Transactions of the Royal Society of Tropical Medicine and Hygiene (2009) **xxx**, xxx–xxx

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/trst

The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update

Hamadou N.M. Ndjemaï[a,b,*], Salomon Patchoké[a], Jean Atangana[a,b], Josiane Etang[c,d], Fréderic Simard[c,e], Charles F. Bilong Bilong[b], Lisa Reimer[f], Anthony Cornel[f], Gregory C. Lanzaro[g], Etienne Fondjo[a]

[a] *National Malaria Control Programme, Ministry of Public Health, P.O. Box 14386, Yaoundé, Cameroon*
[b] *Laboratoire de Parasitologie et Ecologie, Université de Yaoundé I, BP 812, Yaoundé, Cameroon*
[c] *Laboratoire de recherche sur le paludisme, Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), BP 288, Yaoundé, Cameroon*
[d] *Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box 2701, Douala, Cameroon*
[e] *Institut de Recherche pour le Dévelopement (IRD), UR016, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France*
[f] *Department of Entomology and Center for Vector-borne Diseases, University of California Davis, Davis, CA 95616, USA*
[g] *Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, Davis, CA 95616, USA*

Received 29 August 2008; received in revised form 18 November 2008; accepted 18 November 2008

KEYWORDS
Malaria;
Anopheles gambiae s.l.;
Insecticides;
Resistance;
kdr;
Cameroon

Summary Insecticides are a key component of vector-based malaria control programmes in Cameroon. As part of ongoing resistance surveillance efforts, *Anopheles gambiae* s.l. female mosquitoes were exposed to organochlorine (DDT), a carbamate (bendiocarb), an organophosphate (malathion), and three pyrethroids (deltamethrin, lambda-cyhalothrin and permethrin) in WHO bioassay test kits. Results indicated a higher level of resistance (reduced mortality and knockdown effect) to DDT and pyrethroids in populations of *A. gambiae* s.s. than in *A. arabiensis*. The West and East African knockdown resistance (kdr) mutations were found in both species but at much higher frequencies in *A. gambiae* s.s. The West Africa kdr mutant was also more frequent in the *A. gambiae* S form than in the M form. No resistance to bendiocarb and malathion was found. Carbamate and organophosphorous compounds could thus be used as alternatives in locations in Cameroon where pyrethroid-resistant populations are found.

* Corresponding author. Present address: National Malarial Control Programme, P.O. Box 14386, Yaoundé, Cameroon.
Tel.: +237 22 22 39 17; fax: +237 22 22 51 95.
E-mail address: ndjemaihamadou@yahoo.fr (H.N.M. Ndjemaï).

