Anopheles funestus (Diptera: Culicidae) in a Humid Savannah Area of Western Burkina Faso: Bionomics, Insecticide Resistance Status, and Role in Malaria Transmission

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J. Med. Entomol. 44(6): 990–997 (2007)

ABSTRACT An entomological survey was carried out in three humid savannah sites of western Burkina Faso (Bama, Lena, and Soumousso) to 1) update the taxonomy of the *Anopheles funestus* Giles group, 2) examine the role of each species in malaria transmission, 3) characterize the insecticide resistance status of this malaria vector, and 4) determine the distribution of *An. funestus* chromosomal forms in these areas. Polymerase chain reaction identification of the members showed the occurrence of *An. leesoni* Evans in Lena and *An. rivulorum*-like in Soumousso in addition to *An. funestus* s.s. Malaria transmission was ensured mainly by *An. funestus* s.s. both in Soumousso and Lena and by *An. gambiae* s.s. Giles in Bama, the rice-growing area. The insecticide resistance status performed only on *An. funestus* indicated that this mosquito was susceptible to pyrethroids irrespective of the study area, but it was resistant to dieldrin. Furthermore, the occurrence of the two chromosomal forms of *An. funestus*, namely, Kiribina and Folonzo, seemed to follow ecological setups where Kiribina predominated in the irrigated area and Folonzo was more frequent in classic savannah. This study revealed that the problematic of *An. funestus* taxonomy was closer to that of *An. gambiae* requiring more structured studies to understand its genetic ecology.

KEY WORDS *Anopheles funestus*, malaria, insecticide resistance, cytogenetics

Malaria transmission in sub-Saharan Africa is dominated by three widespread vectors: *Anopheles gambiae* s.s. Giles, *An. arabiensis* Patton, and *An. funestus* Giles. Studies on the former two species, especially *An. gambiae*, are abundant, including a wide range of topics such as chromosomal polymorphism (Coluzzi et al. 1985, Touré et al. 1998), molecular characterization (Scott et al. 1993, Favia et al. 2001), ecology (Carnevale et al. 1999), insecticide resistance status (Diabaté et al. 2002), and population genetic structure (Lehmann et al. 2003). Conversely, the biology of *An. funestus* is relatively poorly studied despite its importance in malaria transmission, especially in eastern and southern Africa (De Meillon et al. 1977, Coetzee and Fontenille 2004). Unlike *An. gambiae* s.l., which is a complex of seven morphologically similar sibling species identifiable by fixed rDNA nucleotide substitutions (Scott et al. 1993, Hunt et al. 1998), *An. funestus* belongs to a group of no less than nine species that are difficult to distinguish based solely on morphological characters of a single life stage (Gillies and Coetzee 1987, Harbach 1994). Species identification difficulties have been recently addressed by molecular techniques based on the polymerase chain reaction (PCR) by using a cocktail of species-specific primers permitting identification of the six most common species of the group (Koekemoer et al. 2002). Recent analyses of rDNA sequences (Cohuet et al. 2003) revealed the occurrence in West and Central Africa of a new taxon morphologically related to *An. rivulorum*, which is provisionally named *An. rivulorum*-like, thereby enlarging the number of members of the *An. funestus* group to 10. Among all the members of the funestus group, *An. funestus* s.s. is the most anthropophilic species, and it is considered as the only major malaria vector, although in a Tanzanian village the circumsporozoite protein of *Plasmodium falciparum* was detected by immunological techniques in some *An. rivulorum* Leeson specimens (Wilkes et al. 1996). *An. vaneedeni* Gillies and Coetzee can be experimentally infected with *P. falciparum* in the laboratory, but there is as yet no evidence for its role in malaria transmission in the field (De Meillon et al. 1977), presumably because of its highly zoophilic behavior.

