

Land use change alters malaria transmission parameters by modifying temperature in a highland area of Uganda

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Summary

As highland regions of Africa historically have been considered free of malaria, recent epidemics in these areas have raised concerns that high elevation malaria transmission may be increasing. Hypotheses about the reasons for this include changes in climate, land use and demographic patterns. We investigated the effect of land use change on malaria transmission in the south-western highlands of Uganda. From December 1997 to July 1998, we compared mosquito density, biting rates, sporozoite rates and entomological inoculation rates between 8 villages located along natural papyrus swamps and 8 villages located along swamps that have been drained and cultivated. Since vegetation changes affect evapotranspiration patterns and, thus, local climate, we also investigated differences in temperature, humidity and saturation deficit between natural and cultivated swamps. We found that on average all malaria indices were higher near cultivated swamps, although differences between cultivated and natural swamps were not statistically significant. However, maximum and minimum temperature were significantly higher in communities bordering cultivated swamps. In multivariate analysis using a generalized estimating equation approach to Poisson regression, the average minimum temperature of a village was significantly associated with the number of *Anopheles gambiae* s.l. per house after adjustment for potential confounding variables. It appears that replacement of natural swamp vegetation with agricultural crops has led to increased temperatures, which may be responsible for elevated malaria transmission risk in cultivated areas.

keywords malaria, *Anopheles*, elevation, environment, agriculture, temperature, Uganda

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Introduction

Historically, high elevation areas of Africa (> 1500 m) were considered malaria-free zones. Recently, numerous reports of highland malaria epidemics (Lepers *et al.* 1988; Marimbu *et al.* 1993; Some 1994; Kigotho 1997; Malakooti *et al.* 1998; Kilian *et al.* 1999; Lindblade *et al.* 1999) have generated concern that malaria transmission may be increasing in highland regions. The severe public health burden resulting from malaria outbreaks in populations with little or no immunity to *Plasmodium* spp. has fostered efforts to understand the reasons for the apparent increase in malaria transmission at high elevations. Several hypotheses to explain increased highland malaria transmission have been offered, including

climate change (Loevinsohn 1994; Lindsay & Martens 1998) land use changes (Matola *et al.* 1987; Marimbu *et al.* 1993; Lindsay & Martens 1998; Malakooti *et al.* 1998; Mouchet *et al.* 1998), drug resistance (Malakooti *et al.* 1998), cessation of malaria control activities (Mouchet *et al.* 1998) and demographic changes (Lindsay & Martens 1998; Mouchet *et al.* 1998).

The south-western highlands of Uganda (Kabale District) have experienced 2 serious malaria epidemics in the last 5 years, both of which were associated with *Anopheles gambiae* s.l. (Mouchet *et al.* 1998; Lindblade *et al.* 1999). Previously, this area had been extensively surveyed by scientists from the World Health Organization (WHO) from 1959 to 1963 (Zulueta *et al.* 1964). Through application of DDT,

the WHO team apparently eliminated the only endemic focus of *P. falciparum* > 1500 m (around Lake Bunyonyi) that was associated with *An. funestus*. While the WHO team reported a few small outbreaks of malaria in valleys outside the lake area (one of which was attributed to *An. gambiae* s.l.), malaria transmission did not occur frequently during that period in the valleys that were hit by epidemics in 1994 and 1998. Few specimens of *An. gambiae* s.l. were found in any survey prior to 1963 (Uganda Malaria Eradication Pilot Project 1959–63).

Beginning in the early 1940s in the south-western highlands of Uganda, the British administration encouraged the drainage of swamps containing *Cyperus papyrus* (papyrus) and other swamp grasses to increase the land available for cultivation (Carswell 1996). By 1946, there was speculation that swamp cultivation was responsible for an outbreak of malaria attributed to *An. christyi* (Steyn 1946). Recently, the hypothesis that swamp cultivation has contributed to establishment of malaria transmission in this high elevation region has been revisited (Mouchet *et al.* 1998).

The objectives of this investigation were to determine whether malaria transmission parameters in villages bordering cultivated swamps differed from those bordering natural papyrus swamps. Because vegetation differences may also affect local climate (Stahl 1993; Tinker *et al.* 1996; Hutjes *et al.* 1998), which in turn alters the development rates of the parasite and vector as well as mosquito biting rates and longevity, we also investigated differences in temperature, humidity and saturation deficit between natural and cultivated swamps.

Materials and methods

Climate and geography

The study was conducted in Kabale District, south-western Uganda (1°05′–1°30′S, 29°45′–30°15′E). Elevation in the district ranges from 1500 to 2400 m. Rainfall (850–1200 mm annually) is seasonally bimodal with fairly constant average daily minimum (9.8–12.6 °C) and maximum temperatures (23.2–24.4 °C) throughout the year. Average human population density is high for this region (246 people/km²) (Uganda Ministry of Finance & Economic Planning 1992), in which subsistence farming is the predominant occupation.

Kabale District is composed of steep ridges, narrow valleys and one large body of fresh water (Lake Bunyonyi). Several of the valley bottoms originally contained papyrus swamps. Limited cultivation of sweet potatoes along the edges of swamps occurred prior to the 1940s (Purseglove 1946), but the British administration's concern with the need to feed a burgeoning population facilitated widespread drainage of

swamps for cultivation of diverse crops. Of an estimated 69.7 km² of papyrus swamp surveyed in 1954–55 (Gibb 1956), only about 15% still exists in its natural state. (Only the 4 major swamp systems in present-day Kabale District are included. Estimates of the current area of remaining natural swamp were calculated from land use/land-cover files obtained from the National Biomass Study, Uganda Forest Department.)

