Insecticide Susceptibility Status of *Anopheles gambiae* s.l. (Diptera : Culicidae) in the Republic of Cameroon

JOSIANE ETANG,[1, 2] LUCIEN MANGA,[3] FABRICE CHANDRE,[4] PIERRE GUILLET,[5] ETIENNE FONDJO,[6] REMY MIMPFOUNDI,[7] JEAN-CLAUDE TOTO,[2] AND DIDIER FONTENILLE[8]

Institut de Recherches Médicales et d'étude des Plantes Médicinales/Centre de Recherches Médicales/ Ministère de la Recherche Scientifique et Technique du Cameroun , Yaounde, Cameroon

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ABSTRACT A large-scale survey of *Anopheles gambiae* Giles, 1902 susceptibility to DDT, dieldrin, permethrin, and deltamethrin was conducted in the Republic of Cameroon. 15 field populations from various geographical areas were tested using World Health Organization test kits for adult mosquitoes. The laboratory Kisumu susceptible reference strain was tested as a control. Results showed that dieldrin and DDT resistance was still present in some populations, and indicated permethrin or deltamethrin resistance. Within the *Anopheles gambiae* complex, resistant individuals belonged to *An. gambiae s.s.* and *An. arabiensis* species. Both M and S molecular forms of *An. gambiae* s.s. were found resistant. In most of resistant populations, the knockdown times were 2–5-folds increased. However, none of the surviving mosquitoes was positive to the *kdr* "Leu-Phe" mutation using polymerase chain reaction (PCR) diagnostic test. These results likely suggested involvement of other resistance mechanism(s), such as enzyme detoxification or *kdr* "Leu-Ser" mutation. Researches on *An. gambiae* s.l. resistance should be promoted in Cameroon, to improve malaria vector control programs and to implement resistance management strategies.

KEY WORDS *Anopheles gambiae*, insecticide resistance, Cameroon

RESISTANCE TO PYRETHROIDS in malaria vectors, and particularly in *Anopheles gambiae* s.l., is becoming a real problem for the implementation of malaria vector control programs (Vulule et al. 1994, Elissa et al. 1993). The *kdr* mutation was reported to be the main pyrethroid resistance mechanism in *An. gambiae* s.s. from West Africa (Chandre et al. 1999a, Diabate et al. 2002a). This resistance resulted from a single point mutation of a leucine amino acid to phenylalanine in the sodium channel gene, which is the target site of DDT and pyrethroids (Martinez-Torres et al. 1998). Another mutation was recently identified in East Africa, that involved the replacement of the same leucine amino acid by serine. This mutation conferred a lower level of DDT and pyrethroid resistance than the *kdr* mutation from West Africa (Ranson et al. 2000). Within the *An. gambiae* complex, the *kdr* "Leu-Phe" mutation occurred only in *An. gambiae* s.s. and was first identified in its S molecular form and not in M form, although in savanna areas both forms were sympatric suggesting strong barrier to gene flow (Chandre et al. 1999a). Subsequently, this mutation was also reported in the M molecular form on coastal area from Benin (Weill et al. 2000). However, molecular analysis of the sodium channel polymorphism showed that the *kdr* mutation moved from S to M form through genetic introgression. DDT-resistance in *An. gambiae* complex could also occur by increase of DDT-dehydrochlorinase activity as reported by Prapanthadara et al. (1995) in Zanzibar, Tanzania. However, pyrethroid resistance in other vector species of the complex such as *Anopheles arabiensis*, *Anopheles melas*, and *Anopheles merus* was never detected or not enough investigated. Selection of vector resistance in West Africa partly resulted from the intensive use of insecticides for agricultural purposes (Chandre et al. 1999b, Diabate et al. 2002b). Because bednets impregnated with pyrethroids are strongly advocated for the control of malaria transmission (Lengeler 1998, Lengeler et al. 1996, Zaim et al. 2000), vector resistance to these insecticides has to be constantly monitored. The susceptibility of malaria vec-

