Complexity of the Malaria Vectorial System in Cameroon: Contribution of Secondary Vectors to Malaria Transmission

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ABSTRACT Malaria transmission in Africa is a dynamic and complex system that is so far superficially understood. Further knowledge is required to improve control of the disease. In the present report, we highlight the contribution of the so-called "secondary" malaria vectors to the overall parasite transmission intensity in several sites across Cameroon, through a retrospective analysis of surveys from the Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale database. In total, 48,490 female anophelines belonging to 21 different species were collected between October 1998 and March 2003. *Anopheles gambiae* Giles, *Anopheles arabiensis* Patton, *Anopheles funestus* Giles, *Anopheles nili* (Theobald), and *Anopheles moucheti* Evans represented 89% of the total anopheline fauna. Beside these major vectors, malaria parasites or their circumsporozoite proteins were found in nine secondary malaria vectors: *Anopheles ovengensis* Awono-Ambene et al., *Anopheles carnevalei* Brunhes et al., *Anopheles coustani* Laveran, *Anopheles hancocki* Edwards, *Anopheles marshallii* (Theobald), *Anopheles paludis* Theobald, *Anopheles pharoensis* Theobald, *Anopheles wellcomei* Theobald, and *Anopheles ziemanni* Grünberg. The mean infection rate of secondary vectors (1.36%) was significantly ($P < 0.001$) lower than that of major vectors (3.08%). *An. pharoensis* and *An. ovengensis* were repeatedly found infected by *Plasmodium falciparum* Welch and contributed substantially to the total malaria transmission intensity in some areas where they were abundant. Both species have strong exophilic and/or exophagic habits such that they might elude vector control directed against endophilic and endophagic malaria vectors.

KEY WORDS malaria, Cameroon, vectors, *Plasmodium*, *Anopheles*

Less than 20 of 140 anopheline species present in Sub-Saharan Africa are able to transmit malaria to humans (Hervy et al. 1998). Five species, namely, *Anopheles gambiae* Giles, *Anopheles arabiensis* Patton, *Anopheles funestus* Giles, *Anopheles moucheti* Evans and *Anopheles nili* (Theobald), are considered to be major malaria vectors as these are responsible for >95% of the total malaria transmission on the continent (Mouchet et al. 2004). The remaining (5%) is transmitted by "secondary" or "vectors of local importance" (Mouchet et al. 2004). The so-called secondary malaria vectors play an important role locally, because they may contribute significantly to malaria transmission by increasing or extending the malaria transmission period (Mukiama and Mwangi 1989, Robert et al. 1992, Wanji et al. 2003, Awono-Ambene et al. 2004). Even though malaria occurs throughout Africa, there are significant differences between regions. Great heterogeneities of malaria transmission dynamics are reported between neighboring foci depending on such factors as ecological settings, urbanization, deforestation, and agricultural practices (Trape and Zoulani 1987, Manga et al. 1995, Hay et al. 2000, Fontenille and Simard, 2004, Antonio-Nkondjio et al. 2005). The analysis of such differences is of great interest for the understanding of parameters affecting malaria epidemiology and vector dynamics in the Afrotropical region. Although almost half of the population in Sub-Saharan Africa lives in urban or semi-urban areas, most malaria cases reported occur in rural areas where the vector fauna is abundant and diverse (Hay et al. 2000, Mendis et al. 2000, Robert et al. 2003).

In Cameroon, apart from major malaria vectors, six anopheline species have been found infected with *Plasmodium falciparum* sporozoites: *Anopheles han-*

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cocki Edwards, *Anopheles paludis* Theobald, *Anopheles marshallii* (Theobald), *Anopheles coustani* Laveran, *Anopheles wellcomei* Theobald, and *Anopheles pharoensis* Theobald (Vaucel and Campourcy 1943, Adam 1956, Pajot and Segers 1962, Robert et al. 1992, Fontenille et al. 2000, Wanji et al. 2003, Antonio-Nkondjio et al. 2005). *Anopheles melas* Theobald is present in the country but was never reported infected, although it might transmit malaria elsewhere in its range (Akogbéto and Romano 1999).

Despite the substantial amount of work done on malaria in Africa, knowledge of the vectorial systems and their contribution to malaria transmission remains incomplete. More information on the vector systems will help malaria epidemiology and benefit vector control programs all over the continent. We describe here the role of secondary malaria vectors in parasite transmission in Cameroon, through a retrospective analysis of surveys conducted by the Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC) laboratory between 1998 and 2003.

