Bionomical and cytogenetic heterogeneities of *Anopheles funestus* in Senegal

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Abstract

Malaria transmission by *Anopheles funestus* was investigated from May 1994 to September 1997 in different locations from western to eastern Senegal along the northern border of The Gambia. 10515 *A. funestus* were captured on human volunteers or by indoor pyrethrum spraying. Circumsporozoite protein rates showed that *A. funestus* had a high infection rate, 2–7%, in the whole of the study area. Analysis of feeding behaviour showed great variation of anthropophilic rates from western Senegal, where populations were highly anthropophilic, to eastern Senegal, where they were much more zoophilic. In eastern Senegal many females captured in bedrooms had fed outside on horses. Polytene chromosome analysis showed that the general pattern of karyotype distribution is consistent with the hypothesis of 3 chromosomally differentiated populations of *A. funestus*. In samples from a central part of the study area, analysis showed lack of karyotype intergradation with a deficit of heterokaryotypes, suggesting the presence of 2 genetically differentiated populations in an area of sympatry.

Keywords: malaria, *Plasmodium falciparum*, blood meal, *Anopheles funestus*, genetics, chromosomal polymorphism, Senegal

Introduction

Malaria is one of the major public health problems in Africa. *Anopheles funestus* Giles is one of the 3 major vectors of malaria in Africa, together with *A. gambiae* Giles and *A. arabiensis* Patton of the *A. gambiae* complex. Many studies have been conducted on *A. gambiae*, which is considered to be the main malaria vector in Africa. However, in some areas *A. funestus* is as important in transmitting malaria as the other vectors, and sometimes it can be the main vector (FONTENILLE *et al.*, 1997). Since the 1930s it has been demonstrated by larval morphology that *A. funestus* is a group of the closely related species *A. funestus*, *A. confusus* Evans & Leeson, *A. leesoni* Evans, *A. rivulorum* Leeson and *A. brucei* Service. The sub-group *funestus* was introduced by GILLIES & DE MEILLON in 1968 and consists of 4 species that are indistinguishable as larvae but have slightly different adult morphology: *A. funestus*, *A. parensis* Gillies, *A. aruni* Sobti and *A. vaneedeni* Gillies & Coetzee (GILLIES & COETZEE, 1987). Only *A. funestus* is considered to be a vector of malaria parasites. However, WILKES *et al.* (1996) recently showed by salivary gland dissection that *A. rivulorum* from Tanzania was infected with *Plasmodium falciparum*. Cytogenetic studies of the group have shown that *A. funestus*, *A. rivulorum*, *A. leesoni*, *A. parensis* and *A. confusus* each possess unique chromosome inversion rearrangements that can be used to identify them (GREEN, 1982). *A. vaneedeni*, however, is homosequential with *A. funestus*, differing from it only in the possession of a polymorphic inversion on arm 2 (GREEN & HUNT, 1980). Two species of the group, in addition to *A. funestus*, have been recorded in West Africa: *A. rivulorum* and *A. leesoni* (see CHAUVET *et al.*, 1968; BRENGUES & COZ, 1973). Further chromosome polytene studies of populations of *A. funestus* were carried out by BOCCOLINI and collaborators (1992, 1994, 1998) on various samples from Madagascar and Burkina Faso and one sample from Mali. All these populations share polymorphic inversions 2a, 3a, 3b and 5a described by GREEN & HUNT (1980). In addition, new inversions (s, t, b and u) on chromosome arm 2 were detected in the material from Burkina Faso and Mali (BOCCOLINI *et al.*, 1998). The last 2 inversions are both based on 2a so that they produce arrangements ab and au, respectively. In Madagascar, BOCCOLINI *et al.* (1992) observed significant differences in inversion frequencies between samples from different seasons and geographical areas. Some of the karyotypes appeared to be associated with variation in anthropophily but no evidence of assortative mating was obtained. Studies in Burkina Faso by BOCCOLINI *et al.* (1994) showed wide variations in inversion frequencies among the samples without detectable geographical or temporal clines. Highly significant deviations from Hardy–Weinberg equilibrium were recorded for all the inversions in most samples, showing a uniform trend towards a deficit of the heterokaryotypes at each inversion. A plausible working hypothesis was that *A. funestus* in Burkina Faso could include 2 taxonomic units, one monomorphic standard or nearly so, and the other one polymorphic.

