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Low larval vector survival explains unstable malaria in the western Kenya highlands

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Summary Several highland areas in eastern Africa have recently suffered from serious malaria epidemics. Some models predict that, in the short term, these areas will experience more epidemics as a result of global warming. However, the various processes underlying these changes are poorly understood. We therefore investigated malaria prevalence, malaria vector densities and malaria vector survival in a highland area in western Kenya, ranging from approximately 1550–1650 m altitude. Although only five adult malaria vectors were collected during 180 light traps and 180 resting collections over a 23-month study period, malaria was prevalent among school children (average parasite prevalence: 10%). During an extensive survey of potential larval habitats, we identified only seven habitats containing *Anopheles gambiae* Giles s.l. larvae. Their limited number and low larval densities suggested that their contribution to the adult vector population was small. Experiments on adult and larval survival showed that at this altitude, adult mosquitoes survived inside local houses, but that larval development was severely retarded: only 2 of 500 *A. gambiae* s.l. larvae developed to the pupal stage, whereas all other larvae died prior to pupation. At present, high vector densities are unlikely because of unfavourable abiotic conditions in the area. However, temporary favourable conditions, such as during El Niño years, may increase larval vector survival and may lead to malaria epidemics.

keywords malaria, highlands, *Anopheles gambiae*, *Anopheles arabiensis*, larvae, Kenya

Introduction

During the past decade, several highland areas in eastern Africa suffered from serious malaria epidemics (Malakooti *et al.* 1998; Lindblade *et al.* 1999; Etchegorry *et al.* 2001). Climatic events such as El Niño, climate variability, drug resistance and land-use changes have been identified as major causes of this phenomenon, but their relative contribution remains debatable (Mouchet *et al.* 1998; Hay *et al.* 2002a,b; Patz *et al.* 2002; Zhou *et al.* 2004). Nonetheless, it is expected that in the future relatively subtle changes in climate may have significant effects on the occurrence of malaria epidemics (Lindsay & Martens 1998; Zhou *et al.* 2004).

Both empirical and process-based models have been used to map the distribution of malaria vectors and predict malaria transmission intensity across the African continent (Snow *et al.* 1999; Rogers *et al.* 2002; Smith *et al.* 2004). These models rely on field data that describe the various interactions between the parasite, mosquito and human host. Whereas these data can be readily collected from highly endemic malaria areas, where malaria is stable, data collection from areas that only rarely experience malaria transmission, such as highland areas, is still lagging behind. This discrepancy is most likely because of the comparatively large effort that is needed to collect such data, and as a consequence detailed information about malaria in epidemic situations is rare. Prior studies of malaria epidemics in the east African highlands have therefore mostly used health centre or hospital records of clinical malaria (Malakooti *et al.* 1998; Lindblade *et al.* 1999; Shanks *et al.* 2000, 2002).

In the present study, we investigated malaria prevalence, malaria vector densities of both adult and immature stages and malaria vector survival in a highland area in western Kenya. The objective of the study was to understand malaria transmission dynamics in an area that may be at an increased risk of malaria epidemics as a result of environmental changes.

Materials and methods

Study area

The study was conducted in Fort Ternan (0°12′ S, 35°21′ E), a rural village located on the slopes of the Nandi hills in western Kenya (Figure 1). The area ranged approximately from 1550 m, a river basin, to 1650 m altitude. Temperatures in the area can reach as low as 11 °C during the night and as high as 32 °C during the day with little variation during the year. Rainfall in these elevated areas is higher than that in the adjacent lowlands: 1500–1800 mm/year in the highlands when compared with approximately 1150 mm in the lowlands (White 1972; http://www.worldclimate.com). The majority of people in this area belong to the Kalenjin tribe. Subsistence agriculture and livestock holding are the main economic activities.

Malaria vector collections

From October 1999 to August 2001, adult mosquito collections were performed monthly in 10 selected houses in Fort Ternan. All houses consisted of mud walls and a thatched roof. In the course of the study, each house was replaced by a new one. The occupants did not use bednets. Collections consisted of light trap catches and resting catches. A CDC-light trap (Model 1012; John W. Hock Company, Gainesville, FL, USA) was installed in one house at the foot end of a bed (Mboera *et al.* 1998) and operated from 6 PM to 6 AM. During the night, occupants of the house slept under a bednet provided by the investigators. Family members who could not sleep under a net spent the night elsewhere during the collections. Resting catches were made by two trained collectors using a torch and an aspirator. Each house was examined for 20 min between 7.30 AM and 10 AM. All mosquitoes found were aspirated and transferred to paper cups. Light trap and resting catches were alternated in a house on two consecutive days. Collected mosquitoes were identified to species level (Gillies & Coetzee 1987) and female *Anopheles* mosquitoes were stored individually in labelled microcentrifuge tubes with silica gel for desiccation. Of the *Anopheles gambiae* s.l. females, a leg or small part of the abdomen was used to extract DNA (Collins *et al.* 1987). This was used to determine the sibling species of the *A. gambiae* complex by means of rDNA-PCR (Scott *et al.* 1993). The head and thorax of the female anophelines were used to test for the presence of circumsporozoite protein of *Plasmodium falciparum* by means of the enzyme linked immunosorbent assay (Burkot *et al.* 1984; Beier *et al.* 1987). Specimens were considered sporozoite positive if the absorbance was higher than twice the average absorbance of the negative controls (Beier *et al.* 1988).