Materials and Methods

Collections were made in 32 malaria endemic foci belonging to different bioclimatic regions across Cameroon, ranging from Sahelian savanna in the north to the equatorial forest in the south (Fig. 1). Mosquitoes were collected by human landing catches, indoor pyrethrum spraying, baited bed-net traps, Centers for Disease Control (CDC) light traps, and aspiration in outdoor resting sites and identified to species by using morphological characteristics (Gillies and De Meillon 1968, Gillies and Coetzee 1987) and molecular diagnostic tools for major vector species complexes (Scott et al. 1993, Favia et al. 2001, Koekemoer et al. 2002, Cohuet et al. 2003, Kengne et al. 2003). Infections were detected either by direct observation of *Plasmodium* sporozoites in the salivary glands or by testing for the presence of circumsporozoite proteins (CSP) of *Plasmodium falciparum* Welch, *Plasmodium malariae* Laveran, and *Plasmodium ovale* Stephens by enzyme-linked immunosorbent assay (ELISA), in the head and thorax of female anophelines (Burkot et al. 1984, Wirtz et al. 1987). The CSP rate was calculated as a ratio of mosquitoes infected over moquitoes dissected or tested after ELISA. The 95% confidence intervals (CIs) were calculated as 95% CI = M ± (z*SE), with M being the mean, SE the standard error, and z the score for the confidence interval (z = 1.96). The entomological inoculation rate (EIR) was calculated by multiplying the human biting rate from the landing catches by the circumsporozoite protein rate. Bloodmeal sources of females captured by pyrethrum spraying, baited bed-net traps and outdoor resting sites were identified by ELISA for human, bovine, ovine (sheep and goat), equine (horse and donkey), pig, or chicken hosts (Beier et al. 1988).

Results

In total, 48,490 female anophelines belonging to 21 different species were collected between October 1998 and March 2003 (Table 1). Major vectors including *An. gambiae*, *An. arabiensis*, *An. funestus*, *An. nili*, and *An. moucheti* represented ≈89% of the total anopheline fauna identified. The remaining 11% was composed of *An. pharoensis*, *An. ovengensis*, *An. ziemanni*, *An. paludis*, *An. coustani*, *An. melas*, *An. hancocki*, *An. marshallii*, *Anopheles rufipes* (Gough), *Anopheles smithii* Theobald, *An. wellcomei*, *Anopheles leesoni* Evans, *Anopheles carnevalei* Brunhes et al., *Anopheles pretoriensis* (Theobald), *Anopheles obscurus* (Grünberg), and *Anopheles implexus* (Theobald).

An. gambiae and *An. funestus* were widely distributed in both forest and savanna environments (Fig. 1). The other species were localized in specific bioclimatic foci. *An. moucheti*, *An. nili*, *An. paludis*, *An. coustani*, *An. hancocki*, *A. marshallii*, *An. ovengensis*, *An. smithii*, *An. ziemanni*, *An. wellcomei*, and *An. carnevalei* are present in the forest region and rarely found above latitude 6° N. However, *An. pharoensis*, *An. rufipes*, and *An. arabiensis* are well adapted to drier environments and were scarce south of latitude 6° N (Fig. 1). The highest number of species was recorded in the town of Tibati (13 species collected out of 21) situated close to a dam in a forest savana transition area (Fig. 1). Out of 2,853 bloodmeals tested, 2,610 (91.5%) had been taken on a human host. For the five species considered to be secondary vectors, only 55 out of the 165 bloodmeals tested were from human origin (Table 2).

Malaria parasites or the CS protein were found in 14 species (Table 3). Mean infection rate of secondary vectors (1.36%) was significantly lower than that of major vectors (3.08%) ($\chi^2 = 35.8$, df = 1, $P < 0.001$). Infection rates were similar among secondary vectors ($\chi^2 = 12.9$, df = 8, $P = 0.12$). Only *An. funestus* and *An. gambiae* were infected throughout their ranges.

Discussion

In Cameroon, 48 anopheline species have so far been recorded from the country (Hervy et al. 1998, Brunhes et al. 2003, Cohuet et al. 2003, Kengne et al. 2003, Awono-Ambene et al. 2004). In the current study, only 21 anopheline species were collected, in part because collection methods targeting anthropophilic mosquitoes were used. Highest species richness was observed in rain forest regions with 18 species collected. Less than five species were collected from most sites in savanna zones. Species ranges in this study were similar to previously known anopheline distributions in Cameroon (Adam 1956, Hervy et al. 1998).

