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INFLUENCE OF HOST AND LARVAL HABITAT DISTRIBUTION ON THE ABUNDANCE OF AFRICAN MALARIA VECTORS IN WESTERN KENYA

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Abstract. The abundance of anopheline mosquitoes varies substantially among houses within the same villages. *Anopheles gambiae* sensu stricto is highly anthropophilic, and *Anopheles arabiensis* is zoophilic; thus, it is often hypothesized that the abundance of *An. gambiae* and *An. arabiensis* in a house is associated with the distribution of livestock and humans. In this paper we examined the influence of livestock and human host availability on the distribution and abundance of malaria vectors in the basin region of Lake Victoria in western Kenya. Larvae and adults of *An. gambiae*, *An. arabiensis* and *Anopheles funestus* were collected in the beginning and the end of the rainy season in 1999. *Anopheles gambiae* was the predominant species in both larval and adult samples. Multiple regression analyses found that the ratio of distance between houses and larval habitats to distance between cowsheds and larval habitats had a significant and negative association with the relative abundance of *An. gambiae* larvae for both sampling periods. The ratio of human density to cow density was positively correlated with the relative abundance of *An. gambiae* larvae in the late rainy period. For the adult samples, distance from a house to its nearest larval habitats was the only variable that showed a significant correlation with the *An. gambiae* density in houses in both sample periods. More than 90% of anopheline adults were found in the houses within 300 meters from the nearest larval habitats. Anopheline mosquito density was not correlated to the density of cows or humans, or the distance to cowsheds from houses. These results suggest that livestock and human host availability affect the relative abundance of *An. gambiae* larvae in aquatic habitats, but the distribution of anopheline adults in houses is determined by the distance from houses to larval habitats.

INTRODUCTION

Effective control of malaria through vector management requires information on distribution and abundance of vectors in the targeted area. *Anopheles gambiae* sensu lato (s.l.) is a group of closely related and morphologically indistinguishable species of which two or more coexist in many areas.[1] Because individual species within the species complex differ in host-biting preference, abundance and vector competence, identification of the mosquito vectors to species level and mapping species distribution in heterogeneous environments are critical.[2,3] Simple molecular techniques are now available for identification of anopheline vectors to species level.[4] Through integration of molecular techniques with other tools such as a global positioning system (GPS) and a geographic information system, vector species distribution patterns and the underlying mechanisms can be better studied.[5]

Previous studies have demonstrated high level of heterogeneity in anopheline mosquito species composition at macro-geographic scale. For example, in the basin region of Lake Victoria, there are three malaria vector species, *An. gambiae* (throughout the paper, we refer to *An. gambiae* sensu stricto as *An. gambiae*), *An. arabiensis,* and *An. funestus*, but *An. arabiensis* does not inhabit highland areas in western Kenya.[6,7] The range and relative abundance of *An. gambiae* and *An. arabiensis* are defined by climatic factors such as annual precipitation and annual and wet season temperature. *Anopheles gambiae* usually predominates in moist environments, and *An. arabiensis* is more common in arid areas.[8] Anopheline species composition may also vary between dry and wet seasons.[9] Climatic factor is not the only variable that affects relative abundance of anopheline mosquitoes, as is evident from previous reports that species composition varies significantly among nearby villages where climate is very similar.[9] Thus, other biotic or abiotic factors are involved in causing species composition variation at micro-geographic scale.

In this study, we examined the influence of livestock host availability on distribution and abundance of malaria vectors in the basin region of Lake Victoria in western Kenya. Because *An. gambiae* is highly anthropophilic and *An. arabiensis* is zoophilic,[2,11,12] their abundance may be associated with distribution of cattle and human hosts. On the other hand, mosquito reproduction depends on the availability of aquatic habitats. Thus, it is necessary to evaluate the effect of host availability on distribution of anopheline larvae in aquatic habitats.

