Population Dynamics of Malaria Vectors in Western Kenya Highlands

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ABSTRACT Studies were carried out at three sites in the highlands of western Kenya (Iguhu and Mbale in Kakamega and Vihiga districts, respectively, and Marani in Kisii district) and at one site in the western Kenya lowlands (Kombewa in Kisumu district) to determine the spatial–temporal dynamics of malaria vectors and intensity of malaria transmission from June 2003 to June 2004. At the highland sites, *Anopheles gambiae* Giles predominated, constituting >80% of the vector species, whereas *An. funestus* Giles made up <20%. In contrast, at the lowland site, *An. funestus* made up 68% of the vector species. The mean annual indoor resting densities of *An. gambiae* at Iguhu were 5.0 female mosquitoes per house per night, 14.2- and 26.3-fold greater than those at Mbale and Marani. During the main transmission season, the indoor resting densities of *An. gambiae* increased 4.1-, 10.1-, and 5.0-fold over the dry season period in Iguhu, Mbale, and Marani, respectively. The estimated annual entomological inoculation rate (EIR) at Iguhu was 16.6 infectious bites per person per year (ib/p/yr), 1.1 at Mbale, and 0.4 at Marani. This suggests high spatial variation in vector abundance and malaria transmission intensity. At the lowland site, Kombewa, the total annual EIR was 31.1 ib/p/yr and the indoor resting densities during the transmission season increased 7.1-fold in *An. funestus* and 18.5-fold in *An. gambiae* sensu lato over the dry season. The low level of transmission in the highlands suggests that it may be disrupted by vector control methods such as residual spraying.

KEY WORDS *Anopheles gambiae*, *Anopheles funestus*, highlands, population dynamics

SINCE THE 1920S MALARIA epidemics have been reported periodically in the highlands of East Africa (Garnham 1945, Fontaine et al. 1961). Analysis of clinical data in the East African highlands from the 1990s shows an increasing trend in malaria morbidity and mortality (Malakooti et al. 1998, Shanks et al. 2000, Kiszewski and Teklehaimanot 2004, Ndyomugyenyi and Magnussen 2004). In western Kenyan highlands, the frequency and intensity of malaria outbreaks have increased in the past 15 yr compared with the 1980s (Githeko and Ndegwa 2001, Zhou et al. 2004). Lindsay and Martens (1998) noted that the African highlands should be recognized as an area of special concern when considering the potential effect of climate change on malaria. Worrall et al. (2004) estimated that in Africa >12 million malaria episodes and 155,000–310,000 malaria deaths per year are attributable to epidemics if control options are not implemented or well timed.

Vector abundance is an important determinant of malaria transmission force (Garrett-Jones 1964) and thus factors that increase or decrease vector abundance will have an impact on prevalence of the disease. Intervention using antimalaria drugs has been used as the primary tool for malaria control; however, increasing drug resistance has weakened this strategic tool (Vreugdenhil et al. 2004, Whitty et al. 2004). Recent research on epidemic malaria in the Eastern African highlands has largely relied on hospital outpatient or inpatient time series data because of the paucity of vector dynamics data, and these studies have identified correlations between malaria incidence and climatic factors. For example, increased rainfall in southwestern Uganda during the 1997–1998 El Niño was positively correlated with vector abundance and malaria incidence (Lindblade et al. 1999). In Ethiopia, malaria epidemics in the highlands were positively associated with elevated minimum temperature (Abeku et al. 2003). Zhou et al. (2004) found that in some highland sites of East Africa, climate variability could explain a significant proportion of hospital malaria cases.