In Senegal, *A. funestus* is the most important vector in places such as Dielmo, a village in a holoendemic malaria area. The relative abundance, and the role played by each of the malaria vectors in transmission, depend to a large extent on the time of year. *A. funestus*, even if it is not the most abundant species, transmits malaria more often, due to its higher infection rate (FONTENILLE *et al.*, 1997). If vector control by genetic transformation is to be undertaken, it will be necessary to control the transmission by *A. funestus* as well as by members of the *A. gambiae* complex. In this study, we investigated the variation in circumsporozoite protein rates, anthropophilic feeding rates and chromosomal inversion polymorphisms in different populations of *A. funestus* in Senegal.

Experimental design and Methods

Study sites

Five localities were chosen for this study, from west-

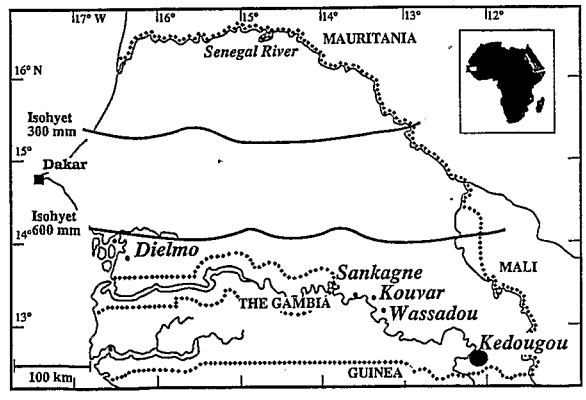

Fig. 1. Map of Senegal showing the 5 study sites (in italics).

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ern to eastern Senegal along the Gambian border (Fig. 1).

The village of Dielmo (13°45′N, 16°25′W) is situated in an area of Sudan-type savannah in the Saloum region of Senegal, 280 km south-east of Dakar and approximately 15 km north of the Gambian border. The rainy season lasts from June to mid-October. Over the last 20 years the average annual rainfall has been approximately 700 mm. Dielmo is situated on the marshy bank of a small permanent stream that permits the persistence of larval habitats all year round. A population of 250 lives in the village. The study site was described in detail by TRAPE and collaborators in 1994.

The villages of Kouvar (13°23′N, 13°37′W) and Sankagne (13°24′N, 13°45′W) are situated in eastern Senegal, in an area of sudanian domain, 3 km from the Gambia river and 1600 m apart. The rainy season lasts from June to October. Kouvar, with 1000 inhabitants, is situated 500 m from a permanent pool. The population of Sankagne is 1600.

The village of Wassadou (13°21′N, 13°20′W) is east of Kouvar, in an area of Sudan-guinean domain in Tambacounda region. It is 500 m from the Gambia river and close to a tributary, the Nieriko. The rainy season lasts from June to November. A population of 800 lives in the village. The study site was described in detail by FAYE and collaborators in 1995.

The Kedougou area (12°33′N, 12°11′W) is situated in the extreme south-east of Senegal in a Sudan-guinean phytogeographic domain, in Tambacounda region. Nine villages were studied within a radius of 15 km of Kedougou city. The Gambia river runs through the area. The rainy season lasts from June to November with an average annual rainfall of 1250 mm. The human population density is fairly low, with 2·5 inhabitants per km^2 living in small dispersed agricultural villages.

Mosquito collections

Adult mosquitoes were collected from May 1994 to September 1997 using the following methods. Hourly outdoor and indoor human bait catches from 19:00 to 07:00 were conducted at the beginning and at the end of the rainy season for 6–12 person-nights each, half outdoors and half indoors, always at the same locations in the village. In Dielmo, where other studies to investigate the relationship between transmission and malaria disease were in progress, catching was conducted for 12–18 person-nights each month. Pyrethrum spray collections were carried out in selected bedrooms, at the beginning and at the end of the rainy season, from 16:00 to 17:00.

Anopheline identification

The identification of adults of the *A. funestus* group was made in the field following the morphological keys of GILLIES & DE MEILLON (1968) and GILLIES & COETZEE (1987).

