Species and Populations of the *Anopheles gambiae* Complex in Cameroon with Special Emphasis on Chromosomal and Molecular Forms of *Anopheles gambiae* s.s.

CHARLES WONDJI,[1, 2, 3] FRÉDÉRIC SIMARD,[1, 2] VINCENZO PETRARCA,[4, 5] JOSIANE ETANG,[1, 6] FEDERICA SANTOLAMAZZA,[4] ALESSANDRA DELLA TORRE,[4] AND DIDIER FONTENILLE[7]

J. Med. Entomol. 42(6): 998–1005 (2005)

ABSTRACT We studied the geographical distribution of species, chromosomal, and molecular forms of the *Anopheles gambiae* Giles (Diptera: Culicidae) complex in 23 sites in Cameroon, Central Africa. Almost all the specimens collected in the four northern-most arid sites were *Anopheles arabiensis*. *Anopheles melas* was found in a rural locality surrounded by mangrove swamps, on the Atlantic Coast. In total, 1,525 *An. gambiae* s.s. females were identified down to their molecular form, and inversion polymorphisms on polytene chromosomes were scored from 186 half-gravid females. The Forest chromosomal form, with standard arrangements almost fixed on both arms of chromosome-2, was the only one observed in the southern, more humid localities. Karyotypes typical of Savanna and Mopti were recorded northwards, in the humid savannas of the Adamawa Province. The molecular forms M and S were widespread throughout Cameroon, and assort independently from the chromosomal forms. S-form populations were characterized by karyotypes typical of Forest and Savanna chromosomal forms, and M-form populations were characterized by karyotypes typical of Forest, Savanna, and Mopti. No M/S hybrid patterns were detected, although M and S mosquitoes were sympatric in 15 sites, providing further evidence for positive assortative mating within molecular forms. The observed ecogeographical distribution of M and S was peculiar: the ecological parameters involved in this distribution still need to be clarified as well as the possible role of competitive exclusion between chromosomally homosequential molecular forms. No difference was observed in host preference or in *Plasmodium falciparum* infection rates between sympatric M and S populations.

KEY WORDS *An. gambiae*, malaria, Cameroon, chromosomal form, molecular form

THE MAJOR AFRICAN MALARIA vector, *Anopheles gambiae* s.s., has been split into five chromosomal forms named under a non-Linnaean nomenclature Forest, Savanna, Mopti, Bamako, and Bissau. Each form has been described by combinations of chromosomal inversions observed on chromosome-2; their geographic distribution was found to be tightly linked to ecological parameters such as aridity and breeding sites patterns (Coluzzi et al. 1985, Touré et al. 1998). The Forest chromosomal form is characterized by the standard arrangement on chromosome-2 or with some inversions such as b and d at low frequencies. It occurs in rain forest and humid savanna and intergrades with the Savanna form known to carry inversions b, cu bcu, and the standard arrangement. The Savanna form is the most widespread form occurring in savanna areas throughout Africa. The Mopti form carries the bc and u inversions. It occurs in riverine or irrigated habitats and is well adapted to artificial sources of water, even where these occur in arid zones. The Bissau form occurs in coastal rice cultivated areas in The Gambia, South Senegal, Guinea Bissau, and Guinea Conakry, and it carries inversion d. The Bamako form carries the j inversion, which may be associated with cu and bcu inversions. This form occurs along the upper Niger River and its tributaries in Mali and northern Guinea Conakry.

Various degrees of gene flow restriction were demonstrated between chromosomal forms, with strong hybrid heterokaryotype deficits in areas of sympatry. Analysis of the rDNA intergenic spacers, located on the X-chromosome, revealed fixed sequence differences between sympatric and synchronous Savanna/Bamako and Mopti populations in Mali and Burkina

[1] Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 288, Yaoundé, Cameroon.
[2] Institut de Recherche pour le Développement (IRD), P.O. Box 1857, Yaoundé, Cameroon.
[3] Current address: Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, United Kingdom (e-mail: c.s.wondji@liverpool.ac.uk).
[4] Sezione di Parassitologia, Dipartimento di Scienze di Sanità Pubblica, Università "La Sapienza", Piazzale Aldo Moro 5, 00185, Rome, Italy.
[5] Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza", Piazzale Aldo Moro 5, 00185, Rome, Italy.
[6] Institut de recheche Médicale et d'étude des Plantes Médécinales (IMPM), P.O. Box 6163, Yaoundé, Cameroon.
[7] Laboratoire de Lutte contre les Insectes Nuisibles (LIN), IRD, BP 64501, 911, Avenue Agropolis, 34 394 Montpellier Cedex 5, France.

