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Ecological transition from natural forest to tea plantations: effect on the dynamics of malaria vectors in the highlands of Cameroon

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ABSTRACT

From October 2002 to September 2003, an entomological survey was carried out in a rural forested fringed village in the highlands of Mount Cameroon region to determine the temporal dynamics of the anopheline population and the intensity of malaria transmission. A total of 2387 *Anopheles* spp. were collected, with *A. funestus* predominating (59.9%), followed by *A. hancocki* (24.4%) and *A. gambiae* s.l. (15.7%). Considerable differences were observed in the nocturnal biting cycles of parous mosquitoes, with peak activity in the latter part of the night. PCR revealed that all specimens of the *A. funestus* group were *A. funestus* s.s. and all specimens from the *A. gambiae* complex were *A. gambiae* s.s. of the S molecular form. *Plasmodium falciparum* sporozoite rates of 17.3% and 8.5% were recorded for *A. funestus* and *A. hancocki*, respectively, with an anthropophilic rate of 96.3%. A strong positive correlation ($r = 0.996$) was found between the human-biting rate and the entomological inoculation rate (EIR). Malaria transmission was very high and perennial, with an estimated annual EIR of 460.1 infective bites per person per year. These results confirm that in high agricultural activity areas, *A. funestus* can be by far the major malaria vector responsible for malaria transmission.

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1. Introduction

The rate of malaria transmission in most parts of Africa is very high, with approximately 60% of clinical cases and >80% of the deaths due to malaria occurring in Africa south of the Sahara.[1] According to the *World Malaria Report*,[2] one-half of the world's population is at risk of malaria. *Plasmodium falciparum* malaria was thought to be responsible for an estimated 247 million cases of malaria in 2006, causing 881 000 deaths each year, mostly in children less than 5 years of age. Description of the epidemiology of malaria is often focused on clinical parameters such as prevalence of parasitaemia, despite the fact that entomological information comprises the key transmission parameters essential to the understanding of the epidemiology of malaria in an area. In Cameroon, little updated information is available on the role of local anopheline species in malaria transmission in the highlands where recent environmental changes, including modified land use, may considerably affect vector distribution and the dynamics of malaria transmission. Hence, after a decade of malaria control, updated knowledge on vector capacity and biology of the major vectors is needed.

At present, the malaria control programme in the highlands of Mount Cameroon is confronted by several technical and administrative difficulties that reduce the effectiveness of the programme. Meanwhile, in this particular study site located in the highlands, the control

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methods applied have failed, probably because of several factors, including: (a) geographic location, with difficult access; (b) sociocultural characteristics of the Bakweri population, with frequent migration, belief in a supernatural aetiology of the disease and housing with incomplete or no walls; (c) possible circulation of *P. falciparum* strains with multiple drug resistance owing to self-medication; and (d) the biting and resting behaviour of the principal vectors.

In southwestern Cameroon, studies on the distribution of malaria vectors have been restricted to the lowlands. To date, no studies have been conducted in the highlands on the role of malaria vectors in the transmission of malaria, which has virtually been overlooked. In this context, in order to fill the gaps in the existing knowledge on malaria vectors in southwestern Cameroon, a longitudinal study was carried out in an isolated forest fringed village in the highlands of Mount Cameroon region to estimate the risk arising from the major malaria vectors occurring in this remarkable ecological landscape. This was the first longitudinal study providing data describing the variation of the entomological inoculation rate (EIR) according to time and season and its role in the transmission of *P. falciparum* in this fragmented forest habitat in a high-altitude ecological zone of southwestern Cameroon where malaria is endemic. This study answers the needs expressed by the National Malaria Control Programs, who are confronted with a complex vector situation in a changing environment where evaluation and possible reassessment of control practices is essential. The findings of this study will help to build up coherent, suitable and cost-effective vector control measures in keeping with new challenges and will contribute to sustaining the success of malaria control programmes in the highlands.

2. Materials and methods

2.1. Study area

This study was carried out from October 2002 to September 2003 in Likoko, a rural forested village located in Fako division (4°10′N and 9°10′E), Cameroon, with a surface area of approximately 14.48 km^2 (Figure 1). The study area is a mountainous wet tropical forest interspersed with streams that increase in number during the rainy season. Most of the original forest in the area has been cleared by logging for tea plantations. Topographically this is a highland village situated at a height of 800 m above sea level at 4°13′N and 9°14′E. The area has a tropical climate with a mean annual rainfall of approximately 2668 mm. Relative humidity remains above 75 ± 5% throughout the year. The study village was comprised of 102 households with 835 inhabitants. The people living in this study site are mostly from the Bakweri tribe and practice subsistence farming, although they serve principally as labourers on the nearby tea estate. They live in traditional houses made of woody walls with thatched or corrugated zinc roofs. The number of occupants sleeping in the selected houses varied from three to eight. Pigs, goats and dogs are the most common large domestic animals bred in the village as well as plenty of chickens. The village lacks public transportation, electricity and running water, and the nearest medical facility is located approximately 25 km away. Meteorological data relating to minimum and maximum temperatures, rainfall and relative humidity were obtained from an agro-industrial company, Cameroon Development Corporation (CDC), at Tole Tea Estate approximately 3 km from the study village. Prior to the study, permission was sought from the village elders, following which village meetings were conducted to explain the purpose of the study and to request participation. Verbal consent was also obtained from house owners and their compound heads for permission to collect mosquitoes from their houses.