Field processing of anophelines

From May 1994 to September 1997, half-gravid females collected in bedrooms were dissected and their ovaries stored in 0·5 mL tubes with Carnoy's fixative for polytene chromosome analysis. The dissected mosquitoes, together with all the fed and unfed mosquitoes from all collections, were stored individually in tubes with dessicant for laboratory processing in Dakar.

Laboratory processing of anophelines

Blood meal sources of all the fed females collected in bedrooms were identified by an enzyme-linked immunosorbent assay (ELISA) (BEIER *et al.*, 1988), which identified human, bovine, ovine or caprine, equine and chicken host.

The heads and thoraces of all females were tested for circumsporozoite protein (CSP) of *P. falciparum*, *P. malariae* and *P. ovale* as described by BURKOT *et al.* (1984) and modified by WIRTZ *et al.* (1987), and the CSP rates were calculated. *P. vivax* is not present in this region of Africa.

The ovaries of half-gravid females were used to obtain squash preparations of the nurse cell polytene chromosomes. After staining (COLUZZI, 1968; HUNT, 1973), these preparations were examined to determine the chromosomal rearrangements. The paracentric inversion karyotypes were scored according to the nomenclature of GREEN & HUNT (1980). The inversions were indicated by lower case letters following the chromosome arm number (e.g., 3a) and the standard by a + sign.

Data analysis

All mosquito data and the results of the various examinations were processed in a computer database. Anthropophily and infection rates were calculated for the different populations using standard methods already described (FONTENILLE *et al.*, 1997; LEMASSON *et al.*, 1997). Genotype frequencies were tested against Hardy–Weinberg expectations for each locus in the pooled population and for each location. Each inversion was considered as an allele on a locus that was the chromosomal arm (TAYLOR *et al.*, 1993). Exact goodness-of-fit tests were performed using Genepop™ V. 2.1 (RAYMOND & ROUSSET, 1995a). The significance level for each test was adjusted to take into account the other tests using the sequential Bonferroni procedure (HOLM, 1979; RICE, 1989). Levels of variation among populations were calculated using Wright's *F* statistic (WRIGHT, 1978; WEIR & COCKERHAM, 1984); significance was assessed using the exact probability test and the exact test of a contingency table testing homogeneity of genotypic frequencies among populations (RAYMOND & ROUSSET, 1995b).

Results

Mosquito collections

A total of 10515 *A. funestus* was captured.

In Dielmo, from May 1994 to September 1997, 6110 *A. funestus* were collected on human volunteers, and 5779 were processed by the CSP ELISA. A total of 2393 specimens was collected in resting sites, and 1962 were processed by the CSP ELISA, 1149 by the blood meal ELISA, and 94 cytogenetically.

In Sankagne and Kouvar, during 6 surveys (end of April, mid-May and end of August in 1996; mid-June, end of August and end of September in 1997), 127 *A. funestus* were captured on human volunteers and 68 were processed by the CSP ELISA. A total of 465 was collected in resting sites and 455 were processed by the CSP ELISA, 179 by the blood meal ELISA, and 57 cytogenetically.

In Wassadou, during 4 surveys (mid-October and mid-November 1994; mid-January and mid-May 1995), only 1 *A. funestus* was collected on a human volunteer and 40 were collected in resting sites, of which 29 were processed by the CSP ELISA, 30 by the blood meal ELISA, and 4 cytogenetically. An impregnated bed net programme was started in July 1995 and no mosquito was collected after this time whatever the method of capture.

In Kedougou region, during 6 surveys (beginning of October and of November 1994; mid-July and mid-October 1996; beginning of July and mid-October 1997), 290 *A. funestus* were collected on human volunteers of which 58 were processed by CSP ELISA. A total of 1089 was collected in resting sites and 791 were processed by the CSP ELISA, 471 by the blood meal ELISA, and 51 cytogenetically.

