

Year to year and seasonal variations in vector bionomics and malaria transmission in a humid savannah village in west Burkina Faso

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ABSTRACT: A longitudinal entomological study was carried out from 1999 to 2001 in Lena, a humid savannah village in the western region of Burkina Faso in order to establish malaria vector bionomics and the dynamics of malaria transmission. In the first year, malaria transmission was mainly due to *An. gambiae* s.s., but during the two later years was due to *An. funestus*, which were observed in high frequency towards the end of the rainy season. PCR identification of samples of *An. gambiae* s.l. showed 93% to be *An. gambiae* s.s. and 7% *An. arabiensis*. *An. funestus* constituting more than 60% of the vectors were identified in PCR as *An. funestus* s.s. The persistence of intense vectorial activity in this village was probably due to the road building in a swampy area creating a semi-permanent swamp that provided large sites for larval mosquitoes. These swampy sites seemed to be more favorable for *An. funestus* than for *An. gambiae* s.s. Thus, land development must be monitored and subjected to planning to minimize vector proliferation. Such a system of planning could lead to the restriction or even elimination of the swamp that is the source of larvae developing in the heart of the village. *Journal of Vector Ecology* 33 (1): 70-75. 2008.

Keyword Index: *Anopheles funestus*, *Anopheles gambiae* s.s., malaria transmission, semi-permanent swamp, Burkina Faso.

INTRODUCTION

Cotton is the main crop in the western region of Burkina Faso. To facilitate the management of this crop, the government and local community, in collaboration with the national cotton management company, have renovated or built road and storage facilities. This caused local changes in the ecological conditions that favored species formerly only considered as secondary vectors of malaria. In such a context, it is crucial to characterize the bio-ecology of such vectors and to evaluate their role in malaria transmission. Based on recent PCR techniques enabling us to address the species and molecular identification of the *Anopheles gambiae* and *Anopheles funestus* complexes (Scott et al. 1993, Favia et al. 2001, Koekemoer et al. 2002), the aim of the present study was to investigate the bionomics of malaria vectors and their role in malaria transmission in an environmentally-modified village in western Burkina Faso formerly dominated by *An. gambiae* s.l. (Robert et al. 1985).

MATERIALS AND METHODS

Study site

Anopheles mosquitoes were collected in Lena (11°18'N; 03°53'W), a humid savannah village in southwestern Burkina Faso from 1999 to 2001. In this region, there are

two distinct seasons: rains occur only from May to October, with a long dry season from November to April. The average annual rainfall ranged from 1,000 to 1,200 mm in the last five years. The larval habitats consist mostly of rain-filled puddles and a semi-permanent swamp on either side of a small roadway suitable for *An. funestus* development. This roadway was built at the end of 1998 to facilitate crossing the swamp that formerly flooded the road.

Mosquito collections

Anopheles mosquitoes were sampled from February 1999 to December 2001 during the rainy season, four times per month by human landing catches and indoor insecticide aerosol spray catches. The human landing catches were carried out by informed volunteers who were provided with free and rapid treatment when they showed fever and *Plasmodium falciparum* parasitemia according to a WHO-recommended regimen. To evaluate human biting rates, pairs of humans sat indoors and outdoors collecting mosquitoes landing on themselves by using flashlights and glass tubes. Collections were carried out between 18:00 and 06:00 h inside and just outside eight houses in various parts of the swamp. To compensate for variation in catching efficiency, collectors rotated between houses on successive nights.

Indoor-resting females were caught by spraying village huts with pyrethroids. Female mosquitoes were knocked

down and immediately retrieved from white sheets laid on the floor of sprayed huts. Mosquitoes were dissected and the head and thorax preserved to determine their infection status. Legs were removed and kept dry for molecular species identification. Blood-fed females were stored in 1.5 ml tubes. Muirhead-Thomson (1958) pit traps were used to catch samples of exophilic mosquitoes in 2000.

Laboratory processing of mosquitoes

Anophelines were morphologically identified using keys of Gillies and Coetsee (1987). Later, some of the females were tested by ELISA for *P. falciparum* CSP (Wirtz et al. 1987) and those positive were processed by PCR for molecular identification of the *An. gambiae* complex species and *An. funestus* group and *An. gambiae* molecular forms (Scott et al. 1993, Favia et al. 2001, Cohuet et al. 2003). Blood meals were identified as human, bovine, sheep/goat, pig, or donkey using the ELISA technique of Beier et al. (1988).