During this survey, human *Plasmodium* infections were recorded in nine secondary vectors: *An. ovengensis*, *An. carnevalei*, *An. coustani*, *An. hancocki*, *A. marshallii*, *An. paludis*, *An. pharoensis*, *An. wellcomei*, and *An. ziemanni*. Most of these species have been

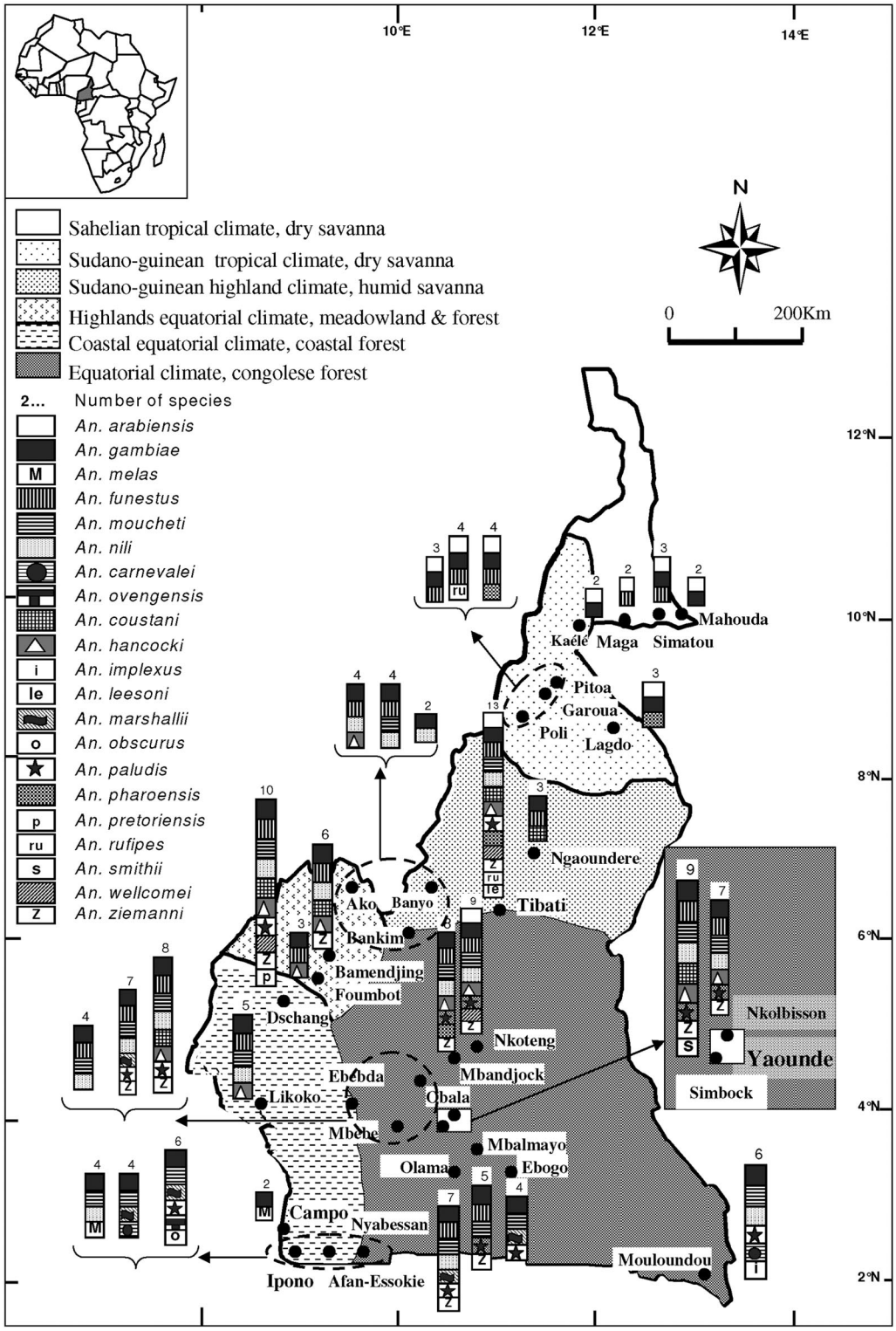

Fig. 1. Map of Cameroon showing anopheline species collected in 32 sites. The inset locates Cameroon in West Africa.

Table 1. Anopheline fauna collected in Cameroon between October 1998 and November 2003