MATERIALS AND METHODS

Study area. The study area (approximately 4.4 km^2) was located in Mbita Point Village, Suba District, Nyanza Province, Kenya (Figure 1). Malaria is the leading cause of morbidity in this area, constituting 42–48% of all clinical cases of local clinics.[13] The area is surrounded by lake Victoria on the east and west sides. Elevation increases gradually toward the hill in the south. The hill is approximately 1,300 meters above sea level (155 meters higher than the water surface of Lake Victoria), and serves as the southern boundary of the study area. Trees (*Aeschynamene elaphroxylon*) were planted along the east lake shoreline to protect crops from hippopotamuses. Water hyacinth is often trapped between the trees near the lakeshore. The shore without the trees is sandy, rocky or covered with short grass. Precipitation was recorded daily at the Mbita Point Field Station (0°26'S, 34°12'E) of the International Center of Insect Physiology and Ecology in the Suba District of western Kenya (Figure 2). The dry season is generally in January and February, and the rainy season starts in March and ends in May.

Mosquito larval sampling. All aquatic habitats in the study area were sampled for anopheline larvae in the beginning of the rainy period (March 2–8, 1999). Aquatic habitats were first inspected for the presence of anopheline mosquito larvae. If anopheline larvae were present, 2–25 dips at each site,

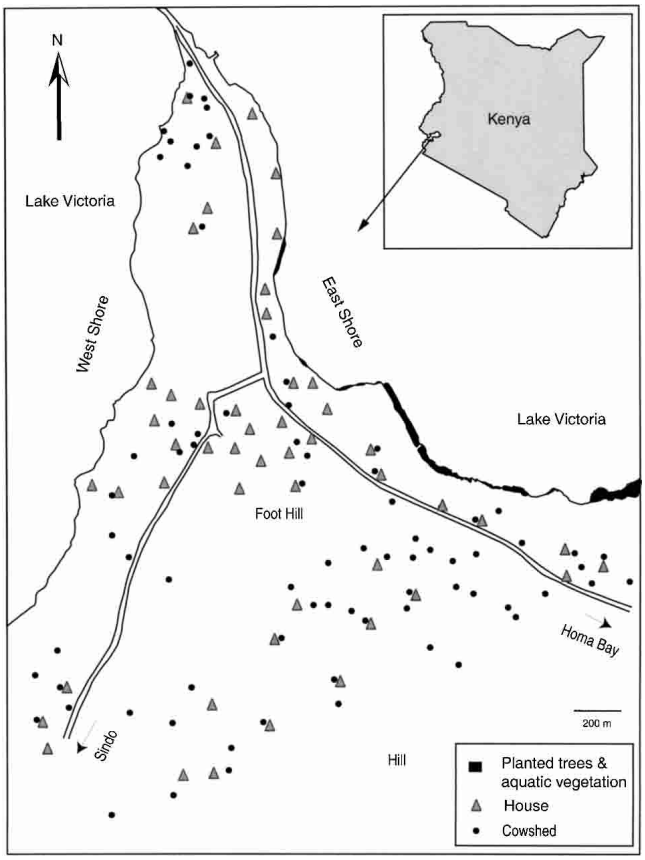

FIGURE 1. Map of the study area (Mbita Point Village, Suba District, in western Kenya). The locations of the sampled houses and cowsheds are shown.

depending on habitat sizes, were taken using a standard mosquito dipper (350 ml).[14] Mosquito larvae were then immediately preserved in 95% ethanol. This sampling method permitted only comparison of relative abundance of each species among the habitats. Absolute abundance could not be estimated because only a proportion of mosquito larvae were sampled from large habitats while all larvae were collected from small habitats.[14] The coordinates of all habitats were recorded with a hand-held GPS unit, and the GPS readings were calibrated by matching the locations on the detailed map (1:1,000) of the study area. The larval survey was repeated in the late rainy period (May 15–20, 1999).

