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Authors
Munga, Stephen
Minakawa, Noboru
Zhou, Guofa
et al.

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ASSOCIATION BETWEEN LAND COVER AND HABITAT PRODUCTIVITY OF MALARIA VECTORS IN WESTERN KENYAN HIGHLANDS

STEPHEN MUNGA, NOBORU MINAKAWA, GUOFA ZHOU, EMMANUEL MUSHINZIMANA, OKEYO-OWUOR J. BARRACK, ANDREW K. GITHEKO, AND GUIYUN YAN*

Kenya Medical Research Institute, Kisumu, Kenya; Faculty of Medicine, Saga University, Saga, Japan; School of Environmental Studies, Moi University, Eldoret, Kenya; Department of Biological Sciences, State University of New York, Buffalo, New York

Abstract. We examined the effects of land cover type on survivorship and productivity of *Anopheles gambiae* in Kakamega in the western Kenyan highlands (elevation = 1,420–1,580 meters above sea level). Under natural conditions, *An. gambiae* sensu lato adults emerged only from farmland habitats, with an estimated productivity of 1.82 mosquitoes/meter^2/week, but not from forest and swamp habitats. To determine the effects of intraspecific competition and land cover types, semi-natural larval habitats were created within three land cover types (farmland, forest, and natural swamp), and three different densities of *An. gambiae* sensu stricto larvae were introduced to the larval habitats. The mosquito pupation rate in farmland habitats was significantly greater than in swamp and forest habitats, and larval-to-pupal development times were significantly shorter. At higher densities, the larvae responded to increased intraspecific competition by extending their development time and emerging as smaller adults, but initial larval density showed no significant effects on pupation rate. Land cover type may affect larval survivorship and adult productivity through its effects on water temperature and nutrients in the aquatic habitats, as shown by the significantly higher water temperature in farmland habitats, enhanced pupation rates and shortened development times from the addition of food to habitats, and a significant negative correlation of the occurrence of *An. gambiae* larvae with canopy cover and emergent plants in natural habitats. These results suggest that deforestation and cultivation of natural swamps in the western Kenyan highland create conditions favorable for the survival of *An. gambiae* larvae, and consequently increase the risks of malaria transmission to the human population.

INTRODUCTION

African highlands are fragile ecosystems under great pressure from increasing populations, deforestation, and increased farming.[1] Population growth in the western Kenya highland is a particularly severe problem, due in part to lack of family planning and migration from other areas.[2] As a consequence of the dramatic human population increase, there have been unprecedented land-use changes in the highlands. For example, since 1965 the Malava Forest in Kakamega district in western Kenya has been reduced from 600 to less than 100 hectares.[3] Most rain forests have been cleared for crop planting, cattle grazing, commercial logging, firewood collection, and housing construction.[1]

These environmental changes can promote vector-borne disease transmission. It has been suggested that increased malaria epidemics in African highlands can be attributed to changes in land cover.[4–8] Recent studies found that larvae of *Anopheles gambiae* sensu lato (s.l.), which is the primary malaria vector in Africa, occur more frequently in temporary pools in cultivated areas than those in forested areas and natural swamps in the western Kenya highland.[9,10] In a Ugandan highland site, a higher adult density of *An. gambiae* s.l. was found in houses near cultivated swamps than in those near natural swamps characterized by tall grasses such as papyrus (*Cyperus papyrus* L.).[7] Since cultivated swamps generally receive more sunlight than natural swamps, the ambient air temperature in the cultivated swamp areas was significantly higher than that in natural swamps.

These observations suggest that the changes in aquatic conditions occasioned by changes in land use practice may enhance larval development and survivorships in cultivated swamps. *Anopheles gambiae* s.l. is a typical r-strategist, colonizing temporary habitats in which selection favors rapid population increase.[11–14] In general, larval predation is less prevalent in temporary habitats than in large, permanent habitats.[15–17] Because small and sunlit habitats have higher water temperatures, mosquito larval-to-pupal development time is shortened, and the warmer habitat produces more food in the form of algae.[18] Larval mortality due to desiccation or poor nutrient conditions may be reduced until water temperatures rise above 30°C.[19,20] Consequently, the enhanced larval habitat conditions may increase productivity of adult mosquitoes, which in turn increases the risk of malaria transmission.