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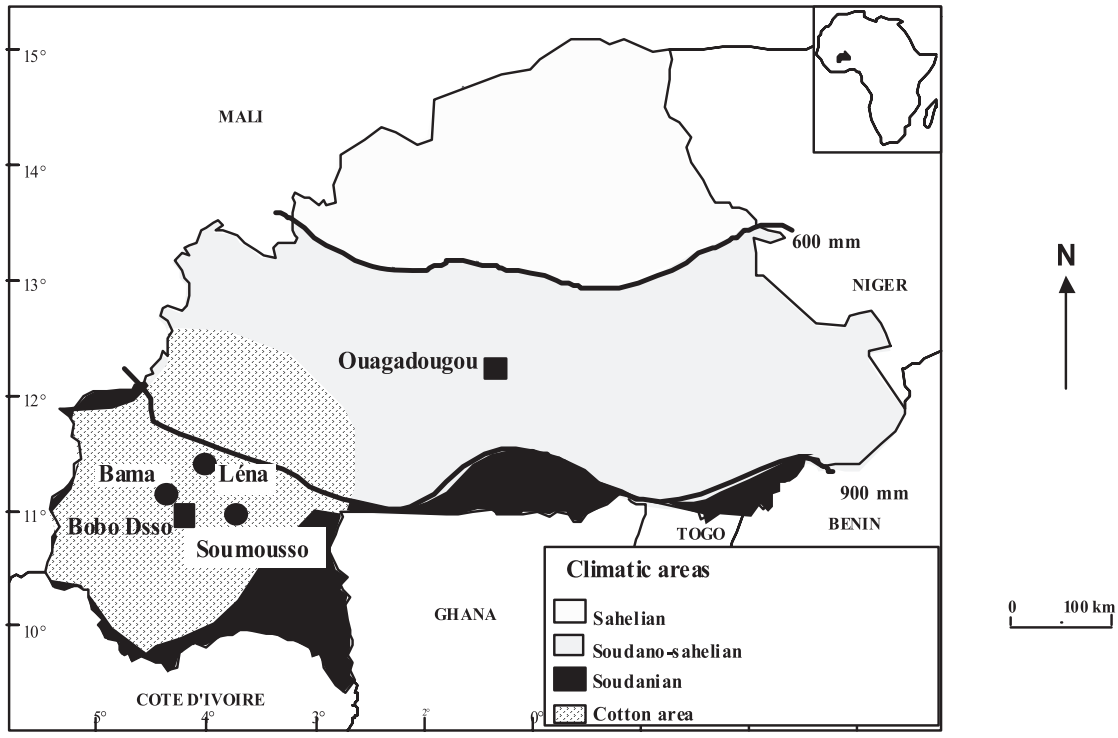

Fig. 1. Location of the study sites.

The genetic polymorphism of *An. funestus* became the subject of investigations only since the early 1980s (Green and Hunt 1980). Even more recently, the chromosomal analysis of polymorphic inversions in sympatric populations of *An. funestus* from Burkina Faso revealed significant departures from Hardy–Weinberg and linkage equilibria, leading to the proposed subdivision of this species in two chromosomal forms, provisionally named Folonzo and Kiribina (Costantini et al. 1999). However, no molecular marker is as yet available to differentiate between these two operational taxonomic units.

In the southwestern region of Burkina Faso, the intensive use of insecticides for agricultural purposes, most notably on cotton, *Gossypium hirsutum* L., is thought to select for insecticide resistance genes in mosquitoes whose breeding sites are exposed to pesticide runoff. The *kdr* allele is one of the main resistance mechanisms involved in anopheline resistance to pyrethroids, occurring mainly in the S molecular form of *An. gambiae* (Diabaté et al. 2002). Resistance to pyrethroids in *An. funestus* has been reported from South Africa (Hargreaves et al. 2000); hence, it is necessary to verify and monitor the insecticide susceptibility status of this species in other areas where insecticides are intensively used.

The objective of the current study was to initiate an in-depth examination of the ecological genetics of the two chromosomal forms of *An. funestus* to 1) establish the vector status of the members of the group living in western Burkina Faso, 2) characterize their bionomics impacting on their capacity to transmit malaria, 3) investigate the insecticide resistance status of *An. funestus* in an area where pesticides are extensively used, and 4) relate the distribution and frequency of the two chromosomal forms of *An. funestus* to varying ecological settings.

Materials and Methods

Study Sites. Anopheline specimens were collected from Bama (11° 24′ N, 04° 24′ W), Lena (11° 18′ N, 03° 53′ W), and Soumousso (11° 00′46″ N, 4° 02′45″ W), three villages located in the humid savannah of southwestern Burkina Faso (Fig. 1). In this area, there are two distinct seasons: the rainy season occurs only from May to October, with a long dry season from November to April. The average annual rainfall ranges from 1,000 to 1,200 mm (records from the latest 5 yr).

Soumousso is a typical Guinean savannah village situated ≈55 km east from Bobo-Dioulasso, the second largest town of Burkina Faso. Three main anopheline malaria vectors are found in this village, including both molecular forms M and S of *An. gambiae*, *An. funestus*, and *An. nili*; *An. arabiensis* is occasionally reported at low frequency (5% of *An. gambiae* s.l. samples). Anopheline breeding sites consist mostly of rain puddles and a semipermanent swamp suitable to the development of *An. funestus* larvae.