Malaria prevalence study

From October 1999 to August 2001, thin and thick blood smears were taken from school children by finger prick. Children aged between 5 and 10 years were enrolled in the

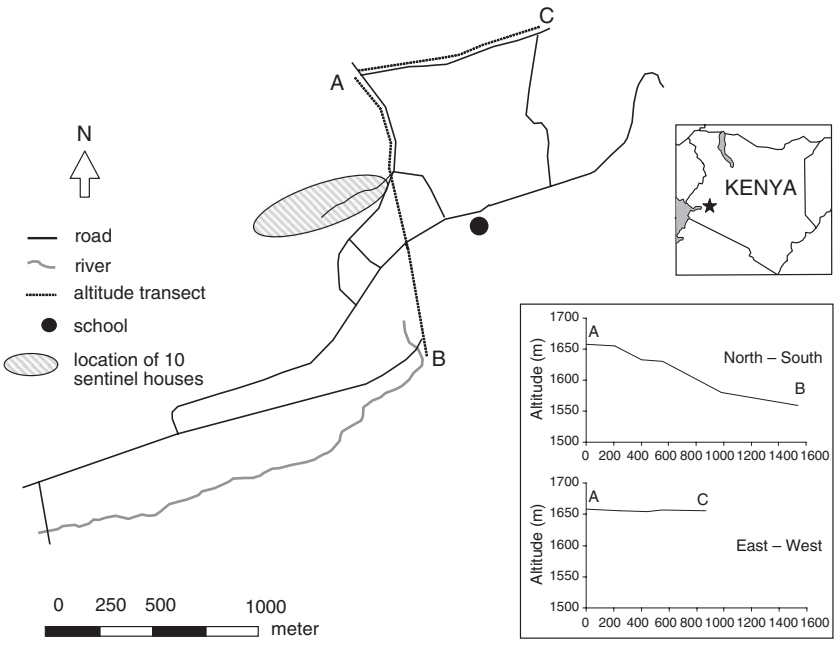

Figure 1 Map of Fort Ternan. The insets show the relative location of the study village in Kenya and the altitude differences on two transects in the area (A–B and A–C). Only the part of the river, which was included in the larval habitat study, is shown on the map.

study and followed monthly. The school was located near the river basin approximately 1 km southeast of the selected houses. Body temperature of the children was measured with a Braun ThermoScan® (Frankfurt, Germany) ear thermometer. Blood smears were stained with Giemsa and thick smears were checked microscopically for the presence of malaria parasites. Children were treated with Fansidar® (sulphadoxine–pyrimethamine) according to prescription guidelines, if they were found *P. falciparum* positive and had a body temperature ≥37.5 °C. In March 2000 and May 2001, the oldest children were excluded from the study and a new group of children were enrolled in such a way that the age cohort remained constant. Monthly malaria prevalence was calculated as the proportion of *P. falciparum*-infected children of the total number of investigated children.

Larval habitat survey

As a result of the extremely low adult vector densities, a larval habitat survey was conducted from August to November 2000 to assess whether actual breeding of vectors took place in the area. During an initial survey, an area of approximately 4 km^2 within Fort Ternan was intensively investigated for the presence of anopheline larval habitats that were either the result of recent rainfall or the result of human activity (cattle drinking places, leaking taps). Many potential habitats were identified with assistance from local villagers. On two occasions, the river bed was inspected for the presence of habitats. All potential habitats were sampled for the presence of larvae. The size of the water body was measured and 10% of the water surface was sampled by calculating the number of dips needed as follows:

$$N = (S/10)/D \quad (1)$$

where N is the number of dips, S the surface of habitat (m^2) and D the surface of dipper (m^2).

Sampling was carried out using a standard white dipper by gently submerging the ladle till just below the water–air interface (Service 1993). All sites found were visited at least three times. The collected anopheline larvae were transferred into plastic bottles filled with habitat water, reared to fourth instar larvae and stored in labelled 1.5 ml microcentrifuge tubes filled with silica gel for desiccation or with alcohol–glycerol fixative. *Anopheles gambiae* s.l. larvae were further identified for species, as described for the adults.

