*Mangifera indica*), and poumuli (*Flueggea flexuosa* Müller) trees (Fig. 1), and twice at one mulberry (*Brousonnetia papyrifera* (L.)) tree, but never at banana (*Musa maclayi* Argent), breadfruit (*Artocarpus altilis* Parkinson), coconut (*Cocos nucifera* (L.)), papaya (*Carica papaya* L.), and royal poinciana (*Delonix regia* (Hook)). When we looked outside of the field station, we saw them at mango and poumuli trees, and once at a breadfruit tree.

All males collected from swarms had rotated terminalia. In total, at 15 sites over 13 sampling days, 111 swarms were sampled yielding a total of 507 male *Ae. polynesiensis* collected. In addition, four female *Ae. polynesiensis* were captured in samples, and one male and two female *Ae. tutuilae* Ramalingam & Belkin (one bloodfed). Once, a pair of mosquitoes was seen flying in copula in a swarm. In addition, cecidomyiids, chironomids, and tipulids were found in swarm samples. For female insemination checks, 29 near females were inseminated and 1 uninseminated, and 31 far females were inseminated and 2 uninseminated.

After rearing larvae to adulthood, in total 129 *Ae. polynesiensis* (71 female, 58 male), 21 *Ae. aegypti* (13 female, 8 male), and 2 *Toxorhynchites* Theobald sp. (both males) were identified from collections of larvae (the *Toxorhynchites* larvae were separated from other larvae and each other upon return to lab); and 1,761 *Ae. polynesiensis* (911 female, 850 male) and 4 *Ae. aegypti* (1 female, 3 male) were identified from adults reared from egg collections. Larvae were collected from aluminum cans, ceramic toilet bowls, coconuts, plastic bottles and buckets, and rubber tires.

For the number of males in a swarm, tree circumference at 0.5 m above the ground ($R^2 = 0.62$; $P \leq 0.002$), diameter ($R^2 = 0.62$; $P \leq 0.002$), and the numbers of total adult ($R^2 = 0.47$; $P \leq 0.02$), adult male ($R^2 = 0.45$; $P \leq 0.02$), and adult female ($R^2 = 0.35$; $P \leq 0.04$) *Ae. polynesiensis* reared from eggs collected in oviposition cups were significant positive predictors. For the likelihood of swarm occurrence (i.e., number of times a site was positive for a swarm), tree circumference ($R^2 = 0.65$; $P \leq 0.002$) and diameter ($R^2 = 0.55$; $P \leq 0.006$) at 0.5 m above ground were significantly positively associated with swarm incidence. Canopy height (F (1,10) = 5.54; $P \leq 0.04$) was significantly negatively associated with swarm incidence (Tables 1 and 2). Swarms most commonly occurred on

Table 1. Physical characteristics of trees that were tested for significance in predicting no. of males in swarms of *Ae. polynesiensis* or swarm occurrence[a]

Site	No. of times with swarms (out of 13)	Avg no. of males sampled from swarm (±1 SD)	Total no. of males collected over study period	Tree (not tested)	Trunk circumference (m) ($R^2 = 0.62$; $P < 0.002$) ($R^2 = 0.65$; $P < 0.002$)	Trunk diam (m) ($R^2 = 0.62$; $P < 0.002$) ($R^2 = 0.55$; $P < 0.006$)	Canopy area (m^2)	Canopy cover (not tested)	Canopy ht (> ≤ 10 m) ($F = 3.89$; $P = 0.08$) ($F = 5.54$; $P < 0.04$)
1	7	2.29 ± 1.80	16	Poumuli	1.08	0.35	36.19	Full	Less
2	9	6.00 ± 4.21	54	Mango	2.74	0.85	211.14	Full	Less
3	12	3.83 ± 2.59	46	Mango	2.10	0.80	126.54	Full	Less
4	0	na	0	Mulberry	1.20	0.28	37.62	Mod	Less
5	9	4.11 ± 2.37	37	Mango	1.80	0.47	157.48	Full	Less
6	2	1.33 ± 1.53	4	Mulberry	2.90	0.88	67.64	Full	Less
7	12	6.82 ± 4.87	75	Poumuli	1.40	0.45	28.05	Full	Less
8	0	na	0	Coconut	1.30	0.46	na	Sparse	More
9	9	1.78 ± 1.20	16	Koa	1.40	0.46	72.09	Mod	More
10	13	9.92 ± 6.40	129	Mango	5.80	1.00	135.70	Full	Less
11	8	1.13 ± 0.35	9	Poumuli	0.90	0.15	15.12	Full	More
12	8	6.89 ± 4.94	62	Poumuli	1.20	0.44	29.76	Full	More
13	7	3.14 ± 2.91	22	Koa	0.85	0.29	34.84	Mod	More
14	9	3.22 ± 2.64	29	Poumuli	1.40	0.37	20.16	Full	Less
15	6	1.33 ± 1.51	8	Poumuli	0.75	0.25	27.56	Full	More