0035-9203/$ – see front matter
doi:10.1016/j.trstmh.2008.11.018

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

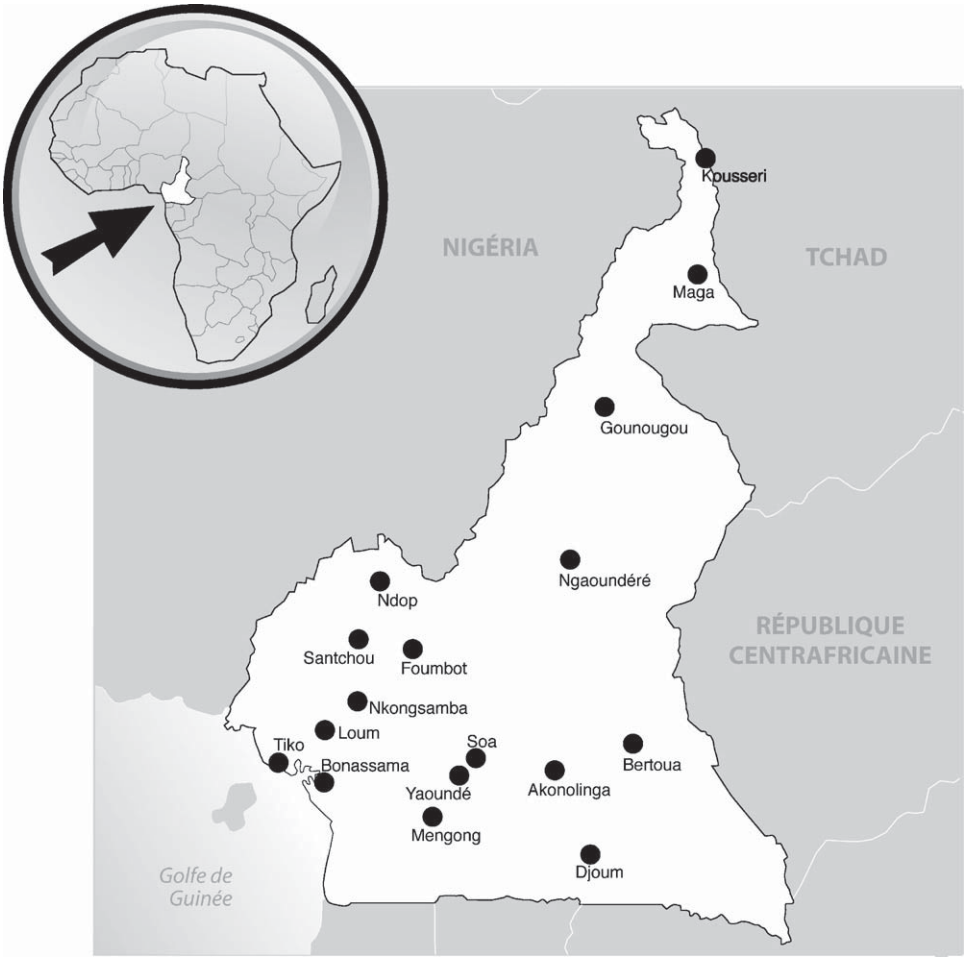

Figure 1 Map of Cameroon showing the study sites.

1. Introduction

The spread of insecticide resistance genes in *Anopheles gambiae* populations across Africa may jeopardize vector-based malaria control programmes, which essentially rely on the use of insecticide-treated materials or indoor residual spraying.[1] In Cameroon, insecticide resistance has been recorded in both *A. arabiensis* and *A. gambiae* s.s.[2] *Anopheles arabiensis* is dominant north of the Adamaoua region (tropical zone), while *A. gambiae* s.s. is almost exclusive in the south (equatorial zone).[3] This latter species is represented by two discrete units known as the M and S molecular forms, which are differentiated on the basis of sequence differences in the X-linked ribosomal DNA. The forms are unevenly distributed in Cameroon.[3]

More recently the M form of *A. gambiae* has been further subdivided into the Mopti-M form and Forest-M form, both of which occur in Cameroon.[4] Although these forms are known to occur in sympatry in several areas in Cameroon[3] and West Africa,[4–8] knockdown resistance (kdr), the major mechanism of resistance to pyrethroids and DDT insecticides in *A. gambiae*, has been found mainly in the S form and only rarely in the M form.[8–10] This resistance is due to a point mutation in the sodium channel gene and is characterized by a leucine–phenylalanine mutation in West Africa[11] or a leucine–serine mutation in East Africa.[12] Studies in West and Central Africa suggest that the kdr mutation first occurred in the *A. gambiae* S form before spreading to the M form and the sibling species *A. arabiensis* through genetic introgression or independent mutation.[8] Both West and East African kdr mutations have recently been reported in *A. gambiae* s.s. populations from Central Africa, including Cameroon,[7,9,10] Equatorial Guinea[13] and Gabon.[7]

Since 2002, the Cameroonian Ministry of Health has made considerable efforts to alleviate the burden of malaria on human populations by freely distributing over one million pyrethroid-treated nets to pregnant women and children under 5 years of age. However, there is concern that this strategy could be compromised by the spread of pyrethroid resistance. This paper presents results gathered from 2002 to 2007 by the National Malaria Control Programme on the status of insecticide susceptibility/resistance in *A. gambiae* s.l. mosquitoes from 17 localities scattered throughout Cameroon's biogeographical domains.

2. Materials and methods

2.1. Study sites

Mosquito populations were collected from 17 localities (Figure 1): Kousseri (12° 04′ N, 15° 02′ E), Maga (10° 34′ N, 15° 00′ E) and Gounougou (09° 07′ N, 13° 55′ E) in the northern

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

savannah zone; Ngaoundéré (07° 19′ N, 13° 35′ E) in the Adamaoua region; Bertoua (04° 54′ N, 12° 31′ E), Yaoundé (03° 51′ N, 11° 31′ E), Soa (03° 97′ N, 11° 60′ E), Akonolinga (03° 57′ N, 12° 04′ E), Mengong (03° 42′ N, 11° 27′ E) and Djoum (02° 4′ N, 12° 41′ E) in the south-eastern forest zone; Ndop (06° 00′ N, 10° 42′ E), Santchou (04° 96′ N, 10° 60′ E) and Foumbot (05° 48′ N, 10° 60′ E) in the western highlands region; Loum (04° 38′ N, 09° 57′ E), Tiko (04° 04′ N, 09° 22′ E), Nkongsamba (04° 96′ N, 09° 93′ E) and Bonassama (04° 05′ N, 09° 44′ E) in the coastal forest zone.

