SPATIAL AND TEMPORAL VARIATIONS OF THE CHROMOSOMAL INVERSION POLYMORPHISM OF *ANOPHELES FUNESTUS* IN SENEGAL

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Summary :

The polymorphism of paracentric inversions of *An. funestus* polytene chromosomes was studied along a transect in Senegal in order to assess their variations at the spatial and temporal level. There was an increase in the degree of chromosomal polymorphism from the West to South-East. At the geographical level the variations in inversion frequencies were highly significant whatever the chromosomal arm considered. However, the variations in the chromosomal inversion frequencies did not change significantly over either seasons or years, except for inversion 3b in the village of Dielmo. Such geographical variability within a relatively limited area, associated to temporal stability, suggest a restricted gene flow between the populations studied, probably due to discontinuities in the *An. funestus* distribution and to its bioecology.

KEY WORDS : *Anopheles funestus*, polytene chromosome, chromosomal polymorphism, Senegal.

Résumé : VARIATIONS SPATIO-TEMPORELLES DU POLYMORPHISME CHROMOSOMIQUE D'*AN. FUNESTUS* AU SÉNÉGAL

*Le polymorphisme des inversions paracentriques des chromosomes polytènes d'*An. funestus *a été étudié le long d'un transect au Sénégal dans le but d'estimer leurs variations spatio-temporelles. Un polymorphisme croissant a été observé de l'ouest vers le sud-est. À l'échelle géographique, les variations des fréquences des inversions ont été significatives quel que soit le bras chromosomique considéré. Cependant ces variations n'ont été liées ni à la saison ni à l'année à l'exception de l'inversion 3b dans le village de Dielmo. De telles variations des fréquences des inversions chromosomiques à l'intérieur d'une aire limitée, associées à une stabilité temporelle, suggèrent un flux génétique restreint entre les différentes populations étudiées dû probablement aux discontinuités observées dans la distribution et la bioécologie d'*An. funestus.

MOTS CLÉS : Anopheles funestus, *chromosome polytène, polymorphisme chromosomique, Sénégal.*

INTRODUCTION

An. funestus Giles is one of the most important vectors of malaria in tropical Africa. Since the thirties, it was found by larval morphology that it belongs to a group of closely related species named *funestus* group, consisting of *An. funestus*, *An. confusus* Evans & Leeson, *An. leesoni* Evans, *An. fuscivenosus* Leeson, *An. rivulorum* Leeson and *An. brucei* Service. Gillies & De Meillon (1968) introduced the term *funestus* sub-group for *An. funestus*, *An. parensis* Gillies, *An. aruni* Sobti and *An. vaneedeni* Gillies & Coetzee which are distinguished on the basis of minor characters of the adult. With a few exceptions, fixed or polymorphic chromosomal inversion rearrangements can reliably identify most species of the section. Only *An. funestus* is a vector of human malaria, but in Tanzania Wilkes *et al.* (1996) recently found *An. rivulorum* infected with *Plasmodium falciparum*. Laboratory experiments have shown that *An. vaneedeni* is susceptible to malaria infection, but its involvement in natural malaria transmission has not been clearly documented (De Meillon *et al.*, 1977). In Senegal, only *An. funestus* is reported (Diagne *et al.*, 1994). During studies on malaria transmission and arbovirus surveys in this country, it was found that *An. funestus* landing on human baits and resting indoors have remarkably high circumsporozoite and anthropophilic rates. A previous study on the chromosomal inversion polymorphism in Senegal showed a high degree of heterogeneity among populations along a latitudinal transect (Lochouarn *et al.*, 1998). This paper provides further information on spatial and temporal patterns of the chromosomal polymorphism from their same sites.

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STUDY AREA

Six villages were chosen along the northern Gambian border. They are located along a transect across the Sudanese and Sudano-Guinean climatic zones (Fig. 1).

The villages of Dielmo (13°45'N, 16°25'W) and Madina Djikoye (13°38'N, 16°18'W) are 12 km apart, and are located in the northern Sudanese zone within the 700–1,000 mm isohyets. The climate is of Sudanese type, annual rainfall falling during six-seven months from April-May to October, with thermal amplitudes often higher than 25°C. Maximum temperatures occur in April-May (40°C) and October (35°C), while minimum temperatures are lower than 10°C in December-January, and between 20 and 25°C the rest of the year. Kouvar (13°23'N, 13°37'W) and the Sankagne area (13° 24' N, 13° 45' W) are 1.6 km apart, and are situated in the southern Sudanese zone within the 1,000-1,300 mm isohyets. The rainy season lasts here from June to October. The vegetation is composed by shrubs and open wooded savanna replacing the climatic forest which formerly covered the area, and whose vestiges are left in relic areas. Degradation of the natural vegetation is due to the combined action of persistent rainfall deficit and the extension of cultivated areas.