[1] Institut of Medical Researches and Studies of Medicinal Plants/ Medical Research Center, Ministry of Scientific Research and Technic, Yaounde, Cameroon (e-mail: josyet@yahoo.fr).
[2] Organisation de coordination pour la lutte contre les endémies en Afrique centrale, BP 288 Yaounde, Cameroon.
[3] World Health Organisation, Regional Office for Africa, Harare, Zimbabwe.
[4] Centre Pierre Richet, 01 BP 1500 Bouake 01, Bouake, Côte d'Ivoire.
[5] World Health Organisation, Head office, Geneva, Switzerland.
[6] National Malaria Program, Ministry of Health, P.O. Box 14306, Yaounde, Cameroon.
[7] Laboratory of Biology, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon.
[8] IRD, Laboratoire de lutte contre les insectes nuisibles, Montpellier, France.

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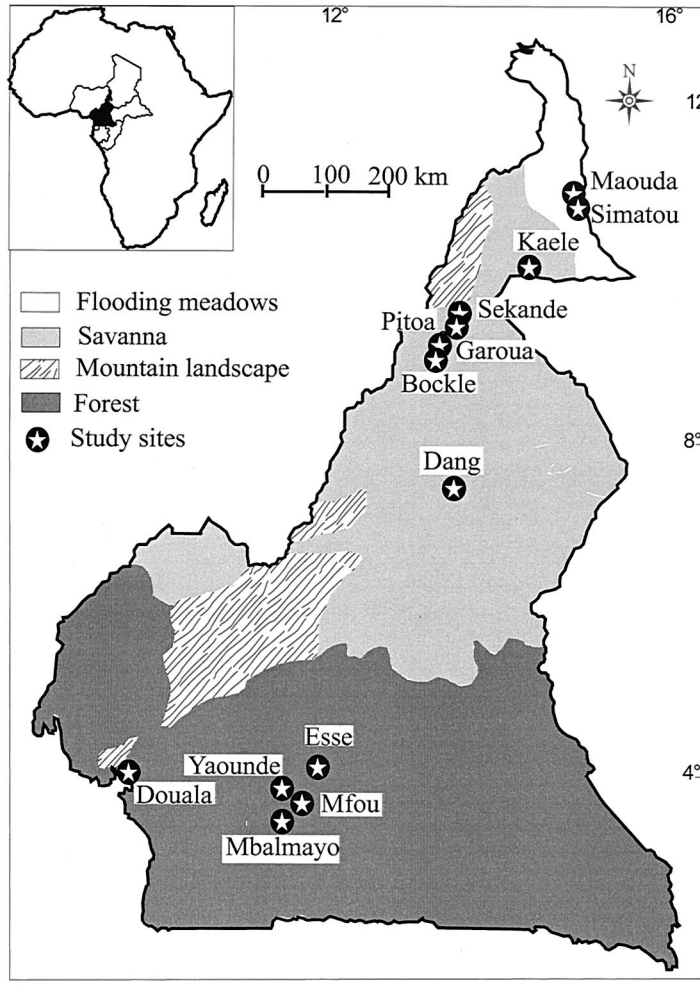

Fig. 1. Map of Cameroon showing study sites in various climatic areas

tors to pyrethroids remains to be documented in many countries, especially in Central Africa where many vector control programs are being implemented. In the Republic of Cameroon, the first report of *An. gambiae* resistance to insecticides is back to the early 1960s and only concerned dieldrin (Mouchet and Cavalié 1959, Gariou and Mouchet 1961). However, no investigation was carried out since this period and no data about pyrethroid resistance was available in this country. The Malaria Research Laboratory, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC, Yaounde, Cameroon), developed a testing and monitoring program from 1997 to 2001 to bridge this gap. This paper presents the baseline situation in various geographical areas from Cameroon.