In the present study, we examined the effects of land cover types on productivity of adult malaria vectors in aquatic habitats in western Kenyan highland. We also identified the important factors related to their production. Because survivorship and development time of larvae are directly related to productivity of adult mosquitoes, we also compared whether development and survivorship of *An. gambiae* Giles (referred to as *An. gambiae* hereafter) larvae differ under the different land cover types. Information from this study is critical for estimating the impact of land use change on productivity of malaria vectors in Kenyan highlands where malaria outbreaks have occurred frequently in the last 15 years.[21,22] Despite its importance, productivity of malaria vectors in various types of aquatic habitats has not been extensively studied in Africa.

MATERIALS AND METHODS

Study area. The study site (approximately 4 × 4 km^2) is located in the western Kenyan highland.[23] The altitude of the study site ranged from 1,420 to 1,580 meters above sea level. The total rainfall and mean daily temperature were 1,840 mm and 20°C, respectively, during the study period (from July 2002 to July 2003). Peak rainfall generally occurs between March and June followed by a short rainy season in October and November. The area is characterized by undulating to-

* Address correspondence to Guiyun Yan, Department of Biological Sciences, State University of New York, Buffalo, NY 14260. E-mail: gyan@buffalo.edu

pography, with both steep and gently sloping hills. Although most of the study area has been cultivated, small patches of indigenous forest still remain along the rivers and streams. A recent study found that larvae of *An. gambiae* occur mostly in aquatic habitats in valley bottoms within the study area.[10] In particular, larvae occur more frequently in cultivated areas than in forests and natural swamps.

Productivity of natural habitats. We determined productivity of *An. gambiae* s.l. in natural aquatic habitats within three different land cover types: farmlands, swamps, and forests. In this study, farmlands refer to natural swamps that were recently converted to agricultural land by draining water, and that were characterized either by the presence of agricultural crops such as maize or by bare ground that had been prepared for planting crops. Aquatic habitats in the farmlands were mainly ditches and temporary pools appearing in depressions created by anthropogenic activities. Swamps were characterized by standing water and the presence of emergent aquatic plants such as *C. papyrus*. Forests were mainly composed of native tree species such as *Ficus lutea* Vahl and *Polyscias fulva* (Hiern). Forests and swamps were the areas with the least anthropogenic modification.

Productivity of the malaria vector was defined as the number of emerging adult mosquitoes/m^2/week from aquatic habitats. We used collapsible emergence traps to collect emerging adult mosquitoes.[24,25] Two sizes of traps, 1 m^2 and 0.5 m^2, were used, depending on the habitat size. The water surface area of all habitats used in this study was greater than 0.5 m^2. The emergence trap prevents adult mosquitoes from ovipositing in the area covered by the trap; therefore, we relocated the emptied trap daily within the same habitat. Service reported that the mortality rate of anopheline larvae generally exceeded 90% under natural conditions.[26,27] Although the emergence trap may not provide an accurate estimation of absolute mosquito productivity of a habitat, it is suitable to compare the relative productivity of different larval habitat types.[24,25,28] The traps were placed in 30 randomly selected sites (10 in each of the three land cover types) to collect emerging mosquitoes for seven consecutive days per month for the period of July 2002 to July 2003. Due to logistic reasons, no collections were made in November 2002. Emergence traps were placed randomly regardless the presence or absence of larvae and pupae in a habitat. Trapped mosquitoes were collected daily by aspiration. Sampled anopheline mosquitoes were identified using morphologic keys.[29] In addition, members of *An. gambiae* s.l. were identified by an ribosomal DNA (rDNA) polymerase chain reaction (PCR) method.[30]

To determine the important environmental factors related to productivity, we recorded the following parameters at each site where a trap was placed on each sampling occasion: canopy cover, occurrence (presence or absence) of emergent plants, occurrence of filamentous algae, water depth, and water temperature. Canopy cover was estimated using a densitometer.[31] Water temperature was recorded using Stowaway Tidbit data loggers (Onset Computers, Bourne, MA) at hourly intervals. Daily rainfall was recorded using one automated rain gauge placed in a fixed central position in the study area (Onset Computers).