Fourteen habitats from this initial survey were selected for further investigations. Seven of these habitats were located on or along a road ('road habitats') and the other seven were found in the immediate vicinity of taps used for supply of water to village households and cattle ('tap habitats'). Road habitats were fed by rainwater, while tap habitats were mostly the result of leakage of tap water or of temporary storage of tap water for cattle or domestic purposes. Tap water was pumped from a nearby river. Larval collections, storage and identification were performed as described earlier. On a few occasions, when the water surface area of the water body exceeded 10 m^2, only 5% (instead of 10%) of the water surface was sampled. In this case, the number of collected larvae was multiplied by 2 in order to enable comparison with the other sampled sites. The larvae were transferred, identified and stored as described earlier. All 14 habitats were visited at 2-week intervals between 3 August 2000 and 26 September 2000 and once during October and November 2000.

Adult survival experiment

To complement the adult and larval field surveys, experimental studies on the survival of the adult and larval stages under the environmental conditions of Fort Ternan were carried out in June 2001. For the adult survival experiment, 3 of the 10 sentinel houses (hereafter referred to as house A, B and C) were selected, which were also involved in the malaria vector studies. Householders did not cook indoors to prevent smoke from killing experimental mosquitoes. We prevented any form of mosquito repellent by supplying bednets to the house owners. In each house, two gauze cages (20 × 20 × 20 cm) containing 1- to 2-day-old adult female mosquitoes were placed: one with 50 *A. gambiae* s.s. females and the other with 50 *Anopheles arabiensis* females. The mosquitoes originated from strains that were set up in April 2001 with blood-fed females collected in the vicinity of the Kenya Medical Research Institute, Kisian, western Kenya. During the experiment, mosquitoes were provided a 6% sugar solution through a drenched piece of cotton wool (10 × 10 cm) placed on the top of the cage. The sugar source was refreshed every other day. The cages were placed on a small table, the legs of which were treated with Vaseline® to prevent ants from disturbing the experiment. Tables were placed in a corner of the living room, opposite to the entrance. The number of dead females was recorded daily, and these were removed and stored in microcentrifuge tubes with silica gel (separated by day, village, house and species). From the females, the length of one of the wings was measured from the axillary incision to the tip (excluding the fringe scales) using an eyepiece micrometer (Lyimo *et al.* 1992).

Larval survival experiment

Twenty batches of 50 newly hatched larvae (0–4 h) of *A. gambiae* s.s. and *A. arabiensis* were collected from the

same strains as used in the adult survival experiment, described earlier. These batches were kept separately in 'transport cups' (7 cm Ø; 10 cm high) filled with 1–2 cm river water. On arrival in Fort Ternan, 10 experimental plastic trays (13.5 cm Ø; 5.5 cm high) were filled with 573 ml river water (depth 4.0 cm). Five trays received 50 *A. gambiae* s.s. larvae and five trays received 50 *A. arabiensis* larvae. The trays were placed on a table, the legs of which were treated with Vaseline to prevent ants from disturbing the experiment. This table was placed in the shade just outside a study house between 7 AM and 7 PM and placed indoors between 7 PM and 7 AM. During periods of rain in day time, the trays were also placed indoors. Trays were covered with a gauze frame. Twenty milligrams of Tetramin® fish food was added daily and the water level was maintained at 4.0 cm. The remaining five *A. gambiae* s.s. and five *A. arabiensis* batches were placed in a lowland village (Miwani, 1211 m altitude; see also Koenraadt *et al.* 2004) as a control and were treated similarly as the larvae placed in Fort Ternan. The experiment was executed in August 2001. In Fort Ternan, the experiment was followed up until all larvae had either died or pupated. In Miwani, the experiment was followed up until day 13.

Meteorological data

One of the three houses selected for the adult survival experiment was equipped with a small meteorological station that measured temperature, relative humidity and rainfall (THERMO-HYGRO, Conrad Electronics, Enschede, The Netherlands; temperature resolution 0.1 °C, accuracy relative humidity 1% (25–95%), rainfall resolution 0.2 mm). In western Kenya, most rainfalls are between March and May, and usually there is a smaller peak of rainfall in November/December. Meteorological data for the months in which the adult and larval survival experiments were carried out (June, July and August 2001) are presented. Temperature data were compared with a biological threshold of 16.0 °C below which the development of the aquatic stages of *A. gambiae* s.l. and *P. falciparum* inside the mosquito host do not occur (Macdonald 1957).

