Chromosomal inversion polymorphism of *Anopheles funestus* from forest villages of South Cameroon

I. Dia[1], D. Boccolini[2], C. Antonio-Nkondjio[3], C. Costantini[4], D. Fontenille[3]

[1] *Laboratoire IRD d'Entomologie Médicale et Institut Pasteur, Dakar, Sénégal;* [2] *Laboratorio di Parassitologia, Istituto Superiore di Sanità, Roma, Italia;* [3] *Laboratoire IRD d'Entomologie Médicale, Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroun;* [4] *Istituto di Parassitologia, Università 'La Sapienza', Roma, Italia.*

Abstract. The polymorphism of paracentric inversions of *Anopheles funestus* polytene chromosomes was studied in three villages (Nkoteng, Obala, and Simbock) located in a forest area of South Cameroon in order to analyse the genetic structure of these populations. A total of 146-210 chromatids could be scored from specimens collected over about two years. A low degree of chromosomal polymorphism was observed with two floating inversions on chromosomal arm 2 (2h and 2d), and three fixed arrangements on arms 3 (3a and 3b), and 5 (5a). Such arrangement of inversions has never been recorded elsewhere so far. The chromosome analysis indicated that the population from Obala was in Hardy-Weinberg equilibrium, whereas the samples from Nkoteng and Simbock showed a significant excess and deficit of heterokaryotypes, respectively. Significant differences in inversion frequencies on chromosomal arm 2 among villages lying in contrasting eco-climatic settings suggested an adaptive role of these inversions.

Key words: *Anopheles funestus*, paracentric inversions, chromosomal polymorphism, Cameroon.

Anopheles funestus Giles is one of the most important vectors of malaria in tropical Africa. Its polytene chromosomes were first described by Green and Hunt (1980) from material collected mainly in southern and East Africa. Ten polymorphic paracentric inversions are described from the autosomes: 2a/+ b/+ c/+ (d/+?) e/+ h/+, 3a/+ b/+, 5a/+ b/+, with arm 4 and the X chromosome showing no variability (Green and Hunt, 1980). The analysis of further material from West Africa showed several new polymorphic inversions in Mali and Burkina Faso: 2s/+ t/+ ab/+ au/+ (Boccolini *et al.*, 1998). In Senegal, Lochouarn *et al.* (1998) found a new inversion based on inversion 2t giving rise to the inverted arrangement 2tz. Recently, based on departures from Hardy-Weinberg equilibrium and linkage disequilibrium between inversions on different chromosomes in sympatric *An. funestus* populations from Burkina Faso, two chromosomal forms provisionally named Kiribina and Folonzo have been defined on the basis of their contrasting degree of chromosomal polymorphism (Costantini *et al.*, 1999). In this paper we report on the chromosomal polymorphism of *An. funestus* from three forest villages of South Cameroon.

Materials and methods

Daytime indoor resting half-gravid *An. funestus* were collected by pyrethrum spray catches from February 1999 to December 2000 in three villages of South Cameroon: Nkoteng (4°30′ N, 12°03′ E),

Obala (4°09′ N; 11°33′ E), and Simbock (3°90′ N, 11°30′ E), distant 160, 40, and 7-8 km from the capital city Yaounde, respectively.

Simbock is 700 m a.s.l. on the slope of a hill in a suburban/rural area in the forest. A small river flows in the valley creating a permanent swamp where several anophelines (*An. moucheti, An. nili, An. funestus*) breed all year round. Borrow pits in the valley provide breeding opportunities for *An. gambiae* as well. Almost 300 inhabitants live in houses mostly built as traditional huts with mud walls and corrugated iron roofs. Domestic animals comprise only some dogs, goats, pigs, and poultry. Obala is a small town of >10,000 inhabitants surrounded by rivers and streams in the Guineo-Sudanian climatic belt. Extensive cattle breeding is practised in this area by Muslim ethnic groups coming from North Cameroon to sell their herds. Anopheline larvae develop on the edge of streams protected by vegetation and in hoofprints created by cattle coming to drink on the muddy borders of rivers. Nkoteng is a larger town in a savanna area with trees and shrubs, hosting a large sugar cane farm and factory (SOSUCAM). The large Sanaga river flows not far away from the farm. Another smaller stream crosses the town, forming a permanent swamp where *An. funestus* breed.

The whole area is characterized by two rainy seasons from March to June and from September to November. Nkoteng and Obala fall under a relatively drier eco-climatic zone than Simbock, experiencing lower annual rainfall (1435-1550 mm *vs* 1654 mm, respectively), and averaging higher min-max monthly temperatures (21-32°C *vs* 20-29°C in February/March, and 19-27°C *vs* 18-25°C in July) (Santoir and Bopda, 1995). During our study,

Correspondence: Dr Ibrahima Dia, Laboratoire IRD d'Entomologie Médicale, Institut Pasteur, BP 220, Dakar, Sénégal, Tel ++221 8 234874, Fax ++221 8 399210, e-mail: diaibra@dakar.ird.sn

annual rainfall totalled 1792 mm in the Simbock area.