Effects of land cover types and larval densities on larval survivorship and mosquito productivity in semi-natural habitats. We examined three land cover types (farmland, forest, and swamp) and three larval densities (20, 60, and 100) in September 2003. In this experiment, no extra food was added to the habitats. A total of 27 semi-natural habitats (3 replicates per land cover type and density combination) were created using plastic washbasins (35 cm in diameter and about 15 cm deep). Two kilograms of dry soil and 2.5 liters of water were added to each washbasin. The dry soil was collected from farmland within the study area. Two holes (3 cm in diameter) were created near the top edge of each washbasin to maintain a constant water level during periods of rain. The holes were covered with a screen (mesh size = 200 μ) to prevent larvae from being washed away. One day before the experiment, the washbasins were flooded with rainwater.

First-instar *An. gambiae* larvae from a colony maintained in an insectary at the Kenya Medical Research Institute in Kisian, Kenya were used. Nine washbasins received 20 newly hatched larvae, each approximately 2 hours old. Three washbasins each with 20 larvae were placed in randomly selected aquatic habitats in each of the three land cover types. Similarly, washbasins that received 60 or 100 larvae were placed in aquatic habitats in each land cover type. Each washbasin was covered with a screen (mesh size = 1 mm) to prevent wild female mosquitoes from ovipositing in the washbasin. We counted the number of larvae surviving in each washbasin daily by picking each larva using a wide mouthed plastic pipette carefully; all larvae were placed back in the same washbasins. Once the larvae began pupating, the washbasin was examined twice a day, from 9:00 AM to noon and from 3:00 PM to 7:00 PM. Pupae were removed from the washbasin and held in cups of water, allowing them to grow to adults. The emerged adults were separated by sex and their wing lengths were measured. Water temperature in each washbasin was monitored hourly with a Stowaway Tidbit data logger.

We carried out a similar experiment for survivorship, which doubled the number of replications (n = 54), in June 2004. A half set of washbasins received a food supplement of 0.05 grams of brewers yeast (Pharmadass Ltd., Harrow, United Kingdom) daily and the other half received no yeast. This experiment was designed to examine the effects of nutrients on mosquito productivity.

Statistical analysis. Logistic regression analysis was used to determine important environmental variables related to occurrence of *An. gambiae* s.l. in the traps. Since most traps (94.5%) contained no mosquitoes, occurrence of mosquitoes was used as the dependent variable instead of the actual numbers of mosquitoes. Independent variables were canopy cover, water depth, maximum water temperature, minimum water temperature, occurrence of filamentous algae, and occurrence of emergent plants. Mean water temperature was not included in the analysis because of its significant correlations with maximum temperature and canopy cover. Occurrences of filamentous algae and emergent plants were treated as categoric variables.

The non-parametric Wilcoxon test was used to examine the effects of land cover type and initial larval density on pupation rate.[32] Pupation rate is defined as the proportion of first-instar larvae that developed to the pupal stage. Analysis of variance (ANOVA) was used to test the effects of these two factors on development time and on wing size of resulting *An. gambiae* in the experiments of September 2003 and June 2004. Development time is the length of time that a first-instar larva requires to emerge as an adult. Development time was logarithm transformed, and wing length data were not trans-

formed. Male and female development time and wing length were analyzed separately. Tukey honestly significant difference tests were used to compare the effects of initial density on development time and wing length. Due to insufficient number of adult mosquitoes emerging from the forest habitats in June 2004 in the treatments without adding additional food, the development time and wing length data from the forest habitats were not included in the ANOVA. Multiple regression analysis was used to determine whether pupation rate, development time, and wing size were associated with maximum water temperature and minimum water temperature. In this analysis, pupation rate and canopy cover were transformed with the arcsine transformation, and the Box-Cox transformation was applied to the other variables except female and male wing sizes.

RESULTS

Species composition and productivity of malaria vectors of natural habitats. A total of 2,264 mosquitoes were collected in the emergence traps. Of these, 117 (5.2%) were *An. gambiae* s.l., and 5 (0.2%) were *An. funestus* Giles (Table 1). Among the 117 *A. gambiae* s.l., 81 were identified as *An. gambiae*, and one was identified as *An. arabiensis* Patton by rDNA PCR analysis. The remaining (35) *An. gambiae* s.l. was not identified because of a failure of the PCR amplification. Other anophelines and culicines composed 80 (3.5%) and 2,062 (91.1%) individuals, respectively. All *An. gambiae* s.l. were trapped in farmlands and the other anopheline species, including *An. coustani* Laveran, *An. implexus* Theobald, *An. maculipalpis* Giles, and *An. squamosas* Theobald, were trapped mainly in forests and natural swamps.