Lena is ≈70 km north from Bobo-Dioulasso. It is ecologically similar to Soumousso, with a semipermanent pool suitable to *An. funestus* larval development.

These two villages lie in the cotton belt of Burkina Faso, where insecticides against agricultural insect pests are intensively used during the cropping period.

Bama is ≈30 km northwest from Bobo-Dioulasso in the valley of the Kou River (Vallée du Kou), a region where extensive rice, *Oryza sativa* L., cultivation has been practiced since the 1970s. This area contains seven villages covering 1,200 ha surrounded by wooded savannah. Both molecular forms M and S of *An. gambiae* are recorded at high densities during the rainy season (especially the M form: ≈200 bites person/night [b/h/n]). *An. funestus* is proportionally less abundant, but its population densities have increased during the last decade (Baldet et al. 2003). Few insecticides are used on the rice, but insecticides are used extensively for cotton located exterior to the rice fields.

Mosquito Collections. Anopheline mosquitoes were sampled from July to December during 2000 rainy season at a frequency of four sessions per month by three sampling methods: human landing catches, indoor insecticide spray-sheet catches, and larval collections. The human landing catches were performed by informed volunteers who were provided free and rapid treatment when suspected clinical signs of malaria according to World Health Organization (WHO)-recommended regimen on the basis of fever and detectable *P. falciparum* parasitemia. To evaluate human biting rates, pairs of human "baits" sat indoors and outdoors collecting mosquitoes that landed on them, by means of a flashlight and glass tubes. Collections were carried out between 1800 and 0600 hours inside and just outside of four houses in each village. To standardize catching efficiency, collectors rotated between houses on subsequent nights.

Indoor resting females were caught by spraying village huts with insecticide aerosols. Female mosquitoes were knocked down onto, and immediately retrieved from, white sheets laid down on the floor of sprayed huts. Mosquitoes were dissected, and the head and thorax were preserved to determine their infectious status. Legs were separated from the carcass and kept dry for molecular species identification. Half-gravid *An. funestus* females were stored individually in 1.5-ml tubes containing Carnoy fixative (3 parts absolute ethanol to 1 part glacial acetic acid), and they were brought to the laboratory for later chromosomal scoring. Larvae of *An. funestus* were collected at the end of the rainy season (September) from Bama and Soumousso and brought to the insectary in Bobo-Dioulasso for rearing. Emerging 2-d-old females were then used in insecticide susceptibility tests.

Laboratory Processing of Mosquitoes. Anophelines were sorted and assigned to species based on morphological characters by using standard identification keys (Gillies and De Meillon 1968). Later, all females tested by enzyme-linked immunosorbent assay (ELISA) (see below) were processed by PCR for molecular identification of species of the *An. funestus* group as described in Cohuet et al. (2003).

The heads and thoraces of anopheline females were tested for the presence of the circumsporozoite protein (CSP) of *P. falciparum*, the major malarial parasite occurring in the study area, by ELISA following the protocol of Beier et al. (1988).

Insecticide Resistance Tests. Two organochlorines (DDT 4% and dieldrin 4%), and two pyrethroids (permethrin 1% and deltamethrin 0.025%) were tested according to the standard WHO vertical tube protocol. Mortality was scored 24 h after an exposure of 100 2-d-old females for 1 h. KDT_{50} and KDT_{95} values corresponding, respectively, to the time that 50 and 95% of ≈100 tested mosquitoes were knocked down were established and compared among three insecticides (permethrin 1%, deltamethrin 0.025%, and DDT 4%), and the susceptible reference strain of *An. gambiae* s.s. (Kisimu) was used as control. The threshold of susceptibility was fixed at 90% for DDT 4% and at 95% for the other three active ingredients, respectively (WHO 1998). In the absence of an *An. funestus* reference strain, controls were established from the same pool of tested mosquitoes kept in insecticide-free WHO test tubes and from *An. gambiae* Kisumu susceptible reference strain. Control mortality was always 0%; therefore, no Abbott correction was necessary during analysis.

