High Malaria Transmission Intensity Due to *Anopheles funestus* (Diptera: Culicidae) in a Village of Savannah–Forest Transition Area in Cameroon

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ABSTRACT An entomological survey was conducted on vectors of malaria in a village of the forest–savannah transition area in Cameroon from February 1999 to October 2000. A total of 2,050 anopheline mosquitoes belonging to eight species were caught 1) after landing on human volunteers, 2) by using pyrethrum spray collections in human dwellings, and 3) in resting sites outdoors. *Anopheles funestus* Giles was the most abundant species (accounting for 91% of anophelines caught) followed by *Anopheles gambiae* Giles (7%). Applying polymerase chain reaction led to the identification of all specimens of the *An. funestus* group as *An. funestus* sensu stricto and mosquitoes from the *An. gambiae* complex were mostly *An. gambiae* sensu stricto of the S molecular form. Malaria transmission was perennial with an entomological inoculation rate estimated at 172 infective bites per person during the period of study. *An. funestus* was responsible for 88% of the total malaria transmission, with a *Plasmodium falciparum* circumsporozoïte rate of 6.8% and an anthropophilic rate of 99.3%. These results confirm that in high agricultural activity areas, *An. funestus* can be, by far, the major malaria vector.

KEY WORDS *Anopheles funestus*, malaria transmission, Cameroon

Anopheles gambiae GILES HAS been studied in depth, but although it can arguably be considered the major vector of *Plasmodium* continent-wise (Gillies and De Meillon 1968), it is frequently associated with other anopheline species that overcome its importance in malaria transmission in certain areas. This is the case in equatorial Africa where the levels of malaria transmission are very high, stable, and perennial. For example, in the rural forested environment of southern Cameroon, at least five species are involved in malaria transmission. From one village to another, sometimes separated by only a few tens of kilometers, malaria transmission can be mainly due to *An. gambiae* (Manga et al. 1997a; Meunier et al. 1999; Wanji et al. 2003), *Anopheles moucheti* Evans (Manga et al. 1995, Antonio-Nkondjio et al. 2002), *Anopheles nili* Theobald (Carnevale et al. 1992), or *Anopheles funestus* Giles (Manga et al. 1997b). *Anopheles hancocki* Edwards also can be of local importance in malaria transmission (Fontenille et al. 2000, Wanji et al. 2003). Among these malaria vectors, *An. funestus* is one of the most important. Its bionomics, closely related to human (anthropophilic and endophilic), and its high susceptibility to human malaria parasites endows *An. funestus* with high vectorial ability, in some cases significantly higher than *An. gambiae* (Fontenille et al. 1997, Manga et al. 1997b). Therefore, any control strategy to be implemented in the field should consider the diversity of this complex vector system. This is particularly crucial if these strategies are to be based on release of genetically transformed mosquitoes with altered vector competence for *Plasmodium.* However, our present knowledge on population dynamics and role in transmission of malaria vectors, other than members of the *An. gambiae* complex, is by far not complete. Importantly, it seems that most of these secondary vectors also belong to species complexes or groups of morphologically very similar species with very different importance as malaria vectors, a feature that seems to be common to several malaria vectors, including *An. gambiae.* Thus, accurate species recognition may be required to target the true vector species and implement suitable (i.e., specific and selective) vector control measures. Accurate species identification within anophelines mosquito species complexes is now possible through the use of straightforward, polymerase chain reaction (PCR)-based, diagnostic tests. Such an assay has been used for more than a decade to identify species within the *An. gambiae* complex

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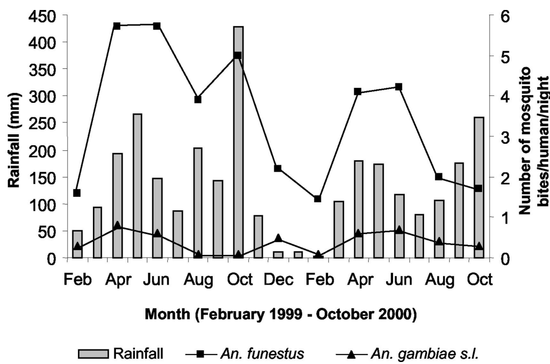

Fig. 1. Rainfall and indoor human biting rates for malaria vector species in Nkoteng, from February 1999 to October 2000.