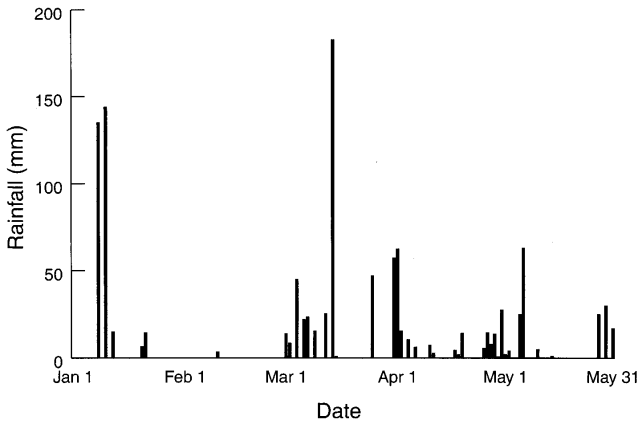

FIGURE 2. Daily precipitation from January 1 through May 31, 1999 in the study area (Mbita Point Village in western Kenya).

Adult sampling. Adult mosquitoes were collected randomly from 50 houses using pyrethrum indoor spray catch method on March 2–8, 1999, the beginning of the rainy season (Figure 1). Adult mosquitoes were collected and preserved in 95% ethanol. The GPS coordinate of each house was recorded using a hand-held GPS unit. The distance to the nearest larval habitats from each house was estimated with a tape measure when the distance was less than 200 meters. When it exceeded 200 meters, the distance was measured from the map. The number of residents was recorded for each house. Adult sampling was repeated on May 15–20, 1999, the end of the rainy season.

Distribution of livestock hosts. Domestic animals in the study area were primarily cattle. The locations of all cowsheds in the study area were mapped (Figure 1), and the number of cows in each cowshed was recorded. Cow density around each larval habitat was estimated by averaging the number of cows in the five nearest cowsheds.[15] Similarly, human density around a larval habitat was estimated by averaging the number of residents in the five nearest houses. Cow density and human density were also estimated by averaging the number of cows in the five nearest cowsheds and the number of residents in the five nearest houses around each house where a mosquito collection was made.

Species identification. All larvae and adults were examined microscopically to distinguish *An. gambiae* s. l. from *An. funestus*, based on the identification keys by Gillies and Coetzee.[16] Larvae and adults were preserved in 95% ethanol. Extraction of DNA followed a standard protocol.[4] Individual species within *An. gambiae* species complex were identified using an rDNA-polymerase chain reaction (PCR) method.[4] If the initial PCR testing failed to amplify a sample, then the PCR analysis was repeated once or twice until successful amplification was achieved. If a sample could not be identified after three PCR amplifications, it was scored as unknown.[14] The unidentified larvae were probably poorly preserved or morphologically misidentified as members of *An. gambiae* s.l..

Statistical analyses. *Relationship between species composition of mosquito larvae and human/livestock distribution.* In our study area, only *An. gambiae* and *An. arabiensis* within *An. gambiae* s.l. were present.[14] Because the two species differ significantly in host-biting preference, we were particularly interested in how human/livestock distribution affects the relative abundance of each species. Multiple regressions with the relative abundance of *An. gambiae* as the dependent variable were used. The relative abundance of *An. gambiae* was calculated as the number of *An. gambiae* divided by the total number of *An. gambiae* s.l. mosquitoes, and was arcsine transformed in the analysis. Independent variables were 1) the ratio of average resident number to average cow number around each larval habitat, 2) the ratio of average house distance to average cowshed distance from the larval habitats, and 3) the distance among larval habitats. Because there were multiple larval habitats in the study area, geographic distances among larval habitats were represented by a distance matrix. Distance matrix of the dependent variable among the sites was computed using the Bary and Curits coefficient.[17] The first two independent variables were transformed using the Box-Cox method,[18] and their Euclidean distance matrices were computed. The distance matrix for the third variable was

computed using geographic coordinates of sampling sites. All distance matrices were computed using the statistical package Progiciel R.[19]