Data analysis

The human biting rate (HBR) was calculated as the number mosquitoes captured per person per night. The rate of endophagy was defined as the proportion of the mosquitoes that were caught indoors with landing collections. The sporozoite rate was defined as the proportion of mosquitoes found positive for *P. falciparum* CS protein. The anthropophilic rate was calculated as the proportion of human blood among all blood meals tested. The entomological inoculation (EIR) was calculated as the product of HBR and the sporozoite rate of mosquitoes caught on landing collections.

RESULTS

Species composition and endophagic rate

In the three years, 1,647 *An. gambiae* s.l., 2,161 *An. funestus*, and 111 *An. nili* were identified. *An. funestus* predominated among malaria vectors with some seasonal and annual variations. *An. funestus* represented 60%, vs 38% for *An. gambiae* s.l. and 2% *An. nili*. However, in 1999, *An. gambiae* s.l. was the main malaria vector with 65% of the total vectors vs 34% of *An. funestus* and only 1% for *An. nili*. During the two later years, the vector population was dominated by *An. funestus* with 74% and 72 % in 2000 and 2001, respectively, with a small increase of *An. nili* from 2 to 3% (Figure 2).

As shown in Table 1, indoor and outdoor catches of *An. gambiae* s.l. did not differ significantly from equality with each other within the same year ($\chi^2= 3$, $df=2$, $P=0.2$). In contrast, *An. funestus* was significantly more endophagic than exophagic in each year and its endophagic rate increased greatly from 1999 to 2001 ($\chi^2= 19.4$, $df=1$, $P<0.001$). *An. nili* tended to exophagy but outdoor catches did not significantly exceed indoor ones in any of the year.

Indoor resting collection and endophilic rate

From July 2000 to December 2001, 1,005 mosquitoes were collected in indoor spray collection of which 187 were *An. gambiae* s.l., 627 *An. funestus*, and 1 *An. nili* as malaria vectors. During 2000, only 14 *An. gambiae* s.l. and 33 *An. funestus* were caught in Muirhead-Thomson traps. Based on these data, it was concluded that *An. gambiae* s.l. and *An. funestus* were endophilic.

Monthly variation of human biting rate

In 1999, biting of *An. gambiae* s.l. began at a low level in July reaching a peak of 25/b/h/n in September and

Table 1. Endophagic rate of vectors caught in Lena village from 1999-2001.

Years	Mosquito species	Indoors		Outdoors	
		N	%	N	%
1999	<i>An. gambiae</i> s.l.	459	48.4	489	51.6
	<i>An. funestus</i>	243	54.2*	205	45.8
	<i>An. nili</i>	9	36	16	64
2000	<i>An. gambiae</i> s.l.	184	52.3	168	47.7
	<i>An. funestus</i>	555	57.6**	409	42.4
	<i>An. nili</i>	16	48.5	17	51.4
2001	<i>An. gambiae</i> s.l.	178	53.6	154	46.4
	<i>An. funestus</i>	500	67.8**	237	32.2
	<i>An. nili</i>	19	36.5	33	63.5

* $P<0.05$.

** $P<0.001$ ($\chi^2=19.4$, $df=1$, $P<0.001$).

Figure 1. Relative frequencies of vector species caught in indoor human landing from 1999-2001.