Statistical analysis

Chi-square tests were performed to compare road and tap habitats from the larval survey. Results of the adult survival experiment were analysed by means of the Cox regression analysis (Cox 1972). The relationship between female size and survival time was tested with Spearman's rank correlation coefficients. Differences in time to pupation from the larval survival experiment were evaluated using the log-rank statistic resulting from the Kaplan–Meier survival procedure (Kleinbaum 1996). All analyses were carried out using SPSS v.11.0 software (SPSS Inc., Chicago, IL, USA).

Informed consent and ethical clearance

Informed consent was obtained from house owners participating in the malaria vector collections and from parents/guardians of children participating in the malaria prevalence study. Ethical clearance was obtained from the Ethical Review Committee of the Kenya Medical Research Institute (SSC PROT. NO. 512).

Results

Malaria prevalence and adult malaria vectors

Malaria, exclusively caused by *P. falciparum*, was prevalent among school children year-round, while malaria vectors were collected in extremely low numbers (Table 1). The malaria prevalence ranged from 0% to 16.9%, with low prevalence in January–March, and the highest prevalence in June–August. During the 18 sampling occasions for adult mosquitoes, spread over a 23-month period, only five malaria vectors were collected. One specimen was *Anopheles funestus*, four were *A. gambiae* s.l. Although only two specimens of *A. gambiae* s.l. could be identified as sibling species, the PCR result indicated that both *A. arabiensis* and *A. gambiae* s.s. occur at this altitude. One *A. gambiae* s.s. contained *P. falciparum* sporozoites.

Of the 81 observed *P. falciparum* infections in school children throughout the study period, at least 15 infections were of children who were also infected on the previous and/or next sampling occasion. Nineteen infected children were *P. falciparum* negative on both the previous and next surveillance occasions. The remainder of the infections could not be tracked because of child absence on the previous/next sampling occasion (35 infections), child taken out of study on the next sampling occasion (1 infection) or incompleteness of data for children sampled on the first and last occasion (11 infections). During the study period, nine infected children (11%) had a body temperature ≥37.5 °C and were treated with Fansidar. All cases with fever were observed during the first year of study.

Larval habitat survey

During the study, 28 potential larval habitats were identified in the 4 km^2 area. Sixteen of them (57%) were tap habitats, while the remaining 12 habitats (43%) were found on or along a road or footpath. In total, 17 habitats

Table 1 Results of the malaria vector collections from 10 sentinel houses and the malaria prevalence study among school children aged 5–10 years. Children were treated with sulphadoxine–pyrimethamine if they had malaria parasites and a body temperature ≥37.5 °C

	Adult mosquitoes collected				Malaria prevalence		
	Resting collections		Light traps				
Month	*Anopheles gambiae* s.l.	*Anopheles funestus*	*Anopheles gambiae* s.l.	*Anopheles funestus*	No. of children tested	Percentage infected (95% CI)	No. of children treated
October 1999	0	0	0	0	95	6.3 (1.4–11.2)	2
November 1999	0	0	0	0	81	4.9 (0.2–9.7)	0
December 1999	–	–	–	–	–	–	–
January 2000	0	1	0	0	82	0.0 (0.0–0.0)	0
February 2000	0	0	0	0	83	1.2 (−1.1–3.6)	0
March 2000	1	0	1	0	75	1.3 (−1.3–3.9)	0
April 2000	0	0	0	0	68	11.8 (4.1–19.4)	0
May 2000	0	0	0	0	46	4.3 (−1.5–10.2)	2
June 2000	0	0	0	0	71	16.9 (8.2–25.6)	4
July 2000	0	0	0	0	66	15.2 (6.5–23.8)	1
August 2000	0	0	0	0	47	2.1 (−2.0–6.3)	0
September 2000	0	0	0	0	80	5.0 (0.2–9.8)	0
October 2000	–	–	–	–	–	–	–
November 2000	0	0	0	0	56	5.4 (−0.5–11.3)	0
December 2000	–	–	–	–	–	–	–
January 2001	–	–	–	–	–	–	–
February 2001	0	0	0	0	–	–	–
March 2001	0	0	0	0	63	07.9 (1.3–14.6)	0
April 2001	–	–	–	–	–	–	–
May 2001	0	0	0	0	59	11.9 (3.6–20.1)	0
June 2001	0	0	2	0	59	13.6 (4.8–22.3)	0
July 2001	0	0	0	0	–	–	–
August 2001	0	0	0	0	54	16.7 (6.7–26.6)	0

(61%) contained anopheline larvae, of which nine were tap habitats (53%) and eight were road habitats (47%). *Anopheles gambiae* s.l. and the non-vector *Anopheles christyi* (Newstead & Carter) were the only anopheline species found, with a much greater abundance of the latter species than the former. *Anopheles gambiae* s.l. was collected from three tap habitats and four road habitats on at least one occasion. PCR analysis indicated that all larvae that showed a positive PCR result were *A. gambiae* Giles s.s. (76/76). Along the river, 10 larval habitats with stagnant water were identified, which were the result of a receding water level in the streambed. On one occasion, one anopheline larva, *A. arabiensis* patton, was identified from a river habitat.