0022-2585/05/0998–1005$04.00/0

Faso (Favia et al. 1997, 2001). A larger geographical survey led to the designation of two nonpanmictic molecular forms, which were named M and S (della Torre et al. 2001). The association of M and S forms with Mopti and Savanna/Bamako, respectively, observed in northern savanna areas (i.e., Mali and Burkina Faso) breaks down in other areas of Africa (Gentile et al. 2002; della Torre et al. 2001, 2002; Wondji et al. 2002). Meanwhile, evidence for incipient speciation or even complete reproductive isolation between molecular forms has accumulated: 1) M/S hybrids are absent or very rare in areas of sympatry (della Torre et al. 2001, Taylor et al. 2001, Wondji et al. 2002). 2) Population genetic studies using microsatellite loci revealed genome-wide differentiation between sympatric M and S populations from the Forest chromosomal form in Cameroon (Wondji et al. 2002) or only a sex-linked differentiation in other studies (Lehmann et al. 2003, Stump et al. 2005) with some X-linked loci showing highly significant differences in allele frequency distribution (Wang et al. 2001). Short interspersed elements insertion polymorphism on the X-chromosome also differentiates M and S molecular forms (Barnes et al. 2005). 3) There is evidence for a premating barrier to gene flow between Mopti-M and Savanna/Bamako-S in Mali (Tripet et al. 2001). 4) The *kdr* allele (conferring resistance to pyrethroids) is found at high frequency in the S form only, and not in sympatric M mosquitoes (Chandre et al. 1999, Diabaté et al. 2003, Fanello et al. 2003), although in Benin the *kdr* allele is present in both forms, probably as a result of introgressive hybridization (Weill et al. 2000). 5) Sequence analysis of the Intron I upstream the *kdr* mutation shows two nucleotide differences associated with M and S, respectively (Weill et al. 2000, Gentile et al. 2003). Such level of genetic and chromosomal heterogeneity within natural populations of *An. gambiae* is expected to translate into biological, behavioral, and epidemiological heterogeneities within this major malaria vector.

Ecological and behavioral differences between M and S molecular forms are actually the subject of several investigations. Studies carried out so far have shown that the M form is better adapted to permanent breeding sites such rice fields, whereas the S form is predominant in temporary habitats (Diabaté et al. 2005). The distribution of chromosomal or molecular forms of *An. gambiae* in Cameroon has been so far barely studied. Robert et al. (1993) indicated the presence of only two chromosomal forms, Forest in the south and Savanna moving to the north. Both M and S molecular forms belong to the Forest and Savanna chromosomal forms and are also found in sympatry or allopatry (della Torre et al. 2001, Wondji et al. 2002).

We have studied the distribution of species, chromosomal, and molecular forms of the *An. gambiae* complex at several sites in Cameroon, covering different ecoclimatic zones. We examined data on malaria transmission dynamics in Simbock, a village where M and S forms coexist, to assess temporal variation in the relative abundance of each molecular form, and compare their feeding preferences and infection rates for *Plasmodium falciparum* across 2 yr.

Materials and Methods

Study Sites. Mosquitoes were collected in 23 locations (Table 1; Fig. 1), covering six bioclimatic domains, as defined in Olivry (1986). Arid savannas in the north gradually turn into rain forest in the south, and highland areas contribute to increased diversity of ecological settings.

Sites 1 and 2 (Simatou and Kaele) were sampled in the dry-tropical climatic zone of northern Cameroon, with <900 mm of annual rainfall and a dry season of >7 mo. Mean annual temperature is 28°C, with large daytime amplitude. Vegetation is typical of the Sudan–Sahelian domain, typical to steppe with thorny shrubs, bushes, and grasses.

Sites 3 to 4 (Pitoa and Djalingo) were sampled in the tropical basin of Benoue River. Mean annual rainfall is 900-1000 mm with a 6-mo dry season, and a mean annual temperature of 26°C. It is the domain of Sudan savanna with locally dense, dry, and open forests. However, both sampling sites were located in the vicinity of the town of Garoua, where forest is highly degraded after intensive exploitation by humans.

Southward, the Adamawa Mountains extend transversally with peak altitudes >2,000 m above sea level (a.s.l.). Two sites, Banyo (5) and Tibati (6), were sampled south of the mountains, within the highland tropical climatic area of the Adamawa Plateau, characterized by mean annual rainfall >1,500 mm, and mean annual temperature ≈22°C. The dry season extends from November to March. Vegetation is of the Sudan–Guinean type, with locally abundant shrubs and bushes spread out over a savanna background. The village of Tibati is located on the edge of a large artificial lake that provides year-round mosquito breeding sites. Bankim (site 7) is in the lowlands bordering the Adamawa foothills (see below).

Site 8 (Dschang) is at an altitude of 1,400 m a.s.l., on the western highlands, and is characterized by a much lower mean annual temperature (18°C) and high annual rainfall (≈1,900 mm) spread over 9 mo. The evergreen highland forest is locally degraded for agricultural use. A few kilometers southeast, Santchou (site 9), is only 750 m a.s.l., separated from Dschang by a high cliff. Mean annual temperature can reach 25°C.

Sites 14, 16 (Douala, the major economic center of Cameroon), and 23 belong to the coastal belt with hot and humid equatorial climate. Annual rainfall is >2,500 mm spread over the year, and mean annual temperature is 26°C. Along the Atlantic shore, the vegetation is locally highly degraded by human activities (industry, urbanization, and agriculture). Site 23 lies within the delta of Ntem River and is surrounded by mangrove swamps.