2.2. Mosquito collection and processing

The entomological survey was conducted for 12 months from October 2002 to September 2003. Two methods were used, which included human-landing catches (HLC) and pyrethrum spray catches (PSC). PSCs were carried out in the day, whilst HLCs were performed at night. For the day collections, a team of three people estimated indoor resting density using the PSC method in 36 randomly chosen houses between 15:00 h and 17:00 h. Collections were performed in rooms that had not used any form of insecticide or repellent during the previous week. The average number of people sleeping in a room ranged from one to ten (mean of three people). Day collections consisted of removing all foodstuff and utensils from the room and covering all exposed surfaces with white sheets. Insecticide aerosol containing tetramethrin, δ-allethrin, dichlorvos and permethrin from a pressurised can was sprayed inside the room for 5–10 s and the room was left with the doors and windows closed for 15 min, after which all the dead and immobilised mosquitoes were collected off the white sheet, identified, counted and put into labelled tubes. To analyse feeding preferences, only blood-fed mosquitoes collected using PSCs had their blood meal squashed on Whatman No. 1 filter paper, which was then stored dry individually in tubes containing desiccant and kept at 20 °C until testing for blood meal analysis. Blood components from the spots were eluted in normal saline overnight and the origin of the blood meal was then determined by ELISA to derive the human blood index.[3]

Night captures were conducted in two randomly selected houses per locality, at least 40 m apart, on the 15th and 16th of each month. Two teams of four volunteers each (a total of eight persons) conducted the HLCs each month in randomly selected houses. The collectors worked in pairs with a personnel change at midnight, with one pair working from 18:00 h to 00:00 h and the next from 00:00 h until 06:00 h. At each house, a collector was posted indoors and another outdoors with a flashlight and a mouth aspirator. Collection teams were rotated among blocks each month in an attempt to limit temporal and/or collector bias. Each month, prior to continuing surveys of non-sampled households, an attempt was made to inspect premises that were previously closed or where access had been refused. Access to these areas was attempted at least three times. A different pair of houses was visited every month. All volunteers received malaria prophylaxis throughout the study period.

Mosquitoes were sorted to species level according to identification of morphological characteristics.[4,5]

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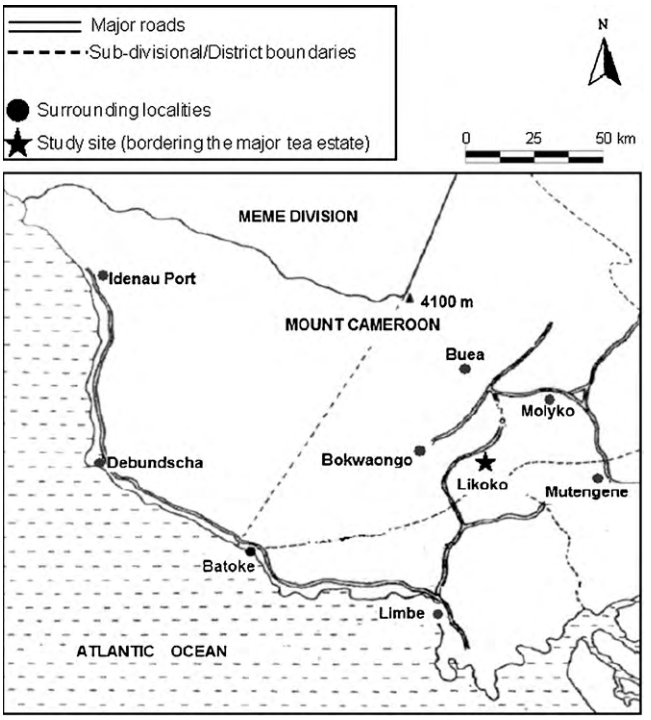

Figure 1. Map of the study area showing Likoko, a forest fringed village in the highlands of the Mount Cameroon region.

All mosquitoes from the HLCs belonging to the *Anopheles* population were dissected for parity determination and the ovaries were examined to establish parity.[6,7] Hourly catches of anopheline mosquitoes were identified as either parous or nulliparous depending on whether the ovarian tracheoles were coiled or uncoiled. Parous mosquitoes were stored over silica-gel desiccant until tested for circumsporozoite protein (CSP).