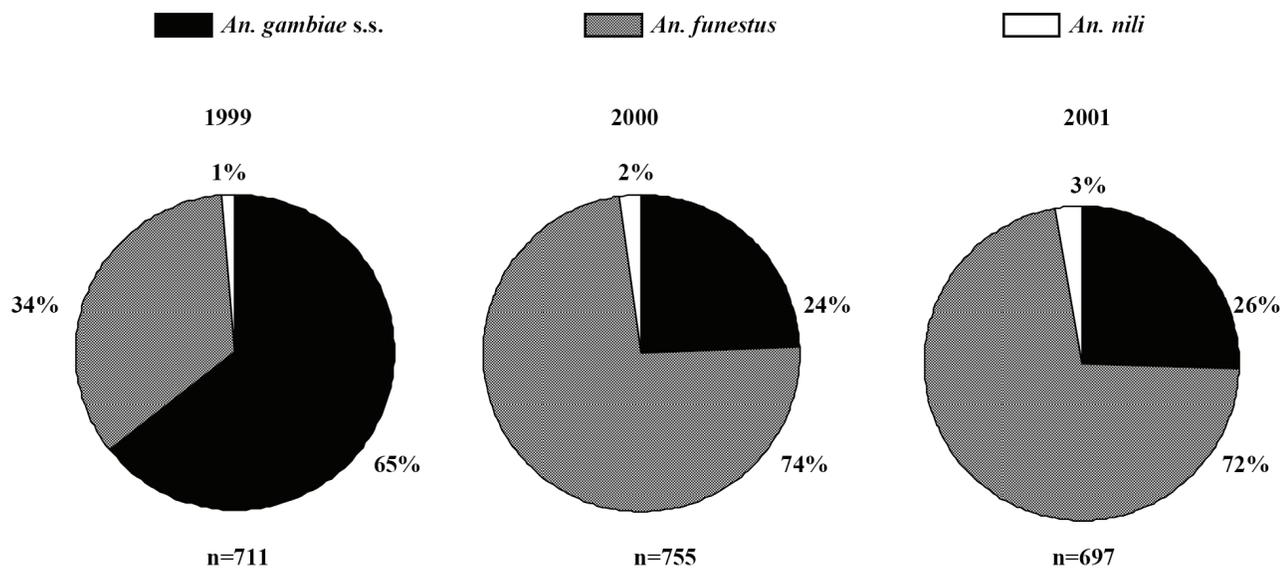


Table 2. Variation among years in entomological inoculation rate (EIR) in Lena village from 1999-2001.

Years	<i>Anopheles gambiae s.s.</i>			<i>Anopheles funestus</i>			
	Parity rate	Sporozoite rate	EIR	Parity rate	Sporozoite rate	EIR	
1999	Indoor	59.1 (77/129)	6.8 (25/366)	119	73.3 (11/15)	16.1 (5/31)	73
	Outdoor	62.1 (77/124)	5.7 (19/333)	112	50 (1/2)	7.1 (1/14)	5
2000	Indoor	74.6 (50/67)	12 (22/184)	85	62.1 (64/103)	4.8 (11/230)	86
	Outdoor	71 (44/62)	10.9 (19/175)	70	59.9 (53/89)	3.0 (7/230)	53
2001	Indoor	82.8 (48/58)	9.8 (18/183)	68	83.5 (86/103)	6.5 (24/370)	123
	Outdoor	74.5 (48/58)	7.9 (12/151) ^o	47	77.1 (37/48)	5.9 (13/222)	51

Table 3. Origin of blood meals of vectors caught in Lena (percentages are between brackets).

Mosquito species	Human	Others (bovine, ovine, or pig)	Mixed (human and others)
<i>An. gambiae s.s.</i>	51 (62)	10 (13.4)	20 (24.6)
<i>An. funestus s.s.</i>	17 (56.6)	11 (36.7)	2 (6.6)