Species	Attractant methods				Resting		Total
	Landing catch		Mosquito nets	CDC light trap	Indoor spraying	Shelters	
	Indoors	Outdoors					
An. moucheti	8,827	3,124	200	8	1,234	12	13,405
An. funestus	4,903	1,784	57	9	5,290	91	12,134
An. gambiae	4,718	3,084	125	6	2,574	5	10,512
An. nili	3,436	1,774	532	8	466	1	6,217
An. ovengensis	361	1,190			36		1,587
An. pharoensis	506	676			131		1,313
An. arabiensis	341	145			236		722
An. paludis	366	136	130		3		635
An. melas	498	20			7		525
An. ziemanni	128	218	151	2	2		501
An. carnevalei		472					472
An. marshallii	65	148	12		2		227
An. hancocki	80	16	6		14		116
An. wellcomei	40	49					89
An. coustani	10	12				1	23
An. obscurus		5					5
An. rufipes	2				1		3
An. smithii						1	1
An. leesoni	1						1
An. pretoriensis					1		1
An. implexus		1					1
Total	24,282	12,854	1,213	33	9,997	111	48,490

reported infected in other areas in Africa (Adam 1956, Hamon and Mouchet 1961, Pajot and Segers 1962, Gillies and De Meillon 1968, Karch and Mouchet 1992, Curtis et al. 1999, Wanji et al. 2003). Meanwhile, *An. ovengensis* (Awono-Ambene et al. 2004) and *An. carnevalei* (P.A.-A., unpublished data) were recently described as malaria vectors, although *An. carnevalei* was incriminated only by CSP ELISA. This technique is known to overestimate by 1.12 sporozoite infection rates in Cameroon compared with salivary gland dissection (Fontenille et al. 2001). Direct observation of sporozoites in the salivary glands remains to be reported for this species.

Among secondary vectors, *An. ovengensis* and *An. pharoensis* had the highest infection rates (1–2%). The recently described *An. ovengensis* of the *An. nili* group occurs in forested environments together with *An. moucheti* and *An. gambiae.* It is rarely found resting indoors but takes blood indoors and outdoors. High biting rates of this species have been recorded on the edges of forested rivers (Awono-Ambene et al. 2004), and its larvae have so far been reported only along the Ntem and Njoh rivers in southern Cameroon. This species also may occur in neighboring countries such as Gabon, Congo, and Equatorial Guinea which share the same bioclimatic zones and hydrographic networks.

An. pharoensis has been reported in Cameroon since 1961 (Cavalié and Mouchet 1961) but was found infected only once (Robert et al. 1992). During our surveys, infected females were recorded in Tibati and Pitoa (northern Cameroon). This vector species, which is very common in Sudanese and Sahelian regions, has been involved in malaria transmission in Egypt (Madwar 1936, Barber and Rice 1937), Nigeria (Barber and Olinger 1931), Mali (Holstein 1951), Senegal (Carrara et al. 1990), Kenya (Mukiama and Mwangi 1989), and Tanzania (Draper and Smith 1957, Gillies 1964). Meanwhile, individuals of this species collected in rice (*Oryza* spp.) cultivation areas in West Africa were never found infected (Hamon et al. 1956, Robert et al. 1985, Faye et al. 1995). These con-

Table 2. Bloodmeal sources detected from anophelines collected in Cameroon (1998–2003)

Species	Tested	Human	Bovine	Ovine	Pig	Mixed[a]
An. funestus	1,209	1,188	7	5	1	8
An. gambiae s.l.[b]	700	683	7	1	2	7
An. moucheti	458	452		3		3
An. nili	321	242	64	1	3	11
An. ziemanni	84	7	31	44	1	1
An. paludis	47	7	11	14	14	1
An. pharoensis	20	20				
An. hancocki	7	7				
A. marshallii	4	4				
An. coustani	3	0	3			
Total	2,853	2,610	123	68	21	31

[a] Bloodmeal taken by a mosquito on at least two different hosts.
[b] Represents *An. gambiae* and *An. arabiensis.*

Table 3. Circumsporozoite rate and annual EIR of anopheline species collected in Cameroon between October 1998 and March 2003

Species	No. tested	Positive	CSP rate (%) (95% CI)	EIR range (infected bites/ human/yr)
Major vectors				
1 *An. funestus*	10,461	466	4.45 (4.06–4.85)	6.5–151.4
2 *An. gambiae*	8,935	342	3.83 (3.43–4.23)	16.0–108
3 *An. moucheti*	11,190	188	1.68 (1.44–1.92)	0.0–303
4 *An. nili*	6,993	154	2.20 (1.86–2.55)	0.0–275
5 *An. arabiensis*	353	19	5.38 (3.03–7.74)	0.0–165
Total	37,932	1,169	3.08 (2.91–3.26)	
Secondary vectors				
1 *An. ovengensis*	1,032	19	1.84 (1.02–2.66)	0.0–70
2 *An. pharoensis*	1,304	14	1.07 (0.51–1.63)	0.0–16.7
3 *An. paludis*	448	5	1.12 (0.14–2.09)	0.0–10.4
4 *An. hancocki*	131	5	3.82 (0.54–7.10)	0.0–12
5 *An. marshallii*	167	3	1.80 (0.05–9.55)	0.0–3.2
6 *An. ziemanni*	475	2	0.42 (0–3.6)	0.0–1.3
7 *An. carnevalei*	84	1	1.19 (0.03–6.46)	0.0–8.6
8 *An. wellcomei*	83	1	1.20 (0.03–6.53)	0.0–1.3
9 *An. coustani*	31	1	3.23 (0.08–16.7)	0.0–3.4
Total	3,755	51	1.36 (0.99–1.73)	
Overall	41,687	1,220[a]	2.93 (276–3.09)	