The estimated productivity of *An. gambiae* s.l. in farmlands was 1.82 mosquitoes/m^2/week over the study period. The highest productivity (7.34 mosquitoes/m^2/week) was recorded in April, 2003, and it was significantly higher than that in the other months ($P < 0.05$) (Figure 1). The estimated productivity of *An. funestus* was 0.05 mosquitoes/m^2/week; this species was trapped only in swamp habitats.

Logistic regression analysis showed that occurrence of *An. gambiae* s.l. was significantly correlated with canopy cover ($\chi^2 = 7.26$, degrees of freedom [df] = 1, $P < 0.01$), occurrence of emergent plants ($\chi^2 = 11.15$, df = 1, $P < 0.001$), and maximum temperature ($\chi^2 = 4.28$, df = 1, $P < 0.05$), but not with occurrence of filamentous algae ($\chi^2 = 0.05$, df = 1, $P > 0.05$) and minimum temperature ($\chi^2 = 0.71$, df = 1, $P > 0.05$). Larval habitats under different land cover types differed in a number of environmental variables characterized (Table 2). For example, maximum water temperature and mean water temperature were significantly higher in farmlands than in the other land covers. Emergent plants were found at more sites within swamps than in the other land covers, but filamentous algae were found at fewer sites within swamps.

TABLE 1

Numbers and species of mosquitoes recovered from emergence traps placed in natural aquatic habitats within farmlands, swamps, and forests

Species	Farmland	Forest	Swamp
Anopheles gambiae s.l.	117	0	0
Anopheles funestus s.l.	0	0	5
Anopheles coustani	1	2	2
Anopheles implexus	23	22	8
Anopheles maculipalpis	0	0	1
Anopheles squamosus	1	1	19
Culicines	433	1,311	318
Total	575	1,336	353

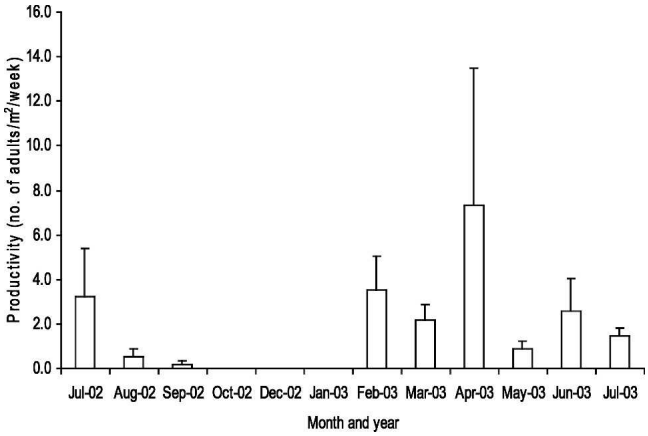

FIGURE 1. Dynamics of productivity (number of individuals/m^2/week) of *Anopheles gambiae* adults in farmland habitats. Error bars show standard errors.

Effects of land cover types and intraspecific competition on pupation rate, development time, and wing length. In the experiment conducted in September 2003 (without adding additional food), no larvae successfully pupated in the semi-natural habitats in forests and natural swamps. However, 24–48% of first-instar larvae developed into pupal stage in habitats in farmland (Table 3). In this experiment, pupation rate was significantly affected by land cover type ($\chi^2 = 43.92$, df = 2, $P < 0.0001$), but not by initial larval density ($\chi^2 = 2.67$, df = 2, $P > 0.05$). Because no adults emerged from forest and swamp habitats, only data from farmland habitats was used in the analysis to test for the effect of initial larval density on development time. Initial larval density significantly influenced female (F = 67.09, df = 2, 146, $P < 0.0001$) and male (F = 7.26, df = 2, 36, $P < 0.0001$) development times. Similarly, initial larval density significantly influenced wing lengths of both females (F = 152.13, df = 2, 124, $P < 0.0001$) and males (F = 20.45, df = 2, 29, $P < 0.0001$). In particular, average development times when the initial density was 20 larvae were significantly shorter than those with initial densities of 60 and 100 larvae, and wing lengths of the females and males in habitats with 20 larvae were longer (Table 3).