The head and thorax of each dissected female anopheline mosquito was kept in a separate well of a 96-well microtitre plate for later analysis of CSP of *P. falciparum*, *P. malariae* and *P. ovale* by ELISA;[8] *P. vivax* is not present in this region of Africa. The EIR (a standard measure of transmission intensity), expressed as the number of infective bites per person per unit time, was calculated as the product of the human-biting rate (HBR) and the sporozoite rate for each sampling period.[9] The HBR was derived directly from HLCs and was expressed as the number of bites/person/night. The annual and per month inoculation rates were derived by multiplying the daily EIR (infective bites/person/night) by 365 days and 30 days, respectively.

Legs and wings of individual *A. gambiae* s.l. mosquitoes were kept in Eppendorf tubes with silica gel desiccant for later molecular identification of sibling species, and ELISA-CSP positive for *A. gambiae* s.s. molecular forms were further analysed for M and S forms in a subset of individuals using PCR-RFLP as previously described.[10,11] Primers were specific for *A. gambiae* s.s., *A. arabiensis* and *A. melas* members of the *A. gambiae* complex. All females in the *A. funestus* group were also analysed by PCR diagnostic assays for species identification.[12]

2.3. Data analysis

Correlations between mosquito abundance, EIR and environmental data (including temperature, relative humidity and rainfall obtained from the meteorological service) were analysed using multiple linear regression. The χ^2 test was also used to test the significance of variations between HBRs with regard to host location (indoor or outdoor), abundance, sporozoite rates and EIR. All statistical analyses were performed in Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Identification and abundance of vector species

During the 96 person-nights of collection, a total of 654 culicines and 2387 female anopheline mosquitoes were captured between October 2002 and September 2003. *Anopheles funestus* was the most abundant, accounting for 59.9% of total anophelines caught, followed by *A. hancocki* (24.4%) and *A. gambiae* s.l. (15.7%) (Table 1). Subfamily Culicinae were represented by different species, principally *Mansonia africana* (Theobald), *Culex quinquefasciatus* Say and *Aedes aegypti*.

Despite the observation that all three *Anopheles* spp. populations showed seasonal variation, with high populations occurring in the peak rainy season or towards the end of the rainy season, there was no significant correlation between monthly rainfall totals and relative abundance of *A. funestus* s.s. ($r = 0.486$, $P = 0.117$) and *A. hancocki*

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Table 1
Relative abundance of indoor and outdoor human bait collections of anopheline mosquitoes at Likoko, southwestern Cameroon (October 2002 to September 2003)

Month	*Anopheles funestus*		*Anopheles gambiae* s.l.		*Anopheles hancocki*		Total
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	
Oct. 2002	160 (17.02)[a]	54 (11.04)	2 (0.88)	–[b]	40 (13.99)	28 (9.43)	284 (11.90)
Nov. 2002	49 (5.21)	15 (3.07)	2 (0.88)	1 (0.68)	23 (8.04)	36 (12.12)	126 (5.28)
Dec. 2002	23 (2.45)	11 (2.25)	–	–	18 (6.29)	27 (9.09)	79 (3.31)
Jan. 2003	31 (3.30)	18 (3.68)	8 (3.52)	7 (4.73)	18 (6.29)	39 (13.13)	121 (5.07)
Feb. 2003	42 (4.47)	28 (5.73)	16 (7.05)	14 (9.46)	35 (12.24)	22 (7.41)	157 (6.58)
Mar. 2003	22 (2.34)	15 (3.07)	7 (3.08)	3 (2.03)	27 (9.44)	26 (8.75)	100 (4.19)
Apr. 2003	10 (1.06)	2 (0.41)	3 (1.32)	1 (0.68)	12 (4.20)	6 (2.02)	34 (1.42)
May 2003	6 (0.64)	2 (0.41)	6 (2.64)	1 (0.68)	4 (1.40)	2 (0.67)	21 (0.88)
June 2003	–	17 (3.48)	7 (3.08)	4 (2.70)	10 (3.50)	2 (0.67)	40 (1.68)
July 2003	161 (17.13)	79 (16.16)	19 (8.37)	18 (12.16)	15 (5.24)	16 (5.39)	308 (12.90)
Aug. 2003	195 (20.74)	89 (18.20)	117 (51.54)	87 (58.78)	29 (10.14)	18 (6.06)	535 (22.41)
Sept. 2003	241 (25.64)	159 (32.52)	40 (17.62)	12 (8.11)	55 (19.23)	75 (25.25)	582 (24.38)
Total	940 (65.78)	489 (34.22)	227 (60.53)	148 (39.47)	286 (49.06)	297 (50.94)	2387 (100)

[a] Values in parentheses represent monthly catches as a percentage of total collection of that species in that collection point, except for the 'Total' row where they represent the percentage of each species collected indoors and outdoors.
[b] Dashes indicate that no sample for the *Anopheles* spp. at that particular collection point was obtained during the survey.