[a] Five and nine *P. ovale* and *P. malariae*, respectively. The remaining were *P. falciparum*.

flicting observations may result from the presence of genetically differentiated forms or sibling species within *An. pharoensis*, as proposed by Miles et al. (1983) on cytological grounds. Further investigations on bioecology and genetic structure are needed to explore the existence of sibling species within *An. pharoensis*.

The low infection rate recorded for most of the secondary vectors was partly attributable to their exophagic and zoophilic behavior, inasmuch as only 55 of the 165 bloodmeals tested had been taken from humans. It also may reflect their lower longevity compared with major malaria vectors (Gillies and De Meillon 1968, Faye et al. 1995).

In most parts of Cameroon, malaria transmission is high and depends mainly on major malaria vectors (Antonio-Nkondjio et al. 2002, Cohuet et al. 2004, Fontenille and Simard 2004). In Sahelian and dry savanna areas, malaria is transmitted by *An. arabiensis* and *An. gambiae*, which are responsible for inoculation rates reaching up to 50 infected bites per person during the rainy season (Fondjo 1996). In wet savanna regions, malaria is mainly vectored by *An. gambiae* and *An. funestus* which are responsible for >90% of malaria transmission, estimated at 100–200 infective bites per man per year (Manga et al. 1997, Cohuet et al. 2004, Fontenille and Simard 2004). In forested areas, four primary malaria vectors, namely, *An. gambiae*, *An. funestus*, *An. nili*, and *An. moucheti*, transmit malaria. Generally, at least three of these species are simultaneously involved in malaria transmission. For example, in the village of Simbock situated close to Yaounde, these species are present year-round and are responsible for inoculation rates ranging from 277 to 368 infective bites per human per year (Antonio-Nkondjio et al. 2002). Apart from a few studies that have highlighted the role of secondary vectors (Robert et al. 1992, Fontenille et al. 2000, Wanji et al. 2003, Awono-Ambene et al. 2004), most entomological studies conducted across the country concluded that secondary malaria vectors are of low epidemiological importance.

The relationship between malaria prevalence and EIR is such that low EIR (0.001 or less) may be found associated with prevalence rates of *P. falciparum* >40% and incidence rates of severe disease >25 per 1,000 (Mbogo et al. 1993, Mbogo et al. 1995, Beier et al. 1999). Thus, despite their low infection rate, secondary vectors might maintain malaria prevalence at a high level, even though in most collection sites their roles are overshadowed by more efficient malaria vectors. In Nyabessan and Tibati where *An. ovengensis* and *An. pharoensis* were abundant, they contributed substantially to the total malaria transmission with, respectively, 34 and 8.5% of malaria transmission estimated at 206 infective bites per person per year in Nyabessan and 194 infective bites per person per year in Tibati (Domfang et al. 2005). However, it is unlikely that secondary vectors are able to maintain on their own permanent malaria transmission due to their low survivorship and limited abundance (Hamon and Mouchet 1961, Faye et al. 1995). Nevertheless, transmission levels recorded for *An. ovengensis* and *An. pharoensis* are well above values recorded in certain endemic areas in Africa (Beier et al. 1999, Nimpaye et al. 2001, Bonnet et al. 2002) and suggest that in certain epidemiological contexts where they are abundant, secondary vectors can be considered as main vectors. It is noteworthy that the co-occurrence of secondary vectors and primary vectors may increase the risk of malaria transmission. High infection rates in secondary vectors also may arise as a consequence of high malaria transmission maintained by primary vectors and increased prevalence of gametocyte carriers in the human population (Quakyi et al. 2000).

Our study sheds light on the complexity of the malaria vectorial system in Cameroon. Although recent efforts on malaria research and control have focused on major vector systems, genetically competent vector species are numerous in Africa and contribute to the overall complexity of malaria transmission dynamics and epidemiology. Substantial information on the epidemiological importance of some of these vectors was documented and should be taken into consideration when dealing with malaria transmission in Africa. National control programs would benefit from additional knowledge on vector population diversity, biology, and genetic structure to implement control measures in the field.

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References Cited

Adam, J. P. 1956. Note faunistique et biologique sur les anophèles de la région de Yaoundé et la transmission du paludisme en zone forestière du sud Cameroun. Bull. Soc. Pathol. Exot. 49: 210–220.