The remaining 11 sites experience a typical four-season equatorial climate, with mean annual rainfall of ≈1,500 mm and mean temperature of 24°C. Except site 22, all were clustered <150 km from the capital city, Yaoundé (site 17). Although rain is recorded

Table 1. Chromosomal and molecular identification of members of the *An. gambiae* complex collected in Cameroon

Site no.	Locality[a]	Geographic coordinates	Date of collection	Sampling method[b]	*An. gambiae* s.s.			*An. arabiensis*	*An. melas*
					Chromosomal form[c]	Molecular form			
						M	S		
1	Simatou	10° 34′ N; 15° 00′ E	Aug. 2001	PSC, NLC		0	0	180	0
2	Kaele	10° 05′ N; 14° 27′ E	June 2000	LC		0	0	27	0
3	Pitoa	09° 24′ N; 13° 30′ E	Oct. 2002	LC	ND	1	0	25	0
4	Djalingo	09° 18′ N; 13° 23′ E	June 2000	LC		0	0	28	0
5	Banyo	06° 45′ N; 11° 49′ E	Sept. 2001	PSC, NLC	Savanna, Forest	0	61	0	0
6	Tibati	06° 28′ N; 12°37′ E	Sept. 2001	PSC, NLC	Savanna, Mopti, Forest	36	63	2	0
7	Bankim	06° 00′ N; 11° 40′ E	Sept. 2001	PSC, NLC	Forest, (Savanna)	0	25	0	0
8	Dschang	05° 25′ N; 10° 10′ E	May 1999–Aug. 2000	PSC, NLC	Forest	1	117	0	0
9	Santchou	05° 17′ N; 09° 58′ E	July 2000	NLC	ND	1	55	0	0
10	Nkoteng	04° 30′ N; 12° 03′ E	Dec. 1998–Feb. 2000	PSC, NLC	Forest	1	66	2	0
11	Ebebda	04° 11′ N; 11° 17′ E	Feb. and April 2001	PSC, NLC	Forest	1	20	0	0
12	Mbebe	04° 10′ N; 11° 00′ E	Feb. and April 2001	PSC, NLC	Forest	1	18	0	0
13	Obala	04° 09′ N; 11° 33′ E	Nov. 98—Mar. 2000	PSC, NLC	Forest	1	51	0	0
14	Buéa	04° 09′ N; 09° 14′ E	Nov. 99	NLC	ND	7	2	0	0
15	Esse	04° 05′ N; 11° 53′ E	May 97	LC	ND	0	15	0	0
16	Douala	04° 03′ N; 09° 44′ E	May 2000	NLC	ND	31	1	0	0
17	Yaoundé	03° 52′ N; 11° 31′ E	April 97	LC	ND[d]	19	3	0	0
18	Simbock	03° 51′ N; 11° 30′ E	Oct. 98–Mar. 2003	PSC, NLC	Forest	661	59	0	0
19	Mfou	03° 44′ N; 11° 38′ E	April 97 and April 2001	PSC	ND	6	9	0	0
20	Mbalmayo	03° 30′ N; 11° 30′ E	June 2000	PSC, NLC	ND	117	3	0	0
21	Olama	03° 26′ N; 11° 17′ E	Feb.–Dec. 2000	NLC	ND	9	6	0	0
22	Nyabessan	02° 23′ N; 10° 24′ E	Feb. 2001	NLC	ND	0	29	0	0
23	Ipono	02° 19′ N; 09° 90′ E	June 2003	NLC	ND	27	2	0	203
	Total					920	605	264	203

[a] Numbers on the left column refer to location position on Fig. 1.
[b] Sampling method: LC, larval collection; NLC, night landing catches; and PSC, pyrethrum spray catches.
[c] Chromosomal form: −, not applicable; ND, not determined.
[d] Robert *et al.* (1993) karyotyped 64 specimens from two sites around Yaoundé, all were forest.

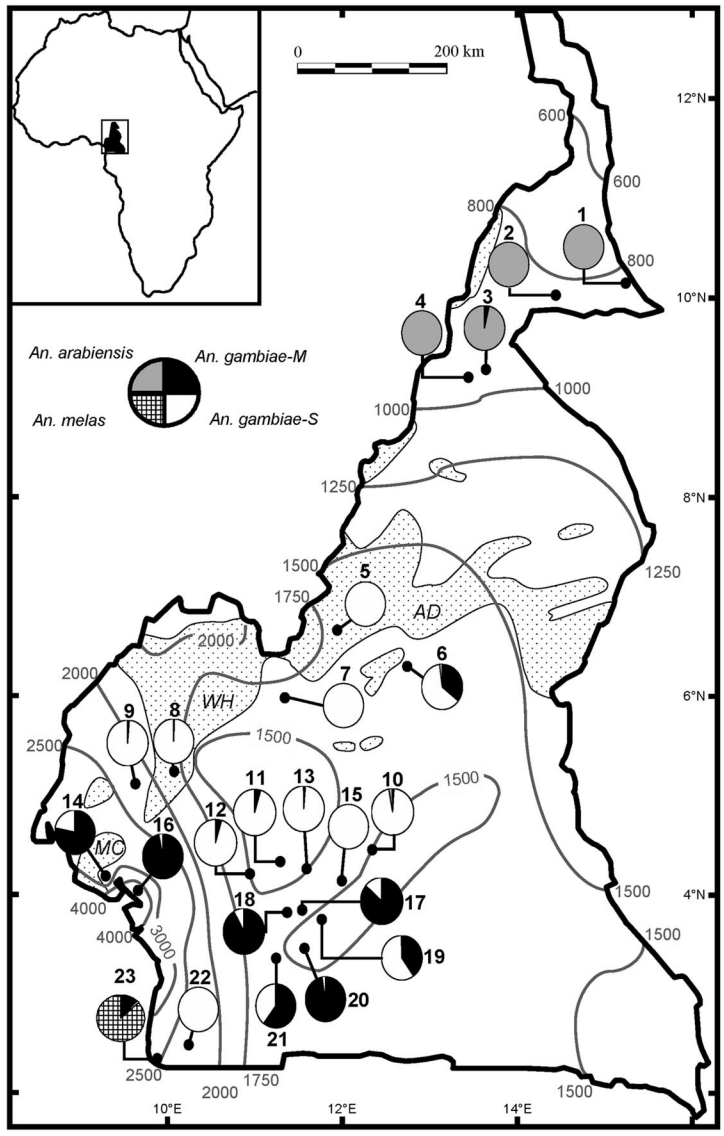

Fig. 1. Relative frequencies of *An. gambiae* M form (black), *An. gambiae* S form (white), *An. arabiensis* (gray), and *An. melas* (hatched) at sites of Cameroon. Numbered sites are listed in Table 1. Isohyets (in millimeters of rainfall per year) are shown as gray lines. Dotted areas: altitude >1000 m a.s.l. showing the Mount Cameroon (MC), Western highlands (WH), and the Adamawa Mountains (AD).