The northern savannah zone is characterized by one long dry season lasting 5—7 months (November to May), with an average annual temperature of 28 °C and total annual rainfall ranging from 400 to 1000 mm.[14] The Adamaoua region (forest-savannah highland area) has an altitudinal climate that differs from that of the northern savannah zone by lower annual average temperatures (22 °C) and higher rainfall (1500 mm).[14] The climate in the south-eastern forest zone has two rainy seasons (late March to June and September to early November) alternating with two dry seasons (late November to early March and July to August), with an annual rainfall of 1500—2000 mm and 25 °C average temperature.[14] The coastal area is characterized by one long rainy season (∼9 months), high annual rainfall (>3000 mm) and 26 °C average temperature.[14] The climate in the western highlands is similar to that of the coast but with less rainfall (1800—2500 mm per year) and an average annual temperature below 22 °C.[14]

Table 1 gives the predominant land cover in the various sample sites, the period (year and season) during which mosquitoes were sampled and the type of habitat from which anopheline larvae were collected. Croplands were found mainly in the northern, Adamaoua, western and coastal regions. All the surveys, except in Maga and Ndop, were conducted during the rainy season. The nature and patterns of pesticides used for personal protection against mosquitoes and pest control in agriculture were investigated in each surveyed locality. This was done by direct observation in households and in the fields, and by oral interviews of residents. The authorities from the local agricultural and animal rearing offices were also consulted in every setting to obtain the list of pesticides in use.[15,16]

2.2. Collection of mosquitoes and bioassays

Larvae of *A. gambiae* s.l. were collected by dipping in larval habitats. In each locality, immature stages were collected from 3—5 breeding sites and pooled. They were brought to the insectary, where they were reared on a diet of Tetra Mikromin fish food until emergence of adults. Bioassays were carried out on 2- to 3-day-old unfed females using WHO test kits and protocols for adult mosquitoes.[17] Papers impregnated with 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 1% permethrin, 4% DDT, 5% malathion and 0.1% bendiocarb were purchased from WHO. Batches of 20—25 females were exposed to impregnated papers in WHO test tubes for 1 h with at least four replicates per bioassay.

The number of mosquitoes knocked down was recorded every 10 min and the final mortality was recorded 24 h post-exposure. Survivors were maintained alive on 10% sucrose solution. Data (knockdown rates per time point) were analysed with the software WinDL[18] to calculate the time of exposure causing 50% and 95% knockdown (KdT_{50} and KdT_{95}, respectively) in the tested population. Tests with untreated papers were simultaneously run as control. Whenever 5—20% mortality was recorded in the control, the mortality rate in test samples was corrected using Abbott's formula.[19] When mortality was above 20% in the control, the test was discarded. Following WHO criteria,[17] mortality rates above 98% in test samples indicated susceptibility to the insecticide being tested, whereas mortality rates below 80% were considered to be evidence of resistance. Mortality rates between 80 and 97% indicated reduced susceptibility, but resistance needs to be confirmed.[17]

2.3. Identification of *Anopheles gambiae* species and molecular forms

Upon emergence, mosquitoes were morphologically identified [20] and only members of the *A. gambiae* complex were used for the bioassays. At the end of the susceptibility tests, random samples drawn from susceptible (dead) and resistant (surviving) mosquitoes from 10 populations (Kousseri, Maga, Gounougou, Ndop, Loum, Bonassama, Tiko, Yaoundé, Mengong and Djoum) were analysed to infer their species and molecular form composition using the PCR-RFLP method described by Fanello et al.[21]

2.4. Detection of kdr alleles

Random samples drawn within the pool of susceptible (dead) and resistant (surviving) mosquitoes from Gounougou ($N = 60$), Djoum ($N = 118$), Ndop ($N = 229$) and Loum ($N = 77$), situated respectively in the northern savannah, south-eastern forest, western highland and coastal regions, were analysed to detect both the East and West African kdr mutations, using the recent high-performance diagnostic PCR assay described by Tripet et al.[22] These locations were picked for three reasons: they were representative of the four main biogeographical domains found in Cameroon; they had not been investigated; and pyrethroid resistance levels were among the highest in these populations. The distribution of genotypes at the kdr locus were tested for conformity to Hardy-Weinberg equilibrium within each site and species, using GENEPOP software version 1.2.[23]

3. Results

3.1. Larval habitats and pesticide use

Larvae of *A. gambiae* s.l. were collected in sunny water collections, both temporary (pools, rice fields, tree holes, hoof prints, road or gutter puddles) and permanent (swamps, cattle watering places and fish ponds) (Table 1). Many of these larval habitats were likely to be contaminated with pesticides from human activities because of their close proximity to human dwellings and agricultural fields. Investigations on pesticide utilization indicated the application of many insecticides (pyrethroids, carbamates, organochlorines, organophosphates and insect growth regulators), herbicides (2,4-D amine salt, isopropyl amine salt, atrazine, chlorine salt), and fungicides (containing heavy metals such as copper) in croplands. Additionally, personal protection

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

Table 1 Description of the main land cover, study period and type of larval habitat in the collection sites