Wassadou (13°21'N, 13°20'W) and the Kédougou region (12°33'N, 12°11'W) are located in the Sudanese-Guinean zone. This is the wettest in the country, with one period of precipitation from June to November, and mean annual rainfall ranging 1,300–1,800 mm. The thermal amplitude is 20°C, with maximum temperatures of 35°C and 30°C in June and October, and minimum temperatures of 15°C and 25°C in December-January and in August, respectively. Natural climatic vegetation is a dense forest. Under the actions of humans this has almost completely disappeared for the benefit of rice and groundnut cultures in the floodplains.

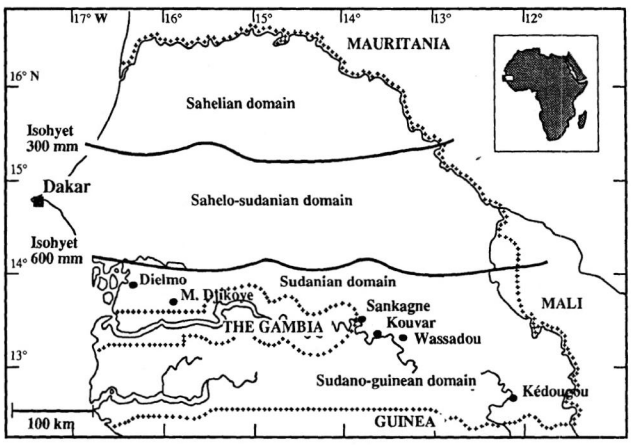

Fig. 1. – Location of the study area.

MATERIALS AND METHODS

Daytime indoor resting adult *An. funestus* were collected from all the six localities by pyrethrum spray catches from April 1994 until November 1998. Upon capture, half-gravid females were sorted and immediately fixed in modified Carnoy's fixative (three parts pure ethanol: one part glacial acetic acid), held for 24 hours at ambient temperature, and then stored at – 20°C until processing. Polytene chromosome preparations from the nurse ovarian cells were obtained following the method of Coluzzi (1968) modified by Hunt (1973). Paracentric inversions were identified by microscopic examination and scored following the nomenclature of Green & Hunt (1980), Boccolini *et al.* (1998), and Lochouarn *et al.* (1998). Inversions frequencies and karyotypic differences among populations were assessed using the Genepop v. 1.2 software (Raymond & Rousset, 1995).

RESULTS

Chromosomal inversion polymorphism

Observations of polytene chromosomes were carried out on 611 specimens. The whole polytene complement could be read in 422 specimens.

In the study area, the analysis of the chromosomal polymorphism has been reported in a previous paper (Lochouarn *et al.*, 1998); this can be summarised as follows: depending on the village, *An. funestus* showed variable levels of polymorphism for ten paracentric inversions producing the following arrangements: six on chromosomal arm 2 (a, s, t, ab, au, tz), three on chromosomal arm 3 (a, b, ab) and one on chromosomal arm 5 (a).

Geographical variation

The chromosomal inversions observed showed clear geographical variations among the various localities, with highly significant differences.

In Dielmo and Madina Djikoye, all the 168 specimens examined were carriers of the standard arrangement on chromosomal arm 2 (Table I). In Kouvar, the inversion 2a was the most frequent (percent frequency: 32.14 %). The inversion 2s was the only one present in Sankagne (96.49 %). The inversions 2a and 2t were predominant in Wassadou and the Kédougou area. In the latter, all inversions were observed but inversion

Locality	Sample size	Chromosomal arm 2							Sample Size	arm 3				Sample Size	arm 5	
		+	a	s	t	ab	au	tz		+	a	b	ab		+	a
Dielmo	320	100	–	–	–	–	–	–	302	81.46	4.30	14.24	–	318	60.69	39.31
M. Djikoye	16	100	–	–	–	–	–	–	12	100	–	–	–	18	50	50
Sankagne	114	3.51	–	96.49	–	–	–	–	112	100	–	–	–	114	71.05	28.95
Kouvar	28	14.29	32.14	28.57	25	–	–	–	32	43.75	–	21.88	34.37	28	75	25
Wassadou	8	–	62.50	–	37.50	–	–	–	8	–	–	12.50	87.50	8	25	75
Kedougou	324	1.23	45.68	–	42.28	5.56	1.85	3.40	374	–	0.53	2.41	97.06	360	46.94	53.06
Total	810	42.96	20	14.57	18.15	2.22	0.74	1.36	840	45.24	1.78	7.62	45.36	844	56.16	43.84

Sample size = number of chromatids.