[a] Total number of males collected (first *P* value reported) or number of swarms (second *P* value reported) at each site over entire study period used as dependent response in ANOVAs and linear regressions. Unless otherwise indicated all independent variables tested for significance, and *P* values only reported if significant.

the north, east, and southeast facing sides of trees (Fig. 2).

The dynamic daily variables (whether any rain had fallen in the last 12 h, barometric pressure, relative humidity, temperature, and amount of cloud cover), tree species, canopy area, and the numbers of hatched and unhatched eggs in ovicups were not significant predictors of number of males or number of swarms (Table 3). The variable of amount of rain in the past 24 h, and all data associated with collections of larvae around swarm trees, did not have large enough sample sizes for testing.

The distribution of the number of males across all sites and days was highly non-normal (i.e., lots of small samples, a few very large ones) and could not be normalized. Thus, when the average numbers of males in early versus late sample times were compared, no significant difference was detectable ($H = 2.33$; $df = 1$; $P \leq 0.13$). However, when the numbers of swarms between early versus late sample times were compared, there were significantly more swarms at later times than early (G-test; $P \leq 0.002$) (Fig. 3). There was a mean of 3.5 (±3.1 SD) males per early sample and 5.2 (±5.0 SD) males per late sample, and the medians for both times were three males per sample.

For the analysis of wing size, four extreme outliers that were >5 SD away from the mean were excluded before analysis to yield a normal distribution (4 out of 481 individuals excluded). Males from the late cohort had significantly longer wings than males from the early cohort (F (1, 475) = 6.82; $P \leq 0.009$), with average wing size of early males being 2.22 (±0.41 SD) mm (range, of 1.59–2.75 mm) and late males being 2.29 (±0.35 SD) mm (range, of 1.54–3.22).

In the early versus late sample cohorts, differences among crop statuses were not significant (G-test; $P \leq 0.11$), but there was an apparent trend toward more empty and less full crops in late cohorts versus early (Fig. 3). However, when the data were analyzed after excluding individuals with partial crops, there were significantly more empty crops during the late cohort as opposed to the early (Fisher Exact Test; $P = 0.04$; Fig. 4).

Discussion

The main outcome of this study was the description of the swarming behavior of male *Ae. polynesiensis* whereby the base of trees appears to serve as a swarm marker. Additionally, we determined that at certain species of trees, as tree size and local oviposition activity increases and canopy height decreases swarm occurrence, and number of males in swarms, increases. Although the total number of eggs, hatched or unhatched, recovered from oviposition sites did not predict male numbers in swarms, the number of adult *Ae. polynesiensis* reared from those collections did, a difference that could be explained by density-dependent mortality operating on immatures. More detailed studies on the relation between *Ae. polynesiensis* breeding sites, larval development, and nearby swarming are warranted to elucidate these processes.

Table 2. Characteristics of oviposition activity, determined by placing oviposition papers at swarm trees, tested for significance in predicting number of males in swarms of *Ae. polynesiensis* or swarm occurrence[a]

Site	Avg no. of eggs in ovicup (±1 SD)	Total no. of eggs collected in ovicup	Total no. of unhatched eggs in ovicup	Total no. of *Ae. polynesiensis* reared from egg papers		No. of habitats w/in 5 m positive for larvae (not tested)	Total no. of *Ae. polynesiensis* from larvae collections (not tested)	
				Female	Male		Female	Male
1	214.0 ± 78.5	642	424	88	74	0	0	0
2	44.3 ± 16.6	133	87	19	13	0	0	0
3	55.7 ± 48.3	167	77	27	19	0	0	0
4	39.0 ± 34.0	114	44	31	34	0	0	0
5	81.3 ± 97.6	244	161	87	92	0	0	0
6	30.0 ± 25.5	90	51	49	42	0	0	0
7	76.7 ± 27.0	230	166	91	59	2	23	10
8	67.0 ± 35.9	201	89	56	80	0	0	0
9	42.7 ± 5.9	128	78	29	36	1	4	2
10	163.7 ± 60.9	491	401	164	154	12	12	20
11	98.0 ± 63.5	294	228	36	40	3	8	4
12	35.0 ± 6.1	105	68	45	89	1	10	11
13	111.3 ± 89.6	334	247	112	22	2	1	0
14	156.7 ± 115.8	470	332	46	74	9	13	11
15	55.7 ± 48.5	167	98	31	22	0	0	0