($r = -0.126$, $P = 0.696$). However, *A. gambiae* s.l. was positively correlated with precipitation ($r = 0.71$, $P < 0.001$) and was negatively correlated with temperature ($r = -0.742$, $P = 0.351$) and humidity ($r = 0.824$, $P = 0.572$) measures.

The HBR (bites/person/night) varied significantly between months ($P < 0.001$). *Anopheles funestus* biting activity was concentrated between July and October, whilst that of *A. gambiae* was in August and that of *A. hancocki* in September. The highest HBRs of 42.6 bites/person/night indoors and 30.8 bites/person/night outdoors were observed in August and September, respectively. For *A. funestus*, peak indoor (30.1 bites/person/night) and outdoor (19.9 bites/person/night) biting rates were recorded in September. For *A. gambiae* s.l., biting rates were generally low throughout the survey in the village (range 0.0–6.5 bites/person/night), except in August with a peak biting activity of 25.5 bites/person/night. *Anopheles gambiae* was the least captured vector in the survey. Table 1 summarises the monthly relative abundance of indoor and outdoor human bait collections of the *Anopheles* spp. at Likoko. A preponderance of *Anopheles* spp. was observed in the indoor collections. Among the total of 2387 *Anopheles*, more than one-half ($n = 1453$; 60.9%) were caught biting indoors. *Anopheles hancocki* was the most common species in outdoor collections, with 50.9% of the catch of this species made outdoors, although there was no significant difference between the indoor and outdoor feeding behaviour of this species. *Anopheles funestus* s.s. was observed to exhibit a strong endophagic biting behaviour ($P < 0.001$). The number of *Anopheles* spp. collected indoors (60.9%; $n = 1453$) and outdoors (39.1%; $n = 934$) in HLCs was significantly different ($P < 0.001$).

All of the *A. gambiae* s.l. ($n = 347$) analysed by PCR to respective sibling species were *A. gambiae* s.s. No *A. melas* were identified from the studied samples. This indicates that *A. gambiae* s.s. is the only sibling species of the *A. gambiae* Giles complex represented in the highlands of southwestern Cameroon. Furthermore, all the 92 specimens of the *A. gambiae* complex identified by PCR were *A. gambiae* s.s. belonging to the S molecular form. PCR identification within the *A. funestus* group revealed that all the specimens tested ($n = 214$) were *A. funestus* s.s.

3.2. Biting activity

Considerable differences were observed in the night biting cycles of parous *Anopheles* females (Figure 2). Each species had different periodicity of human biting activity both indoors and outdoors. For *A. funestus*, two major biting peaks were observed at 01:00–02:00 h and 04:00–05:00 h indoors and at 00:00–01:00 h and 02:00–03:00 h outdoors. *Anopheles gambiae* s.l. had a major peak outdoors before midnight but with two small peaks after midnight. The biting cycle of parous mosquitoes in the indoor and outdoor environment was significantly different with multiple peaks. All mosquitoes were dissected for parity determination and the results are shown in Figure 3.

3.3. Feeding preference

A total of 239 blood meal spots were tested by ELISA for host identification. These were collected both from indoor and outdoor resting females, including 163 *A. funestus*, 19 *A. gambiae* and 57 *A. hancocki*. All specimens had fed on human hosts, recording a high human blood index of 96.3%. A small proportion of mosquitoes in this village had fed on goat and dogs (1.2%). Of all available hosts, humans were the most common blood source for *Anopheles* mosquitoes, followed by chickens (2.5%).

3.4. Circumsporozoite protein rate and entomological inoculation rate

The relationships between sporozoite rate for all three *Anopheles* spp. and the mean monthly rainfall and temperature pattern in the area are shown in Figure 4. The difference between the mean *P. falciparum* sporozoite rate for *A. funestus* (12.5%), *A. gambiae* s.l. (3.1%) and *A. hancocki* (10.6%) over the 12-month study period was significant ($P < 0.001$). No *A. gambiae* s.l. positive for *P. falciparum*