Materials and Methods

Study Sites

The study was conducted in 15 sites, including 10 sentinel sites where insecticides have been intensively used and the National Malaria Control Program, Yaounde, Cameroon, has planed to monitor resistance. Five additional sites were chosen around sentinel sites, to estimate the microgeographic distribution of resistant populations in each region. Among the 15 sites, three were located in the Coastal area, four in the Southern area, and eight in the Northern area (Fig. 1).

Sentinel Sites. In the coastal area, three districts of Douala (3° 48′N, 10° 08′E), named Bonaberi ($Douala_1$), Bonandjo ($Douala_2$) and Bonanloka ($Douala_3$) were selected. The coastal area is characterized by equatorial climatic conditions with a very high annual rainfall (2,000–10,000 mm). The mean temperature is 26°C.

In the Southern part of the country, mosquitoes were collected in Elig-Edzoa, a district from Yaounde (3° 51′N, 11° 30′E) and in Mbalmayo (3° 31′N, 11° 30′E) a small town located at 50 kms from Yaounde. The climate in the southern region is equatorial, characterized by two rainy seasons (March to

June, September to November, 1,500–2,000 mm rainfall), and two dry seasons (November to March and July to August). The mean temperature is ≈25°C.

In the Northern part of the country, study sites were Pitoa (9° 24′N, 13° 31′E), which is an urban area, and four rural areas, Sekande (9°26N, 13°33E), Bockle (9° 18′N, 13° 23′E), Simatou (10° 52′N, 15° 00′E), and Maouda (10° 51′N, 15° 04′E). The climate in the Northern Cameroon is tropical, characterized by one rainy season covering 3–6 mo (March to September) and one dry season of 6–9 mo (November to May). The total rainfall per year ranges from 400 to 900 mm and the main temperature is 28°C.

Additional Sites. In the Southern part of the country, the study was conducted in Esse, a rural area (4° 05′N, 11° 53′E), and Mfou, an urban area (3°44N, 11°38E), located, respectively, at ≈60 kms and 20 kms from Yaounde.

In the Northern part of the country, mosquitoes were collected in Kaele (10° 05′N, 14° 27′E) and Garoua (9° 18′N, 13° 23′E) urban areas and in Dang, a peri-urban area (7° 19′N, 13° 35′E).

Biological Material

Mosquito breeding sites were actively searched in each area, and anopheline larvae collected by dipping. Larvae were brought to the laboratory, where they were fed with fish food (TetraMikromin, Tetra Werke, Germany). Pupae were collected and kept in cages until adult emergence. Adult mosquitoes were maintained with a 10% glucose solution.

A laboratory susceptible reference strain of *An. gambiae* (Kisumu) was used to compare the susceptibility levels of wild populations.

Susceptibility Tests

Bioassays were carried out using WHO test kits for adult mosquitoes (WHO 1981, revised in 1998). Impregnated papers were provided by the "Laboratoire des Insectes nuisibles" of IRD (Montpellier, France), a WHO Collaborating Centre. The following diagnostic concentrations of insecticides were tested: 0.4% dieldrin, 4% DDT, 0.25% permethrin, 1% permethrin, and 0.05% deltamethrin.

Tests were carried out with 2–4-d-old unfed females. Batches of 15–25 females were exposed to impregnated papers for 1 h. The number of knockdown mosquitoes was recorded every 10 min during exposure. The knockdown times for 50% (KdT_{50}) and 95% (KdT_{95}) of tested mosquitoes were calculated using a log-probit software according to Finney (1971). The mortality rate was recorded after 24 h. Tests with untreated papers were systematically run as control. When control mortality was between 5 and 20%, mortality rate in tested samples was corrected using Abbott formula (Abbott 1925).