Partial regression coefficients for each independent variable were computed using the multiple regression on distance matrices method.[20] This method is an extension of the Mantel test; it can use three or more matrices while the Mantel test compares only two matrices.[21] The statistical significance of the partial regression coefficients was determined by the permutation test using the statistical package Permute.[22] We used the multiple regression on distance matrix method for two reasons. First, the density of mosquitoes, humans, and livestock varied among homesteads; our method can control the spatial effect and thus adequately evaluate effects of the non-spatial variables.[23] Second, the mosquito/livestock distribution data violate an important assumption of parametric statistical methods: independence among the observations. However, our method is non-parametric, and is thus not affected by this assumption.[24]

Relationship between density of mosquito adults and human/livestock distribution. The multiple regression analysis described above was also used to determine the relationship between the absolute densities of *An. gambiae* and *An. arabiensis* adults and human/livestock distributions. Like the above analyses, the dependent variables were transformed with the Box-Cox method, and a distance matrix among the sites was computed using the Bary and Curits coefficient. The independent variables were 1) average resident number in each house where mosquito collection was made and in the five nearest houses from the sampled house, 2) average distance to the five nearest houses from each sampled house, 3) average cow number in the five cowsheds nearest from each sampled house, 4) average distance to the five nearest cowsheds from each sampled house, 5) distance to the nearest larval habitat from each sampled house, and 6) distances among the sampled houses. The first five independent variables were transformed using the Box-Cox method, and their Euclidean distance matrices were computed. For the sixth independent variable, the distance matrix was computed using geographic coordinates of sampling sites. We did not conduct this analysis for *An. arabiensis* March samples and for *An. funestus* because a small number of mosquitoes were collected. The analyses were made for only female mosquitoes because males do not bite the hosts and do not transmit malaria parasites.

RESULTS

Relationship between species composition of mosquito larvae and human/livestock distribution. In early March 1999, we found 16 aquatic habitats in the study area. All habitats had mosquito larvae, 12 contained anopheline larvae, and four sites had culicine larvae (Figure 3). All larval habitats were located within 50 meters of the shore of Lake Victoria. For the 12 anopheline-positive habitats, eight were on the lakeshore with planted trees and water hyacinth, and four were human-made habitats, including ditches, concrete holes, and stagnant water in a boat. A total of 522 anopheline larvae was collected from the 12 habitats, and 511 specimens were identified to species. There were 389 *An. gambiae* (74.5%) and 95 *An. arabiensis* (18.2%), but only 16 (3.1%) *An. coustani* and 11 (2.1%) *An. funestus,* of which both were found