every month, the long dry season extends from late November to early March (with 10–30 mm rainfall/mo), and the short dry season includes July and August (80–100 mm rainfall/mo). Rainfall peaks in October at 250–350 mm. However, vegetation is very different on both sides of a line that approximately follows the fourth north parallel. South of this limit (sites 17–22), there is the continental, humid Congolese forest. North of Yaoundé (10–13 and 15) up to Bankim (7), the forest is highly degraded and intertwines with humid savannas in a complex mosaic, shaped by human activities. This is a transition zone between the Congolese forest domain and the Adamawa savannas. Relict gallery forests are present up to Tibati.

Mosquito Sampling. Female anophelines were captured by night landing catches (NLC) on volunteers' legs, and in the afternoon by pyrethrum spray collections (PSC) inside bedrooms. Five samples consisted of collections of larvae (LC) subsequently reared to adults in the insectary. Anophelines were identified using the morphological identification keys of Gillies and de Meillon (1968) and Gillies and Coetzee (1987). Field specimens were stored individually in tubes with desiccant and kept at −20°C until processed in the laboratory. Half-gravid mosquito ovaries were dissected in the field and fixed in Carnoy's solution (3 parts pure ethanol:1 part glacial acetic acid) for subsequent cytogenetic analysis.

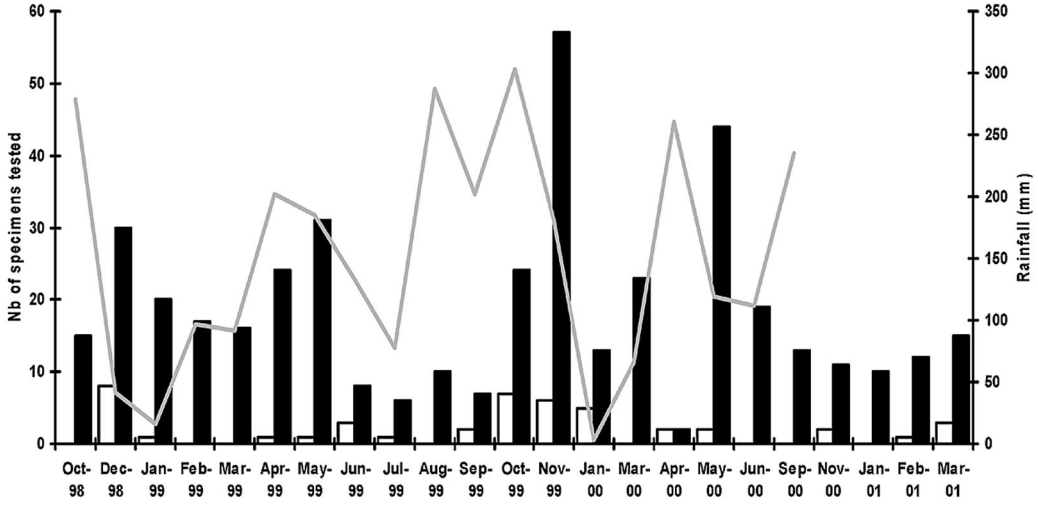

Fig. 2. Proportion of M (black) and S (white) molecular forms in *An. gambiae* samples collected in Simbock from October 1998 to March 2001. Monthly rainfall (in millimeters) for each collection date is given on the right y-axis. No meteorological data were available from November 2000 to March 2001.

Cytogenetic Analysis. All the mosquitoes analyzed for cytogenetic characterization consisted of indoor resting, half-gravid females, collected in the afternoon. Ovaries were processed for chromosomal preparations following della Torre (1997). Paracentric inversion karyotypes were scored according to the nomenclature of Coluzzi et al. (1979) and Petrarca et al. (2000).

Species and Molecular Form Identification. DNA was extracted from two or three legs according to Cornel and Collins (1996) and was resuspended in 100 μl of Tris-EDTA buffer. Specimens were identified to species following the technique of Scott et al. (1993), and all *An. gambiae* s.s. were further processed to attribute them to either the M or S molecular form. At first, the polymerase chain reaction (PCR)-restriction fragment-length polymorphism method by Favia et al. (1997) was used, and then the direct PCR identification method by Favia et al. (2001) was implemented. A set of 161 specimens were processed with both techniques and provided identical results (68 M and 93 S mosquitoes were successfully and consistently identified).

***Plasmodium* Infection Rates and Bloodmeal Identification.** Protocols for *P. falciparum* circumsporozoite protein (Pf-CSP) detection and bloodmeal source identification were given in detail in Antonio-Nkondjio et al. (2002). Our results refer to 546 *An. gambiae* collected in Simbock. Bloodmeal source was determined from 25 blood smears collected from resting females captured by PSC.

