Bionomics of *Anopheles gambiae* Giles, *An. arabiensis* Patton, *An. funestus* Giles and *An. nili* (Theobald) (Diptera: Culicidae) and Transmission of *Plasmodium falciparum* in a Sudano-Guinean Zone (Ngari, Senegal)

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ABSTRACT An entomological study was conducted in a village of Sudano-Guinean savanna in Senegal, during the rainy season from July to November 2001, to investigate the biology and the involvement of each anopheline species in malaria transmission. Mosquitoes were captured when landing on human volunteers and by pyrethrum spray catches. Twelve anopheline species were captured. Four species amounted to 97% of human-bait sampling: *Anopheles gambiae* molecular form S, *An. arabiensis*, *An. funestus*, and *An. nili* s.s. All *An. gambiae* and *An. nili* females were fed on human, whereas the anthropophilic rate was 94.5% for *An. funestus* and 88.9% for *An. arabiensis*. *Plasmodium falciparum* was the only malaria parasite found, and infecting only *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili*. The circumsporozoite rate was 4.5% for *An. gambiae*, 1.6% for *An. arabiensis*, 3.9% for *An. funestus*, and 2.1% for *An. nili*. During the period of study, the entomological inoculation rate was estimated to 264 infected bites. *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* were responsible respectively of 56, 3, 20, and 21% of malaria transmission. This study shows for the first time the implication of *An. nili* in malaria transmission in this area and the complexity of the malaria vectorial system that should be taken into account for any malaria control strategy.

KEY WORDS malaria, transmission, vectors, Sénégal

MALARIA IS STILL NOWADAYS one of the most important diseases in the World (Greenwood and Mutabingwa 2002). In Tropical Africa, malaria parasites are transmitted by primary vectors such as species of the *Anopheles gambiae* complex and *An. funestus* and by secondary focalized species such as species of *An. nili* complex and *An. moucheti* (Fontenille and Lochouarn 1999). In Senegal, many studies on malaria transmission have been undertaken (Faye et al. 1993, 1994,1995, Konate et al. 1994, Robert et al. 1998, Lemasson et al. 1997, Fontenille et al. 1997a,b) mainly in sahelian and sudanian bio-climatic areas. These studies showed that *An. gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles are the main vectors. The two first species are sympatric throughout the country with a variable frequency according to the climatic conditions. *An. funestus* is found almost in all bioclimatic areas near swamps or rivers (Vercruysse and Jancloes 1981, Faye et al. 1995). In contrast there is no published study on malaria vectors in the extreme southeast whereas the anopheline populations species are diversified (Diagne et al. 1994). Contrary to the other regions studied up to now, preliminary studies in this area during the 2000 rainy season have shown the implication of species from *An. nili* complex in malaria transmission (Dia and Fontenille, unpublished data). Four different species belonging to *An. nili* complex exist in Africa. A multiplex polymerase chain reaction (PCR) assay was developed recently for identifying *An. nili*, *An. carnevalei*, *An. somalicus*, and the new *An. nili* molecular form oveng (Kengne et al. 2003). The present work was then conducted in a village situated in Sudano-guinean area to study the anopheline fauna and the implication of each species in malaria transmission.

Materials and Methods

Study Site. The study was carried out from July to November 2001 in the village of Ngari (12° 38′N, 12° 14′W) situated in the extreme southeast of Senegal in a rural area 13 km northeast of Kedougou City. This area belongs to the Sudan-Guinean phytogeographic area and is hilly contrasting with the flat plain that constitutes the rest of the country. Villages around this area are small, agricultural and dispersed. Approximately 100 inhabitants live in this village. The dominant ethnic group is mandingue who are mainly farmers. Main cultures are represented by cotton, millets,

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and peanuts. Most of the houses are of traditional type with spaced and round houses with mud walls and thatched roofs. A small river "The Fangoli" which flows during the rainy season is situated behind the village. The rainy season lasts from June to October–November with an average annual rainfall of 1250 mm. The mean temperature is 25.2°C ranging from 23.5°C in January to 33.0°C in April. Some domestic animals such as sheep, goat, chicken, and cow stay sometimes for the night within the village.