Chromosomal Inversions Analysis. For karyotyping, half-gravid females were removed from Carnoy fixative, and their ovaries were dissected. Polytene chromosomes were squashed and prepared for scoring according to Hunt (1973). The preparations were examined under a phase-contrast microscope at 120× magnification. Paracentric inversions were scored using the chromosomal map and nomenclature of Sharakhov et al. (2004), and karyotypes were assigned to chromosomal form according to the algorithm of Costantini et al. (1999), later modified by Guelbeogo et al. (2005). Two chromosomal forms are determined following this algorithm: the first form, named "Kiribina," is characterized mainly by the standard arranged at all loci; and the second form, named "Folonzo," is mainly polymorphic, with high frequencies of inversions 3R*a*, 3R*b*, and 2R*a*.

Data Analysis. The human biting rate (HBR) was calculated as the ratio of total mosquitoes captured for a period of the total person-night used for the same period. The rate of endophagy was defined as the proportion of mosquitoes caught indoors out of the total number collected both indoors and outdoors from the human landing collections. The circumsporozoite rate was calculated as the proportion of mosquitoes found positive for the CSP. The month entomological rate (EIR) was calculated as the product of the HBR and the CSP rate of mosquitoes collected on the total day of the month. The addition of the monthly EIR during the period of study gave the seasonal EIR. Significance of the test was determined by Fisher chi-square test. Comparisons of different percentages (HBR, CSP rate, and EIR) were done by chi-square test. Chromosomal data were analyzed with Fstat version 2.9.3.2 (Goudet et al. 1996).

Table 1. Total number (relative frequency, %) of anthropophagic anopheline females collected when biting human in three villages of southwestern Burkina Faso

Anopheline females	Bama		Lena		Soumousso	
	Indoors	Outdoors	Indoors	Outdoors	Indoors	Outdoors
An. gambiae s.l.	3,619 (95.2)	2,892 (96.5)	184 (24.4)	168 (28.3)	234 (14.9)	220 (18.7)
An. funestus s.l.	181 (4.8)	98 (3.3)	555 (73.5)	409 (68.8)	1,250 (79.7)	855 (72.5)
An. nili	1 (0.02)	7 (0.2)	16 (2.1)	17 (2.9)	85 (5.4)	104 (8.8)
Total	3,801	2,997	755	594	1,569	1,179

Results

Malaria Vector Species Composition. Of six 798, two 748, and one 349 human-biting malaria vectors collected in Bama, Soumousso, and Lena, respectively, all belonged to the *An. gambiae* s.l., *An. funestus* s.l., or *An. nili* species groups (Table 1). With the exception of the rice-growing area, *An. funestus* was the predominant species, at a frequency of 76.6 and 71.5% in Soumousso and Lena, respectively. Conversely, *An. gambiae* was the prevailing vector species in Bama, representing >95% of the total number of anophelines collected landing on humans. The proportion of exophagic *An. funestus* with respect to the total number collected indoors and outdoors did not differ significantly ($P > 0.05$) between Soumousso and Lena, and it was estimated at 40.6 and 42.4% respectively. Such rate did not differ significantly from that of Bama ($P = 0.07$), which reached 35.1%. Overall, *An. funestus* was the more endophagic of the three malaria vectors, irrespective of collection site.

***An. funestus* Human Biting Rates.** The relative density of *An. funestus* was very low in the rice area (Bama), with trivial density toward the end of the season (Fig. 2). This relative low frequency of *An. funestus* has been shadowed by the high abundance of *An. gambiae*. But, in Lena and Soumousso, *An. funestus* remained the most prevalent mosquito. Indeed, its activities were noted early in August in Lena and 1 mo later in Soumousso, and it was more intensive indoors than outdoors. The peak of the prevalence was observed in September and October in Lena and Soumousso, reaching 45 and 55 b/h/n, respectively. *An. funestus* density decreased quickly in October in Lena, but it remained active in Soumousso where the density was noted in December. The aggressive density outdoors was also important in these two villages where *An. funestus* was observed in human bait up to December with the HBR averaging 20 b/h/n. Finally, *An. funestus* occurred toward the middle of the transmission period from August onward and outnumbered *An. gambiae* until December when this mosquito continued to be active.

Identification of Species of *An. funestus* Group. In total, 864 mosquitoes of the funestus group caught on human bait between August and December 2000 have been identified by PCR. In the Bama rice-growing area, all the 117 mosquitoes analyzed were *An. funestus* s.s. In Lena, 12 *An. leesoni* and 258 *An. funestus* were identified. We found *An. leesoni* at a frequency of 30% in the November outdoor collections, the latest month of malaria transmission in this site. In Soumousso, we found only one specimen of *An. rivulorum*-like among 477 females tested. It was collected outdoors in September (one from 30 successfully identified). According to these data, species other than *An. funestus* s.s. were mostly exophagic, because they were caught exclusively outdoors, late at night (between 0200 and 0300 hours).