Circumsporozoite protein rates

The CSP rate was calculated for each species of *Plasmodium*. A study conducted in 1995 by SOKHNA *et al.* (in press) showed that the ELISA detected 1·5 times

Table 1. Circumsporozoite protein rates determined by enzyme-linked immunosorbent assay of the heads plus thoraces of *Anopheles funestus* in Senegal

Method of capture, locality and date	No. tested	Circumsporozoite protein rate *P. falciparum*	*P. malariae*	*P. ovale*
Landing on human volunteers				
Dielmo				
1992–3[a]	1979	3·44 (2·68–4·34)	0·10 (0·01–0·36)	0·05 (0–0·28)
1993–4[a]	363	2·20 (0·96–4·29)	0	0
1994–5[a]	1329	1·35 (0·81–2·13)	0·08 (0–0·42)	0
1995–6[a]	1886	3·61 (2·81–4·55)	0·11 (0·01–0·38)	0
1996–7[a]	1860	4·19 (3·33–5·21)	0·05 (0–0·30)	0·11 (0·01–0·39)
1997[b]	704	5·82 (4·21–7·82)	0	0
Sankagne				
1997[c]	68	1·47 (0·04–7·92)	0	0
Kedougou region				
1996[c]	58	6·89 (1·91–16·73)	0	0
Resting in bedrooms				
Dielmo				
1992–3[a]	1301	2·00 (1·31–2·91)	0	0
1993–4[a]	151	0	0	0
1994–5[a]	373	3·49 (1·87–5·88)	0	0
1995–6[a]	198	1·52 (0·31–4·36)	0	0
1996–7[a]	1174	2·64 (1·80–3·73)	0	0
1997[b]	217	5·53 (2·89–9·46)	0	0
Kouvar				
1997[c]	47	0	0	0
Sankagne				
1997[d]	38	0	0	0
1997[c]	370	4·86 (2·91–7·58)	0	0
Wassadou				
1994[c]	29	0	0	0
Kedougou region				
1994[c]	223	2·69 (0·99–5·76)	0	0
1996[c]	372	6·45 (4·18–9·45)	0	0
1997[c]	196	2·55 (0·83–5·85)	0	0

[a]April–March.
[b]April–July.
[c]Rainy season.
[d]Dry season.

more *Plasmodium*-infected mosquitoes than did dissection. *P. falciparum* was found in 97·9% of the positive mosquitoes, *P. malariae* in 1·4%, and *P. ovale* in 0·7%. *P. malariae* and *P. ovale* were found only in mosquitoes captured on human bait in Dielmo (Table 1).

The Dielmo results from April 1992 to March 1994 have been extracted from a previous paper (FONTENILLE *et al.*, 1997). In Dielmo, from April 1992 to September 1997, the *P. falciparum* CSP rates in mosquitoes captured on human bait were very different (χ^2=15·82, df=5, P=0·0074). Among resting mosquitoes the difference was also highly significant (χ^2=34·25, df=5, P=2×10^{-6}). Adjusting for years and comparing the infection rate of mosquitoes captured on human bait and the infection rate of resting mosquitoes gave highly significant results (Mantel–Haenszel χ^2 for stratified analysis=7·28, df=1, P=0·0069). In 1992–1993 and 1996–1997 the infection rates were higher among *A. funestus* captured on human volunteers than in those collected by pyrethrum spraying in bedrooms (χ^2=5·83, df=1, P=0·0158 and χ^2=5·07, df=1, P=0·0252, respectively). From April 1994 to March 1995, the CSP rate was lower among mosquitoes captured on human volunteers (χ^2=7·39, df=1, P=0·0066).

In Sankagne, during the 1997 rainy season, the CSP rate in mosquitoes caught on human bait was 1·47 and that for resting mosquitoes was 4·86; this difference was not significant (Fisher's exact test, P=0·3322).

In Kedougou region during the 1996 rainy season, the infection rates were very high and were not significantly different whatever the method of capture (Fisher's exact test, P=0·7806).

No mosquito from Kouvar or Wassadou gave a positive result in the CSP ELISA.

Blood meals

In total, 1829 blood meals from resting female *A. funestus* were tested by ELISA (Table 2).