Field Processing of Mosquitoes. Two classical sampling methods were used: (1) capture of females landing on human at six collection sites, at the frequency of two nights capture each month from 07:00 P.M. to 07:00 A.M. using 12 person-nights per month half indoor and half outdoor and (2) pyrethrum spray catches of resting females each month in the morning after the second night collection. Mosquitoes were sorted and identified morphologically following the key of anopheline species of Senegal (Diagne et al. 1994). Blood meals from fed mosquitoes collected by pyrethrum spray catches were blotted onto filter paper to determine the host source. All mosquitoes were stored individually in numbered vials with dessicant for laboratory processing.

Laboratory Processing of Mosquitoes. The origin of blood meals from fed females captured after pyrethrum spray catches was identified as human, bovine, ovine, and horse using an enzyme-linked immunosorbent assay (ELISA) from the procedure of Beier et al. (1988).

The head-thoraces of all anopheline females were tested by ELISA for circumsporozoite protein (CSP) of *Plasmodium falciparum*, *P. malariae*, and *P. ovale*. The procedure was that of Burkot et al. (1984) and Wirtz et al. (1987). A random sample of at least 30 females belonging to the *An. gambiae* complex was identified to species each month using the PCR technique described by Scott et al. (1993). The likely number of individuals per species for each month and capture method were then calculated by extrapolation. A sample of *An. gambiae* s.s. females was tested by PCR for molecular forms M and S according to Favia et al. (2001). All CSP positive *An. gambiae* s.l. were also processed by PCR for species identification. A sample of *An. nili* was tested by PCR for identifying the species among the complex following the procedure recently developed by Kengne et al. (2003).

Data Analysis. The human biting rate (HBR) was defined as the ratio of total mosquitoes captured for a period to the total person-night used for the same period. The endophagous rate was defined as the proportion of mosquitoes captured indoors among those caught indoors and outdoors from human landing collections. The circumsporozoite rate was calculated as the proportion of mosquitoes found to contain the CS protein. The anthropophilic rate was calculated as the proportion of human blood to the total blood meals determined. The entomological inoculation rate (EIR) was calculated as the product of the human biting rate (HBR) and the CSP rate of mosquitoes collected on night catches.

Table 1. Number of anopheline species collected from July to November 2001 in Ngari village

Mosquito species	Sampling methods			Total
	Human landing collection		Indoor spray	
	Indoor	Outdoor	Catch	
An. brunnipes	0	2	0	2
An. coustani	3	35	0	38
An. domicola	0	12	0	12
An. gambiae s.l.	709	770	430	1909
An. flavicosta	4	9	0	13
An. funestus	181	339	82	602
An. hancocki	2	6	0	8
An. nili	390	641	10	1041
An. pharoensis	1	10	0	11
An. rufipes	0	5	0	5
An. squamosus	0	3	0	3
Total	1290	1832	522	3644

Results

Mosquito Collections. From July to November 2001, 3122 anopheline specimens were captured during 60 person-night collections on human and 522 by pyrethrum spray catches in bedrooms. Anopheline species captured on human are presented in Table 1. *An. gambiae* s.l., *An. funestus*, and *An. nili* s.l. represented overall 97% of anopheline captured. *An. gambiae* s.l. was the most captured species (48.81%) followed by *An. nili* (34.03%) and *An. funestus* (17.16%).

Of the 1479 *An. gambiae* s.l. females captured on human, 224 were processed using the PCR technique. *An. arabiensis* and *An. gambiae* were present in the village. *An. gambiae* was predominant at a frequency of 84.9% (Table 2). From pyrethrum spray catch, 134 females of the 430 collected were identified by PCR. *An. gambiae* was also predominant (percentage 92.5%). The proportions of the two species by the two sampling methods were statistically different (χ^2 = 4.6, df = 1, P = 0.03). The only molecular form of *An. gambiae* s.s. recorded was the S form. All the 50 specimens from *An. nili* complex belonged to *An. nili* s.s.