Sporozoite Rates of *An. funestus*. Of 1,199 *An. funestus* analyzed by ELISA for the presence of the circumsporozoite protein of *P. falciparum*, 98 were positive for the CSP antigen (Table 2). The sporozoite rate was significantly higher (9.7%) in Soumousso than in Lena, which averaged 4.9% ($P < 0.01$) and was even lower in Bama (2.6%). One *A. rivulorum*-like and one *An. leesoni* from Soumousso and Lena, respectively, were tested by ELISA and found negative. Compared with the other vectors, the sporozoite rate did not differ ($P = 0.08$) between *An. gambiae* and *An. funestus* in Bama, Lena, and Soumousso villages.

EIR of *An. funestus*. Malaria transmission was mainly ensured by *An. funestus* in the two savannah sites regardless of the month. Indeed, malaria transmission rate was highest in Soumousso, reaching 472 infected bites per person (Table 2). The dynamic of the transmission showed that the maximum of transmission occurred in Soumousso during the three latest months of the rainy season, reaching 146 infected bites per person in October. Inversely, in Lena, malaria transmission also driven mainly by *An. funestus* occurred early during the two first months and decreased significantly toward the end of the rainy season. In contrast, in Bama, the rice-growing area, malaria transmission was driven by *An. gambiae* irrespective of the period of the transmission. Here, the dynamic of the transmission varied one month to another, but the maximum of transmission also was observed in October with an EIR averaging 90 infecting bites per person.

Insecticide Susceptibility Status. *Knockdown Rate: KDT_{50}.* Except to Bama population for DDT4%, the KDT_{50} value did not differ significantly irrespective of the insecticide tested ($P > 0.05$; Table 3), and it was always faster, occurring during the first quarter (15 min) after the exposure time. In the Bama population tested with DDT 4%, the KDT_{50} value was relatively elevated, corresponding to 27 min after the exposure time.

Knockdown Rate: KDT_{95}. Regardless of the provenance of wild populations of *An. funestus* tested, the KDT_{95} values were more elevated with DDT 4% ($P > 0.05$; Table 3) than that with deltamethrin 0.025%. In