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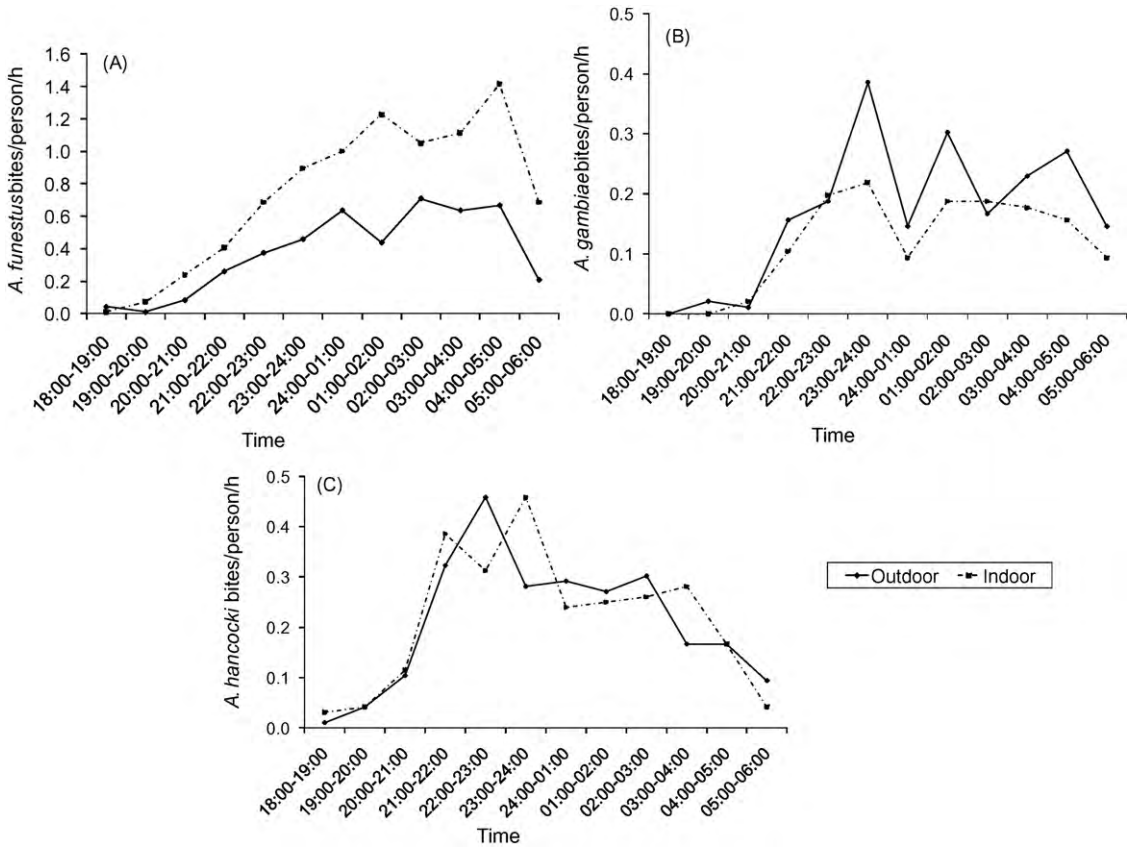

Figure 2. The 12-h biting patterns of parous (A) *Anopheles funestus*, (B) *A. gambiae* and (C) *A. hancocki* mosquitoes captured from indoor and outdoor human bait collections during 96 all-night collections performed from October 2002 to September 2003 in Likoko, southwestern Cameroon.

sporozoite antigens were recorded, except in February, May and September. Generally high sporozoite rates for *A. funestus* (range 0–14.5%) and *A. hancocki* (0–16.7%) were obtained throughout the study period. For *A. funestus*, high positivity rates were recorded, with the peak mean monthly rate occurring in October coinciding with the early dry season, whilst for *A. hancocki* the peak infection rate was recorded in May coinciding with the early rainy season.

There was a very strong positive correlation between the HBR and the EIR for *A. funestus* ($r = 0.984$) and *A. hancocki* ($r = 0.960$). *Anopheles funestus* and *A. hancocki* were the most efficient vectors in the highlands. *Anopheles gambiae*

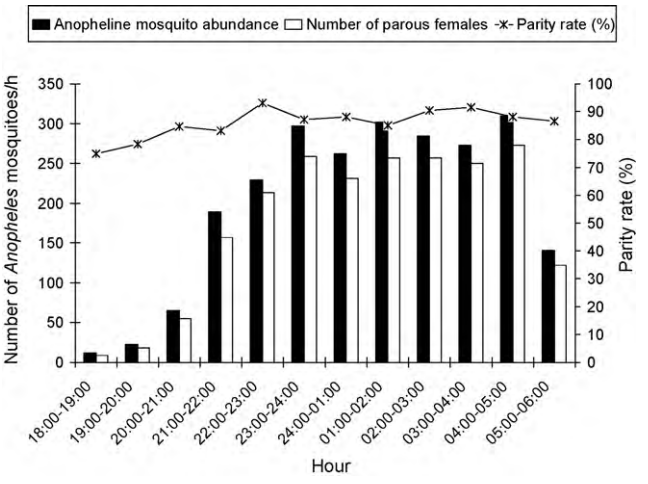

Figure 3. Number and parity rates (%) of hourly collections of *Anopheles* mosquitoes from human landing collections conducted from October 2002 to September 2003 in Likoko, southwestern Cameroon.