Malaria history and vector population

Malaria was investigated in the south-western highlands beginning in the 1930s (Garnham *et al.* 1948). Early research implicated *An. christyi* as a vector of malaria (Steyn 1946). Because *An. christyi* was found to breed in waterlogged ditches in cultivated swamps, it was believed that several outbreaks of malaria were a direct result of swamp cultivation (Steyn 1946). However, no additional sporozoite-positive *An. christyi* have been found since the initial 1944 report. While the WHO was conducting extensive research in the area from 1959 to 1963 as part of a malaria eradication pilot project, a focus of endemic *P. falciparum* around Lake Bunyonyi was noted, with *An. funestus* the only vector in the area (Zulueta *et al.* 1964). There also were occasional small outbreaks of malaria in the valleys outside the lake area, one of which was believed to have been transmitted by *An. gambiae* s.l. (Uganda Malaria Eradication Pilot Project 1959–63). More recently, Mouchet *et al.* (1998) conducted an informal investigation of malaria vectors in the region in response to an epidemic of malaria during June–July 1994. In a number of houses, *An. gambiae* s.s. were found with an overall sporozoite rate (SR) of 14.5%. Subsequently, we reported on a malaria epidemic that occurred while conducting the research reported in this study (Lindblade *et al.* 1999). The vector involved in the 1998 epidemic was *An. gambiae* s.l. with an overall SR of 6.1%.

Site selection

Natural papyrus swamps remain in only 2 valleys in Kabale District (Kashambya, Rwamucucu, Bukinda and Muko Ikumba subcounties), thus limiting the number of villages available for study. To minimize the likelihood of mosquito movement between sites, villages were selected at least 2 km apart. Beginning at one end of each swamp, a 2-km interval was marked and the closest eligible village nearest the end-point was selected for study. Eligible villages were those containing at least 5 grass-thatched houses. If households within a village refused to participate, the next eligible village was selected. Given the limited area of natural swamp, the maximum number of villages that could be selected at least 2 km apart was 8. From among the villages bordering cultivated

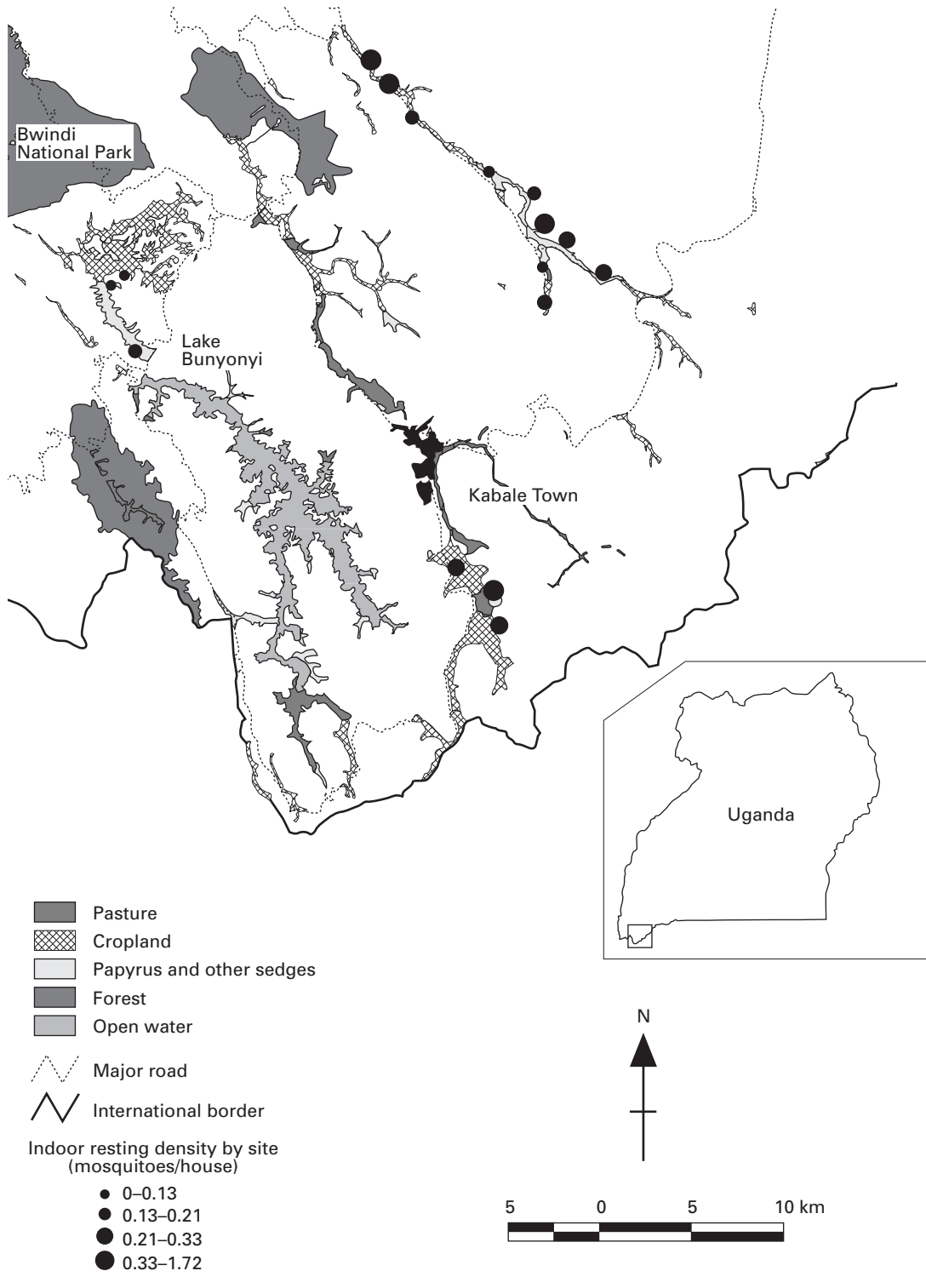


Figure 1 Map of study area indicating sites near natural and cultivated swamps and mean IRD from December 1997 – July 1998.

swamps, 8 villages were selected from 3 valleys (Figure 1), including 1 additional cultivated valley located in Buhara and Kamuganguzi subcounties. The selection method was similar to that for natural swamps. The elevation of the study villages ranged from 1700 to 1960 m.