Results

In total, 1,992 *An. gambiae* s.l. specimens were collected in 23 sampling sites: 203 were *An. melas*, 264 *An. arabiensis*, and 1,525 *An. gambiae* s.s. (Table 1). *An. melas* was observed only in the coastal locality of Ipono (site 23). Virtually all *An. arabiensis* were collected in the northernmost, more arid sites (1–4), whereas *An. gambiae* was sampled southward in more humid areas (Fig. 1).

Distribution of the M and S Molecular Forms. The 1,525 *An. gambiae* females collected in 20 sites were identified to the molecular form (Table 1). M and S forms seemed widespread in the 19 sites south of the Adamawa Mountains (Fig. 1). The S form was the only form present in four locations, whereas both molecular forms were sympatric in 15 locations. In 12 of these locations, the frequency of the dominant form was >88%. The M form was predominant in seven sites, and the S form was predominant in 12 sites. No M/S hybrid pattern was observed.

In the village of Simbock (site 18), where both forms were sympatric, temporal variation in M and S relative abundance was evaluated in subsamples collected from October 1998 to March 2001 (Fig. 2). Excluding the April 2000 sample (n= 4), the relative frequency of the M form ranged from 70 to 100%.

Cytogenetics. Nurse cell polytene chromosomes were scored for 186 half-gravid *An. gambiae* females collected in nine sites (Table 1, 5–8, 10–13, and 18).

In the southern sites (10–13 and 18), the majority of females were represented by standard chromosome-2 homokaryotypes (2R+/+-2L+/+), typical of the Forest chromosomal form. Few inversions were scored at the heterozygous state in samples from Nkoteng (site 19), Obala (site 13), Ebebda (site 11), and Dschang (site 8), mostly as 2Rb/+ (7/63) and 2La/+ (5/63) single heterokaryotypes, with the frequency of 2Rb and 2La arrangements being 6.0% for both heterokaryotypes.

In Bankim (site 7), karyotypes were still to be ascribed mostly to the Forest chromosomal form, with low frequencies of inverted arrangements (2Rb, 16.7%; 2La, 19.1%). In Banyo (site 5), typical Savanna

form karyotypes were scored, with inversions 2Rb and 2La floating at high frequencies (>65%) and arrangements 2Rbc and 2Rd at lower frequencies (<13%). In both villages, only the S form was found.

In Tibati (site 6), we observed the highest degree of chromosomal heterogeneity. We recorded karyotypes with combinations of inversion arrangements typical of Mopti (six 2Rbc/+ and one 2Rbc/u), Savanna (eight specimens: 2Rb/+, 2Rb/b, 2Rcu/+, and 2Rd-carriers), Forest (10 2R+/+), and six Forest/Savanna intermediate karyotypes (mainly single 2Rb/+ or 2La/+ heterokaryotypes). Overall, the frequency of the 2La arrangement was 42.0%, with a significant ($P < 0.01$) heterozygote deficiency. All Mopti specimens were identified as M, whereas carriers of Forest and Savanna karyotypes were identified either as M or S.

***P. falciparum* Infection Rates and Host Preference in Simbock.** In Simbock, 26/502 M (5.2%, confidence interval [CI] = 3.2–7.1) and 3/44 S (6.8%, CI = 0.64–14.2) specimens were found positive for Pf-CSP. Twenty-five bloodmeals of 19 M and six S indoor resting females were analyzed: all had fed on humans. One M specimen had fed on both human and cattle.

Discussion

Three species of the *An. gambiae* complex were recorded in our collection sites: *An. melas*, *An. arabiensis*, and *An. gambiae* s.s.

An. melas is widespread along the Atlantic Coast of Africa where its larvae develop in brackish water (Gillies and de Meillon 1968, Gillies and Coetzee 1987, Coetzee et al. 2000). We found this species only at Ipono (site 23), a rural locality surrounded by mangrove swamps, typical breeding sites for *An. melas.* In this locality, this species accounted for 87.5% of the specimens of the *An. gambiae* complex caught by NLC, confirming its partial anthropophilic behavior, as observed previously in other coastal sites of West Africa (Bryan et al. 1987, Akogbeto and Romano 1999, Diop et al. 2002) and on the nearby island of Bioko, Equatorial Guinea (Berzosa et al. 2002). The exact role *An. melas* plays in human malaria transmission dynamics in this area needs to be further evaluated.

An. arabiensis occurred almost exclusively in the Sudan–Savanna areas of the north (sites 1–4), characterized by a long dry season and a mean annual rainfall <1000 mm, in agreement with literature data for whole sub-Saharan Africa (Gillies and Coetzee 1987, Coetzee et al. 2002) and northern Cameroon (Robert et al. 1992). The finding of few *An. arabiensis* in the northernmost sampling sites of the Yaoundé area (site 6 and 10), characterized by mean annual rainfall >1,500 mm, could suggest an increasing cline in frequency toward the more arid northern localities. Detailed geographical/seasonal sampling along a south–north transect would clarify this point.