Table 2. Numbers of indoor resting *Anopheles funestus* which had fed on different vertebrate hosts in Senegal

Locality and date	No. tested	Blood meal Mixed	Human	Bovine	Ovine	Equine
Dielmo						
May 1994–Sep. 1997	1149	29 (2·52%)	1046 (91·04%)	120 (10·44%)	6 (0·52%)	6 (0·52%)
Kouvar and Sankagne						
Jun.–Sep. 1997	179	7 (3·91%)	133 (74·30%)	10 (5·59%)	4 (2·23%)	39 (21·79%)
Wassadou						
Oct.–Nov. 1994	30	1 (3·33%)	10 (33·33%)	2 (6·67%)	3 (10%)	16 (53·33%)
Kedougou region						
Oct. 1994–Oct. 1997	471	33 (7·01%)	383 (81·32%)	103 (21·87%)	18 (3·82%)	0

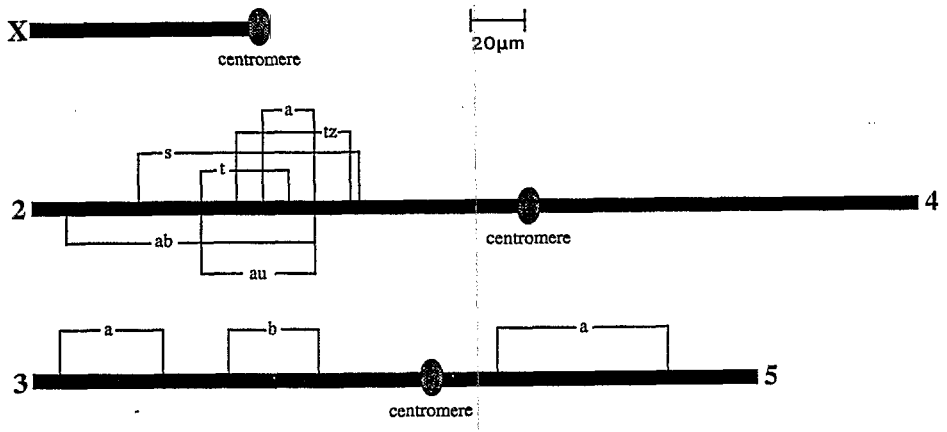

Fig. 2. Diagrammatic representation of the polymorphic chromosomal inversions observed in *Anopheles funestus* in Senegal.

In Dielmo, most mosquitoes had fed on human blood (Table 2); 2·5% of blood meals were taken from 2 different host species; the most frequent combination was human–bovine (1·8%), followed by bovine–equine (0·3%).

The anthropophilic rates were similar (χ^2=0·04, df=1, P=0·8326) in Kouvar and Sankagne (Table 2). In Kouvar, 12·1% of mosquitoes had fed on bovine blood and 9·1% each on ovine and equine; only 2 mosquitoes had fed on 2 different hosts (5·7%), one on human–equine and the other on bovine–ovine. In Sankagne, 24·7% of mosquitoes had fed on equine hosts, 4·1% on bovine and 0·7% on ovine; 5 had mixed blood meals (3·4%), 4 being human–equine and one human–bovine.

In Wassadou, the anthropophilic rate was very low (Table 2). The single mixed blood meal was bovine–ovine.

In Kedougou, the anthropophilic rate was similar throughout the survey (Table 2). The most frequent combinations in mixed blood meals were human–bovine (4·5%), then human–ovine (1·3%) and bovine–ovine (1·3%). None of these mosquitoes had fed on equine blood since there is no horse in the region.

The anthropophilic rates were highly different between the 4 areas (χ^2=121·86, df=3, P=10^{-5}). If Wassadou were excluded from the analysis, χ^2 was lower but nevertheless still significant (χ^2=54·85, P<10^{-6}). When the areas were compared pairwise, the anthropophilic rates were significantly different in Dielmo versus Sankagne–Kouvar (χ^2=43·51, df=1, P<10^{-6}), Dielmo versus Kedougou region (χ^2=30·32, df=1, P<10^{-6}) and Sankagne–Kouvar versus Wassadou (χ^2=19·86, df=1, P=8·3×10^{-6}), but not in Sankagne–Kouvar versus Kedougou region (χ^2=3·89, df=1, P=0·04846).

Polytene chromosome study

A total of 396 half-gravid females was collected in bedrooms for polytene chromosome analysis and 261 suitable preparations were obtained (65·9%). The determination of chromosomal rearrangements showed that the mosquitoes were neither *A. rivulorum* nor *A. leesoni*, the only other species of the *A. funestus* group found in West Africa (GILLIES & DE MEILLON, 1968).