Little information is known about the dynamics of malaria vectors and transmission intensity in the highlands (Lindblade et al. 2000b). Altitude, topography, and land use may be among the most important environmental factors affecting malaria vector abun-

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dance in the highlands. Flat-bottomed valleys in the highlands, where water is likely to accumulate, were associated with a higher risk of malaria in Tanzania (Balls et al. 2004). In southwestern Uganda, malaria indices were higher near cultivated swamps (Lindblade et al. 2000a). In Tanzania, a change from 300 to 1,700 m in altitude was responsible for a >1,000-fold reduction in transmission intensity between the holoendemic lowland and the hypoendemic highland plateau (Bodker et al. 2003). These previous studies were largely based on one-time surveys of malaria vectors, and their spatial–temporal dynamics in the African highlands have not been examined. However, information on the spatial–temporal changes in vectorial systems, vector abundance, and species succession is critical for predicting malaria epidemics in the highlands, for planning effective transmission interventions, and for monitoring the efficacy of vector control measures. The aim of the study is to determine the seasonal and spatial variation in malaria vector species composition and abundance, and the intensity and stability of malaria transmission. This study was carried out in two areas, three sites in epidemic-prone western Kenya highlands and a holoendemic lowland site.

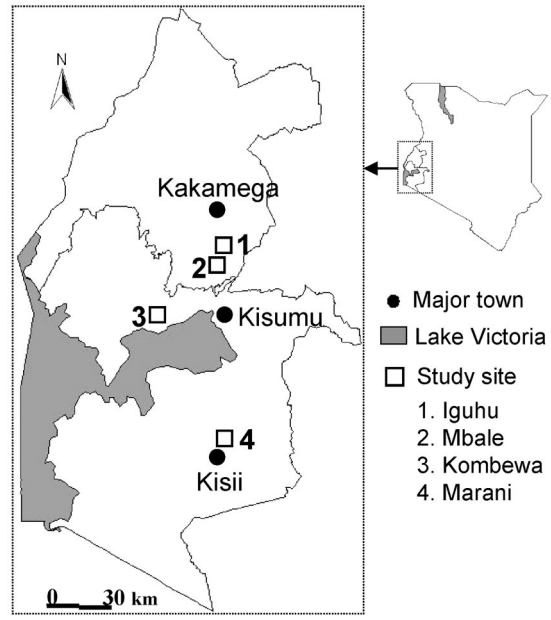

Fig. 1. Map of Kenya showing relative positions of study villages.

Materials and Methods

Study Sites. Indoor resting adult anopheline mosquitoes were collected in three villages in western Kenyan highlands (Fig. 1): Iguhu (1,430–1,580-m elevation) in Kakamega district, Mbale (1,520–1,570 m) in Vihiga district, and Marani (1,520–1,700 m) in Kisii district. A lowland site, Kombewa (1,170–1,300 m), in Kisumu district was used as a reference site for malaria vector abundance and transmission intensity. Each of the three highland sites is characterized by a valley and surrounding hills. The valley in Iguhu is flat-bottomed, prone to flooding, and many of the natural swamps have been reclaimed for agriculture. The Mbale valley has been reforested with *Eucalyptus* trees. The Marani site is in a steep valley where the Marani River flows through a shallow gorge on the western side, and river banks are forested with *Eucalyptus* trees. Maize and tea are the major crops grown in the area. Kombewa lies in the semiarid lowlands with poor drainage and semipermanent swampy streams. No vector control activities exist in any of the study sites, and insecticide-impregnated bed net use is below 10% (A.G., unpublished data). During the period of June 2003 to June 2004, the climate in Iguhu and Mbale was characterized by an average monthly maximum temperature of 27.5°C and a minimum temperature of 15.1°C, with an average monthly rainfall of 157.3 mm. Marani was slightly cooler (average monthly maximum temperature of 26.5°C and minimum temperature of 14.5°C) and wetter (average monthly rainfall of 221.4 mm). Kombewa was warmer (average monthly maximum temperature of 29.1°C and minimum of 18.4°C) and dryer (average monthly rainfall 120.7 mm) compared with the highland sites. Monthly rainfall and maximum temperature in the highland sites varied considerably. Generally, maximum temperatures were highest between January and March and lowest between May and July.