Entomological survey

Within each village, the location of all thatched houses was mapped using a global positioning system (GPS) (Magellan Systems Corporation, San Dimas, CA, USA). The 5 thatched houses situated closest to the swamp edge were sampled throughout the study except when circumstances required a substitute house.

We used a standard pyrethrum spray collection method (WHO 1975) to sample indoor resting mosquitoes in houses of each village twice per month from January through July 1998. (In December 1997, captures of resting mosquitoes were conducted on a limited basis with 1 collector using an aspirator to collect mosquitoes for 30 min). A brief questionnaire was administered at each capture that recorded the time of collection, the number of people occupying the house the night before the spraying, use of any antimosquito measures as well as the number of large animals (cows, sheep and goats) stabled near the house that night. The elevation of the house was determined from its location on a digitized contour map while the width of the swamp adjacent to the village was taken from a digitized land use/land-cover map provided by the National Biomass Study, Uganda Forestry Department. Population densities for each village were calculated using 1991 census population estimates (Uganda Ministry of Finance & Economic Planning 1992) divided by the area of the village obtained by digitizing village boundaries from census maps.

All *Anopheles* mosquitoes were identified morphologically according to Gillies & Coetzee (1987), stored and dried in vials with silica gel until they could be transported back to the USA for further testing. Indoor resting density (IRD) was calculated as the number of mosquitoes per house (m/h) for *An. gambiae* s.l. only. The head and thorax of each anopheline was tested singly for *P. falciparum* sporozoites using a standard enzyme-linked immunosorbent assay (ELISA) (Wirtz *et al.* 1987). The SR was calculated as the number of positive specimens divided by the total number tested from each house-capture.

The abdomens of captured anophelines were also separately tested for the presence of human blood using a standard sandwich ELISA procedure (Chow *et al.* 1993). Daily human biting rates (HBR) were estimated for each house-capture as the total number of mosquito bites on humans from the previous night divided by the number of occupants. The number of bites on humans was taken to be the sum of the number of

mosquitoes with undigested human blood plus half the number of mosquitoes with partially digested human blood; digestion status was determined from the colour of the abdomen solution. Daily entomological inoculation rates (EIR) were calculated as the product of HBR and SR for each house-capture.

Weather data

Three thermometers (HBE Corp., Boca Raton, FL, USA) that measured maximum (T_{\max}) and minimum (T_{\min}) temperatures were placed in each village, 1 at each home of 3 volunteers who manually recorded these temperatures daily throughout the study period. For protection from children and animals, all thermometers were attached approximately 2 m from the ground under the eaves of a house roofed with aluminium sheets (Maximum temperatures were affected by placement of thermometers under roofs covered with aluminium sheets. While measurement of maximum temperature was comparable between villages, the measured value in all sites were higher than the actual maximum temperature in those locations). Volunteers were trained to read and record temperatures; we tested volunteers at the end of the study to determine whether they could correctly read temperature from the thermometers, and the recordings of all volunteers who failed the test were discarded.

Daily T_{\max} and T_{\min} of the 3 recordings in each village were averaged to give a daily village T_{\max} and T_{\min} . Assuming that a large range of temperature values on the same day by the 3 volunteers in each village indicated erroneous measurements, we discarded all daily village temperature measurements for which the range was greater than the mean plus twice the standard deviation of the ranges (determined to be 9.1 °C for T_{\max} and 4.8 °C for T_{\min}). Overall 4.0% of the daily village measurements of T_{\min} and 2.8% of the daily village measurements of T_{\max} were discarded.

One relative humidity (RH) probe (ONSET Computer Corporation, Pocasset, MA, USA) was placed in each of the 16 villages at the same height as the thermometers. Probes were programmed to record RH approximately every hour; data were downloaded every 2–3 months. RH was averaged for periods corresponding to daytime (0700–1900 h) and night-time (1900–0700 h).

Saturation deficit, the difference between the maximum amount of water the atmosphere can hold at the current temperature (saturation vapour pressure) and the actual amount it holds (ambient vapour pressure) was calculated using T_{\max} , T_{\min} and RH. A higher saturation deficit, measured in units of kPa (the Pascal [Pa] is equivalent to kg/m.s²), means greater evaporative water loss, which generally decreases mosquito longevity. Hourly temperatures for each site were estimated from daily T_{\max} and T_{\min} using the method of Campbell and Norman (1998), and then combined with hourly RH readings

from each village to estimate saturation deficit. Saturation deficit was averaged for both day (0700–1900 h) and nighttime (1900–0700 h).

Data analysis

To describe differences in IRD between villages located along natural and cultivated swamps over time, smoothed curves were constructed. Smoothed values of IRD by sampling period were first calculated using a running median followed by several iterations of a moving average until the values stabilized.

Household values for IRD, HBR and EIR in each of the 16 sampling periods were pooled and summary measures were calculated for each swamp type as the arithmetic mean of all house-captures. The Wilcoxon rank-sum test (u) was used to test for differences in summary measures of entomological transmission parameters by swamp type; the sample size for each swamp type was equal to the total number of house-captures over the study period. The assumption of identical distribution shape was tested by comparison of histograms for each variable.