In the experiments conducted in June 2004 without adding food to larval habitats, only a small proportion of first-instar larvae survived and developed into pupal and adult stages in forest (2%) and in swamp (6–33%), but a significantly larger proportion (49–65%) survived and developed in farmland habitats (Table 3). Similar to the findings of Sept. 2003, pupation rates were significantly affected by land cover type ($\chi^2 = 20.81$, df = 2, $P < 0.0001$), but not by the initial larval density ($\chi^2 = 1.36$, df = 2, $P > 0.05$). The effect of land cover types was significant on the development time of females (F = 80.85, df = 1, 155, $P < 0.0001$) and males (F = 228.73, df = 2, 153, $P < 0.0001$), and on the wing lengths of females (F = 3.82, df = 1, 161, $P = 0.05$) and males (F = 0.88, df = 1, 146, $P > 0.05$). In farmlands, the development times were

TABLE 2
Summary of environmental variables in natural and semi-natural habitats under farmland, swamp, and forest land use types*

Variables	Farmland	Forest	Swamp
Natural habitats			
Mean canopy cover (%)	14.6 ± 0.7[a]	90.6 ± 0.5[b]	8.8 ± 0.8[a]
Mean water depth (m)	0.17 ± 0.02	0.57 ± 0.23	0.13 ± 0.01
No. of habitats with emergent plants	47 (10.4%)[a]	12 (2.61%)[a]	332 (72.7%)[b]
No. of habitats with filamentous algae	40 (8.9%)[a]	47 (10.2%)[a]	8 (1.75%)[b]
Maximum temperature (°C)	28.9 ± 0.2[a]	22.3 ± 0.12[b]	26.5 ± 0.19[c]
Minimum temperature (°C)	18.0 ± 0.1[a]	17.8 ± 0.04[b]	18.1 ± 0.07[a]
Mean temperature (°C)	22.1 ± 0.1[a]	19.7 ± 0.04[b]	21.3 ± 0.09[c]
N	452	460	456
Semi-natural habitats			
September 2003 experiment			
Maximum temperature (°C)	39.2 ± 0.23[a]	24.8 ± 0.15[b]	26.4 ± 0.21[c]
Minimum temperature (°C)	17.0 ± 0.09[a]	16.2 ± 0.08[b]	17.9 ± 0.08[c]
Mean temperature (°C)	24.7 ± 0.11[a]	19.8 ± 0.06[b]	20.4 ± 0.05[c]
N	27	27	27
June 2004 experiment			
Maximum temperature (°C)	36.2 ± 0.39[a]	19.5 ± 0.64[b]	20.6 ± 0.78[b]
Minimum temperature (°C)	16.2 ± 0.14[a]	13.4 ± 0.33[b]	13.8 ± 0.58[b]
Mean temperature (°C)	23.3 ± 0.17[a]	15.8 ± 0.46[b]	16.2 ± 0.59[b]
N	54	54	54

* Values are the mean (± SE) except for the proportions (%) of occurrence for emergent plants and filamentous algae. The letters following the numerical values indicate the results of multiple comparison tests; the values with the same letter in a row were not statistically significant.

significantly shorter and wing lengths were significantly longer when the initial larval density was 20, compared with initial density of 60 and 100 (Table 3). Similarly, initial larval density significantly affected the development times of females ($F = 4.78$, $df = 2, 155$, $P < 0.01$) and males ($F = 28.51$, $df = 2, 153$, $P < 0.0001$) and the wing lengths of females ($F = 5.49$, $df = 2, 161$, $P < 0.01$) and males ($F = 5.45$, $df = 2, 146$, $P < 0.01$; Table 3).