Mosquito Sampling. Thirty houses were randomly selected at each site except in Mbale where 10 houses were sampled. House coordinates and elevation were recorded using a global positioning system in differential mode (Hightower et al. 1998). Indoor resting mosquitoes were collected monthly using the pyrethrum spray collection method (WHO 1975) from June 2003 to June 2004. All monthly surveys were conducted in the same houses. Female anopheline mosquitoes collected were classed microscopically as unfed, blood fed, half gravid, and gravid (Gillies and Coetzee 1987). The number of sleepers in these houses was recorded during the surveys.

Species Identification. Individual specimens of the *An. gambiae* complex from three highland sites were identified to species using the rDNA-polymerase chain reaction (PCR) method (Scott et al. 1993) (n = 254 in Iguhu, 50 in Mbale, and 97 in Marani). *An. gambiae* sensu lato (s.l.) specimens from Kombewa were not tested. Species within the *An. funestus* species complex were identified individually using the method developed by Koekemoer et al. (2002) (n = 293 in Kombewa and 120 in Iguhu). *An. funestus* s.l. specimens from Mbale and Marani were not analyzed because insufficient sample size.

***Plasmodium falciparum* Sporozoite Rates and Entomological Inoculation Rate (EIR).** The head and thorax of each female *Anopheles* mosquito were separated from the abdomen, individually placed in a 1.5-ml Eppendorf microcentrifuge tube, processed for circumsporozoite (CS) protein, and then tested for presence of *P. falciparum* CS antigens (Wirtz et al.

1987, Beier et al. 1990). A total of 2,666 *An. gambiae* s.l. and 2,306 *An. funestus* mosquitoes were assayed.

Data Analyses. The average abundance of anopheline mosquitoes in a house was computed from June 2003 to June 2004 for each site. The among-site variation in vector abundance was analyzed using the analysis of variance (ANOVA) with repeated measures for each species. The Tukey–Kramer honestly significant difference (HSD) tests were used to compare the mean densities of *An. gambiae* s.l. and *An. funestus* among the four sites. The proportion of houses that contained one or more anopheline mosquitoes was calculated. The among-site variation in the average proportion of mosquito-positive houses was tested using ANOVA with repeated measures, followed by Tukey–Kramer HSD tests for posteriori means comparisons. The proportion of mosquito-positive houses was arcsine transformed.

The EIR, a standard measure of transmission intensity, was expressed as the number of infective bites per person unit time (e.g., daily or yearly). Annual EIR for each site and each species was calculated as the average number of mosquitoes per house per survey × sporozoite rate × proportion of blood-fed mosquitoes × 365 d/average number of persons who had slept in the house the night before the collections were made. Here, we assume that all blood-fed mosquitoes have fed on humans. *An. gambiae* and *An. funestus* in western Kenya have human blood indices close to 100% (Githeko et al. 1994). The total annual EIR for each site was calculated as the sum of EIRs contributed by *An. gambiae* and *An. funestus.*

Results

Vector Species Abundance. During the study period (June 2003–June 2004), a total of 3,331 *An. gambiae* s.l. and 3,161 *An. funestus* s.l. mosquitoes were caught in the four study sites. *An. gambiae* s.l. was the predominant species at the three highland sites, whereas *An. funestus* was more common at the lowland site, Kombewa. *Anopheles gambiae* s.l. composed 82.3, 83.3, and 79.1% of the malaria vectors in the highlands at Iguhu, Mbale, and Marani, and only 31.8% in Kombewa. *An. funestus* was the minor species in the highland sites (17.7, 16.7, and 20.9% at Mbale, Iguhu, and Marani, respectively). However, it was the predominant malaria vector in the lowland site (68.2%). PCR analysis indicated that 99.2% (252 of 254 specimens within *An. gambiae* s.l.) were *An. gambiae* Giles (referred to as *An. gambiae* hereafter) at Iguhu and 100% at Mbale and Marani. All *An. funestus* s.l. specimens subjected to species identity tests from Iguhu and Kombewa were *An. funestus* Giles.