Chromosomal inversion polymorphisms. No inversion was found on chromosome X and arm 4, as in previous studies on the cytogenetics of *A. funestus* by GREEN & HUNT (1980) and BOCCOLINI *et al.* (1992, 1994, 1998).

Six inversions were detected on chromosome arm 2: a, b, s, t, u and z (Fig. 2), giving rise to 6 rearrangements as follows: a, ab, s, t, au, tz. Inversions a and b have been described by GREEN & HUNT (1980); however, our inversion b originated from 2a, while GREEN & HUNT (1980) referred to b as derived apparently from the standard. Inversions s, t and u have been described previously in Burkina Faso and Mali (BOCCOLINI *et al.*, 1998). Inversion z is a new rearrangement based on chromosome 2t and has been documented up to now only in Senegal. In Dielmo, all 94 specimens had the standard arrangement (Table 3). The most frequently observed inversion in Kouvar was inversion s, which was the only one detected in Sankagne (Table 3). Inversions a and t were found among the 4 mosquitoes from Wassadou with a predominance of inversion a. In the Kedougou area, all inversions were detected but s, a and t were predominant (Table 3).

Two independent inversions were observed on arm 3, giving rise to 3 inverted arrangements: a, b and ab. These chromosomal variations appear to be widespread in *A. funestus* (southern Africa, GREEN & HUNT, 1980; Madagascar, BOCCOLINI *et al.*, 1992; Burkina Faso, BOCCOLINI *et al.*, 1994; Mali, BOCCOLINI *et al.*, 1998). Inverted arrangements ab and b were observed in Wassadou and the Kedougou area, with a predominance of ab (Table 3). The arrangement 3ab was also detected in Kouvar together with the standard. The standard arrangement was remarkably common in Sankagne and Dielmo, and the ab inversion was never observed.

A single inversion, a, was observed on arm 5, in all the population samples (Table 3).

Karyotypic rearrangements. Six rearrangements were observed on chromosome arm 2, giving 13 karyotypes (Table 4). All mosquitoes from Dielmo were carriers of the standard homokaryotype. This karyotype was never observed in the other population samples. The most common karyotype in Sankagne and Kouvar was s/s. The Kedougou populations showed greater polymorphism, with 10 karyotypes but without the s inversion.

Table 3. Frequencies of chromosomal inversions in *Anopheles funestus* from Senegal

Locality	No. of chromatids	Inversions (%) Arm 2 +	a	s	t	ab	au	tz	No. of chromatids	Inversions (%) Arm 3 +	a	b	ab	No. of chromatids	Inversions (%) Arm 5 +	a
Dielmo	188	100·0	–	–	–	–	–	–	184	75·0	6·5	18·5	–	188	54·8	45·2
Sankagne	114	3·5	–	96·5	–	–	–	–	110	96·4	–	3·6	–	114	71·1	28·9
Kouvar	18	16·7	11·1	61·1	11·1	–	–	–	22	81·8	–	–	18·2	20	70·0	30·0
Wassadou	8	–	62·5	–	37·5	–	–	–	8	–	–	12·5	87·5	8	25·0	75·0
Kedougou region	82	2·4	41·5	–	43·9	6·1	2·4	3·7	98	–	–	3·1	96·9	92	48·9	51·1

Table 4. Karyotypic arrangements in *Anopheles funestus* from Senegal

Locality	No. of specimens	Arrangements (%) Arm 2 +/+	a/a	s/s	t/t	s/+	t/+	ab/a	ab/ab	ab/t	au/a	t/a	tz/a	tz/t	No. of specimens	Arrangements (%) Arm 3 +/+	a/+	b/+	a/b	ab/ab	ab/b	b/b	No. of specimens	Arrangements (%) Arm 5 +/+	a/+	a/a
Dielmo	94	100·0	–	–	–	–	–	–	–	–	–	–	–	–	92	58·7	7·6	25·0	5·4	–	–	3·3	94	27·7	54·3	18·1
Sankagne	57	–	–	93·0	–	7·0	–	–	–	–	–	–	–	–	55	92·7	–	7·3	–	–	–	–	57	45·6	50·9	3·5
Kouvar	9	–	–	44·4	–	33·3	–	–	–	–	–	22·2	–	–	11	81·8	–	–	–	18·2	–	–	10	50·0	40·0	10·0
Wassadou	4	–	50·0	–	25·0	–	–	–	–	–	–	25·0	–	–	4	–	–	–	–	75·0	25·0	–	4	–	50·0	50·0
Kedougou	41	–	19·5	–	17·1	–	4·9	2·4	2·4	4·9	4·9	34·1	2·4	7·3	49	–	–	–	–	93·9	6·1	–	46	26·1	47·8	26·1