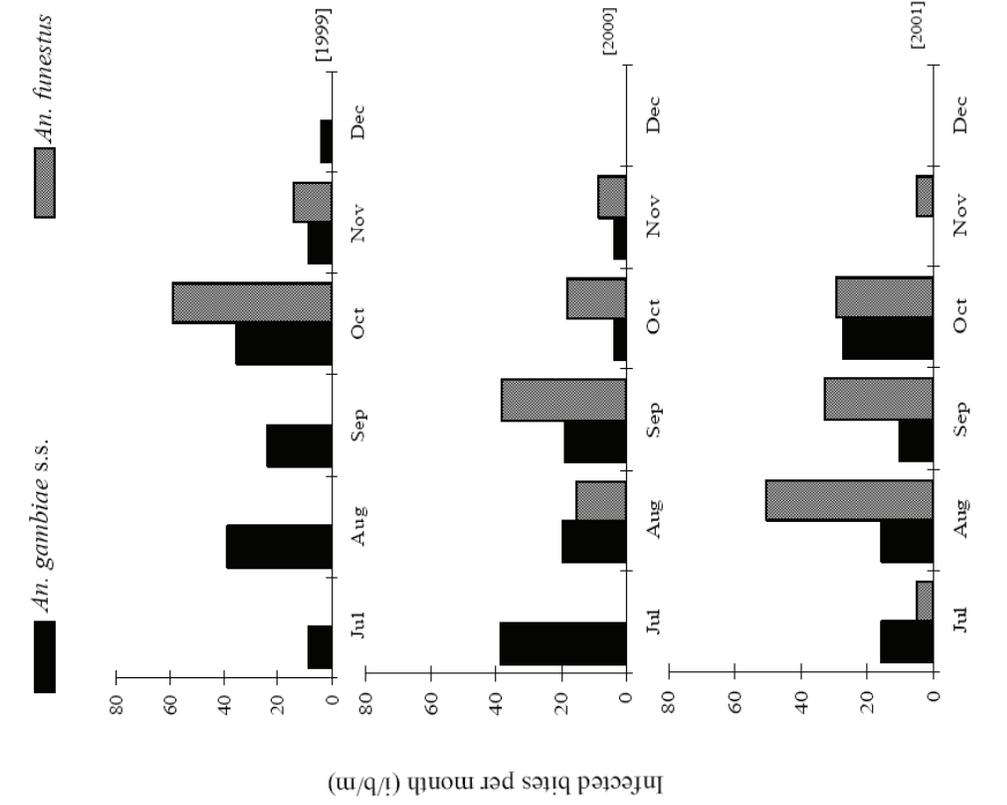


Figure 3: Monthly variation of the EIR from 1999-2001 in Lena.

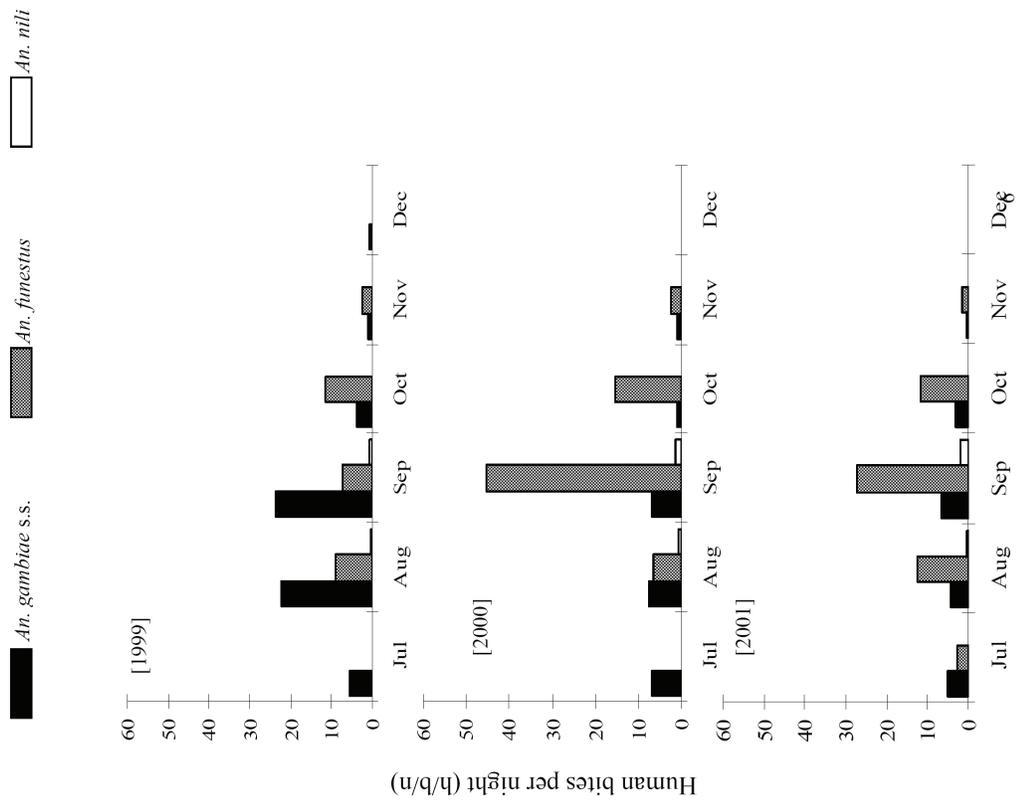


Figure 2: Monthly variation of the human landing rates from 1999-2001 in Lena.