Overall, the indoor resting densities of *An. gambiae* and *An. funestus* varied significantly among the sites ($F_{3, 48} = 5.57$; $P < 0.01$ for *An. gambiae;* $F_{3, 48} = 15.00$; $P < 0.01$ for *An. funestus*). At the highland sites, the mean indoor resting density during the study period for *An. gambiae* was highest in Iguhu and lowest in Marani (Table 1). Kombewa in the lowlands had lower density of *An. gambiae* s.l. than that in Iguhu, but a significantly higher density than Marani or Mbale (Table 1). However, the mean indoor resting density for *An. funestus* was highest in Kombewa, followed by Iguhu with close to one female vector per house. Marani and Mbale showed the lowest vector abundance (Table 1).

Dynamics of Indoor Resting Anopheline Densities. Densities of *An. gambiae* peaked in May to June in the study sites (Fig. 2). These peaks were reached generally ≈1 mo after the onset of the long rainy season. At Iguhu, *An. gambiae* density increased by ≈12-fold from the end of dry season to peak transmission season in May 2004 (Fig. 2A). Mbale showed a similar trend to Iguhu, although vector abundance was consistently lower than in Iguhu (Fig. 2B). At Marani, mosquito abundance was consistently low for both *An. gambiae* and *An. funestus* (Fig. 2C). With *An. funestus,* changes in indoor resting densities were remarkable only in Kombewa (Fig. 2D); they remained fairly unchanged in the highland sites.

Another indicator of vector abundance is the proportion of houses positive for anopheline mosquitoes. At Iguhu and Kombewa, *An. gambiae* was persistent throughout the study period, with >80% of the houses positive for vectors during the long rainy season (Fig. 3). In Mbale and Marani, *An. gambiae* was absent during half of the study period and was present in <20% of the houses in these two villages in most months. There were significant among-site variations in the average proportion of houses with malaria vectors ($F_{3, 48} = 11.48$; $P < 0.0001$ for *An. gambiae;* $F_{3, 48} = 63.68$; $P < 0.0001$ for *An. funestus;* Table 1). The proportion of houses with *An. gambiae* presence was highest in Iguhu and lowest in Mbale. *Anopheles funestus* was most frequently found in Kombewa, the lowland site, but least frequently in Marani (Fig. 3).

Table 1. Average number of *An. gambiae* and *An. funestus* female mosquitoes per house per survey and proportion of houses with either species in western Kenya, June 2003–June 2004

Trait	Species	Iguhu	Mbale	Marani	Kombewa
Mean density	*An. gambiae*	5.00 (2.42–7.59)^a	0.35 (0.05–0.66)^b	0.19 (−0.04–0.42)^b	3.24 (0.40–6.07)^c
	An. funestus	0.86 (0.42–1.30)^a	0.05 (0.01–0.10)^b	0.04 (0.01–0.06)^b	6.96 (3.54–10.38)^c
Mean anopheline-positive houses (%)	*An. gambiae*	67.9 (54.5–81.5)^a	22.5 (5.5–39.5)^b	10.3 (0–21.1)^b	41.7 (26.2–57.2)^c
	An. funestus	34.7 (22.7–46.7)^a	6.7 (1.8–11.5)^b	2.3 (0.6–4.0)^b	71.6 (65.3–77.9)^c

Numbers in parentheses are 95% confidence intervals.

Letters following numbers indicate the results of Tukey–Kramer honestly significant difference tests. Values with the same letter in a row were not statistically significant at $P < 0.05$.