Akogbéto, M., and R. Romano. 1999. Infectivité *d'An. melas* vis-à-vis de *Plasmodium falciparum* dans le milieu côtier lagunaire du Bénin. Bull. Soc. Pathol. Exot. 92: 57–61.

Antonio-Nkondjio, C., P. Awono-Ambene, J. C. Toto, J. Y. Meunier, S. Zebaze-kemleu, R. Nyambam, C. Wondji, T. Tchuinkam, and D. Fontenille. 2002. High malaria transmission intensity in a sub urban area of Yaounde, the capital city of Cameroon. J. Med. Entomol. 39: 350–355.

Awono-Ambene, H. P., P. Kengne, F. Simard, C. Antonio-Nkondjio, and D. Fontenille. 2004. Description and bionomics of *Anopheles* (Cellia) *ovengensis* (Diptera: Culicidae) a new malaria vector species of the *Anopheles nili* group from south Cameroon. J. Med. Entomol. 41: 561–568.

Antonio-Nkondjio, C., F. Simard, P. Awono-Ambene, P. Ngassam, J. C. Toto, T. Tchuinkam, and D. Fontenille. 2005. Malaria vectors and urbanisation in the equatorial forest region of south Cameroon. Trans. R. Soc. Trop. Med. Hyg. 99: 347–354.

Barber, M. A., and J. B. Rice. 1937. A survey of malaria in Egypt. Am. J. Trop. 17: 413.

Barber, M. A., and M. T. Olinger. 1931. Studies on malaria in southern Nigeria. Ann. Trop. Med. Parasitol. 25: 461.

Beier, J., G. F. Killeen, and J. Githure. 1999. Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. Am. J. Trop. Med. Hyg. 61: 109–113.

Beier, J. C., P. V. Perkins, R. A. Wirtz, J. Koros, D. Diggs, T.P.I.I. Gargam, and D. K. Koech. 1988. Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA) tested on *Anopheles* (Diptera: Culicidae) in Kenya. J. Med. Entomol. 25: 9–16.

Bonnet, S., R.E.I. Paul, C. Gouagna, I. Safeukui, J. Y. Meunier, R. Gounoue, and C. Boudin. 2002. Level and dynamics of malaria transmission and morbidity in an equatorial area of south Cameroon. Trop. Med. I. Health 7: 249–256.

Brunhes, J., G. Le Goff, and P. Bousses. 2003. Anopheles afrotropicaux. V. Description du mâle et des stades préimaginaux d'*An. deemingi* et description d'*An. eouzani* n. sp. (Diptera: Culicidae). Ann. Soc. Entomol. Fr. 39: 179–185.

Burkot, T. R., J. L. Williams, and I. Schneider. 1984. Identification of *Plasmodium falciparum* infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. 33: 783–788.

Carrara, G., V. Petrarca, M. Niang, and M. Coluzzi. 1990. *Anopheles pharoensis* and transmission of *Plasmodium falciparum* in the Senegal River Delta. Med. Vet. Entomol. 4: 421–424.

Cavalié, P., and J. Mouchet. 1961. Les campagnes expérimentales d'éradication du paludisme dans le nord de la République du Cameroun. Les vecteurs de l'épidémiologie du paludisme dans le Nord-Cameroun. Med. Trop. 21: 847–869.

Cohuet, A., F. Simard, J. C. Toto, P. Kengne, M. Coetzee, and D. Fontenille. 2003. Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon and evidence for a new species. Am. J. Trop. Med Hyg. 69: 200–205.

Cohuet, A., F. Simard, C. Wondji, C. Antonio-Nkondjio, P. Awono-Ambene, and D. Fontenille. 2004. High malaria transmission intensity due to *Anopheles funestus* (Diptera: Culicidae) in a village of savannah–forest transition area in Cameroon. J. Med. Entomol. 41: 901–905.

Curtis, C. F., H. V. Pates, W. Takken, C. A. Maxwell, J. Myamba, A. Priestman, O. Akinpela, A. M. Yayo, and J. T. Hu. 1999. Biological problems with the replacement of a vector population by *Plasmodium*-refractory mosquitoes. Parassitolologia 41: 479–481.

Domfang, J., C. Wondji, P. Awono-Ambene, C. Antonio-Nkondjio, J. C. Toto, and F. Simard. 2005. Malaria transmission dynamics and the diversity of anophelines vector populations in Tibati, Cameroon. Acta Trop. 95(suppl.): S172.

Draper, C. C., and A. Smith. 1957. Malaria in the Pare area of N.E. Tanganyika. Part I: Epidemiology. Trans. R. Soc. Trop. Med. Hyg. 51: 137.