After each mosquito collection, the ovaries were immediately fixed in modified Carnoy's fixative (3 parts pure ethanol:1 part glacial acetic acid), held for 12-36 hrs at ambient temperature, and then stored at −20°C until processing. Polytene chromosome preparations from the ovarian nurse 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 and Hunt (1980). Genotypic frequencies of the various karyotypes were compared with those expected from estimates of Hardy-Weinberg equilibrium using the Genepop V. 3.2 software (Raymond and Rousset, 1995).

Results

In the samples from the study area we observed 5 inversions: two on chromosomal arm 2 (2h and 2d), two on arm 3 (3a and 3b) and one on arm 5 (5a) (Fig. 1). No inversion was found on chromosomal arm 4 or on the X heterosome. However, only inversions on chromosomal arm 2 were polymorphic, those on arms 3 and 5 being always observed as homokaryotypic inverted, giving rise to fixed 3ab and 5a arrangements (Table 1). As inversion 2d shares one breaking point and completely overlaps inversion 2h, in subsequent population genetic analysis we treated them as different alleles at the same locus.

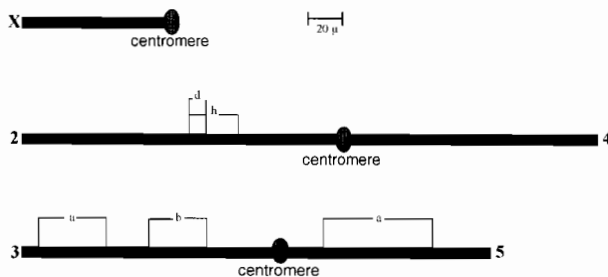Of the polymorphic inversions from the pooled samples, inversion 2h was the most frequently

Fig. 1. Diagrammatic representation of the polytene chromosomes of *Anopheles funestus* and the chromosomal inversions found in the populations from forest villages of South Cameroon of this study.

observed followed by inversion 2d. The standard arrangement (2+/+) was observed only in the village of Simbock at a frequency of 25.0%. In the three villages, inversion 2h was the most prevalent at frequencies of 75.6%, 81.3% and 54.2% for Nkoteng, Obala and Simbock, respectively. Significant differences in the frequencies of inversion 2h between Simbock *vs* Nkoteng ($P=0.04$) and Simbock *vs* Obala ($P=0.03$) were observed. No significant departure from Hardy-Weinberg equilibrium was detected in the population from Obala. However, a slight but significant excess of 2h/d heterokaryotypes was found in the Nkoteng sample ($P=0.04$), whereas a significant deficit of heterokaryotypes was found in the Simbock sample ($P<0.01$) (Table 1).

Discussion

The chromosomal analysis of three *An. funestus* populations from a forest area in south Cameroon showed the presence of five inversions out of the ten which had been observed by Green and Hunt (1980). On chromosomal arm 2, inversions 2h and 2d had been previously described in Southern and East Africa by these authors (Green and Hunt, 1980). However, they reported that their preparations were not good enough to establish precisely the breaking points of inversion 2h. Our samples all had the breaking points shifted by one band towards the telomere as compared to the map presented by Green and Hunt (1980). More samples are needed to clarify whether this represents a new inversion or is indeed the original 2h inversion described by Green and Hunt (1980). To be conservative, we have assumed that the inversion observed in the Cameroonian sample does represent the original 2h inversion of Green and Hunt (1980). In our samples, this was the most frequently observed inversion. However, inversion 2h has never been reported from West African populations (Boccolini *et al.*, 1994, 1998; Lochouarn *et al.*, 1998; Costantini *et al.*, 1999), although it was rarely observed in Madagascar (Boccolini *et al.*, unpublished observations). In a recent study on *An. funestus* polymorphism in Kenya, the 2h inversion was not recorded (L. Kamau, pers. comm.). In Cameroon, inversion 2d was observed only in the heterozygous form h/d. Because we never observed homokaryotypic inverted 2d/d individuals, we cannot exclude the possibility that inversion 2d is in fact based on the 2h inversion to form the 2hd arrangement.

Table 1. Karyotype and inversion frequencies of *Anopheles funestus* from three forest villages in Cameroon. 2n=number of chromatids, P= probability of conformance to Hardy-Weinberg equilibrium, f=inversion percent frequency, F=inbreeding coefficient (F<0 indicates heterozygote excess and F>0 heterozygote deficiency).