When food was added to the habitats in the experiments conducted in June 2004, pupation rates in farmland habitats were highest, followed by swamp habitats and forest habitats ($\chi^2 = 13.92$, $df = 2$, $P < 0.001$; Table 3). Consistent with the findings for experiments made without adding food to larval habitats, pupation rates were similar within the same land cover type, regardless of the number of larvae being introduced (Table 3). The initial larval density and land cover type showed significant effects on the development times of females (initial density: $F = 22.13$, $df = 2, 269$, $P < 0.0001$; land: $F = 345.86$, $df = 2, 269$, $P < 0.0001$) and males (initial density: $F = 22.98$, $df = 2, 269$, $P < 0.0001$; land: $F = 309.29$, $df = 2, 254$, $P < 0.0001$). Mosquito larvae from farmland habitats developed into adults significantly faster than those from forest and swamp habitats. Further, female wing lengths were significant influenced by land cover type ($F = 4.45$, $df = 2, 246$, $P < 0.05$) and initial density ($F = 11.77$, $df = 2, 246$, $P < 0.0001$). However, male wing lengths were only significantly affected by initial larval density ($F = 3.72$, $df = 2, 226$, $P < 0.05$), but not by land cover type ($F = 2.76$, $df = 2, 226$, $P > 0.05$).

Compared with the treatment where food was not added, food supplementation to larval habitats significantly increased pupation rates in forest ($\chi^2 = 6.46$, $df = 1$, $P < 0.01$) and swamp habitats ($\chi^2 = 3.80$, $df = 1$, $P < 0.05$), but not in farmland habitats in the June 2004 experiment. Overall, addition of food significantly shortened development times of females ($F = 18.14$, $df = 1, 463$, $P < 0.0001$) and males ($F = 23.98$, $df = 1, 420$, $P < 0.0001$). Similarly, addition of food significantly increased the wing lengths of females ($F = 18.74$, $df = 1, 420$, $P < 0.0001$) and males ($F = 22.79$, $df = 1, 385$, $P < 0.0001$).

Association between pupation rate, development time, and environmental variables. Maximum, mean, and minimum water temperatures of farmland sites were significantly higher than in the other land covers of the semi-natural habitats during the experiments of September 2003 and June 2004 (Table 2). Multiple regression analysis showed that maximum water temperature was positively correlated with pupation rate ($\beta = 0.66$, $t = 4.60$, $P < 0.001$). Both female and male development times were negatively correlated with maximum (female: $\beta = -0.13$, $t = -2.17$, $P = 0.031$; male: $\beta = -0.16$, $t = -3.13$, $P < 0.001$) and minimum water temperature (female: $\beta = -0.40$, $t = -6.60$, $P < 0.001$; male: $\beta = -0.55$, $t = -10.86$, $P < 0.001$). Standardized beta coefficients indicated that minimum water temperature was relatively important for female and male development times. Female and male wing lengths were also negatively correlated with minimum water temperature (female: $\beta = -0.23$, $t = -3.47$, $P < 0.001$; male: $\beta = -0.25$, $t = -3.57$, $P < 0.001$).

DISCUSSION

This study demonstrated that productivity of malaria vectors was significantly higher in aquatic habitats located in farmlands compared with those in swamps and forests. Pupation rate was significantly greater and development time was shorter in habitats in farmlands compared with other land cover types. It has been suggested that warm water temperatures in open aquatic habitats increases productivity of malaria vectors.[9] We found that more malaria vector mosquitoes emerged from the sites with higher maximum temperature, less canopy cover, and fewer emergent plants. Furthermore, pupation rate was positively correlated with maximum water temperature, and larval development time was negatively correlated with maximum and minimum water temperatures. We found low densities of malaria vectors in the emergence traps.

TABLE 3

Mean ± SE pupation rates, development times, and wing lengths with three different larval densities in semi-natural aquatic habitats within farmlands, forests, and swamps*