Favia, G., A. Lanfracotti, L. Spanos, I. Siden-Kiamos, and C. Louis. 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae s.s.* Insect Mol. Biol. 10: 19–23.

Faye, O., D. Fontenille, O. Gaye, J. F. Molez, L. Konate, G. Hebrard, J. P. Herve, J. Trouillet, S. Diallo, and J. Mouchet. 1995. Paludisme et riziculture dans le delta du fleuve Senegal (Senegal). Ann. Soc. Belge Med. Trop. 75: 179–189.

Fondjo, E. 1996. Etude du comportement du complexe *An. gambiae* et de la transmission du paludisme dans deux faciès eco-climatiques au Mali et au Cameroun. Thèse Doctorat 3e cycle. Université de Bamako (Mali) Bamako.

Fontenille, D., S. Wandji, R. Djouaka, and H. P. Awono-Ambene. 2000. Anopheles hancocki, vecteur secondaire du paludisme au Cameroun. Bull. Liais. Doc. OCEAC 33: 23–26.

Fontenille, D., J. Y. Meunier, C. Antonio-Nkondjio, and T. Tchuinkam. 2001. Use of circumsporozoite protein enzyme-linked immunosorbent assay compared with microscopic examination of salivary glands for calculation of malaria infectivity rates in mosquitoes (Diptera: Culicidae) from Cameroon. J. Med. Entomol. 38: 451–454.

Fontenille, D., and F. Simard. 2004. Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vectors populations. Comp. Immunol. Microbiol. Infect. Dis. 27: 357–375.

Gillies, M. T. 1964. The role of secondary vectors of malaria in north east Tanganyika. Trans. R. Soc. Trop. Med. Hyg. 58: 154.

Gillies, M. T., and B. De Meillon. 1968. The Anophelinae of Africa south of the Sahara, 2ed. South African Institute of Medical Research, Johannesburg.

Gillies, M. T., and M. Coetzee. 1987. A supplement to the Anophelinae of Africa south of the Sahara, South African Institute of Medical Research, Johannesburg.

Hamon, J., J. P. Adam, and A. Grejebine. 1956. Observations sur la répartition et le comportement des anophèles de l'Afrique équatoriale française, du Cameroun et de l'Afrique occidentale. Bull. Organ. Mondiale Santé 15: 549–591.

Hamon, J., and J. Mouchet. 1961. Les vecteurs secondaires du paludisme humain en Afrique. Med. Trop. 21: 643–660.

Hay, S. I., D. J. Rogers, J. F. Toomer, and R. W. Snow. 2000. Annual *Plasmodium falciparum* entomological inoculation rates (EIR) across Africa: literature survey, Internet access and review. Trans. R. Soc. Trop. Med. Hyg. 94: 113–127.

Hervy, J. F., G. Le Goff, B. Geoffroy, J. P. Hervé, L. Manga, and J. Brunhes. 1998. Les Anophèles de la région afrotropicale. CD-ROM, ORSTOM éditions, Paris, France.

Holstein, M. 1951. Note sur l'épidémiologie du paludisme en Afrique occidentale Française. Bull. World Health Organ. 4: 463.

Karch, S., and J. Mouchet. 1992. Anopheles paludis vecteur important de paludisme au Zaïre. Bull. Soc. Pathol. Exot. 85: 388–389.

Kengne, P., P. Awono-Ambene, C. Antonio-Nkondjio, F. Simard, and D. Fontenille. 2003. Molecular identification of the *Anopheles nili* group of African malaria vectors. Med. Vet. Entomol. 17: 67–74.

Koekemoer, L. L., L. Kamau, R. H. Hunt, and M. Coetzee. 2002. A cocktail polymerase chain reaction (PCR) assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am. J. Trop. Med. Hyg. 66: 804–811.

Madwar, S. 1936. A preliminary note on *Anopheles pharoensis* in relation to malaria in Egypt. J. Egypt. Med. Assoc. 19: 616.

Manga, L., J. C. Toto, and P. Carnevale. 1995. Malaria vectors and transmission in an area deforested for a new international airport in southern Cameroon. Ann. Soc. Belge Méd. Trop. 75: 43–49.

Manga, L., B. Bouchité, J. C. Toto, and A. Froment. 1997. La faune anophélienne et la transmission du paludisme dans une zone de transition forêt savane au centre du Cameroun. Entomol. Med. 91: 4.

Mbogo, C. N., R. W. Snow, E. W. Kabiru, J. H. Ouma, J. I. Githure, K. Marsh, and J. C. Beier. 1993. Low level *Plasmodium falciparum* transmission and the incidence of severe malaria infections on the Kenyan coast. Am. J. Trop. Med. Hyg. 49: 245–253.