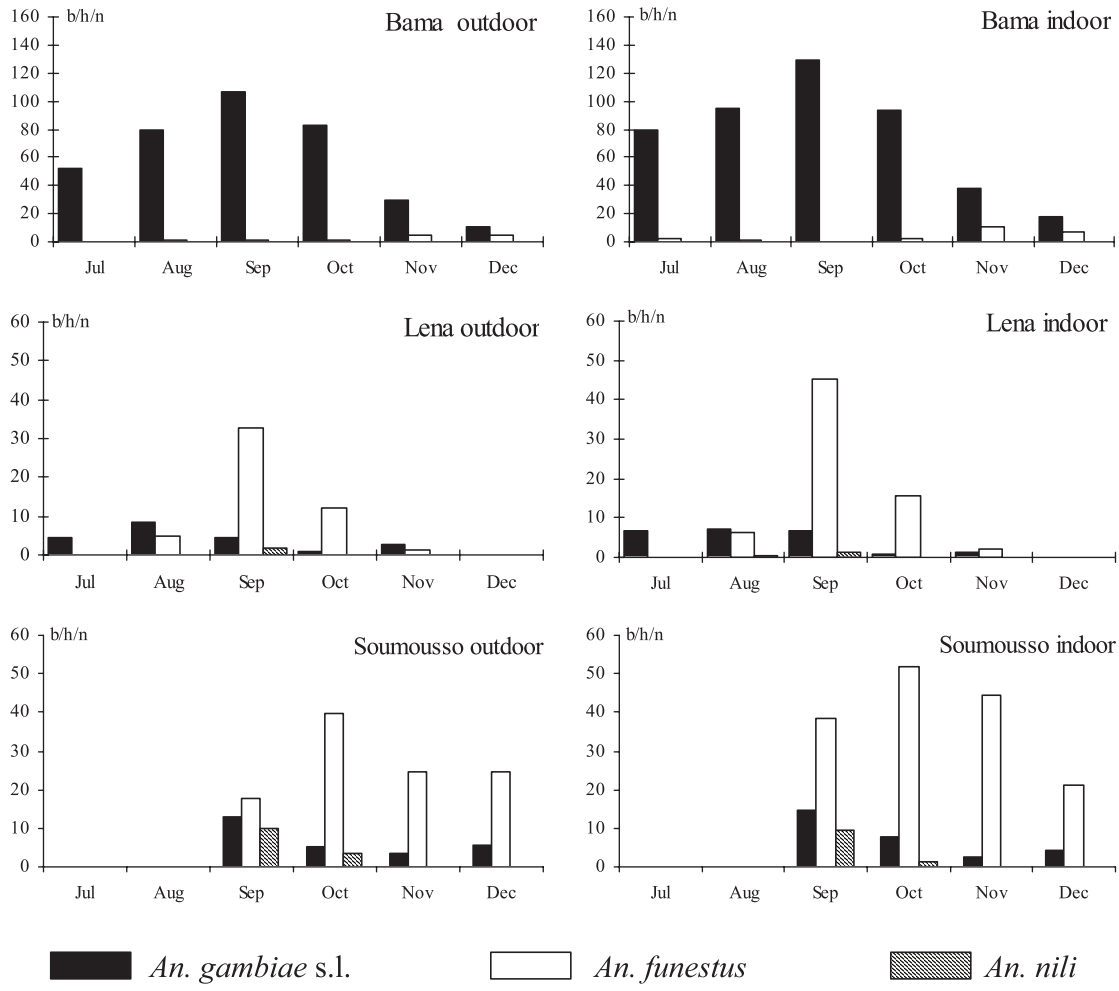

Fig. 2. Monthly variations of biting rates (b/h/n) in the three study sites.

contrast, the KDT_{95} with permethrin 1% from Lena and Bama populations was more elevated than that of Soumousso. In Lena, the KDT_{95} values with DDT 4% were closer than those of permethrin 1%, occurring at 37 and 42 min, respectively, after the exposure time. Globally, the KD effect was fastest with deltamethrin 0.025% compared with permethrin 1% and DDT 4%.

Mortality Rate. The mortality rate was 100% for all populations of *An. funestus* tested to all insecticides, except to dieldrin 4%. Indeed, mortality rates of this

Table 2. Circumsporozoite protein rate calculated by ELISA for *P. falciparum* for three malaria vectors in Bama, Lena, and Soumousso from August to December 2000

Site	Mosquito species	Aug.			Sept.			Oct.			Nov.			Dec.			Total EIR
		HBR	CSPR	EIR	HBR	CSPR	EIR	HBR	CSPR	EIR	HBR	CSPR	EIR	HBR	CSPR	EIR	
Bama	*An. gambiae*	2,948	2.4	70.2	3,885	ND		2,890	3.1	90.2	1,145	3.9	44.3	538	6.7	36	240.5
	An. funestus	35	0	0	300	0	0	81	0	0	303		0	205	4.3	8.8	9
	An. nili	0			0		0	0		0	0		0	0		0	0
Lena	*An. gambiae*	229	10.1	23.1	202	7.7	15.6	31	0	0	34	9.3	3.1	0	0	0	42
	An. funestus	198	20	39.5	1,354	3.7	50	491	3.3	15.85	68	9.5	6.4	0	0	0	112
	An. nili	19	0	0	0		0	0		0	0		0	0	0	0	0
Soumousso	*An. gambiae*	N.D.			443	5.5	24.3	244	6.25	15.3	75	9.1	6.8	128	8	10.2	57
	An. funestus	N.D.			1,159	4.7	54.5	1,604	9.1	146	1,339	9.6	128.5	659	12.6	83	412
	An. nili	N.D.			281	1.2	3.2	39	0	0	0			0		0	3.2

HBR, human landing rate nightly biting rate (ma) × no. of days of the month); CSPR, circumsporozoite protein rate (ratio positive mosquitoes by total no. tested × 100); EIR, entomological inoculation rate (HBR × CSPR/100); N.D., not determined. No entry indicates not possible.