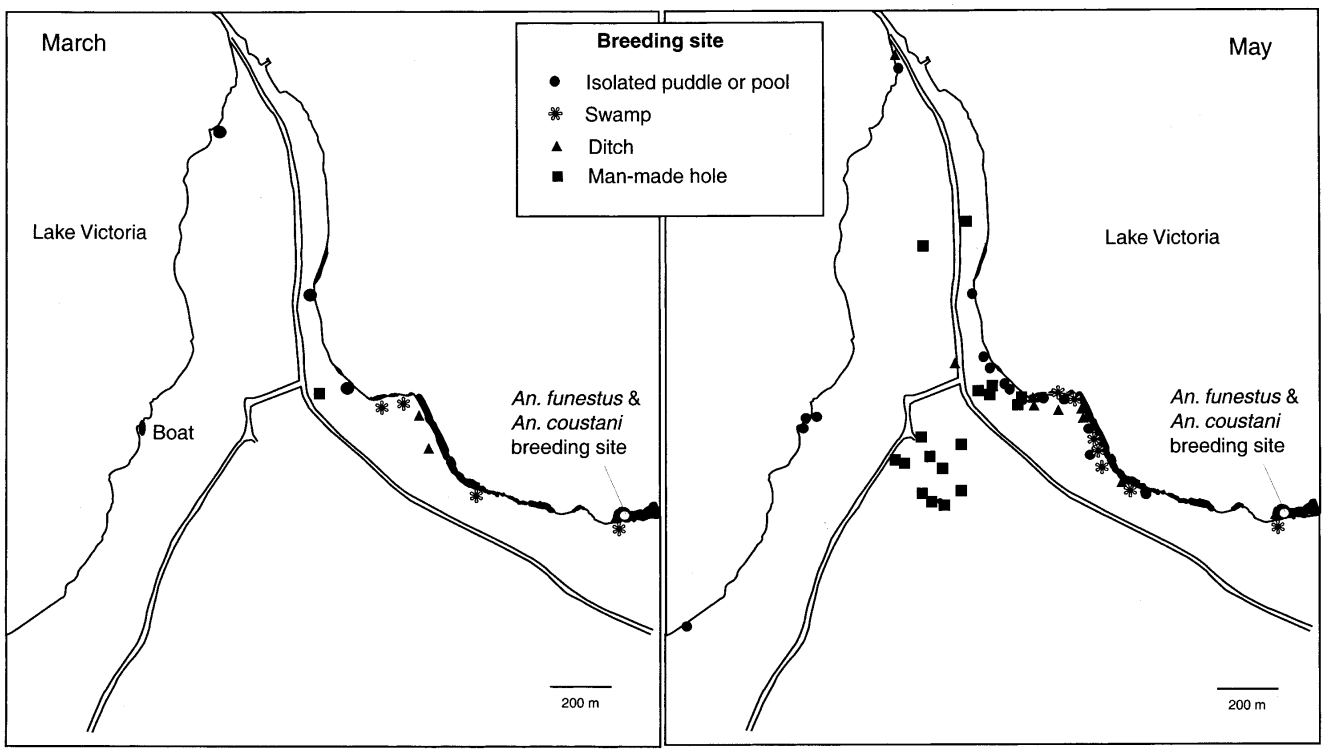

FIGURE 3. Distribution of anopheline larval habitats in the study area at the beginning (March; **left**) and end (May; **right**) of the rainy season, 1999.

only in the lake water with water hyacinth where *An. gambiae* larvae were not found. The relative abundance of *An. gambiae* in a habitat ranged from 14.3% to 97.8%.

In late May 1999, we found 57 aquatic habitats, including 32 on the east shore, 10 on the west shore, and 15 inland (Figure 3). Twenty-three sites were human-made habitats, including irrigation and roadside ditches, and concrete holes. Thirty-four other sites were natural habitats such as puddles and swamps. Among the 57 aquatic habitats, 45 habitats contained *An. gambiae* and *An. arabiensis* larvae, one habitat contained only *An. funestus* and *An. coustani* larvae, and the remaining 11 habitats contained only culicine larvae. A total of 2,024 anopheline larvae was collected, and 1,930 larvae (95.3%) were identified to species by the PCR. The species composition was as follows: *An. gambiae* (80.3%), *An. arabiensis* (17.1%), *An. funestus* (2.0%) and *An. coustani* (0.6%). The relative abundance of *An. gambiae* within a habitat ranged from 18.9% to 94.1%. Overall, the relative abundance of *An. gambiae* did not vary significantly between March and May ($\chi^2 = 2.64$, degrees of freedom [df] $=1$, $P > 0.05$).

Multiple regression analysis detected two variables (the ratio of human density to cow density in a homestead and the ratio of distance to a house from a larval habitat to distance to a cowshed from a larval habitat) significantly associated with the relative abundance of *An. gambiae* larvae for the March samples (Table 1). The correlation coefficient ($r = -0.69$, df $= 9$, $P < 0.01$) indicates a negative association between *An. gambiae* relative abundance and the ratio of distance to a house from a larval habitat to distance to a cowshed from a larval habitat, but a positive association ($r = 0.40$, df $= 9$, $P = 0.02$) for the variable ratio of human density to cow density in a homestead. That is, if a larval habitat is farther away from a house but closer to a cowshed, fewer *An. gambiae* larvae would be found in this habitat. The standard partial regression coefficients suggest that the distance ratio played a more important role than the ratio of human density to cow density (Table 1).