Region	Sample sites		Survey		
	Locality	Land cover[a]	Year	Season	Larval habitat
Northern savannah	Kousseri	Shrubland	2002	Rainy	Gutter puddles, pools
	Maga	Cropland	2003	Dry	Road puddles, hoof prints
	Gounougou	Cropland	2003	Rainy	Rice fields, swamps, pools
Adamaoua	Ngaoundéré	Urban area	2002	Rainy	Gutter and road puddles
South-eastern forest	Bertoua	Urban area	2006	Rainy	Road puddles, pools
	Djoum	Forest area	2005	Rainy	Gutter and road puddles
	Akonolinga	Forest area	2005	Rainy	Road puddles
	Soa	Forest area	2007	Rainy	Gutter and road puddles
	Yaoundé	Urban area	2003	Rainy	Pools, swamps
	Mengong	Forest area	2002	Rainy	Fish ponds, tree holes, pools
Western highlands	Foumbot	Cropland	2007	Rainy	Swamps, road puddles
	Ndop	Cropland	2005	Dry	Cattle watering places
	Santchou	Cropland	2006	Rainy	Gutter and road puddles
Atlantic coast	Nkongsamba	Urban area	2007	Rainy	Swamps, pools, gutters
	Loum	Cropland	2005	Rainy	Gutter and road puddles
	Bonassama	Urban area	2002	Rainy	Gutter and road puddles
	Tiko	Cropland	2003	Rainy	Gutter and road puddles

[a] Land cover defines the predominant landscape in the area.

measures such as mosquito coils and insecticide-treated nets (ITNs) (or long-lasting insecticide nets, LLINs) were being used countrywide, especially by inhabitants in croplands and urban areas. Wooden building materials, furniture and electric poles cut in forest localities were commonly treated with insecticides against wood pests.

3.2. Insecticide susceptibility in *Anopheles gambiae* s.l.

3.2.1. Mortality

None of the studied populations was fully susceptible to DDT (Table 2). Resistance levels were high (<80% mortality) in most *A. gambiae* s.s. populations found south of the Adamaoua region. However, populations of *A. arabiensis* in the northern areas (Kousseri, Maga and Gounougou) expressed only a reduced susceptibility (95—97% mortality).

In most locations, response profiles to deltamethrin (Table 3) and lambda-cyhalothrin (Table 4), which are both type II pyrethroids, were similar. Populations from Maga and Bonassama were susceptible to both compounds (≥98% mortality), while those from Bertoua, Soa, Mengong, Djoum, Ndop and Tiko had similar levels of resistance. Dissimilar responses were observed in samples from Nkongsamba, Ngaoundéré and Kousseri, which were less susceptible to one compound than the other. The level of resistance differed widely for deltamethrin and lambda-cyhalothrin in Gounougou, Foumbot and Santchou populations.

Mosquitoes from Maga, Ndop, Bertoua, Loum, Tiko and Nkongsamba were susceptible, whereas Djoum and Ndop were resistant, to all three pyrethroids, which includes permethrin as the type I and deltamethrin and lambda-cyhalothrin as the type II representatives. Samples from Soa and Foumbot were resistant to both type II pyrethroids but not to permethrin. Mosquitoes from Gounougou were resistant to permethrin and lambda-cyhalothrin but susceptible to deltamethrin and those from Santchou were highly resistant to deltamethrin but not as much to lambda-cyhalothrin and permethrin (Tables 3—5).

All the populations surveyed in the country were fully susceptible (>98% mortality) to bendiocarb and malathion (Table 6).

3.2.2. Knockdown effect

Mosquito populations from the northern area that were susceptible to DDT (95—97% mortality) showed only a slightly longer knockdown time (1.5- to 1.6-fold) compared with the reference strain. Conversely, as expected, knockdown times (KdT) were much longer (2- to 10-fold) in the other, more resistant, populations (Table 2).

The susceptibility of mosquitoes to the knockdown effect of deltamethrin (Table 3) and lambda-cyhalothrin (Table 4) were comparable in several populations and consistent with the mortality rates observed. The increase of KdT was below 1.6-fold in populations from Tiko, Kousseri, Maga, Mengong and Bonassama, which expressed only a reduced susceptibility (>90% mortality) to both deltamethrin and lambda-cyhalothrin. Samples from Soa, Santchou, Djoum, Foumbot and Ndop with higher levels of resistance (<80% mortality) to either one or both compounds recorded a 2- to 5-fold increase in their KdT. However, in samples with comparable resistance level to both compounds (Bertoua, Yaoundé and Nkongsamba), the increase in KdT was higher with lambda-cyhalothrin (2.2- to 3.4-fold) than with deltamethrin (1.0- to 1.7-fold).