The 3 rearrangements observed on arm 3 gave 7 karyotypes. In Wassadou and Kedougou, only the inverted homokaryotype ab/ab and heterokaryotype ab/b occurred, with a high frequency of ab/ab. All karyotypes were observed in Dielmo, except for ab/ab and ab/b, with a predominance of the standard homokaryotype. Mosquitoes from Sankagne and Kouvar were predominantly carriers of the standard homokaryotype with the inverted heterokaryotype b/+ found in Sankagne and the inverted ab/ab in Kouvar.

The 3 karyotypes +/+, a/+ and a/a were observed on arm 5 in all populations except for Wassadou, where the sample size was low.

F statistic values estimated for all loci in the samples from Wassadou and Kedougou region were not significant ($F<0{\cdot}06$, $P>0{\cdot}2426$), so all mosquitoes from these areas were considered to be derived from a single population.

No significant departure from Hardy–Weinberg equilibrium was detected in samples from Dielmo, Sankagne and Wassadou–Kedougou ($P>0{\cdot}4$). However, when the samples were pooled, highly significant deviations from Hardy–Weinberg equilibrium were recorded ($P<10^{-4}$), except for those on arm 5. Significant deviations in the sample from Kouvar were observed on arm 2 (exact probability test, $P=0{\cdot}039$) and on arm 3 the F_{is} value (inbreeding coefficient of the individual relative to the subpopulation) was nearly one, indicating a deficit of heterokaryotypes.

Pairwise comparisons of *F* over the 4 populations, Dielmo, Wassadou–Kedougou region, Sankagne and Kouvar were significant for arm 2 ($P<0{\cdot}0003$). For arm 3 they were all significant ($P<10^{-6}$) except for Wassadou–Kedougou region versus Sankagne. For arm 5, the *F* values were significant only for Dielmo versus Sankagne and Wassadou versus Sankagne ($P<0{\cdot}005$ and $P<0{\cdot}001$, respectively). Over all arms, the *F* values ($0{\cdot}3834<F<0{\cdot}6903$) were highly significant (Table 5). Pooling the 4 populations, the value of *F* was also very significant (0·5908).

Table 5. Values of Wright's *F* statistic (level of variation) for *Anopheles funestus* in different localities in Senegal[a]

	Dielmo	Wassadou–Kedougou	Sankagne	Kouvar
Dielmo	–	–	–	–
Wassadou-Kedougou	0·5897	–	–	–
Sankagne	0·6903	0·4516	–	–
Kouvar	0·3834	0·4326	0·5149	–

[a]All values were highly significant ($P<0{\cdot}0001$).

Discussion

Our studies in 1992–1993 (FONTENILLE *et al.*, 1997) and 1996–1997 in Dielmo, Senegal, showed a higher sporozoite rate among *A. funestus* captured on human volunteers than in those collected by pyrethrum spraying in bedrooms. This result, which was not observed with *A. gambiae* or *A. arabiensis* in 1992–1993 in the same location, suggests the possible existence of 2 different populations of *A. funestus*, one highly anthropophilic with a high infection rate, and another probably less anthropophilic and with a lower infection rate. CSP rates showed that *A. funestus* had a high infection rate throughout all the study area. In Kouvar and Wassadou, no mosquito gave a positive result in the CSP ELISA, but the number of mosquitoes tested was very low. Kouvar was the last village included in the study and an impregnated bed net programme had been set up in Wassadou at the beginning of our study, but O. Faye (personal communication) had found one *A. funestus* infected with *Plasmodium* in 1995.