Table 1. Knockdown times and mortality rates of the susceptible reference strain of *An. gambiae* (Kisumu) to 4% DDT, 1% permethrin, and 0.05% deltamethrin

Insecticides	No.	Knockdown times		Mortality (%)
		KdT_{50} (CI) in min	KdT_{95} (CI) in min	
0.4% Dieldrin	100	–	–	99
4% DDT	100	18.8 (17.6–20.0)	28.7 (25.8–33.7)	100
0.25% Permethrin	100	12.4 (11.2–13.7)	28.8 (24.8–35.4)	94.1
1% Permethrin	99	9.2 (8.6–9.7)	14.3 (13.2–16.0)	100
0.05% Deltamethrin	89	9.4 (8.4–10.2)	17.2 (15.6–20.0)	100

KdT_{50}, Kd time in min for 50% mosquitoes; KdT_{95}, Kd time in min for 95% mosquitoes; CI, confidence interval at 95%; Mortality, mortality rate 24 h post exposure.

Identification of Sibling Species of the *An. gambiae* complex, M and S Molecular Forms of *An. gambiae* s.s., and Detection of the kdr Mutation

All tested mosquitoes were morphologically identified as members of *An. gambiae* complex. After bioassays, surviving mosquitoes were kept at −20°C for polymerase chain reaction (PCR) assays. DNA of surviving specimens was individually extracted according to Collins et al. (1988).

Polymerase chain reaction studies for identification of sibling species of the *An. gambiae* complex were performed according to Scott et al. (1993). DNA of *Anopheles gambiae* s.s. was amplified and digested with *Hha*I enzyme, then restriction profiles were analyzed to determine the M and S molecular forms (Favia et al. 1997). Polymerase chain reaction diagnostic tests for detection of *kdr* "Leu-Phe" mutation were done on all these surviving individuals (Martinez-Torres et al. 1998)

Results

Susceptibility Status

A total of 15 mosquito populations were sampled and 41 susceptibility tests were carried out with DDT, dieldrin, permethrin, and deltamethrin. The resistance status of wild populations was based on decrease of mortality rates according to WHO criteria (1998). In addition, increase of knockdown times during exposure to insecticide was also considered as an early indicator of resistance. For each test, 70–110 mosquitoes were used. Because some WHO diagnostic concentrations changed during our study, bioassays with permethrin were performed at the former concentration (0.25%) and the new one (1% for 25/ 75 cis/*trans* ratio).

The response of Kisumu susceptible strain exposure to 4% DDT, 0.4% dieldrin, 0.25% permethrin, 1% permethrin, and 0.05% deltamethrin is given in Table 1. The mortality rates in most of the tests was 100%, except with 0.25% permethrin where we registered 94%. Because the Kisumu strain is susceptible, fast knockdown effects were observed with $KdT_{95} < 30$ mn for DDT and approximately 15 mn for both pyrethroids.

Table 2. Knockdown times and mortality rates of *An. gambiae* field populations in sentinel sites to 4% DDT, 1% permethrin, and 0.05% deltamethrin