Biting Cycles. All the four species were caught in each month sampled from indoor and oudoor collections. The mean number of bite per human per night was 21.5 for *An. gambiae*, 3.2 for *An. arabiensis*, 8.7 for *An. funestus*, and 17.2 for *An. nili*. The HBR for the four species varied with the rainy season (Fig. 1). The biting rate was maximal in October. The maximum rate

Table 2. Number and percentage (%) of species of the *An. gambiae* complex caught from July to November 2001 in Ngari village

Number percentage	Landing indoor and outdoor	Resting in bedrooms	Total
No. *An. gambiae* s.l. captured	1479	430	1909
No. *An. gambiae* s.l. tested by PCR	224	134	358
% *An. gambiae*	84.9	92.5	86.6
% *An. arabiensis*	15.1	7.5	13.4

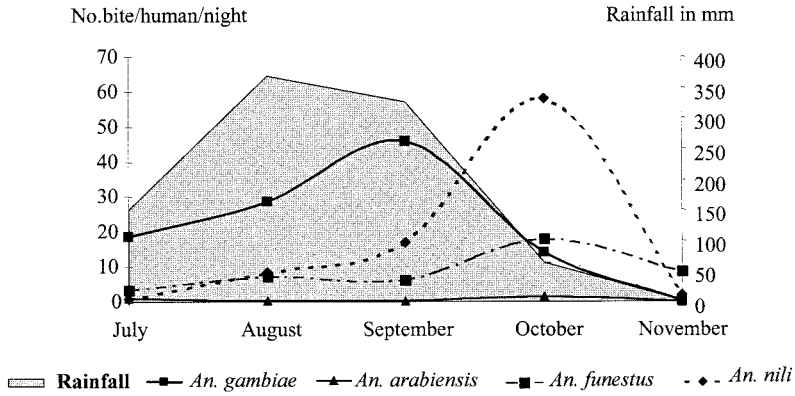

Fig. 1. Monthly density of *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* in relation to rainfall.

was in September for *An. gambiae* and in October for the three other species with respectively an average peak of 46, 8, 58, and 18 bites per person per night. *An. gambiae* density increased from the beginning toward the end of the rainy season from July to September. For the three other species, a regular increase was observed. *An. funestus* was the predominant species in November.

Host-Seeking Behavior. Overall, 48.4% of host-seeking *An. gambiae*, 46% of *An. arabiensis*, 34.8% of *An. funestus*, and 37.8% of *An. nili* were captured indoors. The endophagous rates were highly different between the four species ($\chi^2 = 40.16$, df = 3, $P < 0.0001$).

A total of 156 blood meals from resting females were tested by ELISA to determine the source (Table 3). Mixed blood meals were observed only in *An. funestus*. All the blood meals tested for *An. gambiae* and *An. nili* were taken on human. The anthropophilic rates were 88.9% for *An. arabiensis* and 94.5% for *An. funestus*.

Circumsporozoite Protein Rate. In total, 3117 anopheline specimens belonging to the 12 species collected on human, were processed by ELISA. Only *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* were found to contain the CS protein. *P. falciparum* was the only *Plasmodium* species found. In total, 4.5% [$CI_{95\%}$ = 3.4–5.6] of *An. gambiae*, 1.6% [$CI_{95\%}$ = 0–3.4] of *An. arabiensis*, 3.9% [$CI_{95\%}$ = 2.2–5.6] of *An. funestus* and 2.1% of *An. nili* [$CI_{95\%}$ = 1.2–3] were positive by ELISA (Table 4). The differences were statistically significant between the four species (χ^2 = 12.07, df = 3, $P = 0.007$).

Table 3. Number and percentage (%) of *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* fed on each vertebrate host from July to November 2001 in Ngari village among resting mosquitoes

Mosquito species	No. of mosquitoes	Vertebrate hosts (%)			% mixed
		Human	Bovine	Ovine	
An. gambiae	83	83 (100)	-	-	-
An. arabiensis	9	8 (88.9)	1 (11.1)	-	-
An. funestus	54	51 (94.4)	1 (1.9)	2 (3.7)	3 (5.6)
An. nili	10	10 (100)	-	-	-
Total	156	152 (97.4)	2 (1.3)	2 (1.3)	3 (1.9)

Entomological Inoculation Rate. The mean entomological inoculation rate for the period studied was estimated to 264 infected bites (Table 4). *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* were responsible respectively to 56, 3, 20, and 21% of the transmission. *P. falciparum* transmission by *An. funestus* was observed throughout the study period, this species was responsible of the whole transmission in November. Transmission by *An. gambiae* and *An. nili* was observed during the period studied except at the end of the rainy season in November for *An. gambiae* and July and November for *An. nili*. The implication of *An. arabiensis* was observed only in October when its density is maximal.