Table I. – Geographical variability of chromosomal inversion frequencies in indoor resting samples of half-gravid females of *An. funestus* from various localities in Senegal.

2s; the inversions 2a and 2t were predominant at frequencies of 45.68 % and 42.28 %, respectively (Table I). The variations in inversion frequencies were highly significant ($P < 0.0001$).

On the chromosomal arm 3 in Wassadou and Kédougou the inversions a and b were in linkage disequilibrium. The inverted homokaryotype 3ab/ab was observed in Kouvar together with the standard $3^{+/+}$. In Sankagne, as in Dielmo and Madina Djikoye, the standard arrangement was very common, whereas the inverted arrangement 3ab was never observed (Table I). As found for the chromosomal arm 2, the variations in inversion frequencies on the chromosomal arm 3 were statistically significant ($P < 0.001$). The inversion 5a was observed in all the populations under study, being most prevalent in Wassadou and Kédougou (Table I), and showing an overall highly significant variability ($P < 0.001$).

SEASONAL VARIATION

The only data available to evaluate seasonal variations in the inversion frequencies were obtained from Dielmo, where breeding of *An. funestus* is continuous the year round. The most important samples for this analysis were obtained in 1995 and 1996. During the first year, sampling was carried out by periodic transversal surveys, whereas in 1996 it was performed longitudinally throughout the year. Our analysis, however, relate only to chromosomal arms 3 and 5, since arm 2 was found monomorphic standard in Dielmo. Inversions 3a and 3b were recorded during both the rainy and dry seasons, with the exception of the 1996 rainy season when inversion 3a was not observed. For all the periods considered, the standard arrangement was always the most frequent, followed by inversion 3b (Table II). The differences in inversion frequencies between the dry and the rainy seasons were not significant either in 1995 ($P = 0.21$) or in 1996 ($P = 0.08$). No difference was observed either between the dry seasons 1995-1996 ($P = 0.31$), or between the rainy season samples 1995-1996 ($P = 0.12$). As found for the inversions on chromosomal arm 3, the differences in frequencies for inversion 5a were not significantly different either between the dry and rainy season samples ($P = 0.87$ in 1995, and $P = 0.84$ in 1996), or between samples from successive years ($P = 0.72$ among dry seasons, and $P = 0.73$ among rainy seasons).

Period	Sample size	arm 3				Sample size	arm 5	
		+	a	b	ab		+	a
Dry season 1995	32	71.88	12.50	15.63	–	32	56.25	43.75
Rainy season 1995	54	87.04	5.56	7.41	–	54	55.56	44.44
Dry season 1996	44	63.64	6.82	29.55	–	46	52.17	47.83
Rainy season	40	82.50	–	17.50	–	42	50	50
Total	170	77.06	5.88	17.06	–	174	53.45	46.55

Sample size = number of chromatids.

Table II. – Seasonal variability of chromosomal inversion frequencies in indoor resting samples of half-gravid females of *An. funestus* from Dielmo.

ANNUAL VARIATION

In Dielmo, the standard arrangement was always the prevalent one on arm 3. The inversion 3a was observed at frequencies ranging from 1.22 % in 1998 to 12.50 % in 1994 (Table III). The inversion 3b was observed at frequencies often > 10 % with differences that were statistically significant ($P < 0.01$). On arm 5, the standard arrangement was the prevalent one in all years. The variations in frequency of this inversion were at the threshold of signification ($P = 0.05$).

In the Kédougou area, with the exception of inversion 2ab which was not observed in 1996, and of inversion 2s which was never found, all other inversions were observed on arm 2 during the four years of study (Table IV). Inversions 2a and 2t were always the most frequent whatever the year considered, while inversions 2au and 2tz were always rare (frequencies < 5 %).

Year	Sample size	arm 3				Sample size	arm 5	
		+	a	b	ab		+	a
1994	8	50	12.50	37.50	–	8	62.50	37.50
1995	86	81.40	8.14	10.47	–	86	55.81	44.19
1996	84	72.62	3.57	23.81	–	88	51.14	48.86
1997	42	83.33	2.38	14.29	–	40	75	25
1998	82	92.68	1.22	6.10	–	96	67.71	32.29
Total	302	81.46	4.30	14.24	–	318	60.69	39.31

Sample size = number of chromatids.