When the seven selected road and tap habitats contained water, road habitats were more likely to contain *A. gambiae* s.l. larvae than tap habitats over the entire study period (chi-square test, $P = 0.002$). In all the 11 cases that *A. gambiae* s.l. was collected from a site, *A. christyi* was present, while the reverse was true for 11 of 27 cases (40.7%). This association was highly significant (chi-square test, $P = 0.001$). The total number of anophelines collected from the selected tap sites ranged between 7 and 328 larvae and peaked in week 35 (August 2000). The total number of anophelines in the road habitats also peaked in week 35, but this number rapidly dropped to 0 in week 39 (September 2000; Figure 2a). On subsequent occasions, anophelines were no longer found in road habitats. By contrast, tap habitats contained larvae throughout the entire study period. Figure 2b shows the proportion of *A. gambiae* s.s. that was identified from the total number of anophelines collected. Over the entire study period, the proportion of *A. gambiae* s.s. in the total anopheline population was significantly higher in road sites than in tap sites (chi-square test, $P < 0.001$). With exception of the river habitat (see above), no *A. arabiensis* larvae were identified from the samples.

Adult survival experiment

On average, the daily mortality rate of *A. arabiensis* in the experimental cages was twice as high as that of *A. gambiae*

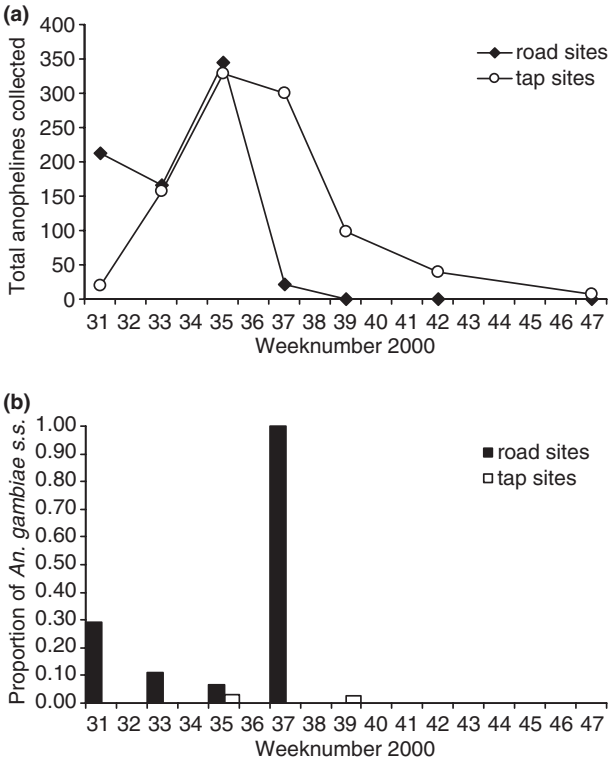

Figure 2 (a) The total number of anophelines collected using the 10% dipping method in road and tap habitats. (b) The proportion of *Anopheles gambiae* s.s. in the total population of anophelines in road and tap habitats.

s.s. (Hazard ratio = 1.95; Table 2). Mosquito mortality rates in houses B and C were significantly higher than that in house A. Figure 3 shows the cumulative hazard rate curves, stratified for the two sibling species. Both curves have a slope increasing with time, which indicates that mortality rate increased with the age of the females. There was no indication that larger females survived longer than smaller females ($r_s = 0.135$, $P > 0.05$ for *A. gambiae* s.s. and $r_s = 0.168$, $P > 0.05$ for *A. arabiensis*). Although their median survival time was shorter, *A. arabiensis* females were significantly larger than *A. gambiae* s.s. females (3.47 ± 0.13 and 3.25 ± 0.12 mm for *A. arabiensis* and *A. gambiae* s.s., respectively, Student's *t*-test, $P < 0.001$).

Larval survival experiment

In Fort Ternan, only 2 of the 500 *A. arabiensis* larvae reached the pupal stage, after 13 and 20 days. All other larvae (*A. arabiensis* and *A. gambiae* s.s.) died prior to pupation. By contrast, from the same batch of newly hatched first instar larvae, pupation of both *A. gambiae* s.s.