Insecticide	Regions	Sites	*N*	Knockdown times			Mortality (%)
				KdT_{50} (CI) (min)	KdT_{95} (CI) (min)	$KdT_{50}R$	
4% DDT	Coastal	$Douala_1$	80	42.9 (27.0–68.1)	97.7 (37.3–255.7)	2.3	**86.2**
		$Douala_2$	88	48.2 (46.1–50.7)	86.2 (76.6–102.5)	2.6	**75**
		$Douala_3$	80	49.3 (41.8–58.7)	125.5 (83.2–197.2)	2.6	**88**
	Southern	Yaounde	80	46.2 (41.9–48.6)	78.5 (70.5–92.1)	2.5	**81.2**
		Mbalamyo	87	53.2 (50.9–56.5)	89.8 (79.8–106.9)	2.8	**63.2**
	Northern	Garoua	75	24.7 (23.1–26.5)	38.3 (34.5–44.6)	1.3	100
		Bockle	71	31.7 (30.0–33.5)	56.2 (51.2–63.4)	1.7	100
		Pitoa	82	32.3 (30.5–34.1)	52.4 (48.1–59.1)	1.7	100
		Sekande	78	29.8 (28.0–38.5)	56.0 (51.3–62.7)	1.7	100
		Simatou	95	48.1 (46.8–49.4)	70.9 (67.0–76.5)	2.5	**90.5**
		Maouda	85	39.4 (38.1–40.5)	56.6 (53.8–60.7)	2.1	98.8
1% Permethrin	Coastal	$Douala_1$	71	13.9 (13.2–14.6)	19.8 (18.5–21.9)	1.5	100
		$Douala_2$	93	16.1 (13.4–16.9)	22.0 (17.7–27.4)	1.6	99
		$Douala_3$	91	16.3 (14.9–17.7)	25.7 (22.4–29.6)	1.7	**72.5**
	Southern	Yaounde	110	10.1 (9.5–10.6)	19.6 (18.2–21.3)	1.1	100
		Mbalmayo	81	13.7 (12.3–15.2)	19.7 (15.7–29.4)	1.5	98.8
	Northern	Bockle	80	14.7 (13.6–15.9)	30.4 (26.0–38.6)	1.6	100
		Sekande	85	14.0 (13.0–15.0)	25.7 (23.1–29.8)	1.5	100
		Simatou	110	19.6 (18.8–20.4)	30.9 (28.9–33.7)	2.1	**96.4**
		Maouda	97	20.4 (19.4–21.4)	32.6 (29.9–36.7)	2.2	**85.6**
0.05% Deltamethrin	Coastal	$Douala_1$	80	12.0 (9.1–15.9)	19.4 (12.0–31.2)	1.3	100
		$Douala_2$	101	23.3 (22.1–24.5)	42.4 (38.1–49.2)	2.7	**95**
		$Douala_3$	86	22.4 (21.2–23.7)	34.8 (31.2–39.0)	2.6	**96.5**
	Southern	Yaounde	86	18.2 (14.5–22.8)	31.2 (20.6–47.5)	2.1	**96.5**
		Mbalmayo	93	17.2 (16.7–17.8)	25.2 (23.3–28.4)	1.8	100
	Northern	Pitoa	80	21.0 (18.6–23.7)	31.7 (25.5–39.4)	2.2	**88.7**
		Simatou	91	20.3 (19.4–21.2)	32.2 (29.9–35.6)	2.1	100
		Maouda	85	18.2 (17.2–19.2)	33.7 (30.7–38.2)	1.9	100

$KdT_{50}R$, KdT_{50} of tested population divided by KdT_{50} of the Kisumu strain; KdT_{50}, Kd time in min for 50% mosquitoes; KdT_{95}, Kd time in min for 95% mosquitoes; CI, confidence interval at 95%; mortality, mortality rate 24 h post exposure.

Bold characters indicate resistant populations.

In sentinel sites, tests were carried out with current WHO recommended diagnostic concentration (Table 2):

With 4% DDT. DDT resistance was detected in $Douala_2$ and Mbalmayo, where the mortality rates were 75% and 63%, respectively. A reduced susceptibility was also observed in $Douala_1$, $Douala_3$, Yaounde, and Simatou with mortality rates ranging from 81% to 90.5%. Considering confidence intervals, all populations showed an significant increase of their knockdown times (KdT_{50} and KdT_{95}) compared with the Kisumu strain. The highest increases in KdT_{50} (≥2.5-fold) were observed for populations with the lowest mortality rates, i.e., $Douala_1$, $Douala_2$, $Douala_3$, Yaounde, Mbalmayo, and Simatou. The most susceptible populations were mainly found in the Northern area (Garoua, Pitoa, Bockle, Sekande), where the mortality rates ranged from 98.8% to 100%, and increase of KdT_{50} was less than 2-fold.