Table 3. KDT values of *An. funestus* s.s. tested with three insecticides (permethrin 1%, DDT 4%, and deltamethrin 0.025%)

Site	DDT 4%			Deltamethrin 0.025%			Permethrin 1%		
	n	KDT_{50}	KDT_{95}	*n*	KDT_{50}	KDT_{95}	*n*	KDT_{50}	KDT_{95}
An. gambiae Kisumu (reference strain)	165	30 (29–32)	50 (48–54)	100	10 (9–11)	13 (12–15)	100	10 (9–11)	15 (12–18)
Bama	100	27 (26–28)	50 (46–56)	104	18 (17–19)	30 (28–32)	106	13 (12–14)	34 (31–39)
Lena	100	17 (16–18)	37 (34–41)	102	12 (11–13)	26 (24–30)	102	13 (12–14)	42 (37–48)
Soumousso	105	14 (13–16)	47 (41–56)	103	14 (13–15)	22 (20–24)	124	10 (9–11)	24 (22–26)

KDT_{50-95}, exposure time (expressed in minutes) corresponding to 50–95% of tested mosquitoes, respectively, knocked down. *n* is number of mosquitoes tested.

insecticide were 48.9 and 59.7%, respectively, in Soumousso and Bama. The insecticide resistance status from Lena for this active ingredient was not determined.

Distribution of Chromosomal Forms. All mosquitoes submitted to cytogenetic analysis were identified by PCR as *An. funestus* s.s. As observed in previous studies, *An. funestus* from this area was polymorphic for inversions 2R*a*, 3R*a*, 3R*b*, and 3L*a*. The frequency of each inversion within and across locales and other population genetics statistics are given in Table 4. Even evidence for Hardy–Weinberg or linkage disequilibria within localities should be perceptible, it had been masked by the small sample of mosquitoes tested, which did not point out a statistical difference, because the Fis value was higher with a P value <0.05. Despite the low sample size, the distribution of karyotypes was significantly different between villages (P value across all loci = 0.001); the locus-by-locus analysis revealed that differentiation was mostly limited to the two inversions on chromosomal arm 3R ($P < 0.001$). Applying the modified algorithm of Guelbeogo et al. (2005) to classify single karyotypes into one of the two chromosomal forms, it seemed that the distribution of the two taxa revealed a contrasting pattern of distribution between villages related to the main ecological conditions of each sampling site: Kiribina predominated in the rice-growing village of Bama (nine of 10 classified specimens; 90%), whereas in Lena and Soumousso Folonzo was the most frequent of the two taxa, with seven of 10 (70%) and seven of eight (88%) individuals, respectively, belonging to this form.

Discussion

Identification of Members of the *An. funestus* Group. In our study area, we recorded three species of the funestus group biting humans: *An. funestus* s.s., *An. leesoni*, and the new taxon provisionally named *An. rivulorum*-like. *An. leesoni* was known to be mostly exophilic and zoophilic (Gillies and Coetzee 1987), whereas in one of the collections of our study (in November at Lena), a significant proportion (30%) of human-biting *An. funestus* s.l. was molecularly identified as *An. leesoni.* Because we do not have independent complementary evidence (e.g., origin of bloodmeals), we do not know whether this population of *An. leesoni* has a specific preference for human feeding. Similarly, only one *An. rivulorum*-like, whose biology and vector status are virtually unknown so far, was collected on human baits. Both *An. leesoni* and *An. rivulorum*-like were exophagic and free of malaria parasites, although our data set is limited in scope and needs to be extended. Thus, only *An. funestus* s.s. was greatly implicated in malaria transmission in the three savannah villages of our study area, with sporozoite rates at times as high as 20%.

Species Composition, Vector Dynamics, and Vectorial Role of *An. funestus.* Toward the end of the rainy season (September), *An. funestus* was found to be the major malaria vector in these two savannah villages, where both its human biting and sporozoite rates were higher than those of other known vectors such as *An. gambiae* complex or *An. nili.* Conversely, in a rice-growing area embedded within this mostly cotton-growing region, *An. gambiae* remained the major malaria vector due to substantially higher population densities. Indeed, *An. funestus* is known to supplement *An. gambiae* s.l. in malaria transmission in the West African savannah, but its major vector role was hardly emphasized from historical studies of Robert et al. (1985, 1988) carried out in the humid savannah of West Burkina Faso. As early as 1985 in two savannah villages from this area, Kongodjan and Karangasso, *An. funestus* EIR reached 64 and 32 infected bites per human per yr, respectively. That number did not differ greatly from those of *An. gambiae*, reaching 69 and 45

Table 4. Frequency of polymorphic chromosomal inversions of *An. funestus* s.s. from three villages of southwestern Burkina Faso