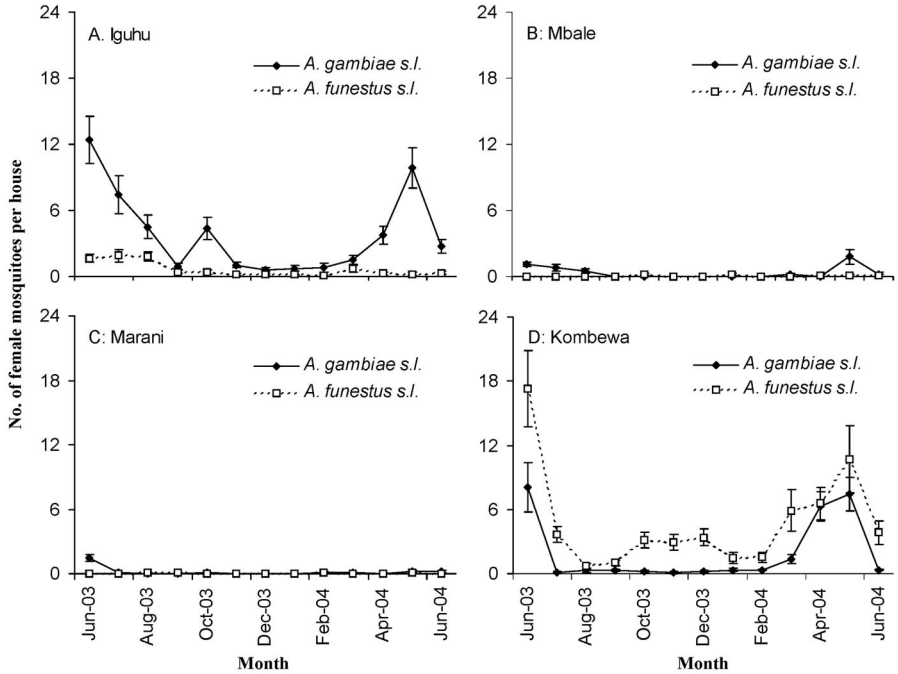

Fig. 2. Mean numbers and standard errors of *An. gambiae* and *An. funestus* per house in three western Kenya highland sites (Iguhu [A], Mbale [B], and Marani [C]), and a lowland site (Kombewa [D]).

Sporozoite Rates and Entomological Inoculation Rates. The mean sporozoite rates of *An. gambiae* from Iguhu, Mbale, Marani, and Kombewa were 3.6, 4.0, 1.5, and 3.6%, respectively (Table 2). For *An. funestus*, mean sporozoite rates were 5.2, 0.0, 5.6, and 3.9% for Iguhu, Mbale, Marani, and Kombewa, respectively. At Iguhu, annual *P. falciparum* EIR by *An. gambiae* was 13.1 infectious bites/person/yr (ib/p/yr) and 3.5 ib/p/yr from *An. funestus.* At Mbale, EIR by *An. gambiae* was 1.1 ib/p/yr and 0 from *An. funestus.* The EIR at Marani was 0.2 ib/p/yr and 0.2 from *An. gambiae* and *An. funestus*, respectively. In the lowland site, Kombewa, EIR was 10.1 ib/p/yr from *An. gambiae* and 21.0 from *An. funestus.* The combined EIR for both species were 16.6, 1.1, 0.4, and 31.1 ib/p/yr at Iguhu, Mbale, Marani, and Kombewa, respectively (Table 2).