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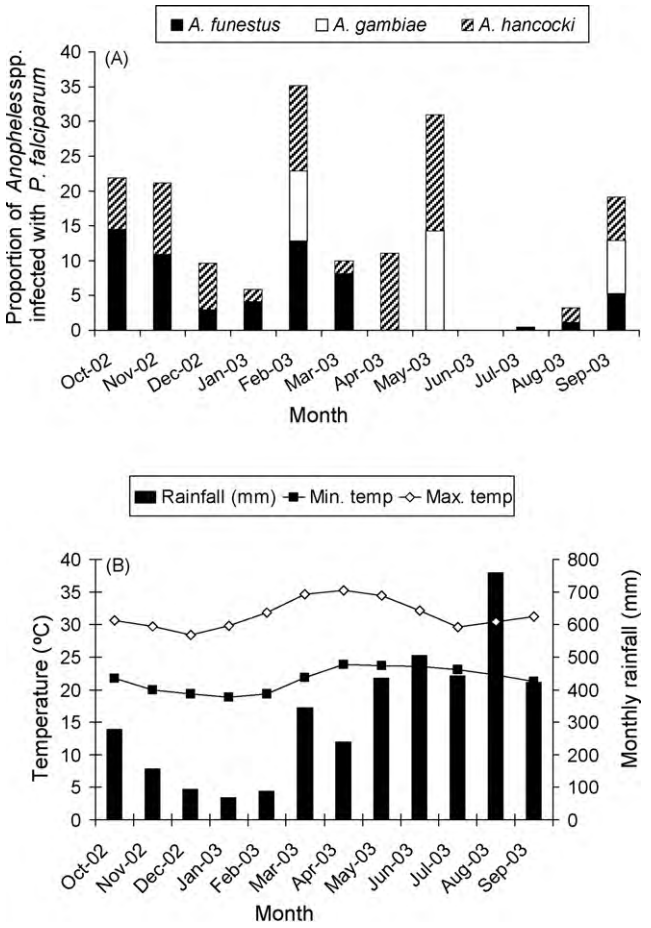

Figure 4. Seasonal variation in (A) circumsporozoite protein rate (i.e. proportion of *Anopheles* spp. infected with *Plasmodium falciparum*) and (B) mean monthly rainfall and minimum and maximum temperatures over the 1-year period of the longitudinal study from October 2002 to September 2003 in Likoko, southwestern Cameroon.

was infective only for 3 months throughout the 12-month study period (0.01–0.08 infective bites/person/night). The mean EIR in this highland village over the 12-month study period (365 days) was 296.5 infective bites/person/year for *A. funestus* and 133.1 infective bites/person/year for *A. hancocki*, giving a ratio of 2.2:1 for the two species. For *A. gambiae* s.l., the annual EIR was 30.4 infective bites/person/year. The combined EIR for the three species was 460.1 infective bites/person/year for this site. The number of infective bites/person/night for *A. funestus* was observed to increase progressively from June to October, which then dropped gradually between November to December with decreased rainfall amounts and then showed a small increase in the dry season (December to February) giving a double peak of transmission (Figure 5). *Anopheles gambiae* had three peaks, whilst *A. hancocki* had a double peak. Despite the apparent seasonal variation in EIR rates observed, correlation analysis revealed a positively significant linear relationship between EIR and HBR for *A. funestus* ($r = 0.984$, $P < 0.001$) and *A. hancocki* ($r = 0.959$, $P < 0.001$). There was no correlation between EIR and HBR for *A. gambiae* s.l. The highest EIRs for *A. funestus* were experienced in August and September, corresponding to the peak rainy season, and in October, which is the beginning of the dry season. The number of days it would take for an individual to receive an infective bite from an infected mosquito was 1.7 days for *A. funestus*, 5.1 days for *A. hancocki* and 12.2 days for *A. gambiae* during September.

4. Discussion

The intensity of malaria transmission is heterogeneous in this rural forested village in the highlands of Mount Cameroon region and is greatly influenced by the mosquito species composition, vector competence, and underlying demographic and environmental factors.[13] High levels of transmission frequently occur where both *A. gambiae* s.l. and *A. funestus* are present, as they tend to exploit different breeding habitats and peak at different times, thereby prolonging the transmission period. Generally, *A. gambiae* s.l. is most abundant during the rainy season, and *A. funestus* is predominant at the end of the rains and the beginning of the dry season.[14,15] The extent to which these species are influenced by the same environmental factors is largely unknown, as very few studies have examined them simultaneously over a wide geographical range.