Variable	Farmland			Forest			Swamp		
	20 larvae	60 larvae	100 larvae	20 larvae	60 larvae	100 larvae	20 larvae	60 larvae	100 larvae
Experiment in September 2003 without adding food to habitats									
Pupation rate	0.48 ± 0.08^{a}	0.46 ± 0.07^{a}	0.24 ± 0.04^{a}	0	0	0	0	0	0
Female development time (day)	11.16 ± 0.32^{a}	14.71 ± 0.23^{b}	16.46 ± 0.24^{c}	–	–	–	–	–	–
Male development time (day)	11.37 ± 0.46^{a}	14.77 ± 0.50^{b}	15.00 ± 0.81^{b}	–	–	–	–	–	–
Female wing length (mm)	2.86 ± 0.03^{a}	2.35 ± 0.02^{b}	2.35 ± 0.03^{b}	–	–	–	–	–	–
Male wing length (mm)	2.64 ± 0.06^{a}	2.28 ± 0.04^{b}	2.31 ± 0.03^{b}	–	–	–	–	–	–
Experiment in June 2004 without adding food to habitats									
Pupation rate	0.65 ± 0.03^{a}	0.49 ± 0.03^{b}	0.51 ± 0.08^{b}	0.02 ± 0.00	0.02 ± 0.1	0	0.33 ± 0.17^{a}	0.11 ± 0.03^{b}	0.06 ± 0.02^{b}
Female development time (day)	12.04 ± 0.28^{a}	14.78 ± 0.20^{b}	16.72 ± 0.25^{c}	20.11 ± 1.70	27.63 ± 2.82	–	19.50 ± 1.64^{a}	28.00 ± 1.53^{a}	23.38 ± 2.36^{a}
Male development time (day)	12.53 ± 0.47^{a}	14.97 ± 0.27^{b}	16.17 ± 0.22^{b}	–	–	–	19.40 ± 1.13^{a}	27.45 ± 1.96^{b}	26.57 ± 3.06^{b}
Female wing length (mm)	2.81 ± 0.03^{a}	2.66 ± 0.03^{b}	2.66 ± 0.03^{b}	2.60 ± 0.00	2.46 ± 0.00	–	2.73 ± 0.05^{a}	2.56 ± 0.05^{b}	2.59 ± 0.05^{b}
Male wing length (mm)	2.68 ± 0.04^{a}	2.48 ± 0.03^{b}	2.53 ± 0.02^{b}	–	–	–	2.67 ± 0.04^{a}	2.53 ± 0.06^{a}	2.60 ± 0.04^{a}
Experiment in June 2004 with adding food to habitats									
Pupation rate	0.70 ± 0.08^{a}	0.61 ± 0.05^{a}	0.62 ± 0.14^{a}	0.17 ± 0.17^{a}	0.23 ± 0.16^{a}	0.10 ± 0.03^{a}	0.43 ± 0.20^{a}	0.32 ± 0.10^{a}	0.24 ± 0.03^{a}
Female development time (day)	9.00 ± 0.16^{a}	11.49 ± 0.14^{b}	12.10 ± 0.17^{b}	18.42 ± 1.12^{a}	20.14 ± 0.91^{b}	24.15 ± 1.00^{b}	21.75 ± 1.75^{a}	19.33 ± 0.91^{b}	23.70 ± 1.14^{b}
Male development time (day)	8.86 ± 0.17^{a}	11.11 ± 0.16^{b}	12.33 ± 0.18^{b}	20.50 ± 0.89^{a}	16.95 ± 0.89^{b}	22.89 ± 1.35^{b}	17.08 ± 1.10^{a}	21.62 ± 1.10^{b}	23.24 ± 0.89^{b}
Female wing length (mm)	3.21 ± 0.03^{a}	2.82 ± 0.02^{b}	2.71 ± 0.03^{c}	2.86 ± 0.21^{a}	2.82 ± 0.05^{b}	2.70 ± 0.08^{b}	2.93 ± 0.07^{a}	2.78 ± 0.06^{b}	2.70 ± 0.03^{c}
Male wing length (mm)	2.96 ± 0.03^{a}	2.72 ± 0.03^{b}	2.58 ± 0.02^{c}	2.65 ± 0.05^{a}	2.77 ± 0.05^{b}	2.75 ± 0.15^{b}	2.71 ± 0.05^{a}	2.69 ± 0.09^{b}	2.59 ± 0.04^{b}

* Values with the same letter in a row were not statistically significant within the same land cover type.

This is expected because of low abundance of malaria vectors in the highlands.[33,34]

It is interesting to note that the emergence traps did not collect any *An. gambiae* s.l. adults in swamps and forests even though the mean water temperatures in these habitats were within the optimal temperature range indicated in previous studies.[19,20] Furthermore, the differences in minimum water temperature among the three land cover types were insignificant. These results suggest that factors other than water temperature also influence mosquito productivity. A previous study in the same area found that the sizes of swamps and aquatic habitats in forests are larger than those in farmlands.[10] Larger habitats are likely to remain in place long enough for predators to establish.[15–17] Thus, in natural conditions abundant predators in habitats in swamps and forests may reduce survivorship of *An. gambiae* s.l., and longer development time in these habitats increases the chance of larvae to be preyed upon.