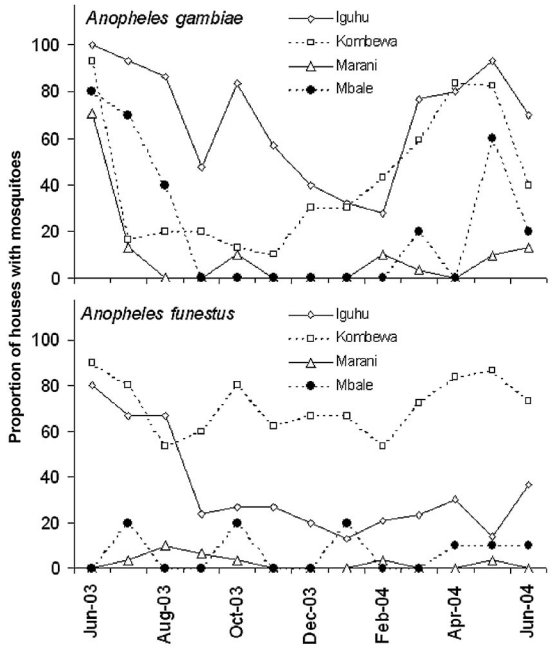

Fig. 3. Monthly proportions of *An. gambiae*- and *An. funestus*-positive houses in three western Kenya highland sites (Iguhu, Mbale, and Marani) and a lowland site (Kombewa).

Discussion

Results showed that during the study period *An. gambiae* made up >80% of the vector population in three highlands sites in western Kenya, whereas *An. funestus* predominated in the lowland site. Mean indoor resting densities within the highland sites varied by a factor of 12.7 during the long rainy season (June 2003) and by a factor of 23.5 during the dry seasons (January–March) at two highland sites of similar elevation—Iguhu and Mbale. Mean indoor resting density varied by 10.0-fold between Iguhu and Marani during peak seasons. Similarly, although 68% of the houses in Iguhu harbored the major vector *An. gambiae*, only 10% of the houses in Marani were vector-ridden. During the main transmission season, indoor vector resting densities in the three highland sites increased by 9.6- to 19.0-fold, but they remained low

Table 2. Mean sporozoite rates and annual EIR in western Kenya highlands and lowland, June 2003–June 2004

Site	Species	Mean no. of sleepers/ house	Total no. of mosquitoes collected	No. mosquitoes tested by ELISA	No. sporozoite-positive mosquitoes	Sporozoite rate (%)	% mosquitoes bloodfed	Annual EIR by species	Total EIR by site
Iguhu	*An. gambiae*	2.6	1,951	1,595	57	3.6	52.3	13.1	16.6
	An. funestus		420	420	22	5.2	44.4	3.5	
Mbale	*An. gambiae*	3.1	50	50	2	4.0	62.0	1.1	1.1
	An. funestus		10	10	0	0.0	10.0	0.0	
Marani	*An. gambiae*	2.8	68	68	1	1.5	64.7	0.2	0.4
	An. funestus		18	18	1	5.6	44.4	0.2	
Kombewa	*An. gambiae*	2.3	1,262	953	34	3.6	55.3	10.1	31.1
	An. funestus		2,713	1,858	73	3.9	48.6	21.0	

in other seasons. At the lowland site, indoor resting densities during the transmission season increased 5.2-fold for *An. funestus* and 13.0-fold for *An. gambiae* s.l. over the dry season, which indicates a rapid vector population increase during rainy seasons. The total annual EIRs at Iguhu (16.6 ib/p/yr) were lower than those at the lowland site (31.1 ib/p/yr) but were 15- to 41-fold higher than the other two highland sites, Mbale and Marani. Therefore, these results suggest that there are large spatial and temporal variations in malaria vector abundance and transmission intensity in western Kenya highlands.

The vectorial system in the western Kenyan highlands is dominated by *An. gambiae*, whereas *An. funestus* plays a minor role in transmission. These results are consistent with those reported by Shililu et al. (1998) that *An. gambiae* comprised 84% of the vector population in Mumias, western Kenya at an altitude of 1,500 m above sea level. Minakawa et al. (2002) observed that *An. gambiae* was the only member of the *An. gambiae* complex collected >1,400 m above sea level. However, in the lowland site, Kombewa, studied here, *An. funestus* was the predominant malaria vector species. Vector species distribution in the lowlands is spatially heterogeneous and dynamic. For example, in Suba district, western Kenya, *An. gambiae* constituted 67.4% of anophelines (Shililu et al. 2003). In other lowland sites in western Kenya (Kisian and Saradidi), >85% of human malaria vectors collected from human landing collections were *An. gambiae* s.l. (Beier et al. 1990), whereas a previous study found that samples of *An. gambiae* s.l. collected in areas close to Kisian consisted of 75.3% *An. gambiae* (Joshi et al. 1975). A survey performed in October–November 1988 at Kombewa with the pyrethrum spray collection method found that *An. gambiae* was the major malaria vector (Githeko et al. 1996). The current study found that the major malaria vector in Kombewa during 2003–2004 was *An. funestus*. The change in malaria vector species composition in Kombewa may be because of larval habitat changes in this area. We observed numerous swamps in the area where *An. funestus* tend to thrive (White 1972) but which are not very suitable for *An. gambiae* larval development (Minakawa et al. 1999). In addition, interannual variation in rainfall also could contribute to reduced abundance of *An. gambiae* in Kombewa. Reduced *An. gambiae* abundance likely contributed to a lower EIR estimate for this site compared with other malaria endemic lowland sites in western Kenya reported in the literature (Beier et al. 1990, Githeko et al. 1993).