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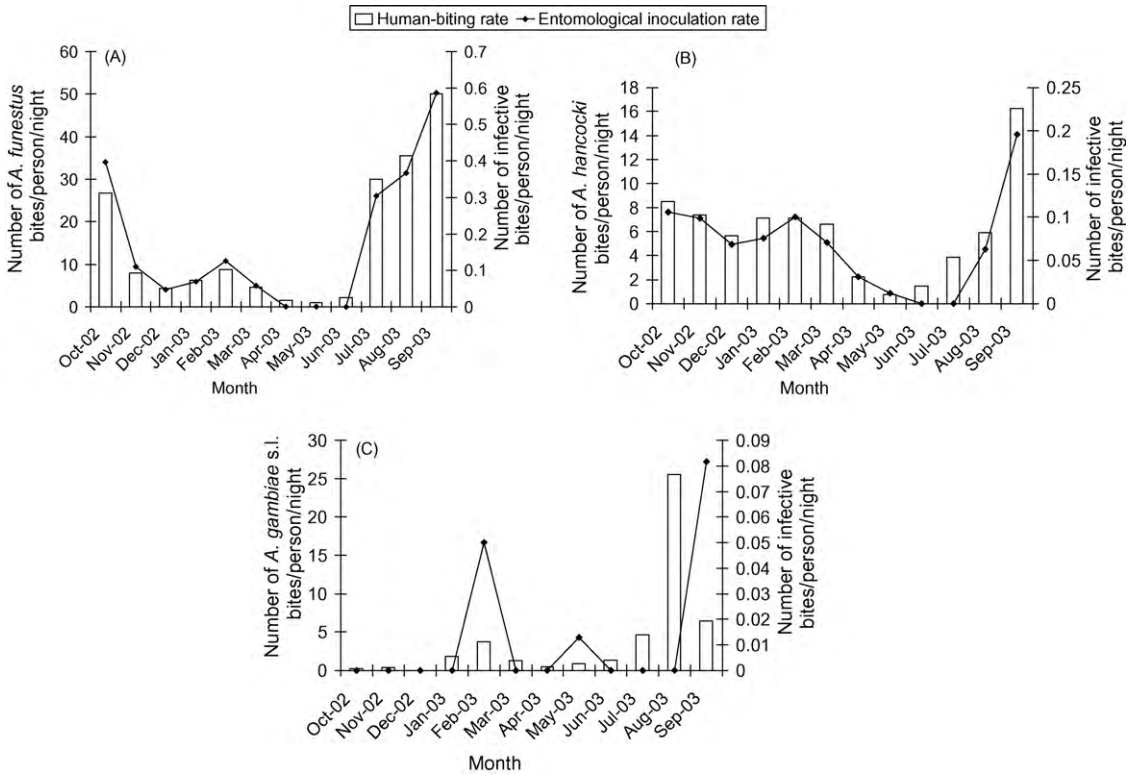

Figure 5. Human biting rate (number of bites/person/night) and entomological inoculation rate (number of infective bites/person/night) calculated by ELISA for all three *Plasmodium falciparum*-infected vectors from October 2002 to September 2003 in Likoko, southwestern Cameroon. (A) *Anopheles funestus*, (B) *A. hancocki* and (C) *A. gambiae*.

Although *A. gambiae* s.l. has been well studied in depth and uncertainly reported as the major vector of *Plasmodium* continent-wide, it is frequently associated with other anopheline species that overcome its importance in malaria transmission in certain areas.[4] This is the case in the rural forested environment in the highlands of southwestern Cameroon where the levels of malaria transmission are very high, stable and perennial, with three anopheline species aggressively involved simultaneously in malaria transmission. Among these malaria vectors, *A. funestus* and *A. hancocki* are the primary and most important vectors of malaria in this area. The bionomics of *A. funestus*, closely related to their ability to take blood from humans (anthropophilic), their indoor resting (endophilic) and their high susceptibility to human malaria parasites endows *A. funestus* in the highlands with a high vectorial ability, which is significantly higher than that of *A. gambiae* s.l.[16,17] These findings corroborate other studies carried out in Senegal and in a forested area of southern Cameroon, although these studies were not in the highlands.[16,17] Contrary to the findings from our study, the vectorial system in the western Kenyan highlands is dominated by *A. gambiae*, whereas *A. funestus* plays a minor role in transmission.[18,19] Malaria vector species distribution in the highlands where massive deforestation of virgin forest has been carried out for establishment of large tea plantations is temporally heterogeneous and dynamic, implying that several factors may have contributed to the large temporal variation in their abundance. Land use change has been reported to influence malaria transmission by increasing the temperature and decreasing the humidity of vector mosquito habitats, which in turn affects biting, survival and reproductive rates of malaria vectors.[20–23]

All of the sporozoite-positive samples of *A. gambiae* s.l. analysed by PCR were identified as *A. gambiae* s.s., showing that this could be the only member of the *A. gambiae* Giles complex represented in the highlands of southwestern Cameroon. The absence of *A. melas* in the highlands could be attributed to the cooler temperatures and the extremely low salinity of the freshwater streams (usually < 0.5 parts per thousand), both of which do not appear to favour this species. PCR-RLFP performed to distinguish between the molecular forms revealed that the S form is the sole form found in the highlands.[24] However, our study indicates that despite the absence of the M form in the highlands, the S form contributes significantly to levels of malaria in the population. Although the status of these molecular forms as incipient species is still a controversial issue, this study is the first to underline their geographical occurrence in the highlands of southwestern Cameroon and therefore contributes to an increase in the body of data available on the distribution, relative prevalence and role in human malaria transmission.[25] In West Africa, the chromosomal forms of *A. gambiae* s.s have been shown to have differing spatial distributions and environmental parameters, and distinct differences