For the samples in the late rainy season, only one variable (the ratio of distance to a house from a larval habitat to distance to a cowshed from a larval habitat) showed a significant and negative association ($r = -0.75$, df $= 43$, $P < 0.01$) with the relative abundance of *An. gambiae* larvae (Table 1). This result was consistent with the finding in the early rainy season when larval habitat distribution was more restricted.

Relationship between adult mosquito densities and human/livestock distribution. During the early March survey, 228 adult anopheline mosquitoes, including both males and females, were collected from 31 houses. Nineteen houses did not have any mosquitoes. The specimens included *An. gambiae*, *An. arabiensis* and *An. funestus*, and their relative abundance was 68.0%, 7.5%, and 16.7%, respectively. The species identity of 18 specimens (7.9%) could not be established because the PCR failed to amplify. Adult species composition was significantly different from the larval samples collected in the same dates ($\chi^2 = 9.34$, df $= 1$, $P < 0.01$). The average densities per house were 3.1 for *An. gambiae,* 0.3 for *An. arabiensis,* and 0.8 for *An. funestus*. Significantly more females than males were collected because females prefer to rest indoors after ingesting a blood meal.

In late May, 2,245 anopheline adults, including both males and females, were collected from 48 houses. Two houses had no mosquitoes. The relative abundance was 88.0% for *An. gambiae,* 6.3% for *An. arabiensis,* and 1.4% for *An. funestus*. The PCR analysis failed to identify 96 specimens (4.3%). There was a significant difference in species composition between larval and adult specimens ($\chi^2 = 291.53$, df $= 1$, $P < 0.001$). The differences in anopheline mosquito species composition were not significant between March and May ($\chi^2 = 2.31$, df $= 3$, $P > 0.05$). The average density was 39.5 mosquitoes per house for *An. gambiae,* 2.8 for *An. arabiensis,* and 0.6 for *An. funestus*. The average densities of *An. gambiae* and *An. arabiensis* in May were more than 10 times higher than those in March. However, *An. funestus* densities did not vary significantly between early and late rainy seasons ($t = 0.83$, df $= 1$, $P > 0.05$).

For the adult mosquito samples collected in March, of the six independent variables analyzed, distance from a house to the nearest larval habitat was the only variable significantly associated with *An. gambiae* density (Table 2). For the May adult mosquito samples, distance to the nearest larval habitat from a house was also the only significant factor for *An. gambiae* density, and none of the six independent variables was significantly associated with *An. arabiensis* density (Table 2). The negative association ($r = -0.50$, df $= 22$, $P < 0.05$ for March; $r = -0.51$, df $= 47$, $P < 0.01$ for May) between *An. gambiae* density in a house and distance from the house to its nearest larval habitat in both samples suggests that there would be more *An. gambiae* mosquitoes in houses near larval habitats than in houses far from larval habitats. The relationship between mosquito density and distance from the houses to the nearest larval habitats is shown in Table 3. More than 90% of *An. gambiae* adults were found in the houses within 300 meters from the nearest larval habitat in both sampling periods.