Studies on feeding behaviour of indoor resting *A. funestus* revealed a high anthropophilic rate in Dielmo and a lower rate in Wassadou in eastern Senegal. In Wassadou and Sankagne, 53% and 25%, respectively, of the females captured in bedrooms had fed outside on horses. This behaviour is not typical of *A. funestus* and suggests the existence of at least one population with a lower anthropophilic rate than those observed anywhere else during previous studies on this species (GITHEKO *et al.*, 1994). In Wassadou and Sankagne, the availability of the human host was high and the density of horses was similar to that in the other villages. This surprising behaviour had already been reported from Wassadou by FAYE *et al.* in 1995.

The polytene chromosome study showed the presence of 3 very different populations without significant departures from Hardy–Weinberg equilibrium. The genetic characteristics of these populations were maintained during the period of the survey and corresponded to the following prevailing chromosomal formulae; X, 2, 3b/+ a/+, 4, 5a/+ for Dielmo; X, 2s, 3, 4, 5a/+ for Sankagne and Kouvar; and X, 2a t/+ ab/+ au/+ tz/t, 3ab, 4, 5a/+ for Wassadou and the Kedougou area. In August 1997 a small peculiar sample of 4 *A. funestus* was obtained from Kouvar including 2 homozygous specimens (2s/s and 3+/+) and 2 specimens with the karyotypes 2t/a and 3ab/ab. In spite of this small sample, there is a strong indication that we were dealing with 2 gene pools, one prevailing in Sankagne the other one characteristic of Wassadou and Kedougou, without any indication of intergradation. This could be evidence of reproductive isolation between 2 sympatric taxa of the *A. funestus* complex. However, we have no proof that the 2 chromosomal forms were actually sympatric at the mating site. The village of Kouvar could in fact be the site of post-mating contact between 2 populations which were ecotypically and chromosomally differentiated. Further sampling in the area of Kouvar is needed to clarify the allopatry or sympatry between the 2 chromosomal forms.

While the hypothesis of 3 populations provides a coherent explanation for the pattern of behavioural and chromosomal variations observed in the study area, it also raises many questions. The most important concerns the mechanism of isolation in Kouvar between the 'Sankagne' and 'Wassadou–Kedougou region' populations. Their taxonomic status remains to be defined. In addition, the question of whether the horse-feeding population of *A. funestus* in eastern Senegal is a different species from the anthropophilic population remains to be answered.

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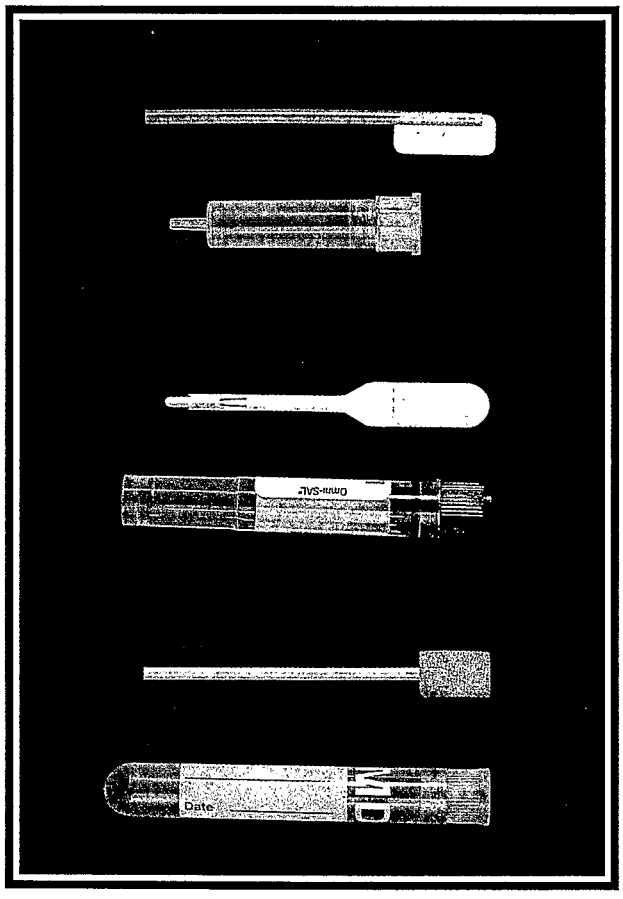

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