Because repeated measurements taken on houses within each village are probably correlated, thereby affecting standard error calculations, we used a generalized estimating equation. (GEE) approach to Poisson regression (for count data) to account for the correlation between repeated measurements of mosquito density in the same village. To examine the impact of swamp type and other variables on IRD and number of human bites, we used PROC GENMOD of SAS (SAS System for Windows 6.12, Cary, NC, USA) and assumed an exchangeable correlation matrix (i.e. the dependence between 2 observations from the same house is the same no matter how far apart in time they occur). This model is appropriate when the correlation between measures is considered a nuisance (i.e. the correlation itself is not of interest) (Diggle *et al.* 1994). Coefficients are interpreted as for Poisson regression, i.e. the natural antilog of the coefficient gives the odds ratio (OR), which approximates the rate ratio, for the effect of the variable on the outcome measure. 95% confidence intervals (CI) for parameters were produced as likelihood ratio-based confidence intervals.

We used IRD (m/h) and the number of bites taken on humans per house (b/h) as outcome variables. Predictor variables for these outcome measures were the swamp type, mean elevation for the sampled houses in the village, the village population density, width of the swamp adjacent to the village, mean T_{\max} for the village, mean T_{\min} for the village, mean daily RH and saturation deficit for the village, mean nightly RH and saturation deficit for the village, number of occupants, presence or absence of animals, number of animals, season (wet, December–March; dry, April–July) and hour of

capture. In the unadjusted analysis, each variable was introduced singly into the model to determine the effect on number of *An. gambiae* s.l. per house and the number of human bites per house. Adjusted models included all variables from the crude analysis with statistically significant ($P < 0.05$) coefficients.

Daily mean T_{\max} and T_{\min} , daytime and nighttime RH and saturation deficit for all villages were computed for each swamp type and compared using a 2-sided t -test for independent samples (t); an approximation to the t -test was used if variances were found to be unequal. 95% CI were calculated using the t distribution.

Results

Entomological survey

From December 1997 through July 1998, we conducted 489 mosquito captures in 8 villages located along natural swamps and 458 captures in 8 villages located along cultivated swamps. The average number of houses surveyed in each sampling period was 3.9 from villages located along natural swamps and 3.7 from those along cultivated swamps. None of the households reported use of personal protection measures against mosquitoes.

A total of 287 anophelines was captured, of which 258 (90.0%) were identified as *An. gambiae* s.l. Other anophelines captured included *An. implexus* ($n = 11$), *An. coustani* ($n = 2$), *An. kingi* ($n = 2$) and others too damaged to identify ($n = 14$). The greater proportion (64.7%) of the captured *An. gambiae* s.l. were collected from villages located along cultivated swamps (Table 1). The average IRD of *An. gambiae* s.l. throughout the study period varied considerably from village to village, but the summary IRD was almost twice as high in the villages near cultivated swamps as in villages near natural swamps; this difference, however, was not statistically signifi-

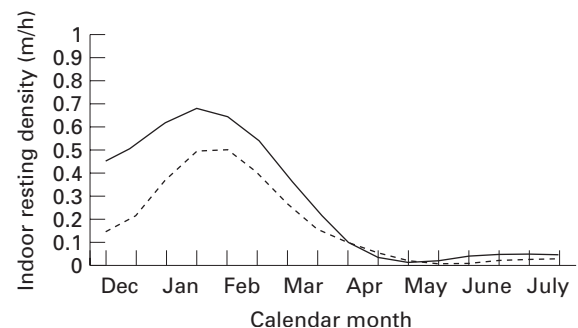


Figure 2 Smoothed mean indoor resting density of *Anopheles gambiae* s.l. by swamp type, December 1997 – July 1998. ----- natural swamp; — cultivated swamp.

Table 1 Results of the entomological survey by village with summaries for villages located near natural and cultivated swamps, December 1997–July 1998, Kabale, Uganda

Village (Parish)	No. of house captures	No. of <i>Anopheles gambiae</i> s.l.	No. of other anophelines	Crude SR* % (no. tested)	Mean IRD* m/h	Mean HBR* b/p/n	Mean EIR* ib/p/n
Natural swamp							
Summary **	489	91	22	4.1 (73)	0.19	0.0153	0.0004
Kahoko (Kitanga)	65	4	0	0 (3)	0.06	0.0036	0.0000
Rubirizi (Nyakagabagaba)	64	11	0	0 (10)	0.18	0.0179	0.0000
Rwempisi (Burime)	52	30	14	4.8 (21)	0.57	0.0286	0.0022
Kitwe (Kitojo)	63	7	0	0 (4)	0.12	0.0271	0.0000
Nyakafura (Burime)	70	16	2	0 (15)	0.24	0.0214	0.0000
Kabimbiri (Kyerero)	62	15	1	8.3 (12)	0.22	0.0092	0.0013
Bugunga (Nyarurambi)	73	3	1	0 (3)	0.05	0.0000	0.0000
Habutobere (Butare)	40	5	4	20.0 (5)	0.13	0.0222	0.0000
Cultivated swamp							
Summary**	458	167	5	7.0 (157)	0.36	0.0367	0.0030
Kanyabugunga (Kafunjo)	40	78	1	4.0 (75)	1.72	0.1389	0.0018
Kazoooha (Rutengye)	49	22	1	15.0 (20)	0.47	0.0488	0.0071
Kantare (Rutengye)	60	8	0	0 (8)	0.14	0.0339	0.0000
Sindi (Mparo)	58	11	1	0 (8)	0.19	0.0184	0.0000
Rushunga (Nyarurambi)	70	1	0	0 (1)	0.02	0.0130	0.0000
Kyase A (Buhara)	60	23	2	4.8 (21)	0.42	0.0573	0.0119
Kashaki A (Buhara)	48	9	0	33.3 (9)	0.22	0.0134	0.0056
Nyamurindira B (Kicumbi)	73	15	0	6.7 (15)	0.23	0.0306	0.0020

*SR, sporozoite rate; IRD, indoor resting density; m/h, mosquitoes/house; HBR, human biting rate; b/p/n, bites/person/night; EIR, entomological inoculation rate; ib/p/n, infectious bites/person/night. **Summary measures were calculated as arithmetic means of all house-captures in each swamp type.

cant (w , $P = 0.4430$).