Table III. – Annual variability of chromosomal inversion frequencies in indoor resting samples of half-gravid females of *An. funestus* from Dielmo.

No statistically significant difference was found in the variation in frequency of these inversions (P = 0.52). On chromosomal arm 3, both inversions 3b and 3ab were observed at high frequency during all years of the study (Table IV). The variation in frequency was not significant between the four years of the study (P = 0.95). The inversion 5a was more frequent than its corresponding standard arrangement during every year, except in 1997 when it was observed at a frequency of exactly 50 % (Table IV). These changes in frequency were not significantly different (P = 0.13).

DISCUSSION

The chromosomal analysis showed that contrary to what is generally assumed (Gillies & De Meillon, 1968), *An. funestus* is a very polymorphic species. On the chromosomal arms carrying inversions, i.e. 2, 3 and 5, we observed seven, four and two arrangements, respectively. Most of these arrangements were already reported in the pioneer work of Green & Hunt (1980) from samples collected in Nigeria, South Africa, Zimbabwe, Namibia and Kenya. Indeed, of the six inversions on arm 2 that were described by these authors, only the inversions 2a and 2b were found in our Senegalese samples, whereas the inversions c, d, e and h were not observed. Among the three inversions described on arm 3, only the 3a and the 3b were observed, whereas the 3c was not found. On arm 5, only the inversion 5a was found, whereas the 5b was not observed.

No inversion was observed on the X heterosome and on arm 4, as found in previous studies on the cytogenetics of *An. funestus* (Green & Hunt, 1980; Boccolini *et al.*, 1992, 1994, 1998). Our study, therefore, further supports the evidence of a monomorphism of these two portions of the polytenic complement.

The inversions 2s, 2t and 2u were previously described from specimens collected in Mali and Burkina Faso (Boccolini *et al.*, 1998). Inversion 2z is a new inversion based on inversion 2t reported so far only from Senegal (Lochouarn *et al.*, 1998). In Burkina Faso, the inversion 2s was observed in some villages at a frequency of 3.7 % (Boccolini *et al.*, 1994). The inversion 2t was observed there at a very low frequency: only two heterozygotes were found out of 186 specimens examined from Diarabakoko, southern Burkina Faso. In the village of Banambani, in southern Mali, the inversions 2a and 2t were the most prevalent in 78 readable preparations at frequencies of 62.1 % and 25.0 %. The inversions 2ab and 2au accounted for another 6.4 % and 5.7 %, respectively. In Madagascar, only the inversion 2a was observed at frequencies ranging from 2.0 % in Tulear to 32.5 % in Mandoto (Boccolini *et al.*, 1992). On chromosomal arm 3, the standard arrangement was the prevailing one in Dielmo, Madina Djikoye, and Sankagne. These observations are comparable to those of Green (1982) who reported that at Wallikunda, in The Gambia, the arm 3 was monomorphic except for inversion 3a found at a relative frequency of 6.0 %. In Kédougou and Wassadou, the inverted arrangement 3ab was always prevalent. These two inversions occur at greatly different frequencies throughout Africa (in South Africa, Green & Hunt, 1980; in Madagascar, Boccolini *et al.*, 1992; in Mali, Boccolini *et al.*, 1998; and in Burkina Faso, Costantini *et al.*, 1999). In Banambani, inversion 3b is apparently fixed and inversion 3a nearly so, most of the speci-

Year	Sample size	Chromosomal arm 2							Sample size	arm 3				Sample size	arm 5	
		+	a	s	t	ab	au	tz		+	a	b	ab		+	a
1994	46	4.35	32.61	–	45.65	10.87	2.17	4.35	56	–	–	3.57	96.43	52	42.31	57.69
1996	32	–	56.25	–	37.50	–	3.13	3.13	38	–	–	2.63	97.37	36	61.11	38.89
1997	174	1.15	48.85	–	40.80	4.60	1.72	2.87	196	–	0.51	2.04	97.45	190	50	50
1998	72	–	41.67	–	45.83	6.94	1.39	4.17	84	–	1.19	2.38	96.43	82	36.59	63.41
Total	324	1.23	45.68	–	42.28	5.56	1.85	3.40	374	–	0.53	2.41	97.06	360	46.94	53.06

Sample size = number of chromatids.

Table IV. – Annual variability of chromosomal inversion frequencies in indoor resting samples of half-gravid females of *An. funestus* from the Kedougou area.