[a] Unless otherwise indicated, all independent variables tested for significance, and P values only reported if significant. Number of total adult ($R^2 = 0.47$; $P \leq 0.02$), adult male ($R^2 = 0.45$; $P \leq 0.02$), and adult female ($R^2 = 0.35$; $P \leq 0.04$) *Ae. polynesiensis* reared from ovicup collections at swarm trees were significant predictors of no. of male *Ae. polynesiensis* sampled from swarms at the same trees.

From our observations and analyses, we think that males found in a particular swarm were recruited from nearby emergence sites, rest near (or sometimes at the base of) swarm trees during the day, and forage for nectar at night in the nearby habitat to fuel swarm flights. We hypothesize that swarms are used as mating assemblies for *Ae. polynesiensis.* We recommend follow-up studies that investigate the influence of hosts (both blood and nectar) and resting sites near swarms.

The earliest signs of swarming on a given day were typically observed around 1630 hours, although weather conditions did affect this, and swarming was not observed after sunset. From the comparison between early and late swarming activity, it was clear that swarming became more prevalent and activity increased from the early to the late cohort. Males captured in the late period were also significantly larger, as measured by their wing size, and tended to have less expanded clear space (presumed to be nectar) in their crops. Studies on the behavior of *Anopheles freeborni* Aitken showed that males consume >50% of their available energetic reserves while swarming (Yuval et al. 1994). The larger male size in the late cohort may therefore reflect the ability to compete for females for a longer duration. An alternative interpretation is that smaller males may initiate swarming behavior earlier to avoid competition with larger males, perhaps before female mating activity reaches an optimum or when predation risk is more intense, as suggested to be the case for *An. freeborni* (Yuval et al. 1993, Yuval and Bouskila 1993). More detailed studies on the swarming behavior of males, their energetic reserves, and the distribution of suc-

Table 3. Dynamic environmental characters and sampling dates with numbers of male *Ae. polynesiensis* collected and number of swarms[a]

Date	Sky	Rain in past 12 h?	Amount of rain in past 24 h (cm) (not tested)	Temp. (°C)	Barometric pressure (in Hg)	Relative humidity (%)	Total no. of males collected over all trees	Total no. of trees with swarms
25 May 2012	Overcast-grey	y	5.5	26.9	29.56	86.9	46	11
27 May 2012	Clear	n	0	28.8	29.62	75.0	38	10
29 May 2012	Clear	n	0	30.0	29.62	70.8	42	9
30 May 2012	Clear	n	0	27.9	29.67	80.8	39	7
31 May 2012	Overcast-grey	y	0	26.3	29.70	84.9	30	8
1 June 2012	Overcast-grey	y	1.0	26.4	29.73	80.7	23	7
5 June 2012	Partly cloudy	y	2.5	27.4	29.67	89.1	40	7
7 June 2012	Partly cloudy	y	3.0	27.9	29.71	88.7	7	5
8 June 2012	Partly cloudy	y	0.5	27.7	29.70	88.6	39	11
9 June 2012	Partly cloudy	n	0	29.2	29.69	84.6	56	9
18 June 2012	Partly cloudy	y	1	28.4	29.72	90.0	66	10
19 June 2012	Clear	n	0	28.9	29.75	78.5	29	8
20 June 2012	Clear	n	0	30.6	29.76	76.7	49	9

[a] No variables were significant predictors of swarm presence or number of males.

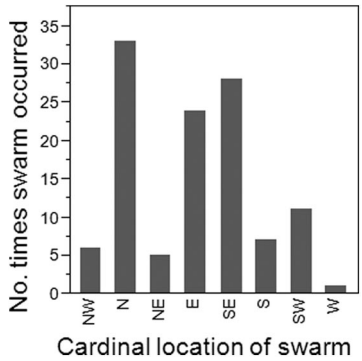

Fig. 2. Number of times swarms were observed at cardinal locations on tree trunk.

cessful copulations over the duration of the swarm would provide much needed insight into what constitutes male mating competitiveness under field conditions.