TABLE 1

Regression analysis results for association between relative abundance of *Anopheles gambiae* larvae and host availability in the early rainy period (March 1999) and late rainy period (May 1999)

Month	Variable	Standard partial regression coefficient	*P*
March	Distance from larval habitats to houses/distance from larval habitats to cowsheds	0.601	<0.001
	Human density/cow density	0.278	<0.01
	Distance matrix among larval habitats	–0.276	>0.05
May	Distance from larval habitats to houses/distance from larval habitats to cowsheds	0.673	<0.01
	Human density/cow density	–0.066	>0.05
	Distance matrix among larval habitats	0.062	>0.05

TABLE 2

Regression analysis results for association between anopheline adult mosquito density and host availability in the early rainy period (March 1999) and late rainy period (May 1999)*

Species	Variables	March		May	
		b†	*P*	b†	*P*
Anopheles gambiae	Average human density in the 5 houses surrounding the sampled house	0.163	> 0.05	0.326	> 0.05
	Distances to the 5 nearest houses from the house where mosquitoes were sampled	–0.075	> 0.05	0.259	> 0.05
	Average cow number in the 5 nearest cowsheds from the sampled house	–0.017	> 0.05	0.062	> 0.05
	Distance to the 5 nearest cowsheds from the sampled house	–0.020	> 0.05	0.034	> 0.05
	Distance to the nearest breeding site from the sampled house	0.183	< 0.05	0.144	< 0.05
	Distance matrix among larval habitats	–0.114	> 0.05	–0.036	> 0.05
Anopheles arabiensis	Average human density in the 5 houses surrounding the sampled house			–0.225	> 0.05
	Distances to the 5 nearest houses from the house where mosquitoes were sampled			0.301	> 0.05
	Average cow number in the 5 nearest cowsheds from the sampled house			0.155	0.06
	Distance to the 5 nearest cowsheds from the sampled house			–0.043	> 0.05
	Distance to the nearest breeding site from the sampled house			–0.125	> 0.05
	Distance matrix among larval habitats			0.026	> 0.05

* The analyses were not conducted for *An. arabiensis* in the March sampling and for *An. funestus* in the March and May samples due to small sample sizes.
† b = standard partial regression coefficient.

DISCUSSION

This study has examined the ecologic factors underlying species composition variation in anopheline larval and adult mosquitoes at the village level. It is well known that *An. gambiae* is anthropophilic, and *An. arabiensis* is zoophilic, but how the distribution of livestock and human affects the species composition of larvae in aquatic habitats and adults in resident's houses is unknown. Here we have demonstrated that density ratio of humans to cows in homesteads and the ratio of distance between larval habitats and houses to the distance between larval habitats and cowsheds were significantly associated with the relative abundance of *An. gambiae* larvae in aquatic habitats. More *An. gambiae* larvae would be found in a habitat closer to houses and farther from cowsheds. For adult mosquitoes, distance from houses to larval habitats was the only variable significantly associated with *An. gambiae* adult density. *Anopheles gambiae* density in a house was not correlated with either human and cow densities in the homestead or with the distances to the cowshed from the house. More *An. gambiae* would be found in houses near larval habitats than in houses farther from larval habitats. More than 90% of *An. gambiae* adults were found in the houses within 300 meters from the nearest larval habitats in the two samples collected in the beginning and the end of the rainy season.

Anopheles gambiae was the predominant species in both adult and larval samples in our study area. The temporal change in anopheline species composition between the beginning and the end of the rainy season was not significant. Gimnig and others reported that *An. arabiensis* was the predominant species in larval habitats in Asembo Bay (approximately 40 km from our study site),[6] and temporal changes of species composition were observed in sites near Kisumu and approximately 80 km from our study site.[3,9] Over a large geographic scale, the relative abundance of *An. gambiae* and *An. arabiensis* is defined by climatic factors such as precipitation and temperature.[8] The discrepancy between our results and re-

TABLE 3

Distribution of anopheline adults in relation to the distance from the nearest larval habitat in the early rainy period (March 1999) and the late rainy period (May 1999)