(Scott et al. 1993) and has recently been developed for other African malaria vectors such as *An. funestus* (Koekemoer et al. 2002, Cohuet et al. 2003) and *An. nili* (Kengne et al. 2003). By allowing more precise identification and characterization of vector populations in the field, these tests will undoubtedly be essential for the study of malaria transmission dynamics and vector species turnover and will help building a comprehensive view of transmission heterogeneities commonly observed in sub-Saharan Africa. For example, the use of such diagnostic tool recently revealed that *Anopheles parensis* Gillies, which is not a vector of human malaria, was almost the only member of the *An. funestus* group that was found resting inside human dwellings in a central area of Kenya (Kamau et al. 2003).

Three members of the *An. funestus* group have been identified in Cameroon (Mouchet and Gariou 1961, Cohuet et al. 2003). Here, we investigated the role of *An. funestus* in malaria transmission in a village where high densities of this species have been reported (Dia et al. 2000).

Materials and Methods

Study Area. The study was carried out in the village of Nkoteng (4° 30′ N, 12° 03′ E) located in a rural area of the central province of Cameroon, in the forest–savannah transition. Several thousands of inhabitants live in this village in traditional houses with mud walls and roofs of corrugated iron. Most people work at the local sugar cane plantation. The climate is equatorial, with two rainy seasons, extending from March to June and September to November. Mean annual rainfall averaged over the period 1965–1996 was 1416 mm. In 1999, we recorded 1,719 mm of total rainfall and 1,213 mm in 2000 (Fig. 1). The mean monthly temperature between 1975 and 2000 was 24.8°C, ranging from 23.4°C in July to 27°C in February. Pigs, sheep, goats, chickens, and cows were bred in the village.

Field Sampling and Processing of Mosquitoes. Entomological surveys were conducted every 2 mo from February 1999 to October 2000. Adult mosquitoes were collected by human volunteers when landing on legs, for two consecutive nights, from 7 p.m. to 6 a.m. in 10 different indoor locations in the village. The human biting rate was expressed as the average number of mosquito bites per person per night.

Table 1. Number of anophelines collected from February 1999 to October 2000 in Nkoteng village by three methods

Mosquito species	Indoor feeding	Indoor resting	Outdoor resting	Total
An. funestus	767	968	133	1,868
An. gambiae s.l.	86	57	3	146
An. hancocki	22	1	0	23
An. moucheti	5	1	0	6
An. nili	5	0	0	5
An. wellcomei	1	0	0	1
An. zeimanni	1	0	0	1
Total	887	1,027	136	2,050

Indoor-resting mosquitoes were collected in the afternoon inside bedrooms by using pyrethrum spray, and outdoor-resting mosquitoes were collected by using mouth aspirators in a pit shelter and an empty barrel.

Anophelines were sorted according to the morphological identification keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). To analyze feeding preferences, blood meal spots were collected on filter paper after dissecting the midgut of freshly fed resting females. Each specimen was stored individually in tubes containing desiccant and kept at −20°C until processed in the laboratory.

Laboratory Processing of Anophelines. The origin of the blood meal was determined by enzyme-linked immunosorbent assay (ELISA) as described in Beier et al. (1988). The technique identified human, bovine, ovine, equine, pig, and chicken blood.

The head and thorax of female anophelines were tested for detection of the circumsporozoïte protein (CSP) of *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale* by ELISA according to Burkot et al. (1984) and Wirtz et al. (1987). *Plasmodium vivax* is not present in this region of Africa. The entomological inoculation rate (EIR) was calculated as the product of the human biting rate by the CSP rate for each sampling period and overall.

Representative samples of females from the *An. funestus* group and the *An. gambiae* complex, including all the specimens collected resting outdoors, were analyzed by PCR diagnostic assays described by Cohuet et al. (2003) and Scott et al. (1993), respectively, for species identification. Female *An. gambiae* s.s. were further analyzed for their molecular form (M or S) according to Favia et al. (2001).

Results

Identification and Abundance of Vector Species. A total of 2,050 anopheline mosquitoes were caught. Seven anopheline species or species complex were identified on morphological basis: *An. funestus* s.l., *An. gambiae* s.l., *An. hancocki*, *An. moucheti*, *An. nili*, *Anopheles wellcomei* Theobald, and *Anopheles ziemanni* Grünberg (Table 1). *An. funestus* s.l. was the

Table 2. Monthly circumsporozoïte protein rate (CSPR) (%) and entomological inoculation rate (EIR) (number of infected bites per person per month) for *An. funestus* and *An. gambiae* in Nkoteng from February 1999 to October 2000