Permethrin knockdown results (Table 5) were generally consistent with deltamethrin and lambda-cyhalothrin data. Populations that were susceptible to both types of pyrethroids (those from Maga and Tiko) showed no increase in their KdT, while those with higher levels of resistance (12—86% mortality; those from Djoum, Ndop, Foumbot and Santchou) recorded an increase in their KdT by factors higher than 2. Incongruences were, however, observed in Gounougou and Soa populations, which showed a

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

Table 2 Knockdown times and mortality rates of *Anopheles gambiae* populations to 4% DDT

Region (species/form)	Site	*N*	Knockdown time (min)		$KdT_{50}R$	Mortality (%)	Status
			KdT_{50} (95% CI)	KdT_{95} (95% CI)			
Reference strain	Kisumu	100	18.8 (17.6–20.0)	31.2 (28.7–33.7)		100	S
Northern savannah (*A. arabiensis*)	Kousseri	100	30.3 (28.7–31.7)	50.4 (47.1–54.8)	1.61	95	Sr
	Maga	97	29.2 (25.8–32.5)	48.0 (41.6–60.6)	1.55	95	Sr
	Gounougou	100	28.3 (24.9–31.8)	46.4 (40–59.5)	1.50	97	Sr
Adamaoua (*A. gambiae* S form)	Ngaoundéré	99	43.3 (39.0–48.4)	83.4 (68.9–118.9)	2.30	83.8	Sr
South-eastern forest (*A. gambiae* S or M form)	Bertoua	99	62.5 (56.5–68.5)	168.3 (131–191.3)	3.32	50.5	R
	Yaoundé	90	44.9 (42.7–47.5)	84.3 (75.4–98.2)	2.39	80	R
	Soa	100	88.3 (74.1–126.8)	198.4 (135–455.1)	4.70	30	R
	Akonolinga	99	40.6 (30.1–65.6)	165.2 (89.9–911.1)	2.16	94.9	Sr
	Mengong	84	29.2 (27.9–30.4)	39.8 (37.5–43.3)	1.55	83.3	Sr
	Djoum	100	196.4 (91.3–4587)	2220 (410^2–$3\,10^6$)	10.45	48	R
Western highlands (*A. gambiae* S form)	Ndop	96	69.9 (62.9–-76.9)	149.9 (116–183.8)	3.72	33.3	R
	Foumbot	100	100.3 (77.7–292.8)	190.4 (115.9–1619)	5.33	7	R
	Santchou	98	46.7 (44.6–49.1)	81.4 (73.5–94.0)	2.48	73.4	R
Atlantic coast (*A. gambiae* M form)	Bonassama	100	58.0 (53.3–64.7)	142 (115–194)	3.08	32.3	R
	Tiko	88	37.8 (35.9–39.8)	67.8 (62.1–76.1)	2.01	92.5	Sr
	Nkongsamba	100	40.6 (39.1–42.0)	59.1 (55.9–63.5)	2.16	96	Sr
	Loum	100	36.6 (34.8–38.6)	72.8 (66.2–82)	1.95	79.3	R

KdT_{50}: knockdown time for 50% mosquitoes; KdT_{95}: knockdown time for 95% mosquitoes; $KdT_{50}R$: KDT_{50} of the tested population divided by KDT_{50} of the Kisumu strain; Mortality (%): mortality rate 24 h post-exposure; *N*: sample size; R: resistant; S: susceptible; Sr: reduced susceptibility.

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

Table 3 Knockdown times and mortality rates of *Anopheles gambiae* populations to 0.05% deltamethrin

Region (species/form)	Site	*N*	Knockdown time (min)		$KdT_{50}R$	Mortality (%)	Status
			KdT_{50} (95% CI)	KdT_{95} (95% CI)			
Reference strain	Kisumu	89	9.4 (8.4–10.2)	17.8 (15.6–20.0)		100	S
Northern savannah (*A. arabiensis*)	Kousseri	100	7.3 (6.7–7.8)	15.8 (14.2–17.9)	0.78	91	Sr
	Maga	97	8.6 (6.3–10.4)	16.4 (13.1–26.5)	0.91	97.9	S
	Gounougou	94	16.8 (15.7–18.0)	39.7 (35.9–45.0)	1.78	86.2	Sr
Adamaoua (*A. gambiae* S form)	Ngaoundéré	95	9.4 (8.4–10.2)	21.3 (18.9–25.3)	1.22	97.9	S
South-eastern forest (*A. gambiae* S or M form)	Bertoua	99	13.9 (11.8–15.2)	33.7 (31.2–36.8)	1.48	87.9	Sr
	Yaoundé	100	9.8 (7.6–12.1)	28.9 (21.9–45.4)	1.04	96	Sr
	Soa	99	22.9 (21.7–24.2)	44.9 (41.3–49.6)	2.44	67.7	R
	Akonolinga	98	8.2 (7.7–8.7)	16.2 (14.2–18.2)	0.87	98.9	S
	Mengong	87	9.6 (6.3–14.8)	18.5 (9.9–34.3)	1.02	97.7	Sr
	Djoum	100	29.6 (27.6–31.6)	75 (65–85)	3.15	60	R
Western highlands (*A. gambiae* S form)	Ndop	98	24.7 (23.3–26.1)	52.4 (47.9–56.9)	2.63	64.3	R
	Foumbot	100	21.4 (18.8–24.4)	60.1 (51.6–84.1)	2.28	64	R
	Santchou	100	25.7 (24.4–26.9)	47.6 (44.1–52.2)	2.73	28	R
Atlantic coast (*A. gambiae* M form)	Bonassama	100	7.3 (6.7–7.8)	15.7 (14.2–17.9)	0.78	100	S
	Tiko	86	11.8 (10.9–12.9)	31.1 (27.7–35.7)	1.25	97.4	Sr
	Nkongsamba	100	16.1 (13.7–18.4)	42.4 (35.4–55.0)	1.71	99	S
	Loum	100	16.6 (14.6–18.7)	34.1 (28.8–44.4)	1.77	92	Sr