Activities of farmers also influence habitat conditions in farmlands. During the short rainy season, emergent aquatic plants grow considerably and increase shade over aquatic habitats in farmlands, which make the habitats unsuitable for mosquitoes to breed. The most common aquatic habitats in farmlands are drainage ditches, and farmers clear vegetation and stagnant water from the ditches in February and March, at the end of the dry season. This activity enhances habitat conditions for the malaria vector by reducing shade and predators.

Our experiments suggest that two factors are important for the successful development of *An. gambiae* larvae in the highlands. The first factor is the land cover type of larval habitats. High canopy cover in forests and tall grass in swamps reduces the amount of solar radiation reaching larval habitats, which maintains lower water temperatures. In the laboratory, larvae of *An. gambiae* failed to pupate at constant temperatures below 16°C.[19,20] In the experiments conducted in June 2004 in semi-natural habitats without adding food, mean water temperature in the artificial habitats in forests and swamps was approximately 16°C, and a small proportion of larvae (2–33%) pupated although they took a much long time to develop into pupae. Conversely, in September 2003 when the average water temperature was higher than in June 2004 (19.6°C in forest habitats and 20.5°C in swamp habitats), no larvae successfully developed into pupae, When food was added to the habitats, pupation rates were substantially improved in June 2004. Therefore, land cover affects the temperature of larval habitats directly and food conditions and other factors indirectly, but the synergistic effects of these factors may be more significant to larval survivorship and adult mosquito productivity in highlands than individual factors.

The second factor is intraspecific competition. Overall, the larvae responded to increasing intraspecific competition by extending their development times and subsequently emerging as smaller adults. For example, within the same land cover types, mosquito larvae in habitats with a density of 20 larvae in 2.5 liters of water showed significantly faster larval-to-pupal development time and larger emerging adults than larvae in habitats with initial densities of 60 or 100 larvae in the same volume. Our results are consistent with the findings of Gimnig and others using artificial habitats in lowland areas of western Kenya.[18] In the current study, we found that adding

food to larval habitats increased pupation rate and adult body size and shortened development time in forest and swamp habitats. This suggests that food resources were limited in swamps and forests. Conversely, maize pollen may enhance food availability in farmland habitats. A study in Ethiopia found that larvae of *An. arabiensis* feed on pollen grains of maize, and that pollen enhances pupation success and adult body size and shortens development time.[35,36] Maize is the major crop in our study area. It is possible that maize cultivation in western Kenya highlands enhances *An. gambiae* productivity by improving nutrient condition of larval habitats.

The findings from this study support the hypothesis that recent land use changes in African highlands augment habitat conditions for malaria vectors.[7] Clearing forests and tall grass in swamps creates suitable habitats for malaria vectors to breed.[9,10] Such land modifications enhance vector production and subsequently increase the risk of malaria transmission to humans.[8,37,38] The information from this study improves our understanding of the effects of ongoing ecologic processes on malaria transmission and may be useful to develop new, effective malaria vector control programs in African highlands.

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Authors' addresses: Stephen Munga, Emmanuel Mushinzimana, and Andrew K. Githeko, Kenya Medical Research Institute, Kisumu, Kenya, E-mails: smunga@kisian.mimcom.net, emushinzimana@kisian.mimcom.net, and agitheko@kisian.mimcom.net. Noboru Minakawa, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan, E-mail: minakawa@post.saga-med.ac.jp. Guofa Zhou and Guiyun Yan, Department of Biological Sciences, State University of New York, Buffalo, NY 14260, E-mails: gzhou2@acsu.buffalo.edu and gyan@buffalo.edu. Okeyo-Owuor J. Barrack, School of Environmental Studies, Moi University, Eldoret, Kenya, E-mail: viredresearch@yahoo.com.

Reprint requests: Guiyun Yan, Department of Biological Sciences, State University of New York, Buffalo, NY 14260.

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