Several factors may have contributed to the large spatial variation in the abundance of malaria vectors at the three highland sites. Temperature difference among the sites may explain some difference in vector densities between Kakamega sites (Iguhu and Mbale) and Kisii site (Marani). That is, Marani exhibits lower temperatures than Iguhu and Mbale; lower temperatures are known to delay anopheline larval development and increase larval mortality (Bayoh and Lindsay 2003, Tuno et al. 2005). However, the observed climate differences among the sites cannot entirely explain the magnitude of differences observed in vector densities. For example, Iguhu and Mbale have similar climates, but the malaria vector density in Iguhu is 14-fold higher than Mbale. The large among-site differences in vector abundance may be partially related to the characteristics of the valleys, which contain most of the vector larval habitats. The valley at Iguhu is broad and contains several reclaimed swamps which create stable larval mosquito habitats. In the Usambara Mountains, Tanzania, water accumulation in valley bottoms was associated with an increased risk of malaria transmission (Balls et al. 2004). At Mbale, the valley has been reforested and land cover has increased. *An. gambiae* immature stages survive poorly in forested areas because of lack of direct sunlight (Tuno et al. 2005). At Marani, water drainage is very efficient leading to unstable larval habitats.

Our data indicate that the risk of malaria transmission is dramatically different among sites in the western Kenyan highlands. Although the malaria transmission intensity in Iguhu is high, it is much lower than in the adjacent Lake Victoria basin region. For example, Beier et al. (1990) reported total annual EIRs varying between 237 and 299 in two lowland sites, Saradidi and Kisian, in the Lake Victoria Basin region. Githeko et al. (1993) observed EIRs of 416 and 91 in two other lowland sites in western Kenya, Ahero and Miwani, respectively. Vector control in the highland region—where malaria transmission intensity is low—may yield a significant reduction in malaria morbidity. Beier et al. (1999) suggest that a substantial reduction in malaria is only likely when EIRs are less that one infective bite per person per year. In the highlands

where transmission is relatively low, malaria transmission could be successfully interrupted by a control method such as indoor residual spraying. Fontaine et al. (1978) suggested that in areas of low malaria transmission, even a single adult vector control method could achieve a high impact on disease incidence. Indoor residual spraying and insecticide-impregnated bed nets have been shown to reduce EIR by >90% (Fontaine et al. 1978, Gimnig et al. 2003). In western Kenyan lowlands, however, EIRs of 100 or more have been reported. This suggests that multiple vector control strategies, such as indoor residual spraying, insecticide-impregnated bed nets, mosquito larval control, and larval habitat source reduction (Killeen et al. 2002, Shililu et al. 2003), are likely needed to interrupt malaria transmission to significantly reduce malaria morbidity and mortality.

We conclude that large among-site variation exists in the abundance and temporal dynamics of malaria vector populations in the western Kenyan highlands, where the magnitude of malaria transmission intensity varies from modest to very low. The relatively low level of transmission in the highlands suggests that malaria transmission may be disrupted by malaria vector control measures.

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