Mbogo, C. N., R. W. Snow, C. P. Khamala, E. W. Kabiru, J. H. Ouma, J. I. Githure, K. Marsh, and J. C. Beier. 1995. Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. Am. J. Trop. Med. Hyg. 52: 201–206.

Mendis, C., J. L. Jacobsen, A. Gamage-Mendis, E. Bule, M. Dgedge, R. Thompson, N. Cuamba, J. Barreto, K. Begtrup, R. E. Sinden, and B. Hogh. 2000. *Anopheles arabiensis* and *An. funestus* are equally important vectors of malaria in Matola coastal suburb of Maputo, southern Mozambique. Med. Vet. Entomol. 14: 171–180.

Miles, S. J., C. A. Green, and R. H. Hunt. 1983. Genetic observations on the taxon *Anopheles pharoensis* Theobald (Diptera: Culicidae). J. Trop. Med. Hyg. 86: 153–157.

Mouchet, J., P. Carnevale, M. Coosemans, J. Julvez, S. Manguin, D. Richard-Lenoble, and J. Sircoulon. 2004. Biodiversité du paludisme dans le monde Editions John Libbey Eurotext, Montrouge, France.

Mukiama, T. K., and R. W. Mwangi. 1989. Seasonal population changes and malaria transmission potential of *Anopheles pharoensis* and the minor anophelines in Mwea irrigation scheme, Kenya. Acta Trop. 46: 181–189.

Nimpaye, H., M. Van Der Kolk, D. Fontenille, and C. Boudin. 2001. Le paludisme urbain à Yaoundé (Cameroun) en 2000. Etude entomologique dans le quartier central "Dakar". Bull. Liais. Doc. OCEAC. 34: 11–14.

Pajot, F. X., and L. G. Segers. 1962. Notes sur la biologie d'*Anopheles hargreavesi* Evans 1927 et d'*Anopheles paludis* Theobald 1900 dans le sud de la zone d'entraînement de Yaoundé (Cameroun), le long du fleuve Nyong. Cahier ORSTOM, Ser. Entomol. Med. Parasitol. 2: 3–15.

Quakyi, I. A., R. G. Leke, R. Befidi-Mengue, M. Tsafack, D. Bomba-Nkolo, L. Manga, V. Tchinda, E. Njeungue, S. Kouontchou, J. Fogako, et al.. 2000. The epidemiology of *Plasmodium falciparum* malaria in two Cameroonian villages: Simbok and Etoa. Am. J. Trop. Med. Hyg. 63: 222–230.

Robert, V., P. Gazin, C. Boudin, J. F. Molez, V. Ouedraogo, and P. Carnevale. 1985. La transmission du paludisme en zone de savane arborée et en zone rizicole des environs de Bobo Dioulasso (Burkina Faso). Ann. Soc. Belge Med. Trop. 55: 201–214.

Robert, V., K. Macintyre, J. Keating, J. F. Trape, J. B. Duchemin, W. McWilson, and J. C. Beier. 2003. Malaria transmission in urban sub-Saharan Africa. Am. J. Trop. Med. Hyg. 68: 169–176.

Robert, V., Van den Broek, P. Stevens, R. Slootweg, V. Petrarca, M. Coluzzi, G. Le Goff, M. A. Di Deco, and P. Carnevale. 1992. Mosquitoes and malaria transmission in irrigated rice-fields in the Benoue valley of northern Cameroon. Acta Trop. 52: 201–204.

Scott, J. A., W. G. Brogdon, and F. H. Collins. 1993. Identification of single specimens of the *Anopheles gambiae* complex by polymerase chain reaction. Am. J. Trop. Med. Hyg. 49: 520–529.

Trape, J. F., and A. Zoulani. 1987. Malaria and urbanization in Central Africa: the example of Brazzaville. Part III: Relationships between urbanization and the intensity of malaria transmission. Trans. R. Soc. Trop. Med. Hyg. 81: 19–25.

Vaucel, M., and A. Campourcy. 1943. L'anophélisme au Cameroun français. Rev. Sci. Med. Pharm. Vet. Afr. Libre Brazzaville 2: 85–88.

Wanji, S., T. Tanke, S. N. Atanga, C. Ajonina, N. Tendongfor, and D. Fontenille. 2003. *Anopheles* species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. Trop. Med. Int. Health 8: 643–649.

Wirtz, R. A., F. Zavala, Y. Charoenvit, G. H. Campbell, T. R. Burkot, I. Schneider, K. M. Esser, R. L. Beaudoin, and R. G. Andre. 1987. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. Bull. World Health Organ. 65: 39–45.

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