Mosquito species	*An. funestus*			*An. gambiae* s.l.		
	Nt	CSPR	EIR	Nt	CSPR	EIR
1999						
Feb.	102	5.9	2.6	15	6.7	0.6
April	274	5.8	10.0	22	4.6	1.1
June	214	7.5	12.8	14	14.3	2.6
Aug.	198	5.6	6.7	2	0	0
Oct.	307	5.2	8.1	2	50	0.8
Dec.	73	6.9	4.7	8	0	0
2000						
Feb.	54	9.3	3.9	2	0	0
April	117	9.4	11.6	12	25	4.1
June	142	7.0	8.9	16	0	0
Aug.	27	7.4	4.6	1	0	0
Oct.	39	5.1	2.7	5	0	0
Total[a]	1.547	6.5	153.2	99	8.1	18.4

Nt, no. of mosquitoes tested by ELISA.

[a] Total no. of mosquitoes tested, overall CSP rate, estimated EIR across 22 mo (January 1999–October 2000).

most abundant, accounting for 91% of total anophelines caught, followed by *An. gambiae* s.l. (7%). PCR identification within the *An. funestus* group revealed that all the specimens tested ($N = 352$, including 133 specimens collected outdoors), were *An. funestus* s.s. Furthermore, 76 females of the *An. gambiae* complex were identified by PCR. Two of those specimens were *Anopheles arabiensis* Patton, representing ≈3% of *An. gambiae* s.l. All others 74 specimens were *An. gambiae* s.s., 73 of which belong to the S molecular form and only one showed a M profile. The three specimens collected outdoors were *An. gambiae* s.s. of the S molecular form.

The overall indoor human biting rate, averaged over the period of study, was 3.4 bites per person per night for *An. funestus* and 0.4 for *An. gambiae* s.l. Both species were present all year long, with marked seasonal fluctuations in abundance (Fig. 1). Peak abundance was observed during the rainy seasons in April–June (4–5.6 bites per person per night for *An. funestus* and 0.8–1 bite per person per night for *An. gambiae*), whereas lowest densities were observed during the "long" dry season, in December–February. However, the human biting rate for *An. funestus* was always >1 bite per person per night (Fig. 1).

Feeding Preference. A total of 440 blood meal spots were tested by ELISA for host identification. These were collected from both indoors and outdoors resting females, including 418 *An. funestus*, 20 *An. gambiae*, and two *An. hancocki*. All specimens had fed on human hosts, but three *An. funestus* females had taken mixed blood meals and also contained ovine (one) or bovine (two) blood. Among these, one specimen was collected resting outdoors.

Circumsporozoïte Protein Rate and Entomological Inoculation Rate. In total, 1,672 anopheline specimens belonging to the eight species collected, were processed by ELISA. Only *An. funestus* and *An. gambiae* were found infected with *P. falciparum*. *P. ovale* was found in one *An. funestus*, together with *P. falciparum*. No *P. malariae* infection was detected.

Plasmodium-infected *An. funestus* specimens were found at each bimonthly sampling. In total, 6.5% (95% CI, 5.3–7.8) of *An. funestus* and 8.1% (95% CI, 3.5–15.3) of *An. gambiae* were positive by ELISA (Table 2). The difference was not statistically significant between both species ($\chi^2 = 0.39$, df = 1, $P > 0.05$). In *An. funestus* and in *An. gambiae*, no significant differences of infection rate were found between samples of specimens indoor feeding, indoor resting, or outdoor resting (χ^2 test; $P > 0.05$).

From February 1999 to October 2000, the overall entomological inoculation rate was estimated at 172 infective bites per human. Malaria transmission occurred all year long (Table 2). Transmission intensity reached its peak in April 2000, with an average of 0.52 infective bites per human per night observed indoors. *An. funestus* was the major vector of *P. falciparum*, accounting for 88% of total transmission. *An. gambiae* also played an active role in the transmission of malaria parasites in this location, although its importance is far less than that of *An. funestus*.

Discussion

A study of malaria transmission for 20 mo in the village of Nkoteng (southern Cameroon) revealed that two common African mosquito vector species, *An. funestus* and *An. gambiae* s.s., are involved and sustain perennial parasite inoculation to the local human population. The total entomological inoculation rate was estimated at 172 infective bites per human across the whole period of study, which, even if CSP ELISA overestimates the true transmission level by a factor of 1.1–1.9 (Fontenille et al. 2001), remains high.