Zone	Chromosomal arm 2											arm 3		arm 5		References
	a	b	c	d	e	h	s	t	ab	au	tz	a	b	a	b	
East and South Africa	+	+	+	+	+	+						+	+	+	+	Green & Hunt, 1980
Madagascar	+											+	+	+		Boccolini *et al.*, 1992
Burkina Faso	+						+	+				+	+	+		Boccolini *et al.*, 1994 Costantini *et al.*, 1999
Mali	+							+	+	+		+	+	+		Boccolini *et al.*, 1998
Senegal	+						+	+	+	+	+	+	+	+		Lochouarn *et al.*, 1998

Table V. – Summary of the different inversions described in *An. funestus* from South and East Africa, West Africa and Madagascar island.

mens analysed so far being homozygotes 3ab/ab. In our study, this arrangement was observed only in the south-east of Senegal, in particular in Kouvar, Wassadou, and Kédougou. In Madagascar the inversion 3a was the only one observed at frequencies varying between 21.9 % at Ankotrofotsy to 59.1 % at Anjiro.

The chromosomal arm 5 is polymorphic for the inversion 5a; the inverted arrangement was prevalent in Wassadou and Kédougou, whereas in Dielmo, Madina Djikoye, Kouvar, and Sankagne the standard arrangement was prevalent. All the different inversions described in *An. funestus* are summarized in Table V.

At the geographical level, the variations of the relative frequencies of the chromosomal inversions observed on arms 2, 3 and 5 were all significant. Along our transect, there was an increase in the degree of chromosomal polymorphism from the West to the South-East, i.e. from Dielmo to the Kédougou area. These results differ from those obtained by Costantini *et al.* (1999) in Burkina Faso where no clinal variability was observed. In Dielmo, the seasonal variations in the chromosomal inversion frequencies were not significant either for the chromosomal arm 3 or 5. In Madagascar, however, temporal differences in the frequency of the inversions 2a and 3a were observed by Boccolini *et al.* (1992) in Mandoto. On arm 3, the year-to-year changes in frequencies were significant in Dielmo, whereas in the zone of Kédougou no variation was observed on arms 2, 3 and 5. In the chromosomal form Mopti of *An. gambiae s.s.* Giles, Touré *et al.* (1994) reported how in Mali the chromosomal inversions 2Rbc and 2Ru show consistent and clinal geographical and temporal fluctuations, suggesting an adaptive role of these arrangements for more arid and humid conditions, respectively. Similarly, we observed geographical heterogeneities in the frequency of *An. funestus* inversions on chromosomal arms 2 and 3 in the two different climatic zones, but temporal stability for most of the arrangements in spite of the large degree of seasonality. The stability of the chromosomal polymorphism at seasonal and annual levels in spite of the heterogeneity at the geographical level could be explained by the patchy distribution of *An. funestus*, as suggested by Collins *et al.* (1994). Limitations in dispersal ability could constitute a barrier to the diffusion of genes of resistance to insecticides, as well as genes of interest for disease control using transgenic mosquitoes.

An alternative explanation, not mutually exclusive, is that proposed by Costantini *et al.* (1999) who suggested that *An. funestus* in Burkina Faso is constituted by two taxa that are sympatric in the strictest sense, and have limited, if any, genetic exchange. They were provisionally named with a non-Linnean nomenclature chromosomal forms Kiribina and Folonzo. The former would be characterized uniquely by inversion 2s and by a low degree of polymorphism at all inversions, whereas the latter would be characterized by a much higher degree of polymorphism at all inversions and by inversions 3a and 3b nearly fixed. According to their hypothesis, therefore, our samples could be interpreted as pure (or nearly so) populations of one or the other of the two chromosomal forms. The Dielmo, Madina Djikoye, and Sankagne populations would fall under the definition given for the Kiribina form, whereas populations from Wassadou and the Kédougou area would fall under the definition of the Folonzo form. Lochouarn *et al.* (1998) demonstrated that by pooling these two groups together, there was a significant departure from Hardy-Weinberg equilibrium due to a deficit of heterokaryotypes. The village of Kouvar, however, may represent a zone of sympatry of both forms, where departures from the Hardy-Weinberg equilibrium have been difficult to detect so far due to the low sample size available. The temporal stability in the degree of polymorphism might then be associated to the more stable nature of the *An. funestus* larval environment. The use of more polymorphic tools such as microsatellite markers currently under development (Besansky, personal communication) could help to discern between these hypotheses.

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