decreased sharply in October. *An. funestus* remained below 15/b/h/n in all months (Figure 3). These two vectors were found biting until December at low frequencies. In 2000, *An. gambiae* s.l. biting predominated until August after which *An. funestus* increased and reached a peak of 45 b/h/n in September. Thereafter, the *An. gambiae* s.l. biting rate declined and neither *An. funestus* nor *An. gambiae* s.l. were observed in December. In 2001, *An. funestus* predominated as early as August and remained predominant until the end of the rainy season. Overall, the relative proportion of *An. gambiae* s.l. decreased and *An. funestus* increased.

Parity, circumsporozoite, and entomological inoculation rates

Data on parity and sporozoite rates of each species in each year are shown in Table 2. Because there were no major changes in parity rate, variation in EIR (Figure 4) resembled those in sporozoite rates, i.e., predominance of *An. gambiae* until September in 1999 with *An. funestus* thereafter. In 2000 and 2001, *An. funestus* predominated even in the earlier months (Table 2). *P. falciparum* CS protein was analyzed in 330 females of *An. gambiae* s.l. for species and molecular form identification. Only 7% (n=23) were identified as *An. arabiensis* vs 93% (n=307) as *An. gambiae* s.s. No *An. arabiensis* were found to be infected with *P. falciparum*. Among 210 females identified as *An. gambiae* s.s., 28% (n=60) were characterized as the M molecular form vs 72% of the S (n=150) molecular form. Three hundred female *An. funestus* (100 in each year) were analysed by PCR and identified as *An. funestus* s.s.

The anthropophilic rate

Eighty-two *An. gambiae* s.s. and 30 *An. funestus* randomly sampled from indoor resting females were tested for their origin of blood meal. These two species had anthropophilic rates reaching up to 55%, with a few cases of mixed meals (Table 3).

DISCUSSION

Because Lena is a typical savannah village, mosquito larval habitats are mainly rain-dependent and vector activities coincide with the rainy season. Thus, vector populations were dominated by *An. gambiae* s.l. during most of the rainy season. However, we observed some residual and intense vector activity occurring toward the end of the rainy season resulting from the appearance of *An. funestus* concomitantly with *An. gambiae* s.l., two major anthropophilic vectors. The first dominated the vector distribution especially in the years 2000 and 2001. Indeed the occurrence of *An. funestus* did not appear to modify overall vector abundance, but the proportion of each species. That led to a specific vector dynamic and malaria transmission pattern resulting in a high inoculation rate toward the end of the rainy season where the natural larval sites were drying. This increase of *An. funestus* apparently resulted from a semi-permanent swamp that favored *An.*

gambiae s.l. at the beginning of the rainy season before the vegetation grew abundantly and covered the water surface. As *An. gambiae* developed better in sites exposed to the sun and without vegetation (Carnevale et al. 1999), they had less ability to colonize this kind of breeding site and *An. funestus* had the opposite requirements. Indeed, there was more rain in 2000 than the other two years which might lead one to expect more vectors there, but this was not the case. In each year, August had the most rainfall but there was not a corresponding peak of the two vectors. The presence of a swamp is apparently the main cause of increased vector abundance toward the end of the rainy season. The dumping in the swamp of waste soil for road construction made the site more favorable to *An. funestus*. Furthermore, the volume of rain that fell between June and August was relatively higher and might contribute first, to leach away the breeding sites colonized by *An. gambiae* and second, to fill the swamps favorable to *An. funestus*.

In conclusion, the modification of the natural landscape by human activities can greatly change vector composition, shifting the malaria transmission pattern as suggested by our study. This landscape modification must be monitored and controlled by a system of planning permission to prevent vector proliferation that could interfere with local efforts to reduce the malaria burden. This consent action could lead to the restriction or even the elimination of the swamp which now allows vector breeding in the heart of the village.