An. gambiae s.s. occurred virtually only in localities south of the Adamawa Mountains, characterized by humid savanna and forested areas and mean annual rainfall >1,500 mm (sites 5–23). The single *An. gambiae* specimen (M form) found in one northern locality (site 3) represents the first record of this species in the area, where only *An. arabiensis* was reported previously (Robert et al. 1992). In the localities south of latitude 5° N (sites 10–13 and 18), *An. gambiae* samples showed a low degree of chromosomal diversity and could be all assigned to the Forest chromosomal form, as reported previously (Awahmukalah et al. 1992, Robert et al. 1993). However, in the area extending roughly between latitude 5° and 7° N (sites 5–8), which is characterized by a mosaic of degraded forest islands within a savanna background, *An. gambiae* showed increasing frequencies of 2R and 2L inversions, typical of intergrading Forest–Savanna populations. In Tibati (site 6), karyotypes typical of the Mopti chromosomal form were found together with Forest–Savanna karyotypes; the observed significant 2La heterokaryotype deficiency also supports the coexistence of different chromosomal forms. It is worth noting that Tibati is situated on the edge of a large artificial lake that may provide permanent breeding opportunities for the Mopti form (Coluzzi et al. 1985). In summary, 1) chromosomal diversity in *An. gambiae* increases northward; and 2) the observed pattern of distribution of the chromosomal forms and of their chromosomal variants is consistent with the ability of these forms in exploiting different habitats, as hypothesized by Coluzzi et al. (1985), Touré et al. (1994, 1998), and Powell et al. (1999).

Both M and S seemed widespread south of the Adamawa Mountains and are sympatric in most localities (Fig. 1); nevertheless, no M/S hybrids were observed throughout Cameroon, providing strong support for reproductive isolation between molecular forms, as suggested by a number of recent studies (della Torre et al. 2002, and references therein). It is particularly noteworthy that, in most instances, where both molecular forms were found in sympatry, one of them outnumbers the other (in a ratio 7–9:1). This is true even where all specimens can be assigned to a single, chromosomally homogenous population. Indeed, in such an area, all M and S populations sampled were characterized by karyotypes typical either of the Forest form or of intergrading Forest–Savanna populations. The only exception to this pattern came from the M-form population from Tibati, where karyotypes typical of the Mopti chromosomal form were found. The role of environmental selection pressure acting differentially on alternative chromosomal arrangements could not explain uneven distribution of molecular forms, as is the case in Mali and Burkina Faso (Touré et al. 1998, Powell et al. 1999). Indeed, the pattern observed in southern Cameroon would suggest that geographical distribution of molecular forms is associated more with local (microgeographical) than with regional environmental conditions. There is, however, a trend for the M form to be prevalent in the southern part of the area, where vegetation cover is deeper, whereas the S form seems to predominate in the northern part, where the Congolese forest is highly degraded. It should be noted that in Nyabessan (site 22), where only S-form mosquitoes were found, deforestation is currently taking place as a result of

intensive wood exploitation. Alternatively, the M form seems to be predominant in urban or peri-urban settings (e.g., sites 14, 16, 17, 18, and 20), whereas the S form is usually more abundant in rural settings (e.g., sites 8–13, 19, and 22), as observed in Nigeria (Kristan et al. 2003) and in Angola (V.P., unpublished data). Furthermore, competitive exclusion between molecular forms with identical chromosomal constitution, thus a priori exposed to similar environmental pressure, could well be the key factor shaping composition of local *An. gambiae* populations in terms of molecular forms. The mechanism by which this exclusive competition is acting is not yet known but this probably happens at the larval level (Diabaté et al. 2005). A difference in preference for breeding sites between these molecular forms (Diabaté et al. 2005) also may explain this pattern of distribution. The longitudinal follow-up showed temporal stability of the imbalance between M and S molecular forms in Simbock, where both forms were sympatric and synchronous yearround, with few exceptions probably because of sample size constraints, rather than seasonal variation in the S-form prevalence.

The results obtained during this study are in accordance with the general pattern of relation between molecular and chromosomal forms of *An. gambiae* as observed throughout Africa (della Torre et al. 2001). All Mopti specimens belong to the M form, whereas the Forest and Savanna chromosomal forms both comprise M and S molecular forms. But because of the tight link between the environment and the distribution of *An. gambiae*, these results cannot be generalized throughout Africa, especially in some countries such as Mali where M and S forms always belong to different chromosomal forms. Nevertheless, we think that this study constitutes a good basis to understand the pattern of geographical repartition of molecular forms of *An. gambiae* in an environment similar to that of Cameroon.

Regarding vectorial ability, our results, although very preliminary with relatively small sample sizes, seem to suggest similar feeding preferences and susceptibility to *P. falciparum* infection in the molecular forms of *An. gambiae*, at least in Simbock, where both forms present homosequential standard chromosome arrangements. Overall, the Pf-CSP index was not significantly different from that recorded in previous observations, i.e., ≈7% in the southwestern provinces (Awahmukalah et al. 1992).

In conclusion, the pattern of distribution of *An. gambiae* molecular forms observed in Cameroon suggests that these may have different abilities in exploiting differentiated habitats, independently from their chromosomal inversion patterns. A deeper scale assessment of biological, ecological, and behavioral traits of both molecular forms is being conducted to identify factors shaping their geographical distribution within the forest block and to monitor and better understand their interactions as well as their contribution to the vector system.

Acknowledgments

We are grateful to Christophe Antonio-Nkondjio, Parfait Awono-Ambene, Samuel Wanji, Jean Claude Toto, Sylvie Zebaze-Kemleu, Rose Nyambam, Isabelle Morlais, Matthieu Boulesteix, Maria Calzetta, and Giovannino Petrangeli for laboratory and field help. We acknowledge two anonymous reviewers for useful comments. Part of this study was funded by a WHO-MIM grant no. 980101, WHO-RTG mo. A10827 (to C.S.W.), the Pal+ Program from the French Ministry of Research, the Institut de Recherche pour le Développement, and Italian MIUR/COFIN funds (to A.d.T. and V.P.).