Table 2 Hazard ratios of the adult survival experiment, calculated with Cox regression analysis, with 95% CI and significance levels for each covariate (species and house). Empty cells indicate the species or house that was taken as the baseline when determining the hazard ratio

	Hazard ratio	95% CI	*P* value
Fort Ternan			
Species			
Anopheles gambiae			
Anopheles arabiensis	1.95	1.52–2.51	<0.001
House			
A			
B	2.74	1.96–3.85	<0.001
C	3.29	2.32–4.66	<0.001

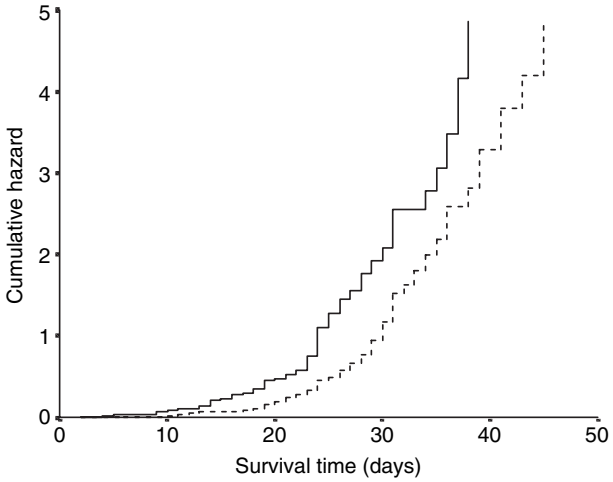

Figure 3 Cumulative hazard rates for *Anopheles arabiensis* (solid line) and *Anopheles gambiae* s.s. (dashed line).

and *A. arabiensis* began after 7 days in the control group placed at lower altitude. In this group, median time to pupation was significantly shorter for *A. arabiensis* than that for *A. gambiae* s.s. (log-rank test, $P < 0.001$). The total proportion that pupated within 13 days was 64% for *A. arabiensis* and 36% for *A. gambiae* s.s.

Meteorological data

The mean outdoor minimum temperature was 13.5 °C on almost each day of the study period. Indoor minimum and maximum temperatures were 17.4 and 22.6 °C, respectively (Table 3). Outdoor maximum temperatures could not be recorded because of mechanical failure of the temperature probe. At these outdoor temperatures, *A. gambiae* s.l.

Table 3 Meteorological data from Fort Ternan for the months during which the adult and larval survival experiments were carried out (June, July and August 2001)

Location	Parameter	Value (±SD)
Outdoor	T_{min} (°C)	13.5 ± 1.3
	No. of days with $T_{min} \leq 16.0$ °C	88
	No. of days with rain	54
	Average rainfall for rain days (mm)	10.1 ± 9.4
Indoor	T_{min} (°C)	17.3 ± 1.1
	T_{max} (°C)	22.6 ± 1.3
	RH_{min} (%)	63.2 ± 6.5
	RH_{max} (%)	76.8 ± 3.1
	No. of days with $T_{min} \leq 16.0$ °C	12
	Total days	92

cannot develop. This temperature is also too low for the extrinsic development of *P. falciparum* that has a threshold temperature of 16 °C (Macdonald 1957). Indoor temperatures were suitable for the development of both mosquitoes and parasites.

Discussion

We found significant levels of malaria prevalence in a highland area in western Kenya: on average 10% of a selected group of school children aged between 5 and 10 years from this area was infected with *P. falciparum*. However, very few malaria vectors were collected throughout the same study period. Based on a total of five adult anophelines found, of which one contained *P. falciparum* parasites, the malaria risk estimate for the study area using the entomological inoculation rate (Smith *et al.* 2001) was insignificantly low.

In a highland area nearby our study area, Brooker *et al.* (2004) reported *P. falciparum* cases among school children. In this study, 129 incident cases were found during a 10-week period between May and July in 2002, which coincides with the end of the long rains. In the same months in 2001 and 2000, we recorded high prevalence rates for our study area, but, remarkably, only seven children had clinical symptoms of malaria in the year 2000 and none had clinical symptoms in 2001. Akhwale *et al.* (2004) report a *P. falciparum* prevalence of 18–25% in children <5 years old in villages at 1600–1660 m near Kisii, western Kenya. This area is 50 km southwest of our study area with an altitude similar to that of Fort Ternan. The malaria prevalence found in the children in these villages appears to be common at this altitude throughout the region, although it remains questionable whether this will be sufficient to build up protective immunity (Day & Marsh 1991). From these and our studies, we conclude that malaria is widely present in the western highlands of Kenya and that a considerable proportion of children is annually infected, but only a small proportion is affected by the disease. This raises the question how malaria transmission can be sustained with the observed low vector densities. We discuss two explanations: low sensitivity of the used collection methods and differences in vector survival between high and low elevation sites.