A surprising find was the near absence of swarming activity near breadfruit and mulberry trees, both in the family Moraceae. However, breadfruit trees are known to have a repellency action on mosquitoes (Jones et al. 2012), and another tree in the same family is known to have mosquito larvicidal effects (Govindarajan et al. 2011). We think the reason that male swarms were found only one time at a breadfruit and twice at a mulberry tree could be because of a previously described repellency effect of breadfruit and an unreported repellency effect of mulberry. To explain the single mulberry and breadfruit trees with swarms, we suspect this could be because of a transitory increase in density of local breeding sites causing mosquitoes to use a more diverse set of swarm trees. No sugar sources (e.g., rotting mangoes) were noticed near any positive tree over the entire study.

Although we did not find any significant weather variables, we think these might merit further investigation. For instance, swarms appeared to disperse at the start of rain and reform upon cessation of rain, and

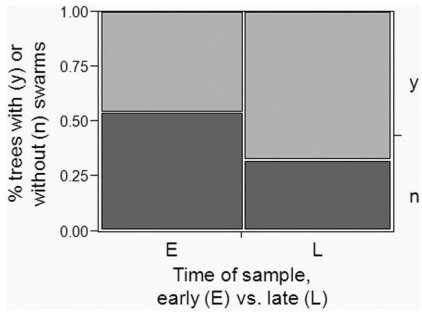

Fig. 3. Percentage of times trees were positive (y) or negative (n) for swarms during early (E = 1645–1710 hours) or late (L = 1725–1750 hours) sampling times was different, with significantly more swarms during the late cohort (Fisher Exact Test; $P = 0.002$).

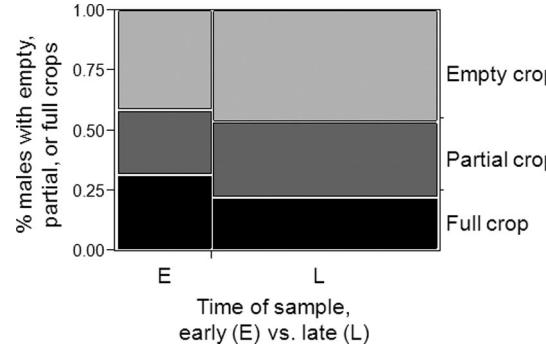

Fig. 4. Percentages of males with empty, partial, or full crops sampled during early (E = 1645–1710 hours) or late (L = 1725–1750 hours) times were not significantly different (G-test; $P \leq 0.11$). However, when partial crop data were excluded, there were significantly more empty crops during the late cohort versus the early (Fisher Exact Test; $P = 0.04$). Relative width of x-axis indicates number of males in each cohort (E vs. L).

no swarms were ever seen during heavy rain; thus, swarm presence could be affected if there is an actively falling barometric pressure. Rainfall amount over time should have an effect on swarm presence and size, as this will directly affect the number of local oviposition sites. Anecdotally, we noticed that the ideal weather conditions for swarms seemed to consist of an overcast sky all day, with intermittent rain, and very slight mist of rain during swarm time. We suspect that some microclimate variation is important (e.g., relative humidity and temperature at base of tree), but this could not be assessed with the instruments at our disposal.

Questions remain regarding *Ae. polynesiensis* male ecology and potential implications both for applied autocidal strategies, and for insight into the evolution of host-based mating systems, but were beyond the scope of the current study. Our results should, however, facilitate further studies and lead to refinements of applied strategies. An example is the resulting recommendation that the ideal swarm tree for the purposes of releasing males into the field or monitoring males during a release would be a mango tree at least a half meter in diameter, with a full canopy <10 m in height, near an abundance of larvae development habitats. Sampling should occur within 1.5 h of sunset to maximize sampling effort. An important applied question for autocidal strategies is how likely males are to be recruited to the nearest marker, and how much dispersal of males and females occurs between such trees (e.g., mark-release-recapture studies would be illuminating). Further studies on whether the use of trees as markers shifts to the use of blood hosts as markers at higher densities of mosquitoes, or whether there is variation in the degree to which different populations display this behavior could serve to elucidate phenotypic and genetic components of swarming behavior in this primary vector of lymphatic filariasis.

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