References Cited

Akogbeto, M., and R. Romano. 1999. Infectivity of *Anopheles melas* vis-à-vis *Plasmodium falciparum* in the coastal lagoon of Benin. Bull. Soc. Pathol. Exot. 92: 57–61.

Antonio-Nkondjio, C., H. P. Awono-Ambene, J. C. Toto, J. Y. Meunier, S. Zebaze-Kemleu, R. Nyambam, C. S. Wondji, T. Tchuinkam, and D. Fontenille. 2002. High malaria transmission intensity in a village close to Yaounde, the capital city of Cameroon. J. Med. Entomol. 39: 350–355.

Awahmukalah, D.S.T., M. Coluzzi, and V. Petrarca. 1992. Osservazioni sul complesso *Anopheles gambiae* (Diptera: Culicidae) nel Cameroon Occidentale. XVII Congresso SOIPA. Parassitologia 34 (Suppl): 18–19.

Barnes M. J., N. F. Lobo, M. B. Coulibaly, N′. F. Sagnon, C. Costantini, and N. J. Besansky. 2005. SINE insertion polymorphism on the X chromosome differentiates *Anopheles gambiae* molecular forms. Insect Mol. Biol. 14: 353–363.

Berzosa, P. J., J. Cano, J. Roche, J. M. Rubio, L. García, E. Moyano, A. Guerra, J. C. Mateos, V. Petrarca, V. Do Rosario, et al. 2002. Malaria vectors in Bioko Island (Equatorial Guinea): PCR determination of the members of *Anopheles gambiae* Giles complex (Diptera: Culicidae) and pyrethroid knockdown resistance (*kdr*) in *An. gambiae* sensu stricto. J. Vector Ecol. 27: 102–106.

Bryan, J. H., V. Petrarca, M. A. Di Deco, and M. Coluzzi. 1987. Adult behaviour of members of the *Anopheles gambiae* complex in The Gambia with special reference to *An. melas* and its chromosomal variants. Parassitologia 29: 221–249.

Chandre, F., S. Manguin, C. Brengues, J. Dossou Yovo, F. Darriet, A. Diabaté, P. Carnevale, and P. Guillet. 1999. Current distribution of pyrethroid resistance gene (kdr) in *Anopheles gambiae* complex from West Africa and further evidence for reproductive isolation of the Mopti form. Parassitologia 41: 319–322.

Coetzee, M., M. Craig, and D. Le Sueur. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. Parasitol. Today 16: 74–77.

Coluzzi, M., V. Petrarca, and M. A. Di Deco. 1985. Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae.* Boll. Zool. 52: 45–63.

Coluzzi, M., A. Sabatini, A. Petrarca, and M. A. Di Deco. 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. Trans. R. Soc. Trop. Med. Hyg. 73: 483–497.

Cornel, A., and F. H. Collins. 1996. PCR of the ribosomal DNA intergenic spacer regions as a method for identifying mosquitoes in the *Anopheles gambiae* complex. Methods Mol. Biol. 50: 321–332.

della Torre, A. 1997. Polytene chromosome preparation from Anopheline mosquitoes, pp. 329–336. *In* J. M. Crampton, C. B. Beard, and C. Louis [eds.], Molecular

biology of insect disease vectors: a method manual. Chapman & Hall, London, United Kingdom.

della Torre, A., C. Fanello, M. Akogbeto, J. Dossou-yovo, G. Favia, V. Petrarca, and M. Coluzzi. 2001. Molecular evidence of incipient speciation within *Anopheles gambiae* s.s. in West Africa. Insect Mol. Biol. 10: 9–18.

della Torre, A., C. Costantini, N. J. Besansky, A. Caccone, V. Petrarca, J. R. Powell, and M. Coluzzi. 2002. Speciation within *Anopheles gambiae* –the glass is half full. Science (Wash. DC) 298: 115–117.

Diabaté A., T. Baldet, F. Chandre, K. R. Dabire, P. Kengne, R. Guiguemde Tinga, F. Simard, P. Guillet, J. Hemingway, and J. M. Hougard. 2003. Kdr mutation, a genetic marker to assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in the tropical savannah area of West Africa. J. Med. Entomol. 40: 195–198.

Diabaté A., K. R. Dabire, E. H. Kim, R. Dalton, N. Millogo, T. Baldet, F. Simard, J. E. Gimnig, W. Hawley, and T. Lehmann. 2005. Larval development of the molecular forms of *Anopheles gambiae* (Diptera: Culicidae) in different habitats: a transplantation experiment. J. Med. Entomol. 42: 548–553.

Diop, A., J. F. Molez, L. Konate, D. Fontenille, O. Gaye, M. Diouf, M. Diagne, and O. Faye. 2002. Role of *Anopheles melas* Theobald (1903) on malaria transmission in a mangrove swamp in Saloum (Senegal). Parasite 9: 239–246.

Fanello, C., V. Petrarca, A. della Torre, F. Santolamazza, A. Allouèche, M. Coulibaly, G. Dolo, C. Curtis, Y. T. Touré, and M. Coluzzi. 2003. The pyrethroid knock-down resistance gene in *Anopheles gambiae* complex in Mali and further evidence of incipient speciation within *An. gambiae* s.s. Insect Mol. Biol. 12: 241–245.

Favia, G., A. della Torre, M. Bagayoko, A. Lanfrancotti, N′. F. Sagnon, Y. T. Touré, and M. Coluzzi. 1997. Molecular identification of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation. Insect Mol. Biol. 6: 377–383.