Throughout our studies, substantial numbers of *Culex* spp. were recovered (data not shown), indicating that the low density of adult *Anopheles* spp. was not a result of malfunctioning traps or inefficient resting collections. Besides, light traps were always checked for the presence of other flying insects to make sure that they had been working properly. Some studies have shown that traps are, in some situations, more efficient at higher mosquito densities (Mbogo *et al.* 1993; Service 1993; Magbity *et al.* 2002). With the low vector densities in our study area, as confirmed with the resting catches, we may thus have less efficient collections. However, others argue that light traps are considered to collect proportional numbers at all densities (Lines *et al.* 1991; Smith 1995). Alternative collection techniques, such as pyrethrum spray collections may be more sensitive to low densities in highland areas (Lindblade *et al.* 2000). However, our results from the larval surveys and larval survival study do not lend support to a local production of *A. gambiae* s.s. or *A. arabiensis*, the only vector species reported in the study area.

We identified several larval habitats of *A. gambiae* s.s., but their limited number (seven habitats over a 4-month period) and low larval densities suggest that their contribution to the adult population was low. By contrast, many larval habitats contained larvae of *A. christyi* that is not considered as a malaria vector (Gillies & Coetzee 1987). Brooker *et al.* (2004) report the presence of anopheline larvae in streams at all altitudes. These authors do not mention the anopheline species, but based on our results their findings may have been non-vector species such as *A. christyi*. In north east Tanzania, Bødker *et al.* (2003) also found very low adult *Anopheles* densities at high altitudes. However, they did not carry out a systematic larval survey, and therefore they could not verify whether these mosquitoes originated from local breeding or dispersal from lower areas.

The outcome of the larval survival experiment indicated that it is unlikely for wild larvae to pupate and develop into adults at this altitude. Although our experimental larvae developed in artificial trays and were kept out of direct sunlight, we believe that survival of wild larvae would even be more compromised than that of the experimental larvae, as our larvae were not exposed to the cold outdoor night

temperatures. Garnham (1948) reported on the presence of anopheline larvae and malaria in the same study area and described an unusually long larval development period of 24 days, which would have certainly led to nutritionally compromised adults. Garnham (1948) similarly described the presence of *A. christyi*, and it seems likely that the anopheline population has not changed much over >50 years.

The results from the adult survival experiment show that adult vectors survived indoors under the environmental conditions of Fort Ternan. *Anopheles gambiae* s.s. was the better survivor when compared with *A. arabiensis*, consistent with earlier findings on the adult distribution of *A. gambiae* s.l. in the highlands of western Kenya, whereby *A. gambiae* s.s. was the dominant species (White 1972; Highton *et al.* 1979; Shililu *et al.* 1998; Minakawa *et al.* 2002).

The observed differences in adult survival between the study houses may reflect random variation, but more likely differences in the number of occupants in a house (Garnham 1948) and amount of light or shade during the day (for which we did not control) influence temperature and relative humidity conditions indoors, affecting the survival of both sibling species.

In the adult survival experiment, mosquitoes probably died of senescence, which is unusual in natural populations, where death is normally caused by predators, disease or other hazards (Jenkins 1964; Clements & Paterson 1981). In addition, wild female mosquitoes blood-feed every 2–3 days, while our experimental females had access to a sugar source only. In contrast with earlier studies, we did not find an effect of body size on survival within or between species (Ameneshewa & Service 1996; Takken *et al.* 1998). Nevertheless, daily mortality rates increased with age of the females consistent with mark–release–recapture and parity rate studies from the field (Gillies & Wilkes 1965; Clements & Paterson 1981; Charlwood *et al.* 2000).

We propose the following conceptual framework that may explain our findings for the Fort Ternan area. As a result of dispersal from the lower areas where malaria transmission is perennial, some adult mosquitoes, of which a proportion is infected with malaria parasites, will arrive at higher altitudes through natural or mechanical (by vehicles) dispersal. Here, they are able to survive inside local houses, as supported by our adult survival experiment, take blood meals and transmit malaria as a consequence. Egg batches will be laid in local water bodies, but stable vector populations will not be maintained because of highly retarded development and low survival.

Although the malaria infections in the study children may have been contracted at lower altitudes while visiting, an oral survey among our study households (K. Derks, unpublished data) found that our investigated age group (5–10 years) rarely moves outside the study area. This suggests that the children became infected in their own village. As the environmental conditions for anopheline development appear unfavourable, the infections are most likely because of immigrant mosquitoes. Manga *et al.* (1993) stated that slope might limit anopheline dispersal, but Smith (1959) concluded that populations in a mountainous area in Tanzania descended from populations in the lower areas, as residual house spraying in the plains directly affected mosquito numbers in huts in the mountains. In the immediate vicinity of Fort Ternan, areas with much lower altitudes than 1550 m are present within a few kilometres, a distance that can be readily covered by *A. gambiae* s.l. (Gillies 1961). As the ambient temperature in these lower altitude areas is higher [0.6 °C per 100 m altitude difference (Linacre 1992)], immature development of the mosquitoes may take place in these areas, followed by random dispersal of the adults also to higher altitudes. Additional studies are required to investigate to what extent dispersal from lower areas contributes to maintaining malaria transmission in higher elevated areas.