With 1% Permethrin. Permethrin resistance was observed in $Douala_3$ and Maouda, where mortality rates were 72.5% and 85.6%, respectively. Sample from Simatou showed a decrease of mortality (96.4%), suggesting possible resistance. The six other samples were fully susceptible. A low but significant increase of KdT_{50} was also detected in most populations except Yaounde.

With 0.05% Deltamethrin. Deltamethrin resistance was detected in Pitoa, with 88.7% mortality rate. In three other sites ($Douala_2$, $Douala_3$, and Yaounde), the mortality rates decreased at 95–96%, suggesting possible resistance. As seen with permethrin, a low but significant increase of KdT_{50} was observed for most samples.

Additional tests were carried out with dieldrin 0.4%, and with former WHO permethrin diagnostic dose 0.25% (Table 3):

With 0.4% Dieldrin. The mortality rate in the Kisumu strain was 99%. Dieldrin resistance was observed in Mbalmayo and Kaele, where the mortality rates were 2% and 61.2%, respectively.

With 0.25% Permethrin. Eight populations were tested, i.e., three from sentinel sites ($Douala_3$, Yaounde, and Pitoa) and four from additional sites (Esse, Mfou, Dang, and Garoua). For all samples, the mortality rate did not exceed 97%, even with the Kisumu-susceptible strain. The highest mortality rate was recorded in Mfou (96.2%). The $Douala_3$ population previously found resistant to 1% permethrin was also resistant to 0.25% permethrin (78.7% mortality). In Yaounde, the mortality rate was 100% to 1% permethrin but only 89% with 0.25% permethrin. In the Esse, Dang, Garoua, and Pitoa sites, tests with 1% permethrin were not performed; however, the mortality rates to 0.25% permethrin were 73%, 73.3%, 43.5%, and 57.5%, respectively. These values indicated that the four populations were likely resistant to per-

Table 3. Knockdown times and mortality rates of field populations to 0.4% dieldrin and 0.25% permethrin

Insecticide	Regions	Sites	No.	Knockdown times			Mortality (%)
				KdT_{50} (CI) (min)	KdT_{95} (CI) (min)	$KdT_{50}R$	
0.4% Dieldrin	Southern	Mbalmayo	100	–	–	–	**2**
	Northern	Kaele	80	–	–	–	**61.2**
0.25% Permethrin	Coastal	Douala3	80	64.1 (59.0–73.9)	110.5 (90.0–166.4)	5.2	**78.7**
	Southern	Mfou	80	34.1 (31.7–36.4)	62.7 (56.4–72.5)	2.7	96.2
		Yaounde	100	28.3 (22.7–35.3)	60.0 (40.1–90.1)	2.3	89.0
		Esse	73	25.6 (24.3–26.9)	49.9 (46.0–55.1)	2.1	**73.0**
	Northern	Dang	60	37.7 (35.1–40.1)	56.7 (52.0–64.7)	3.0	**73.3**
		Garoua	50	38.9 (35.6–41.9)	59.9 (54.1–70.7)	3.0	**43.5**
		Pitoa	80	41.2 (38.1–44.3)	83.1 (72.4–101.1)	3.3	**57.5**

KdT_{50}, Kd time in min for 50% mosquitoes; KdT_{95}, Kd time in min for 95% mosquitoes; CI, confidence interval; at 95%; Mortality, mortality rate 24 h post exposure. Bold characters indicate resistant populations.

methrin. A significant increase of knockdown times (2.5-fold) was also observed for most samples.

Species and Molecular Forms of Surviving Mosquitoes and kdr Mutation Prevalence

Surviving mosquitoes were analyzed by PCR, for species and molecular forms of *An. gambiae* s.s. identification, and detection of *kdr* "Leu-Phe" mutation.

Only *An. gambiae* s.s. was found in the equatorial region (Table 4), while two species of the complex were detected in the tropical region with 77.9% *An. arabiensis* and 22.1% *An. gambiae* s.s. No specimen of *An. melas* was observed in surviving mosquitoes from the coastal area (Douala).