Village	2*N* (range)	2R*a*	F_{is}	*P*	3R*a*	F_{is}	*P*	3R*b*	F_{is}	*P*	3L*a*	F_{is}	*P*	F_{is}	*P*
Bama	18–20	0.10	1.00	0.06	0.10	1.00	0.06	0.00	–	–	0.10	1.00	0.08	1.00	**0.0042**
Lena	20	0.50	0.25	0.38	0.55	0.44	0.23	0.30	0.10	0.67	0.05	0.00	1.00	0.26	0.1125
Soumousso	14–16	0.50	0.22	0.52	0.81	0.63	0.18	0.69	0.19	0.62	0.19	0.63	0.20	0.38	**0.0417**
Unweighted frequencies		0.37			0.49			0.33			0.11				

Probability values of Wright's F values (values in bold denote significance level $P < 0.05$ after Bonferroni correction) test for departures from Hardy–Weinberg equilibrium. The last two columns report the same test across all loci within a sample.

infected bites per human per yr, respectively, in these two villages for the same period. Nowadays in this area, the *An. funestus* EIR has increased considerably; however, it differs significantly between Lena and Soumousso with 110 and >400, respectively, infected bites per mo during malaria transmission season. Thus, the vector status of *An. funestus* in this region has presumably increased in importance during the last decade, where now it plays a major role, especially toward the end of the rainy season. Similarly, *An. funestus* also has been shown to be a major malaria vector in other African savannah regions, as observed in Cameroon and Senegal (Manga et al. 1997, Dia et al. 2000).

Insecticide Susceptibility Status of *An. funestus*. No resistance to two pyrethroids, deltamethrin and permethrin, nor to DDT was observed in *An. funestus* in our study area. However, the KD effect (KDT_{95}) of wild populations of *An. funestus* from the three study sites (Bama, Lena, and Soumousso) was relatively more elevated to DDT 4% and permethrin 1% compared with the reference *An. gambiae* s.s. susceptible strain. In Kwazulu/Natal, South Africa, and in the southern region of Mozambique, *An. funestus* has developed resistance to pyrethroids (Hargreaves et al. 2000). The South African government has since been obliged to switch back to DDT, despite the worldwide restrictions of this active ingredient (Coetzee and Fontenille 2004). In the southern African populations, resistance mechanisms other than *kdr* were involved, with mixed function oxidases conferring cross-resistance to carbamates such as propoxur (Brooke et al. 2001). Although no cross-resistance to DDT and pyrethroids was observed in our study area, *An. funestus* in Soumousso and Bama was highly resistant to dieldrin. Data from Lena were not available, but previous data collected in a village near Lena indicated that *An. funestus* there was resistant to dieldrin. Lena is close to and ecologically similar to Soumousso, and resistance to dieldrin was found irrespective of ecological settings, we can infer that resistance to this active ingredient is common and widespread in our study area. Insecticide susceptibility tests performed in 1967 in the same villages (Bama and Soumousso) already reported a lower degree of resistance to dieldrin in *An. funestus* (11% survival in Soumousso; Hamon et al. 1968). After the huge use of dieldrin in Africa in the 1970s, the level of resistance has greatly increased, reaching 51% survival in Soumousso, and >30% in Bama.

Distribution of *An. funestus* Chromosomal Forms. The Kiribina form was almost the only form recorded from Bama, the rice-growing village, whereas the mostly polymorphic Folonzo form (showing high frequencies of the 3R*a*, 3R*b*, and 2R*a* inversions) was observed in higher relative frequencies in Lena and Soumousso, where it represented 70% of the karyotyped specimens. These findings are in accordance with the distribution pattern reported by Costantini et al. (1999), whereby Kiribina was found predominantly in irrigated areas. It must be stressed that our results are preliminary due to the small size of our samples, and they must be gauged accordingly. Such a contrasting pattern of distribution, giving rise to almost "pure" alternative populations of each chromosomal form, in combination with small sample sizes, probably hindered the possibility to point out significant departures from Hardy–Weinberg and linkage disequilibria in each locale. Conversely, such a pattern is in accordance with the significant differences in the distribution of genotypes among the three villages.

As in *An. gambiae* s.s., where some chromosomal inversions are clearly correlated with ecological variables (Touré et al. 1998), it is likely that the distribution of the chromosomal forms in *An. funestus* follows a pattern affected by specific environmental conditions. It will be interesting to precisely identify the ecological characteristics leading to the alternative distribution of the two chromosomal forms of *An. funestus* in different environments (e.g., rice-growing areas versus classic savannah).

Acknowledgments

We are grateful to the volunteers of Soumousso, Lena, and Bama who made this study possible. This study was supported by a grant from the program Pal+ of the French Ministry of Scientific Research.

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Received 15 July 2006; accepted 1 February 2007.