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REFERENCES CITED

- Antonio-Nkondjio, C., P. Awono-Ambene, J. C. Toto, J.Y. Meunier, S. Zabaze-Kemleu, R. Nyambam, C.S. Wondji, T. Tchuimkam, and D. Fontenille. 2002. High malaria transmission intensity in a village close to Yaounde, the capital city of Cameroon. *J. Med. Entomol.* 39: 350-355.
- Antonio-Nkondjio, C., F. Simard, P. Awono-Ambene, P. Ngassam, J.C. Toto, T. Tchuimkam, 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.
- 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.

- Beier, J.C., P.V. Perking, R.A. Wirtz, J. Koros, D. Diggs, T.P.I.I. Gargan, and D.K. Koetch. 1988. Blood meal identification by direct enzyme-linked immunosorbent assay (Elisa) tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J. Med. Entomol.* 25: 9-16.
- Carnevale, P., P. Guillet, V. Robert, D. Fontenille, J. Doannio, M. Coosemans, and J. Mouchet. 1999. Diversity of malaria vectors in rice growing areas of the Afrotropical region. *Parassitologia* 41: 273-276.
- 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. Hyg.* 69: 200-205.
- Coetzee, M., M. Craig, and D. Le Sueur. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitol. Today* 16: 74-77.
- Dabiré, K.R., T. Baldet, A. Diabaté, I. Dia, A. Cohuet, C. Costantini, T.R. Guiguemdé, and D. Fontenille. 2007. *Anopheles funestus* s.l. Giles (Diptera: Culicidae) in a humid savannah area of western Burkina Faso: bionomics, insecticide resistance status and role in malaria transmission. *J. Med. Entomol.* (in press).
- Diabaté A., T. Baldet, F. Chandre, M. Akogbeto, F. Darriet, C. Brengues, T.R. Guiguemdé, P. Guillet, J. Hemingway, and J.M. Hougard. 2002. The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. *Am. J. Trop. Hyg.* 67: 617-622.
- Favia, G., A. Lanfrancotti, L. Spanos, I. Sideén-Kiamos, and C. Louis. 2001. Molecular characterisation of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. *Insect Mol. Biol.* 10: 19-23.
- Gillies, M.T. and M. Coetzee. 1987. A supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region), vol. 55. Publications of the South African Institute of Medical Research, Johannesburg, South Africa, 143pp.
- Koekemoer, L.L., M.M. Weeto, 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.
- Mouchet, J., P. Carnevale, M. Coosemans, J. Julvez, S. Manguin, D. Richard-Lenoble, and J. Sircoulon. 2004. Biodiversité du paludisme dans le monde. John Libbey Eutotext, 428 pp.
- Muirhead-Thomson RC. 1958. A pit shelter for sampling outdoor mosquito populations. *Bull. Wld. Hlth. Org.* 19: 1116-1118.
- Robert, V., P. Carnevale, V. Ouedraogo, V. Petrarca, and M. Coluzzi. 1985. La transmission du paludisme humain dans un village de savane du Sud-Ouest du Burkina Faso. *Ann. Soc. Belge Med. Trop.* 68: 107-121.
- Scott, J.A., W.G. Brogdon, and F.M. Collins. 1993. Identification of single specimens of *Anopheles gambiae* complex by the polymerase chain reaction. *Am J. Trop. Med. Hyg.* 49: 520-529.
- Touré Y.T., V. Petrarca, S.F. Traoré, A. Coulibaly, H.M. Maiga, O. Sangaré, M. Sow, M.A. Di Decco, and M. Coluzzi. 1998. The distribution and inversion polymorphism of chromosomally recognised taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia* 40: 477-511.
- Tripet, F., T. Thiemann, and G. C. Lanzaro. 2005. Effect of seminal fluids in mating between M and S forms of *Anopheles gambiae*. *J. Med. Entomol.* 42: 596-603.
- Wirtz, R.A., F. Zavala, Y. Charoenvit, G.H. Campbell, T.R. Burkot, I. Schneider, K.M. Esser, R.L. Beaudoin, and G. Andre. 1987. Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for Elisa development. *Bull. Wld. Hth. Org.* 65: 39-45.