Both M and S molecular forms of the *An. gambiae* s.s. species were found among the surviving individuals (Table 5). Only the M form was found in the Coastal area. In the Southern area, the distribution of the two forms was 93% M and 7% S. Only S form was detected in the area between Yaounde and Pitoa.

The kdr "Leu-Phe" mutation was missing in all of the 274 resistant mosquitoes (Table 4).

Discussion

In a recent review on organochlorine and pyrethroid resistance in African malaria vectors, *An. gambiae* s.s. was reported to be resistant to DDT and dieldrin in Cameroon, whereas *An. arabiensis* developed only resistance to dieldrin (Coetzee et al. 1999). However, no resistance to pyrethroids has been reported neither in *An. gambiae* s.s. nor in *An. arabiensis.* Data of the current large-scale survey confirmed dieldrin resistance in *An. gambiae* s.s. and *An. arabiensis,* DDT resistance in *An. gambiae* s.s. and pointed out DDT resistance in *An. arabiensis* from Simatou. These insecticides have been used in Cameroon for 7 yr during the 1950s in the framework of malaria control campaigns (Livadas et al. 1958, Cavalié and Mouchet 1961). Resistance might have been selected during that time and alleles conserved throughout many years, either by selection from agricultural or household insecticides.

Moreover, our data clearly indicated permethrin resistance in *An. gambiae* s.s. and *An. arabiensis.* Susceptibility tests carried out with 0.25% permethrin showed a decreased mortality, even with the Kisumu susceptible strain. As mentioned by Chandre et al. (1999b), the diagnostic concentration of 0.25% permethrin (isomeric ratio 25%cis/75%trans) was too low to assess *An. gambiae* resistance. Then we considered that populations with >80% mortality to permethrin 0.25% were susceptible, and those with <80% mortality were resistant. Diagnostic concentrations for susceptibility tests were recently revised for *An. gambiae* by WHO (1998), taking into account the variability

Table 4. Distribution of *An. gambiae* sibling species and *kdr* allelic frequency within surviving mosquitoes

Regions	Sites	4% DDT & 0.4% Diel*			1% Per			0.25% Per			0.025% Del			F(kdr)
		No.	Ag	Aa	No.	Ag	Aa	No.	Ag	Aa	no.	Ag	Aa	
Coastal	$Douala_1$	11	11	0	0						0			0
	$Douala_2$	22	22	0	1	1	0				5	5	0	0
	$Douala_3$	10	10	0	25	25	0	17	17	0	3	3	0	0
Southern	Yaounde	15	15	0	0		11	11	0	3	3	0	0	
	Mbalmayo	15 & 32*	15 & 32*	0 & 0	1	1	0				0			0
	Mfou	0						3	3	0				0
	Esse	0						20	20	0				0
Northern	Dang	0						11	3	8				0
	Garoua	0						28	2	26				0
	Pitoa	0						23	14	9	5	2	3	0
	Kaélé	17*	0*	17*										
	Simatou	9	0	9	4	0	4				0			0
	Maouda	1		1	14	0	14				0			0

*, Number with stars indicate specimens that survived to dieldrin; Ag, *Anopheles gambiae*; Aa, *Anopheles arabiensis*; F(kdr), kdr allelic frequency; 1% Per, 1% permethrin; 0.25% Per, 0.25% permethrin; 0.05% Del, 0.05% deltamethrin; Diel*, 0.4% dieldrin.