Mean IRD varied over time during the study period, with higher levels reported during and following the rains (December through March); the difference in IRD between natural and cultivated swamps was most marked when overall densities were at the highest levels (Figure 2). During the dry season, there was little difference in IRD between swamp types. We previously reported an epidemic of malaria that occurred while we were collecting data for this study due, in part, to the elevated IRD during the first half of the study period (Lindblade *et al.* 1999).

The circumsporozoite antigen of *P. falciparum* was detected in 14 of 230 (6.1%) *An. gambiae* s.l. tested. No other *Anopheles* species was found positive for sporozoites. The summary SR was higher among mosquitoes captured along cultivated swamps than papyrus swamps (Table 1) although the difference was not statistically significant (w ; $P = 0.1071$). Human blood was found in 127 of 192 (66.1%) *An. gambiae* s.l. with partially digested or undigested blood meals. Mean HBR of *An. gambiae* s.l., calculated for each house sampled and averaged for each village, were very low, ranging from 0 to 0.1389 bites/person/night (b/p/n). The summary HBR was more than twice as high in

villages located along cultivated swamps than those along natural swamps (Table 1), although this difference was not

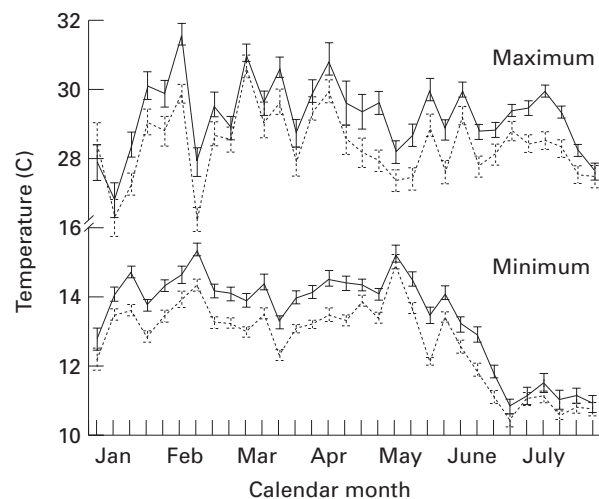


Figure 3 Weekly mean maximum and minimum temperatures by swamp type, January – July 1998. ----- natural swamp; — cultivated swamp. Error bars are ± 1 standard error.

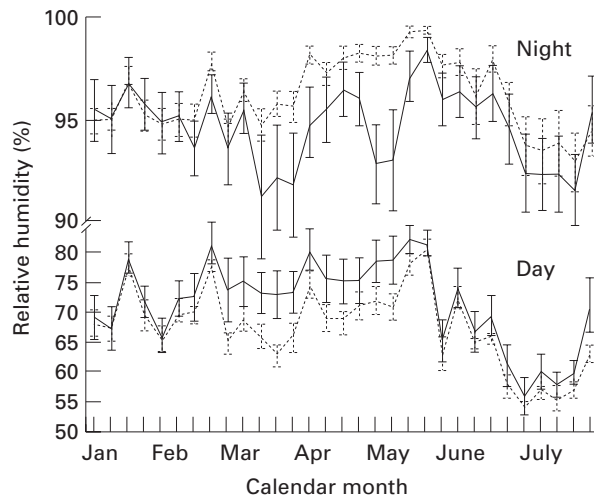


Figure 4 Weekly mean relative humidity by time of day and swamp type, January – July 1998. ----- natural swamp; — cultivated swamp. Error bars are ± 1 standard error.

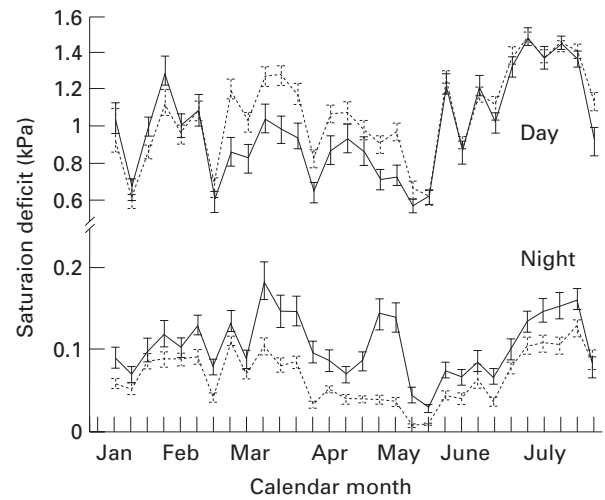


Figure 5 Weekly mean saturation deficit by time of day and swamp type, January – July 1998. ----- natural swamp; — cultivated swamp. Error bars are ± 1 standard error.

statistically significant ($w, P = 0.7983$). The theoretical number of days to receive 1 bite from *An. gambiae* s.l. is equal to the inverse of the HBR: in villages located along cultivated swamps, individuals on average received 1 bite from *An. gambiae* s.l. every 27.3 days while individuals living alongside natural swamps were bitten once every 65.4 days.