KdT_{50}: knockdown time for 50% mosquitoes; KdT_{95}: knockdown time for 95% mosquitoes; $KdT_{50}R$: KDT_{50} of the tested population divided by KDT_{50} of the Kisumu strain; Mortality (%): mortality rate 24 h post-exposure; *N*: sample size; R: resistant; S: susceptible; Sr: reduced susceptibility.

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

Table 4 Knockdown times and mortality rates of *Anopheles gambiae* populations to 0.05% lambda-cyhalothrin

Region (species/form)	Site	*N*	Knockdown time (min)		$KdT_{50}R$	Mortality (%)	Status
			KdT_{50} (95% CI)	KdT_{95} (95% CI)			
Reference strain	Kisumu	100	12.6 (11.4–13.8)	36.9 (31.8–44.8)		100	S
Northern savannah (*A. arabiensis*)	Kousseri	100	16.6 (15.8–17.5)	27.5 (25.4–30.5)	1.32	98	S
	Maga	98	11.0 (10.3–11.7)	20.4 (18.7–22.8)	0.87	98.9	S
	Gounougou	91	21.6 (17.7–25.4)	40.4 (32.9–60.1)	1.71	69.2	R
Adamaoua (*A. gambiae* S form)	Ngaoundéré	99	22.2 (21.2–23.3)	34.2 (31.8–37.5)	1.76	89.9	Sr
South-eastern forest (*A. gambiae* S or M form)	Bertoua	99	28.4 (26.3–31.2)	74.9 (66.9–86.9)	2.25	83.9	Sr
	Yaoundé	99	32.4 (30.8–34.1)	57.8 (53.6–63.4)	2.57	86.8	Sr
	Soa	100	48.5 (45.2–52.7)	121.1 (102–153.2)	3.85	56	R
	Mengong	87	13.7 (12.7–14.8)	30.7 (26.3–38.3)	1.09	97.7	Sr
	Djoum	100	55.8 (50.8–60.8)	126.8 (107–146.8)	4.43	66	R
Western highlands (*A. gambiae* S form)	Ndop	97	36.7 (34.3–39)	86.7 (76.6–97.8)	2.91	56.7	R
	Foumbot	99	61.9 (56.1–70.7)	165.0 (129–238.6)	4.91	12.1	R
	Santchou	88	32.0 (30.1–34.1)	72.5 (64.8–83.3)	2.53	86.4	Sr
Atlantic coast (*A. gambiae* M form)	Bonassama	100	11.7 (11.0–12.3)	18.3 (17–20.3)	0.93	98	S
	Tiko	86	20.2 (19.0–21.5)	39.7 (36.6–43.8)	1.60	97.7	Sr
	Nkongsamba	100	42.8 (41.2–44.3)	63.9 (60.1–69.3)	3.40	90	Sr
	Loum	100	16 (12.4–19.7)	37.9 (28.9–63.4)	1.27	80	R

KdT_{50}: knockdown time for 50% mosquitoes; KdT_{95}: knockdown time for 95% mosquitoes; $KdT_{50}R$: KDT_{50} of the tested population divided by KDT_{50} of the Kisumu strain; Mortality (%): mortality rate 24 h post-exposure; *N*: sample size; R: resistant; S: susceptible; Sr: reduced susceptibility.