An. funestus is the major vector in the area, accounting for 88% of the total malaria transmission. *An. gambiae* s.s. was the only species of the *An. gambiae* complex found infected with malaria parasites but *An. arabiensis* is present in the area, although at a very low density. Five other anopheline species were collected biting humans or resting inside human dwellings dur-

ing our survey: *An. hancocki*, *An. moucheti*, *An. nili*, *An. wellcomei*, and *An. ziemanni.* The three former species have been found infected with malaria parasites in other areas of Cameroon (Le Goff et al. 1993, Njan Nloga et al. 1993, Manga et al. 1995, Fontenille et al. 2000, Antonio-Nkondjio et al. 2002, Wanji et al. 2003). *An. nili* and *An. moucheti* in particular were shown to be major human malaria vectors of local importance in Cameroon (Carnevale et al. 1992, Njan Nloga et al. 1993, Antonio-Nkondjio et al. 2002) and in other western and central African countries (Elissa et al. 1999, Dia et al. 2003).

The seasonal abundance of *An. gambiae* was fairly low (always <1 bite per person per night) but also varied along the year with a maximum during the first wet season (April–June). The *An. funestus* population density cycle of *An. funestus* depended partly on rainfall with a lower human indoor biting rate at the end of the dry season, around February. *An. funestus* has been described to run relay with *An. gambiae* and *An. arabiensis*, mostly in Savannah areas, reaching its peak of abundance in the early dry season (Gillies and De Meillon 1968). Interestingly, in our study, *An. funestus* densities seemed to positively correlate with rainfall. Because typical breeding sites of *An. funestus* are large and more or less permanent swamps, *An. funestus* is less dependent on rainfall than *An. gambiae* and *An. arabiensis.* The most important feature of breeding site to allow the development of *An. funestus* would be the presence of emergent vegetation (Gillies and De Meillon 1968). In certain areas as Savannah, vegetation at the edge of breeding site begins to grow at the rainy season with the extension of the swamp. The time of the vegetation growth could explain the subsequent increase of *An. funestus* density in the later rainy season and early dry season. In more humid areas, such as Nkoteng, the vegetation is more abundant and borders the breeding sites almost permanently. When the swamp increases its size during the rainy season, it floods vegetation and thus immediately extends the breeding sites for *An. funestus*, explaining the correlation between rainfall and *An. funestus* density in our study. The configuration of the breeding sites and the vegetal environment are therefore important factors in *An. funestus* densities.

Three members of the *An. gambiae* s.l. complex are found in Cameroon: *Anopheles melas* Theobald colonizes mangrove swamps along the Atlantic shore (southwest of the country), *An. arabiensis* is the predominant species of the complex in the dry savannas of the north (southern border of Lake Chad) and extends down to the evergreen forest's edge, and *An. gambiae* s.s. is widespread in the southern, more humid, part of the country. The detection of a few *An. arabiensis* specimens in Nkoteng represents the most southern report of this species in Cameroon and probably points out the southern border of its distribution in the country. In agreement with the acknowledged ecotypic adaptation of chromosomal forms of *An. gambiae* s.s. (Coluzzi et al. 1985, Toure et al. 1998), all specimens identified so far from Nkoteng belong to the Forest chromosomal form, presenting mainly standard chromosome-2 arrangements (unpublished data). Both recently described M and S molecular forms of *An. gambiae* s.s. were represented in our sample, the S form being largely predominant. It has been advocated that these molecular forms represent incipient species but granting them specific status is still a moot point (Black and Lanzaro 2001, della Torre et al. 2002, Wondji et al. 2002, Lehmann et al. 2003). Factors underlining their geographic distribution are still unclear, and deserve further investigation. Studies such as ours will contribute to increase the body of data available on the distribution, relative prevalence and role in human malaria transmission of each form, providing baseline data for thorough assessment of the biological and epidemiological consequences of this genetic subdivision.

An. funestus s.s. is the only member of the *An. funestus* group identified in Nkoteng. All *An. funestus* s.s. from this village previously observed for chromosomal inversions (Dia et al. 2000) belong to the Folonzo chromosomal form after assignment following Costantini et al. (1999). *Anopheles leesoni* Evans and the recently identified *Anopheles rivulorum*-like are known from Cameroon (Mouchet and Gariou 1961, Cohuet et al. 2003), but they were not collected in Nkoteng.

An. funestus has been previously found from south to north of the country (Mouchet and Gariou 1961, Robert et al. 1992, Manga et al. 1997, Antonio-Nkondjio et al. 2002, Wanji et al. 2003) and was the main vector in some localities within the forest block (Manga et al. 1997b). Our study showed that *An. funestus* also could have a major role in malaria transmission in a forest–savannah transition area. These findings underline the influence of local ecology on malaria transmission and the importance of breeding sites availability.

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