We argue that slight increases in temperature, as a result of true climate change, temporary favourable conditions during El Niño years or changes in microhabitat (e.g. by deforestation or changes in agricultural practices), may facilitate the completion of the mosquito's life cycle through increased survival and development of the aquatic stages. Additionally, temperatures may reach above the threshold of parasite development in the mosquito. As a small human parasite reservoir is already present, but probably not sufficient to build up large herd immunity, the resulting increase in vectors may initiate an outbreak of malaria in highland areas. After an outbreak in 1990, associated with El Niño, 50 *A. gambiae* s.l.and 26 *An. funestus* vectors were collected from 16 houses in Fort Ternan using pyrethrum spray collections (A.K. Githeko, unpublished data). This supports our conclusion that temporarily favourable conditions may increase larval vector survival and precipitate a malaria epidemic.

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La faible survie de la larve du vecteur explique l'instabilité de la malaria dans les montagnes de L'ouest du Kenya

Plusieurs régions de montagnes en Afrique de l'est ont récemment souffert de sérieuses épidémies de malaria. Certains modèles prédisent qu'à court terme, ces régions subiront encore plus d'épidémie à cause du réchauffement climatique globale. Cependant, les différents aspects responsables de ces changements sont peu compris. Dès lors, nous avons investigué la prévalence de la malaria, la densité du vecteur et sa survie dans une région de montagnes dans l'ouest du Kenya, située entre environ 1550 et 1650 m d'altitude. Bien que seuls 5 vecteurs adultes de la malaria aient été collectés au moyen de 180 pièges de lampes et 180 collectes de repos sur une période d'étude de 23 mois, la malaria était prévalente chez les écoliers (prévalence moyenne du parasite: 10%). Au cours d'une surveillance extensive d'habitats potentiels des larves, nous avons identifié seulement 7 habitats contenant des larves de *An. gambiae* Giles s.1. Leur nombre limité et la faible densité larvaire suggèrent que leur contribution à la population de vecteurs adultes est faible. Des expériences sur la survie des adultes et des larves a montré qu'à cette altitude, les moustiques adultes survivaient à l'intérieur des habitations locales mais, que le développement larvaire y était largement retardé: seuls 2 sur 500 larves de *An. gambiae* s.1. se sont développés au stade de chrysalides et toutes les autres larves sont mortes avant ce stade. Il est improbable qu'en ce moment, de fortes densités du vecteur soient dues à des conditions abiotiques non favorables dans la région. Cependant, des conditions temporairement favorables telles que les années El Niño peuvent accroître la survie des larves du vecteur et conduire à des épidémies de malaria.

mots clés malaria, montagnes, *Anopheles gambiae*, *Anopheles arabiensis*, larve, Kenya

Una baja supervivencia larval del vector explica la instabilidad de la malaria en las tierras altas de Kenia Occidental

Varias áreas en tierras altas del este de África han sufrido recientemente de epidemias serias de malaria. Algunos modelos predicen que, a corto plazo, estas áreas experimentarán más epidemias como resultado del calentamiento global. Sin embargo, los diferentes procesos que subyacen a estos cambios se entienden muy poco. Por lo tanto, hemos investigado la prevalencia de malaria, las densidades del vector de la malaria y la supervivencia del mismo en un área de tierras altas del oeste de Kenia, que se encuentra entre aproximadamente 1550 a 1650 m de altura. Aunque solo se recolectaron cinco vectores de malaria adultos en 180 trampas de luz y 180 capturas en reposo, durante el período de estudio de 23 meses la malaria estuvo presente entre los niños en edad escolar (prevalencia parasitaria promedio: 10%). Durante un estudio extensivo de hábitats potenciales de larvas, se identificaron solamente 7 hábitats con larvas de *An. gambiae* Giles. Su número limitado y las bajas densidades larvales sugerían que su contribución a la población vectorial adulta era baja. Experimentos sobre la supervivencia adulta y larval demostraron que en esta altitud, los mosquitos adultos sobreviven dentro de las casas locales, pero que el desarrollo larval esta severamente retardado: solo dos de 500 larvas de *An. gambiae* s.l. se desarrollaban hasta el estado de pupa, mientras que todas las demás morían antes de la pupación. Actualmente es poco probable que las altas densidades vectoriales se deban a condiciones abióticas no favorables en el área. Sin embargo, condiciones temporales favorables, como El Niño, pueden aumentar la supervivencia larval del vector y resultar en epidemias de malaria.

palabras clave malaria, tierras altas, *Anopheles gambiae*, *Anopheles arabiensis*, larvas, Kenia