Localities	Chromosomal arm 2									Chromosomal arm 3				Chromosomal arm 5				
	2n	+/+	h/d	h/h	f(+)	f(d)	f(h)	F	P	2n	ab/ab	f(ab)	F	P	2n	a/a	f(a)	F	P
Nkoteng	90	–	22	23	–	24.4	75.6	−0.31	0.04	94	47	100	–	–	140	70	100	–	–
Obala	32	–	6	10	–	18.7	81.3	−0.20	1.0	34	17	100	–	–	34	17	100	–	–
Simbock	24	3	5	4	25.0	20.8	54.2	+0.35	0.01	32	16	100	–	–	36	18	100	–	–

On chromosomal arm 3, inversions 3a and 3b showed the highest level of linkage disequilibrium as the homokaryotypic inverted arrangement 3ab was the only one found in the study area out of 160 chromatids examined. High frequencies of this arrangement were also observed in South-Eastern Senegal (Lochouarn *et al.*, 1998; Dia *et al.*, 2000), in a village of Southern Mali (Boccolini *et al.*, 1998) and in South-Western Burkina Faso (Costantini *et al.*, 1999). Inversion 5a was found fixed inverted in the samples from our study area. Similar findings were obtained in Madagascar (Boccolini *et al.*, 1992), whereas this inversion floats usually at low frequencies in West African populations (Boccolini *et al.*, 1998; Lochouarn *et al.*, 1998; Costantini *et al.*, 1999).

Keeping in mind our sample sizes were small, the population sample from Obala was found in Hardy-Weinberg equilibrium, suggesting a situation of panmixia. Conversely, a significant excess and deficit of heterokaryotypes 2h/d were observed in the Obala and Simbock samples, respectively, but it must be noted that the total sample was collected over almost two years and departures from Hardy-Weinberg equilibrium may be due to temporal changes in the population frequency of the two inversions concerned. In comparison to West African populations of *An. funestus* which display a high degree of polymorphism (Boccolini *et al.*, 1994, 1998; Lochouarn *et al.*, 1998, Costantini *et al.*, 1999), our samples from South Cameroon were more homogeneous. However, the population from Simbock showed significant heterogeneities on chromosomal arm 2 with respect to the other two villages, as the standard arrangement was observed only in this village.

Significant changes in gene and genotype frequencies suggest an adaptive role of chromosomal arm 2 inversions to local ecological conditions as inferred from the observed differences between villages lying in contrasting eco-climatic settings. Costantini *et al.* (1999) attributed the chromosomal pattern observed in West Africa to two chromosomal forms: Kiribina characterized by a low degree of polymorphism at all inversions, and Folonzo characterized by a much higher degree of polymorphism at all inversions and by inversions 3a and 3b nearly fixed. Cameroonian populations showed a frequency of 100% for arrangement 3ab. Based only on chromosomal arm, the populations from South Cameroon seem close to the chromosomal form Folonzo. However, the high frequencies of the 2h and 2d inversions, rare or absent elsewhere in Africa, suggest that these central African populations can not be easily classified into the West African groups. Additional studies are needed to investigate the poly-

morphism on a larger scale and with larger samples in order to confirm these results, particularly in more humid and dryer areas. Moreover, studies on the temporal variability in inversion frequencies, their relationship with *Plasmodium falciparum* infectivity and mosquito trophic behaviour are to be further elucidated in our study area.

References

Boccolini D, Rakotoson R, Ralisoa O, Sabatini A, Randrianarisoa E, Coluzzi M (1992). Polimorfismo cromosomico di *Anopheles funestus* in Madagascar. Parassitologia 34, Suppl 1: 14-15.

Boccolini D, Sabatini A, Sanogo E, Sagnon NF, Coluzzi M, Costantini C (1994). Chromosomal and vectorial heterogeneities in *Anopheles funestus* from Burkina Faso, West Africa. Parassitologia 36, Suppl 1: 20.

Boccolini D, Sagnon NF, Toure YT (1998). Chromosomal polymorphism in *Anopheles funestus* and description of new inversions in Burkina Faso and Mali. Parassitologia 40, Supplement 1, 14.

Coluzzi M (1968). Cromosomi politenici delle cellule nutrici ovariche nel complesso *Anopheles gambiae*. Parassitologia 10: 179-183.

Costantini C, Sagnon NF, Sanogo E, Coluzzi M, Boccolini D (1999). Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso. Parassitologia 41: 595-611.

Dia I, Lochouarn L, Boccolini D, Costantini C, Fontenille D (2000). Spatial and temporal variations of the chromosomal inversion polymorphism of *Anopheles funestus* in Sénégal. Parasite 7: 179-184.

Green CA, Hunt RH (1980). Interpretation of variation in ovarian polytene chromosomes of *Anopheles funestus* Giles, *A. parensis* Gillies, and *A. arun?* Genetica 51: 187-195.

Hunt RH (1973). A cytological technique for the study of *Anopheles gambiae* complex. Parassitologia 15: 137-139.

Lochouarn L, Dia I, Boccolini D, Coluzzi M, Fontenille D (1998). Bionomical and cytogenetic heterogeneities of *Anopheles funestus* in Senegal. Trans R Soc Trop Med Hyg 92: 607-612.

Raymond M, Rousset F (1995). GENEPOP (version 1.2.): a population genetics software for exact tests and ecumenicism. J Hered 86: 248-249.

Santoir C, Bopda A, coordinators (1995). Atlas régional sud Cameroun. Editions de l'ORSTOM, 53 pp.