Table 5. Distribution of *An. gambiae* s.s. molecular forms M and S within surviving mosquitoes

Regions	Sites	No.	M form	S form
Coastal	$Douala_1$	11	11	0
Coastal	$Douala_2$	28	28	0
	$Douala_3$	30	30	0
Southern	Yaounde	10	9	1
	Mbalmayo	45	43	2
	Mfou	3	0	3
	Esse	15	0	15
Northern	Pitoa	6	0	6
	Dang	1	0	1

observed between susceptible reference strains reared in different laboratories throughout the world. The permethrin concentration was increased threefold from 0.25% to 0.75% for the cis/*trans* isomeric ratio 40/60 and fourfold from 0.25% to 1% for the isomeric ratio 25/75. Deltamethrin concentration was increased from 0.025% to 0.05%. At these new concentrations, some populations from West Africa with 18% *kdr* allelic frequency displayed 95% mortality rate (Diabaté et al. 2002). The threshold of mortality rate for resistant populations for these new diagnostic concentrations was therefore defined below 95%. Subsequently, most of the tested populations were susceptible to permethrin 1%. Resistance was observed in samples from $Douala_3$ and Maouda and were suspected in the Simatou rice field sample. Using 0.25% permethrin, resistance was detected in $Douala_3$, Esse, Dang, Garoua, and Pitoa. Using 0.05% deltamethrin, one sample was found resistant (Pitoa), whereas those from $Douala_2$, $Douala_3$, and Yaounde were suspected to be resistant. It is important to note that, during the sampling, no larvae were found in potential breeding sites within cotton fields that are heavily treated with insecticides. At the same period, larvae were abundant in rice fields (Simatou, Maouda) and swamps ($Douala_3$), which received few or no treatments. Because of the high level of mosquito nuisance around rice fields and swamps, people usually use personal protection tools as coils, aerosols, mats. These observations reveal the use of pyrethroids for domestic purposes and agriculture in Cameroon, but the selection process of insecticide resistance in vector populations needs further investigations.

Within the *An. gambiae* complex, *An. arabiensis* and the two M and S molecular forms of *An. gambiae* s.s. developed insecticide resistance. Only *An. gambiae* s.s. was found in equatorial area, whereas both *An. gambiae* s.s. and *An. arabiensis* were present in tropical areas. This eco-geographic distribution of *An. gambiae* and *An. arabiensis* in surviving specimens is consistent with Coz (1973). Within the *An. gambiae* s.s. species, the distribution of M and S molecular forms showed also a geographical divergence, in agreement with Wondji (2002). Only the M form was found in the coastal area, both M and S forms colonized the Southern area, and only the S was represented in the Northern area. The distribution of knockdown resistance allele in West Africa has provided evidence of strong barrier to gene flow between M and S forms (Chandre et al. 1999a). Because the two forms displayed insecticide resistance in Cameroon, it would be interesting to determine whether the same mechanism(s) are involved in these forms and resulted from independent events, or from a single event which was spread within the complex through introgression. In tested samples, the "Leu-Phe" *kdr* mutation was not found, although DDT and permethrin resistance was detected. Nevertheless, the knockdown times were not strongly increased compared with resistant populations from West Africa, and some samples were resistant to one insecticide but not to the others. These results suggested that another mechanism such as "Leu-Ser" *kdr* mutation or detoxification enzymes could be involved. Detoxification through cytochrome P450 monooxygenases or esterases is often involved in pyrethroid resistance (Scott 1996, Vulule et al. 1999), and does not systematically confer resistance to organochlorines that are preferably metabolizesd by glutathion-S-transferases (Yu 1996). Ongoing studies will help to identify such resistance mechanisms.

This study is the first large-scale survey of malaria vector susceptibility to insecticides in Central Africa. Because pyrethroid resistance in the *An. gambiae* complex has early been observed in West Africa (Elissa et al. 1993, Chandre et al. 1999a), East Africa (Vulule et al. 1994, 1999; Ranson et al. 2000), and in *An. funestus* from South Africa (Hargreaves et al. 2000), this report from Central Africa strengthened the wide distribution of resistance in malaria vectors all over the continent. These data will be taken into account, as a baseline for National Malaria Control Programs and vector resistance management in Cameroon.

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