Month	Distance (meters)	*Anopheles gambiae*			*Anopheles arabiensis*			*Anopheles funestus*		
		Female	Male	Total	Female	Male	Total	Female	Male	Total
March	100	36.3	42.9	38.1	0.0	0.0	0.0	0.0	0.0	0.0
	200	67.3	59.5	65.2	25.0	0.0	23.5	7.1	10.0	7.9
	300	93.8	90.5	92.9	75.0	100	76.5	75.0	100	81.6
	400	94.7	90.5	93.5	93.8	–	94.1	78.6	–	84.2
	> 500	100	100	100	100	–	100	100	–	100
	Samplesize	113	42	155	16	1	17	28	10	38
May	100	51.5	49.7	50.9	45.5	52.4	47.5	0.0	0.0	0.0
	200	83.0	90.4	85.3	63.6	81.0	68.8	23.5	0.0	12.5
	300	91.8	94.4	92.6	84.8	85.7	85.1	82.4	46.7	65.6
	400	95.8	96.0	95.9	88.9	85.7	87.9	82.4	46.7	65.6
	> 500	100	100	100	100	100	100	100	100	100
	Sample size	1,352	624	1,976	99	42	141	17	15	32

sults of other studies may be due to climatologic differences in the study sites or due to temporal changes in species composition.

Although *An. gambiae* was the predominant species in both adult and larval samples, significantly more *An. gambiae* was found in the adult samples than in the larval samples. Such a difference is most likely due to differences in feeding and resting behavior between the two species. *Anopheles gambiae* is anthropophilic and prefers to rest indoors after a blood meal.[2,11] *Anopheles arabiensis* is zoophilic and prefers to rest outdoors.[25,26] Therefore, species composition based on adult samples is biased toward *An. gambiae*. Although the larval population sampling reflects mosquito species composition more precisely in an area compared to adult indoor collections, the epidemiologic inference from adult sampling substantiates the vector importance of *An. gambiae* in malaria transmission and target control.

Our results showed that distance from houses to larval habitats was the only variable significantly associated with *An. gambiae* adult density in houses. Other variables such as human and cow densities and distances to the cowsheds from a house had no significant association with *An. gambiae* density. Charlwood and Edoh reported that anopheline adult density is negatively correlated with the distance to larval habitats from houses,[27] and Shidrawi[10] found no correlation between *An. gambiae* density and cattle density.[11] We observed that more than 90% of anopheline adults were found in houses less than 300 meters from larval habitats, suggesting that anopheline mosquitoes tend to inhabit houses around larval habitats.[28,29] Conversely, availability of larval habitats is strongly affected by human activities. Human-made larval habitats were often found in our study area. For example, most larval habitats found in the late rainy season were human-made holes and roadside ditches created by vehicles and irrigation. The footprints of cows and humans are often suitable larval habitats for anopheline mosquitoes.[30,31] Human-made environmental changes may render aquatic habitats previously unsuitable for anopheline mosquito breeding into suitable habitats. For example, the shore of Lake Victoria is generally not a suitable habitat for anophelines. However, tree planting along the lakeshore reduces wind action and water waves, and leads to stagnant water and rapid growth of water hyacinths in which we observed *An. funestus* larvae breeding at both the beginning and end of the rainy season. Similar adult density of *An. funestus* in both sampling periods suggests that the *An. funestus* larval habitats remained stable throughout the year. *Anopheles funestus* tends to breed in large permanent waters with aquatic vegetation, such as swamps, river edges, and large ponds.[30,32,33] The aquatic plants provide effective shelter for *An. funestus* larvae from predators.

Our results have several interesting implications on malaria vector control in the basin region of Lake Victoria. First, vector control should be community-based. Elimination of mosquito larval habitats in one's homestead is important, but is not sufficient for reducing mosquito densities in a community. Adult mosquitoes in a house may be originated from larval habitats of several hundred meters apart. Thus, larval control should target larval habitats in a community. Second, zooprophylaxis may not be effective. Our data showed anopheline adult mosquito density had no correlation with cow density or distance to cowsheds. Perhaps environmental management through elimination of larval habitats and larval control using bioinsecticides may be a more effective approach for reducing adult mosquito densities. Our results may be specific to the ecologic conditions in our study area, but the validity of these results needs to be determined in different areas under different ecologic conditions.

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