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between the M and S molecular forms have been described in Mali.[26–29]

The observed increase in malaria transmission in the highlands of southwestern Cameroon where tea plantations have been well established is not an unusual situation because tea plantations, for example in the Kericho District of Kenya, have kept some of the most intensive long-term time series for malaria records in Africa.[30] Although the relationship between the rise in temperature and its impact on malaria transmission in the highlands has been extensively debated, it remains an important factor of concern because the effects of climate change on malaria risk are not geographically uniform and are the most sensitive at the edge of the distribution of the disease.[30] Some authors in an earlier attempt to explain malaria transmission in the highlands strongly recognised the potential importance of indoor resting habits of the vectors and the higher ambient temperatures indoors, and this is in accordance with our findings where the most common and efficient African vectors (*A. funestus*, *A. hancocki* and *A. gambiae*) were observed both indoor and outdoor throughout the longitudinal study period.[31,32] This indoor resting habit of the vectors indeed provided indoor temperature conditions that are more conducive for *Plasmodium* parasite development, and this phenomenon is more striking in the highlands because the residences are inhabited and overcrowded.[33]

The source of blood meals is a critical determinant of the potential of a species to be a vector of disease and it is important to medical entomologists and epidemiologists for understanding host–vector relationships and the dynamics of disease transmission.[34] The effect of species' human blood-feeding rates for *Anopheles* mosquitoes collected from the village site was significant ($P < 0.001$), with the rate for *A. funestus* significantly higher than that for *A. gambiae* s.l. and *A. hancocki*. Although the human blood-feeding rate can vary depending on where the resting mosquitoes are collected, several authors report that a high human blood index of 36.0–93.4% can be recorded from indoor-resting mosquitoes, which corroborates the present study.[35–37] However, the results of this study indicate that differences in host-feeding patterns do exist in sympatric *Anopheles* spp. and thus imply that these species are the most likely malaria vectors in the study area and will readily feed on humans irrespective of the availability of other domestic animals.[38] The higher human blood-feeding rates for *A. funestus* suggest that these females seek blood when the potential for human contact is probably greatest as people are presumed to be in bed. The degree of human feeding influences the probability that mosquitoes will come into contact with gametocyte carriers and thus acquire *Plasmodium* infections. The present study provides a better understanding of the influence of local ecology on the intensity and seasonal patterns of malaria transmission in the highlands and represents important baseline data essential for implementing control programmes. With the local human population in the study area usually staying in bed between 22:00 h and 06:00 h, only 19.8% (24/121) of the infective bites would occur before they retire to bed. Thus, if insecticide-treated bed nets (ITN) are widely distributed in the highland rural villages, approximately 80.2% (97/121) of the infective bites would probably be prevented. Consequently, the late biting behaviour of the most dangerous parous *A. funestus*, *A. hancocki* and *A. gambiae* s.l. is a significant finding with respect to increased use of ITNs for the reduction of malaria in the southwest province of Cameroon.

Some authors report that a method of vector control which offers an alternative to house spraying is pyrethroid impregnation of bed nets with respect to the reported operational cost (including labour, transportation, insecticide and consumables) per person of insecticide house spraying.[39] The saving in costs by introducing impregnated bed nets, the attractiveness of the method to householders as observed in other countries and in a small trial in southern Venezuela, and its effectiveness for exophilic and endophagic mosquito suggest that this measure could be feasible and effective in this rural forested village in the highlands of Mount Cameroon region.[40,41] Against *A. gambiae* s.l. and *A. funestus*, communitywide use of impregnated bed nets has been shown to be effective in reducing population density, survival and sporozoite rate.[42,43] The same could be expected with *A. hancocki* so that biting indoors and outdoors by this species during the evening would become less risky. However, it can be presumed that impregnated nets would not be effective for controlling other potential vector species that bite early and mainly outdoors. Therefore, it would be appropriate to motivate the human population to use supplementary protective measures such as insect repellents on the skin or on clothing.

Authors' contributions: MCT identified data sources and conceived the experimental design of the study, conducted field work, carried out data analysis, interpreted the results and wrote the first draft of the manuscript; WIN contributed substantially to conception, study design, interpretation of data and critically reading the manuscript for important intellectual content. Both authors read and approved the final manuscript and are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: Permission to carry out this study was obtained from the Human Resources and Health Departments of the Cameroon Development Corporation (CDC), and ethical clearance was properly sought from the National Ethical Review Committee of Cameroon but was deemed unnecessary. A sensitisation rally was organised with the population, during which the purpose of the study was clearly explained. Participation in the study was voluntary.

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