EIR was calculated for each house sampled as the product of the SR and the HBR. An arithmetic average was computed for each village, and a summary value was calculated for each swamp type as the arithmetic average of all house-captures. Because so few mosquitoes were infected, EIR was 0 infected bites/person/night (ib/p/n) for many villages (Table 1). The summary EIR was almost an order of magnitude higher in villages located along cultivated swamps than in villages located along natural swamps but this difference was not statistically significant ($w, P = 0.1307$). Sporozoite-positive mosquitoes were detected from mid-December through the end of

March. Assuming that this 14-week period comprised the only transmission season during the study, and that rates held constant during this period, individuals along cultivated swamps would have received a total of 0.29 ib/p while those living along natural swamps would have received 0.04 ib/p. In percentage terms, up to 29.4% of the population along cultivated swamps would have received an infectious bite compared to only 4.0% of the population living near natural swamps.

Weather associations

T_{\max} and T_{\min} were recorded daily in each village from January 1 through July 30 1998, using up to 3 max/min thermometers per village. Due to breakage of the thermometer or failure of volunteers to accurately record the temperature, the daily average T_{\max} and T_{\min} from some villages was computed from

Table 2 Summary weather data for natural and cultivated swamps, January – July 1998, Kabale, Uganda

	Natural swamp		Cultivated swamp	
	Mean	(95% CI)	Mean	(95% CI)
Temperature (°C)				
Maximum	28.4	(28.2, 28.6)	29.3	(29.1, 29.6)
Minimum	12.8	(12.7, 13.0)	13.6	(13.4, 13.8)
Relative humidity (%)				
Day	66.8	(65.6, 68.1)	69.6	(68.3, 70.8)
Night	95.6	(95.2, 96.1)	93.8	(93.4, 94.3)
Saturation deficit (kPa)				
Day	1.0742	(1.0708, 1.0772)	0.9936	(0.9904, 0.9968)
Night	0.0687	(0.0683, 0.0691)	0.1041	(0.1037, 0.1045)

Table 3 Characteristics of villages located near natural and cultivated swamps, Kabale, Uganda

Village (Parish)	Elevation (m)	Population density (people/km ²)	Width of swamp (km)	Mean max temperature (°C)	Mean min temperature (°C)	Mean daily relative humidity (%)	Mean nightly relative humidity (%)	Mean daily saturation deficit (kPa)	Mean nightly saturation deficit (kPa)
Natural swamp									
Kahoko (Kitanga)	1770	174.2	0.45	30.94	12.63	65.09	96.25	1.31	0.07
Rubirizi (Nyakagabagaba)	1811	82.5	0.26	30.11	12.48	62.49	93.07	1.38	0.14
Rwempisi (Burime)	1802	75.5	0.42	27.97	12.78	67.70	96.73	1.05	0.07
Kitwe (Kitojo)	1768	189.6	0.31	28.79	13.45	69.99	97.93	1.02	0.04
Nyakafura (Burime)	1812	94.5	0.20	28.24	12.86	62.49	93.95	1.22	0.10
Kabimbiri (Kyerero)	1794	144.0	0.23	28.60	13.94	63.80	97.17	1.20	0.04
Bugunga (Nyarurambi)	1917	228.3	0.58	27.53	12.15	71.45	96.54	0.92	0.06
Habutobere (Butare)	1958	237.1	0.58	26.00	12.35	73.38	96.99	0.73	0.03
Cultivated swamp									
Kanyabugunga (Kafunjo)	1753	403.3	0.25	29.73	13.94	66.42	91.42	1.19	0.17
Kazooha (Rutengye)	1712	430.4	0.18	31.70	14.90	61.29	92.61	1.35	0.14
Kantare (Rutengye)	1712	140.6	0.15	30.49	13.90	87.59	92.77	0.41	0.17
Sindi (Mparo)	1768	929.7	0.25	28.62	14.19	67.65	94.49	1.10	0.17
Rushunga (Nyarurambi)	1938	302.6	0.43	27.67	10.77	61.82	95.78	1.16	0.07
Kyase A (Buhara)	1829	235.2	0.91	30.30	13.75	76.92	97.99	0.85	0.04
Kashaki A (Buhara)	1815	1111.2	1.66	28.10	13.70	67.94	89.92	0.99	0.16
Nyamurindira B (Kicumbi)	1715	257.5	1.20	28.93	14.19	79.29	99.15	0.63	0.01

less than 3 observations, and 1 village contributed no temperature recordings until February 1998.

Both mean T_{\max} and T_{\min} were approximately 0.9 °C higher in villages located along cultivated swamps than in villages located along natural swamps (t , $P < 0.0001$) (Table 3). Differences remained statistically significant when only the 10 sites located in the same valley (Figure 1) were considered, as well as when all daily village samples with ranges > 4 °C were removed. Differences in temperature between natural and cultivated swamps were consistent over time (Figure 3).

Mean daily RH, measured using data loggers, did not differ between villages located along cultivated swamps (mean = 81.2%; 95% CI 80.4–82.0) and those located along natural swamps (mean 81.7%; 95% CI 80.9–82.5). However, calcu-

lation of the daily mean masked differences between sites during day and night (Table 2). RH was lower during daylight hours in villages near natural swamps while the opposite was true during nighttime hours (Figure 4).

The difference in saturation deficit between natural and cultivated swamps was similar to that for RH, with higher deficits (i.e. greater evaporative loss) occurring during the day and lower deficits at night (Figure 5). Villages located along natural swamps had deficits that were higher during the day and lower at night than villages near cultivated swamps (Table 2). However, the RH and saturation deficit of villages near both natural and cultivated swamps were similar from January-March and June-July; differences were only apparent from March-June.