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

Table 5 Knockdown times and mortality rates of *Anopheles gambiae* populations to 1% permethrin

Region (species/form)	Site	*N*	Knockdown time (min)		$KdT_{50}R$	Mortality (%)	Status
			KdT_{50} (95% CI)	KdT_{95} (95% CI)			
Reference strain	Kisumu	99	9.2 (8.6–9.7)	14.3 (13.2–15.4)		100	S
Northern savannah (*A. arabiensis*)	Maga	97	8.8 (5.5–11.2)	19.8 (15.3–35.6)	0.96	100	S
	Gounougou	98	17.1 (15.9–18.3)	42.9 (38.7–48.7)	1.86	34.7	R
South-eastern forest (*A. gambiae* S or M form)	Bertoua	99	30.2 (26.7–33.5)	136.3 (106.4–170)	3.28	76.8	R
	Yaoundé	98	9.2 (8.6–9.7)	14.3 (13.2–15.4)	1.25	87.7	Sr
	Soa	100	19.2 (16.0–22.3)	55.2 (44.0–78.5)	2.08	97	Sr
	Djoum	100	65.9 (58.9–72.9)	180.6 (130–230.6)	7.16	27	R
Western highlands (*A. gambiae* S form)	Ndop	100	35 (26.5–43.5)	216.2 (121.5–311)	3.80	61	R
	Foumbot	99	28.3 (26.5–30.2)	69.8 (62.3–80.4)	3.07	82.8	Sr
	Santchou	100	21.4 (18.3–24.7)	60.1 (48.3–84.1)	2.33	78	R
Atlantic coast (*A. gambiae* M form)	Tiko	87	11.5 (10.8–12.2)	19.6 (18.0–21.8)	1.00	100	S
	Nkongsamba	100	12.0 (11.4–12.5)	18.3 (17.1–20.1)	1.30	93	Sr
	Loum	100	14.4 (10.3–18.6)	32.6 (23.9–66.4)	1.56	81	Sr

KdT_{50}: knockdown time for 50% mosquitoes; KdT_{95}: knockdown time for 95% mosquitoes; $KdT_{50}R$: KDT_{50} of the tested population divided by KDT_{50} of the Kisumu strain; Mortality (%): mortality rate 24 h post-exposure; *N*: sample size; R: resistant; S: susceptible; Sr: reduced susceptibility.

comparative increase in KdT for both type I and II pyrethroids, despite huge differences in resistance level (Tables 3–5).

No KdT was recorded with bendiocarb and malathion, which lack a knockdown effect.

3.3. Distribution of insecticide resistance in *Anopheles gambiae* s.l. species and molecular forms

North of the Adamaoua region, all specimens tested from Kousseri (*N* = 28) and Maga (*N* = 99) collections were *A. arabiensis*. However, the sample from Gounougou contained a majority of *A. arabiensis* (125/136, 92.0%) together with the *A. gambiae* S form (11/136, 8.1%) (Table 7). Moreover, resistant mosquitoes (survivors) in this village were mainly found within *A. arabiensis* individuals (41/42, 97.6%). South of the Adamaoua highlands, samples from Ndop (*N* = 229) and Djoum (*N* = 118) comprised exclusively the *A. gambiae* S molecular form. Those from Loum, Yaoundé, Mengong and Tiko were composed of a mixture of both the M and S forms of *A. gambiae*, with the M form occurring at frequencies ranging from 64.9 to 93.2% (50/77 in Loum, 55/59 in Yaoundé, 40/58 in Mengong, 92/106 in Tiko). Among resistant individuals from these sympatric sites that were further identified by PCR, more than 50% belonged to the M form. The Bonassama sample was exclusively made up of *A. gambiae* M molecular form (*N* = 80).

3.4. kdr distribution

The West African kdr mutation was detected in a few *A. arabiensis* specimens (2/54) and *A. gambiae* M forms (1/50) from Gounougou and Loum, respectively (Table 8). The majority of *A. gambiae* S form populations from Djoum (113/118), Ndop (221/229) and Loum (22/27) were carrying this mutation. The distribution of genotypes at this locus conformed to the Hardy-Weinberg equilibrium within *A. arabiensis* and *A. gambiae* S forms in Gounougou and Loum populations, respectively, whereas heterozygote excess was found in *A. gambiae* S form populations from Ndop and Djoum. The susceptibility status of mosquitoes in some cases assorted independently of their genotype at the kdr locus. Respectively, 95.2% (20/21), 0% (0/76), 3.1% (5/163) and 6.4% (3/47) individuals from Gounougou, Djoum, Ndop and Loum populations with the resistant phenotype were homozygous for the susceptible allele, whereas 7.7% (3/39), 80.3% (53/66), 88.1% (37/42) and 7.5% (3/40) individuals with the susceptible phenotype carried at least one copy of the kdr allele. Among the few mosquitoes that carried the East African kdr mutation in either a homozygous state (one *A. gambiae* S form from Ndop) or associated with the West African type (one *A. arabiensis* and two *A. gambiae* S form individuals from Gounougou and Djoum, respectively; Table 8), only the *A. arabiensis* individual had a susceptible phenotype.