Discussion

During this study from July to November 2001, 12 anopheline species out of the 20 already recorded in Senegal were collected by the two sampling methods in Ngari village. This observation confirms those of Diagne et al. (1994) in their review on the anopheline fauna of Senegal. The variety of the anopheline populations species has to be linked to the number and the diversity of the biotopes in this area. However, only the predominate species *An. gambiae*, *An. arabiensis*, *An. funestus*, and *An. nili* were involved in *P. falciparum* transmission. Resting populations from these species were highly antropophilic in accordance with Konate et al. (1999) and Dia et al. (2001) in the same area. None of the other species was involved in malaria transmission.

Only *An. gambiae* represented by its S molecular form and *An. arabiensis* of the *An. gambiae* complex are present in the study area.

An. gambiae was predominant contrary of sahelian and sudanian areas where *An. arabiensis* is the major vector (Fontenille et al. 1997a,b, Lemasson et al. 1997, Faye et al. 1993, Robert et al. 1998). The endophagous rates calculated from landing collections of the two species were similar. However, the proportion of *An. gambiae* to *An. arabiensis* was higher among resting than among host-seeking collections. Therefore *An.*

Table 4. Circumsporozoite protein and entomological inoculation rates calculated by enzyme-linked immunosorbent assay for *P. falciparum* for each vector species during the period of study

Mosquito Species	July			August			September			October			November			Total		
	Nt	CSR	EIR	Nt	CSR	EIR	Nt	CSR	EIR	Nt	CSR	EIR	Nt	CSR	EIR	Nt	CSR	EIR
An. gambiae	225	2.2	12.9	344	4.9	43.9	549	3.1	42.5	168	11.3	49.1	2	0	0	1288	4.5	148.4
An. arabiensis	65	0	0	11	0	0	13	0	0	97	3.1	7.7	5	0	0	191	1.6	7.7
An. funestus	39	2.6	2.6	88	4.5	10.2	74	2.7	5	215	5.6	31.1	103	1	2.5	519	3.9	51.4
An. nili	8	0	0	100	6	15.5	204	2.5	12.5	697	1.6	28.4	22	0	0	1031	2.1	56.4

Nt, number of mosquitoes tested by ELISA; CSR, circumsporozoite protein rate; EIR, entomological inoculation rate.

gambiae seems to have a higher degree of endophily. Nevertheless, because of the moderate number of mosquitoes captured, and the potential bias inherent to mosquito sampling, the comparison between these two species in this area would need further investigation.

Density fluctuations of *An. gambiae* in this bio-climatic area of Sudano-Guinean savanna are related to the rainfall as what is generally found in the other bio-climatic areas in Senegal. A regular increase was observed with the rainfall toward the end. A similar observation was also observed with *An. funestus* and *An. nili* whose maximal densities were observed at the end of the rainy season in October.

An. gambiae was the principal vector in this area followed by *An. nili*, *An. funestus*, and *An. arabiensis*. *An. gambiae*, *An. arabiensis*, and *An. funestus* are already found to be involved in malaria transmission in Senegal. *Plasmodium* positive *An. nili* was previously observed in this region (Fontenille and Dia, unpublished data). This study evaluates for the first time the exact role of this species in malaria transmission and it appears that it must not be considered as a secondary vector. A lower infection rate was observed compared with *An. gambiae* and *An. funestus* but this species had a very high HBR. Among the four species or forms usually described within *An. nili* complex, only *An. nili* s.s. was observed in eastern Senegal. Species of this complex have a major implication in malaria transmission in central African regions (Carnevale et al. 1992, Elissa et al. 1999, Antonio-Nkondjio et al. 2002). This study shows clearly a complex vectorial system with four vector species. Hence, any strategy for controlling malaria vectors in this area should take in account this heterogeneity.

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