Table 4 Poisson regression of swamp type and other variables on the number of mosquitoes per house using generalized estimating equations for 181 houses in 16 villages, December 1997 – July 1998, Kabale, Uganda

	Unadjusted model				Adjusted model*		
	No. of samples	Regression coefficient	OR‡	95% CI‡	Regression coefficient	OR	95% CI
Village-specific variables							
Swamp type	947						
Cultivated		0.76	2.14	0.74–6.16			
Natural		1.00					
Elevation (m)	947	0.0004	1.00	0.997–1.004			
Population density (people/km ²)	947	0.0003	1.00	0.997–1.004			
Width of adjacent swamp (km)	947	– 0.62	0.54	0.18–1.57			
Temperature (°C)†	947						
Maximum		0.20	1.23	0.98–1.54			
Minimum		0.55	1.73	1.18–2.52	0.57	1.77	1.23–2.54
Relative humidity (%)†	947						
Day		– 0.02	0.98	0.94–1.02			
Night		– 0.18	0.84	0.66–1.06			
Saturation deficit (kPa)†	947						
Day		1.04	2.83	0.72–11.11			
Night		8.63¶					
House-specific variables							
Number of occupants	945	0.22	1.25	1.12–1.40	0.19	1.21	1.10–1.33
Any animals	946	0.47	1.60	1.01–2.52			
Number of animals	946	0.01	1.01	0.93–1.10			
Sample-timing variables							
Season	947						
Wet		2.57	13.04	7.34–23.15	2.61	13.62	6.88–26.94
Dry		1.00	1.00				
Time of capture	947						
Before 0800 h		– 0.37	0.69	0.37–1.28			
0800–0900 h		– 0.14	0.87	0.56–1.34			
0900–1000 h		– 0.14	0.87	0.54–1.42			
1000–1030 h		1.00					

*Adjusted model contains minimum temperature, number of occupants and season. ‡OR, odds ratio; CI, confidence interval †Weather data are arithmetic averages for each village of daily village temperatures, relative humidity or saturation deficit for the period January 1–July 31, 1998

¶Point estimate gives very large OR and extremely wide CI.

Analysis of swamp type other variables on IRD and number of human bites

Villages differed in several characteristics that may have affected the mean mosquito density in their area (Table 3). We examined the effects of characteristics of the village, house and timing of the sample on IRD of *An. gambiae* s.l. using a generalized estimating equation. (GEE) approach to take into account the correlation between repeated measures on the same village. In the unadjusted analysis, we observed that houses located alongside cultivated swamps had 2.14 times the IRD of houses located alongside natural swamps, although the effect of this variable was not statistically significant (Table 4). For every increase of 1 °C in the average minimum temperature of the village, IRD increased by 73%. The presence of any animal stabled outside the house increased IRD by 60%. The number of occupants in the house the night before spraying appeared to raise the IRD by 25% for each additional person. The wet season was associated with a 13 times increase in IRD over the dry season while the time of day when the capture was made was not associated with differences in IRD.

The final adjusted model for the number of *An. gambiae* s.l. per house included minimum temperature, number of occupants and season (Table 4). The presence of animals was found to have no effect after adjustment for other variables and was therefore not included in the final model. Coefficients for the variables were not much altered after adjustment.

Results for the number of human bites per house are similar to that of IRD above and therefore are not presented. Minimum temperature, wet season, and number of occupants all were associated with an increased rate of human bites per house when adjusted for other variables.

Discussion

The endemic focus of malaria around Lake Bunyonyi in the south-western highlands of Uganda, monitored by the WHO team from 1959 to 1963, demonstrated that malaria transmission at elevations > 1800 m was possible. In contrast, malaria outbreaks in the valleys outside the lake area were uncommon, limited in time and place, and rarely associated with *An. gambiae* s.l. (Zulueta *et al.* 1964). However, in the last 5 years, these valleys have experienced 2 serious malaria outbreaks with thousands of cases and uncounted deaths; transmission appears entirely due to *An. gambiae* s.l. (Mouchet *et al.* 1998; Lindblade *et al.* 1999). Results of our investigation help to explain, in part, how *An. gambiae* s.l. appears to have become more widespread and abundant in the highland valleys of Kabale District since 1963.

The enormous transformations that have occurred in the south-western highlands of Uganda during the past half century have included more than a doubling of the population,

increased transportation and communication with other regions, shifts in land use patterns, introduction of new crops, a decline in basic public health services, and an increase in mean annual temperature. Although any or all of these changes could have affected malaria transmission patterns, we chose to investigate the impact of land use changes, which previously have been blamed for malaria outbreaks. Cultivation of valley papyrus swamps, which began 60 years ago, has produced major changes in land cover and land use in this area. From the mid-1940s, there was concern that ditches formed between ridges constructed for agricultural purposes in the former swamps would encourage mosquito breeding. Several reports noted that anophelines would not breed in papyrus swamps but could be found in the ditches formed during cultivation, and attributed several malaria outbreaks to mosquitoes breeding in these habitats (Steyn 1946; Goma 1958).

Although longitudinal data on malaria before, during, and after cultivation of papyrus swamps began are not available, sufficient natural swamp remains to serve as spatial comparison with the changed area. Thus, we compared malaria transmission parameters between communities located near both natural and cultivated swamps, and found that entomological indices of malaria risk, including IRD, SR, HBR and EIR, were elevated near the latter. Mosquito density during the wet season was consistently higher in cultivated swamps than in natural swamps, although densities in the 2 areas were more similar in the dry season. While differences in transmission parameters between cultivated and natural swamps were not statistically significant, they amounted to an estimated seven-fold increase in the proportion of the population near cultivated swamps that received an infectious bite compared to the population living near natural swamps.