Favia, G., A. Lanfrancotti, L. Spanos, I. Siden-Kiamos, and C. Louis. 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s. Insect Mol. Biol. 10: 19–23.

Gentile, G., A. della Torre, B. Maegga, J. R. Powell, and A. Caccone. 2002. Genetic differentiation in the African malaria vector, *Anopheles gambiae* s.s., and the problem of taxonomic status. Genetics 161: 1561–1578.

Gentile, G., F. Santolamazza, C. Fanello, A. Caccone, and A. della Torre. 2003. Nucleotide differences in an intron sequence of the voltage-gated sodium channel gene as markers of genetic differentiation between *Anopheles gambiae* s.s. molecular forms. Insect Mol. Biol. 13: 371–377.

Gillies, M. T., and M. Coetzee. 1987. A supplement to the Anophelinae of Africa South of the Sahara. Publ. South Afr. Inst. Med. Res. 55.

Gillies M. T., and B. de Meillon. 1968. The Anophelinae of Africa south of the Sahara, 1st ed. Publ. South Afr. Inst. Med. Res. 54.

Kristan, M., H. Fleischmann, A. della Torre, A. Stich, and C. F. Curtis. 2003. Pyrethroid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and *An. gambiae* s.s. malaria vectors in Nigeria and Ghana. Med. Vet. Entomol. 17: 326–332.

Lehmann, T., M. Licht, N. Elissa, B.T.A. Maega, J. M. Chimumbwa, F. T. Watsenga, C. S. Wondji, F. Simard, and W. A. Hawley. 2003. Population structure of *Anopheles gambiae* in Africa. J. Hered. 94: 133–147.

Olivry, J. C. 1986. Fleuves et rivières du Cameroun. Collection Monographies Hydrologiques ORSTOM No. 9, Edition ORSTOM, Paris, France.

Petrarca, V., A. D. Nugud, M. A. Elkarim Ahmed, A. M. Haridi, M. A. Di Deco, and M. Coluzzi. 2000. Cytogenetics of the *Anopheles gambiae* complex in Sudan, with special reference to *An. arabiensis:* relationships with East and West African populations. Med. Vet. Entomol. 14: 149–164.

Powell, J. R., V. Petrarca, A. della Torre, A. Caccone, and M. Coluzzi. 1999. Population structure, speciation, and introgression in the *Anopheles gambiae* complex. Parassitologia 41: 101–113.

Robert, V., A. Van den Broek, P. Stevens, R. Slootweg, V. Petrarca, M. Coluzzi, G. Le Goff, M. A. Di Deco, and P. Carnevale. 1992. Mosquitoes and malaria transmission in irrigated rice-fields in the Benoue valley of northern Cameroun. Acta Trop. 52: 201–204.

Robert V., V. Petrarca, G. Le Goff, and L. Manga. 1993. Quelques données cytogénétiques sur le complexe *Anopheles gambiae* au Sud-Cameroun. Bulletin de liaison et de documentation de l'OCEAC 26: 99–101.

Scott, J. A., W. G. Brogdon, and F. H. Collins. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49: 520–529.

Stump, A. D., J. A. Shoener, C. Costantini, N. F. Sagnon, and N. J. Besansky. 2005. Sex-linked differentiation between incipient species of *Anopheles gambiae.* Genetics 169: 1509–1519.

Taylor, C., Y. T. Touré, J. Carnahan, D. E. Norris, G. Dolo, S. Traoré, F. E. Edillo, and G. C. Lanzaro. 2001. Gene flow among populations of the malaria vector, *Anopheles gambiae,* in Mali, West Africa. Genetics 157: 743–759.

Touré Y. T., V. Petrarca, S. F. Traoré, A. Coulibaly, H. M. Maiga, O. Sankaré, M. Sow, M. A. Di Deco, and M. Coluzzi. 1994. Ecological genetic studies in the chromosomal form Mopti of *Anopheles gambiae* s.str. in Mali, West Africa. Genetica 94: 213–223.

Touré Y. T., V. Petrarca, S. F. Traoré, A. Coulibaly, H. M. Maiga, O. Sankaré, M. Sow, M. A. Di Deco, and M. Coluzzi. 1998. The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. Parassitologia 40: 477–511.

Tripet, F., Y. T. Touré, C. E. Taylor, D. E. Norris, G. Dolo, and G. C. Lanzaro. 2001. DNA analysis of transferred sperm reveals significant levels of gene flow between molecular forms of *Anopheles gambiae.* Mol. Ecol. 10: 1725–1732.

Wang, R., L. Zheng, Y. T. Touré, T. Dandekar, and F. C. Kafatos. 2001. When genetic distance matters: measuring genetic differentiation at microsatellite loci in whole-genome scans of recent and incipient mosquito species. Proc. Natl. Acad. Sci. U.S.A. 98: 10769–10774.

Weill, M., F. Chandre, C. Brengues, S. Manguin, M. Akogbeto, P. Guillet, and M. Raymond. 2000. The kdr mutation occurs in the Mopti form of *Anopheles gambiae* s.s. through introgression. Insect Mol. Biol. 9: 451–455.

Wondji, C., F. Simard, and D. Fontenille. 2002. Evidence for genetic differentiation between the molecular forms M and S within the Forest chromosomal form of *Anopheles gambiae* in an area of sympatry. Insect Mol. Biol. 11: 11–19.

Received 28 April 2005; accepted 18 August 2005.