Changes in vegetation generally alter evapotranspiration systems and may modify local climate (Stahl 1993; Tinker *et al.* 1996; Hutjes *et al.* 1998). We found both T_{max} and T_{min} were uniformly higher near cultivated swamps, while RH and saturation deficit patterns also differed according to swamp type. Replacement of dense papyrus stands and other swamp grasses with agricultural crops is the only logical explanation for observed differences in temperature, RH and saturation deficit between swamp types. Temperature is a key determinant of several malaria transmission parameters, including the rate of parasite and mosquito development and mosquito biting rates (Molineaux 1988). The parasite extrinsic incubation period (EIP) equation for *P. falciparum* demonstrates that at temperatures near the lower limit for parasite survival (i.e. 16 °C), small temperature changes have substantial effects on the number of days required for parasite development (Detinova 1962). For example, EIP is reduced by 17.3 days (from 55.5 to 38.2 days) when temperature is increased from 18.0 to 18.9 °C. A similar relationship with temperature exists for larval mosquito development (Clements 1992) and adult biting rates (Lindsay &

Birley 1996). Although RH and saturation deficit are known to affect mosquito longevity (Pal 1943) and, thus, potentially to increase the infective lifespan of a mosquito, the exact relationships between these parameters, and interactions with temperature, are not clear (Clements 1963).

Small increases in temperature near the lower limit for parasite and mosquito development would probably produce greater mosquito densities, higher biting rates and more rapid parasite development in the mosquito. While there may also be a negative effect of temperature on survival of adult mosquitoes at higher temperatures (Clements 1963), the net effect should be a larger proportion of mosquitoes positive for sporozoites and, thus, greater EIR. These theoretical results are consistent with our observations in the cultivated swamps, where higher temperatures were noted.

While swamp type itself was not a significant predictor of IRD or the number of human bites, in a Poisson regression using generalized estimating equations, the mean minimum temperature of the village was associated with both outcome variables. For every 1 °C increase in average minimum temperature, the model indicated an increase of 77% in the number of *An. gambiae* s.l. per house, after adjustment for other variables. Earlier studies hypothesized that swamp cultivation contributed to the entrenchment of malaria in the highlands by creating larval habitat. Our results, however, suggest that malaria transmission indices near cultivated swamps also may have increased due to local temperature changes. The dichotomous variable (natural/cultivated swamp) that we used to examine the impact of cultivation on malaria transmission probably fails to reflect the variation in the extent and type of vegetation that now exists in these areas. The average minimum temperature of a village, which appears to be lower where stands of papyrus still exist, may better capture this variation.

Other characteristics of villages besides location next to a natural or cultivated swamp may have been responsible for observed differences in temperature and malaria transmission parameters. Because of the selection method, however, all houses sampled were similar in construction material as well as distance from the valley bottom. Other variables such as elevation, human population density and extent of adjacent swamp were measured but found not to be significant predictors of IRD or number of human bites. Factors such as general wind direction, average slope, soil type, or use of agricultural insecticides were not measured, but we do not expect these to be associated with swamp type.

While swamp cultivation appears to have affected local temperature and, thus, altered malaria transmission parameters, this is unlikely entirely to explain the increased abundance and distribution of *An. gambiae* s.l. in the area. Annual mean maximum and minimum temperatures have increased 1.0 and 1.2 °C, respectively, since 1960 (unpublished data from the

Kabale District meteorological station 1980 m elevation). These figures are similar to the increase in mean annual temperatures reported for neighbouring Rwanda (Loevinsohn 1994). It is highly probable that this overall temperature increase has improved both parasite and *An. gambiae* s.l. development in the region, although this hypothesis is debated (Mouchet *et al.* 1998).

While it is evident that conditions in Kabale District around Lake Bunyonyi in the middle of this century were suitable for malaria to be transmitted by *An. funestus*, it is also clear that those same conditions were not conducive to malaria transmission by *An. gambiae* s.l. A population of *An. funestus* may have become established around the lake, despite the lower temperatures and subsequent lower development rates, because *An. funestus* larval habitat (i.e. the lake margin) persisted throughout the time required for adult emergence. In the valleys outside the lake area, the slow rate of *An. gambiae* s.l. development, due to the cooler temperatures, may have extended the time to emergence past the lifetime of the preferred habitat of *An. gambiae* s.l. (i.e. small, temporary pools of water), thereby preventing large numbers of mosquitoes to survive to emergence. It is possible that over the years, as mean annual temperature began to rise, the rate of mosquito development also increased, and small pools of water may have become productive *An. gambiae* s.l. larval habitat. Excessive rainfall may help perpetuate suitable *An. gambiae* s.l. larval habitat, thus fostering malaria outbreaks (Lindblade *et al.* 1999), especially in combination with increased temperature. Swamp cultivation may have contributed to this process by providing larval habitat of longer duration (i.e. water-logged ditches between cultivated rows) and increasing local temperature, thereby intensifying both mosquito and parasite development rates.

In summary, our findings reported here and elsewhere (Lindblade *et al.* 1999) suggest the following hypothesis for increased malaria transmission in the highland valleys of south-western Uganda: temperature increases over the last 40 years, including locally elevated temperature near cultivated swamps, combined with occasional excessive precipitation, have sporadically encouraged populations of *An. gambiae* s.l. as well as *Plasmodium* transmission. Whether this scenario is generalizable to other highland areas of Africa where climatic, ecological, epidemiological and entomological profiles differ is unknown. This scenario does provide an outline for further research that would test hypotheses aimed at addressing this emerging problem.

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