4. Discussion

In the northern region of Cameroon, all the anopheline samples except those from Gounougou were susceptible to

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

Table 6 Mortality rates of *Anopheles gambiae* populations to 0.1% bendiocarb and 5% malathion

Region (species/form)	Locality	Bendiocarb 0.1%			Malathion 5%		
		N	% mortality	Status	*N*	% mortality	Status
Northern savannah (*A. arabiensis*)	Gounougou	100	100	S	—	—	—
South-eastern forest (*A. gambiae* S or M form)	Bertoua	100	100	S	100	100	S
	Yaoundé	98	99	S	100	100	S
	Soa	100	99	S	ND	ND	ND
Western highlands (*A. gambiae* S form)	Foumbot	100	98	S	ND	ND	ND
Atlantic coast (*A. gambiae* M form)	Tiko	100	100	S	100	100	S
	Nkongsamba	100	100	S	ND	ND	ND

% mortality: mortality rate 24 h post-exposure; —: not done; *N*: sample size; S: susceptible.

Table 7 Proportion of *Anopheles gambiae* species and molecular forms within dead and surviving mosquitoes 24 h post-exposure to insecticide-treated papers

Region	Locality	Phenotype[a]							
		Dead				Survivors			
		N	% Ar	% S	% M	*N*	% Ar	% S	% M
Northern savannah	Kousseri	12	100	—	—	16	100	—	—
	Maga	91	100	—	—	8	100	—	—
	Gounougou	94	89.4	10.6	—	42	97.6	2.4	—
Western highlands	Ndop	66	—	100	—	163	—	100	—
Atlantic coast	Loum	30	—	13.3	86.7	47	—	46.8	53.2
	Bonassama	45	—	—	100	35	—	—	100
	Tiko	96	—	14.6	85.4	10	—	—	100
South-eastern forest	Yaoundé	35	—	5.7	94.3	24	—	8.3	91.7
	Mengong	40	—	40	60	18	—	11.1	88.9
	Djoum	42	—	100	—	76	—	100	—

% Ar: proportion of *A. arabiensis*; % M: proportion of *A. gambiae* M form; % S: proportion of *A. gambiae* S form; —: not found; *N*: sample size.
[a] The status of the mosquito 24 h post-exposure to insecticide-treated papers.

pyrethroids. *Anopheles arabiensis*, the predominant species in this region,[3] was previously shown to exhibit reduced susceptibility to pyrethroids in cotton-growing areas, but not in other settings.[2,24] Rice and cotton are the main crops cultivated in this area. Several studies in Africa have shown that rice fields are generally treated with pesticides less intensively than are cotton fields.[2,5,8,22] The study sites in northern Cameroon included two cotton-free areas (Kousseri

Table 8 Distribution of West and East African *kdr* mutations in *Anopheles gambiae* species and molecular forms

kdr genotype	Locality (region) and species					
	Gounougou (Northern savannah)		Ndop (Western highlands)	Loum (Atlantic coast)		Djoum (south-eastern forest)
	A. arabiensis	*A. gambiae*	*A. gambiae*	*A. gambiae*		*A. gambiae*
		S form	S form	S form	M form	S form
SS	51	6	7	5	49	5
SRw	2	0	202	20	1	98
SRe	0	0	0	0	0	0
RwRe	1	0	0	0	0	2
RwRw	0	0	19	2	0	13
ReRe	0	0	1	0	0	0
All	54	6	229	27	50	118
P(HW)*	0.046	ND	$<10^{-4}$	1.0	ND	$<10^{-4}$

ND: not determined because only one allele present or the frequency of the second allele too low; P(HW): goodness of fit to Hardy-Weinberg equilibrium; S: susceptible allele; Re: East African kdr allele; Rw: West African kdr allele.

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

and Maga), and a rice-growing area surrounded by cotton fields (Gounougou). Hence, a possible explanation of the high susceptibility of mosquitoes from Kousseri and Maga is that the level of insecticide pressure on mosquito populations is too low to select for resistance.

In Gounougou, where pyrethroid resistance was higher, the pesticides sprayed onto cotton plants might be responsible for higher selection pressure exerted on mosquitoes. Similarly, in Burkina Faso, Diabaté et al.[5] suggested that resistance in rice fields was due to the immigration of resistant mosquitoes coming from the neighbouring cotton fields. The resistance of the Gounougou mosquito population to most pyrethroids contrasted with the important susceptibility observed for DDT (97% mortality). This finding is probably the result of the past use of DDT during the 1950s, with resistance being maintained at low frequency after the interruption of DDT-based vector control programmes in the 1960 s.[2] Low frequency of the kdr alleles (<3%) in *A. arabiensis* from Gounougou, coupled with uneven susceptibility to DDT and pyrethroids and slight KdT_{50} increase (1.5-fold) compared with the reference strain, suggest that kdr, although present, is not the major mechanism responsible for the resistance observed in this area. Consistently, several genes with antioxidant functions, including superoxide dismutases, glutathione S-transferase, thioredoxin-dependent peroxidase and cytochrome P450 were found over-expressed in mosquito families from cotton-growing areas in northern Cameroon.[25,26]

Anopheles gambiae s.s., the predominant species of the *A. gambiae* complex found south of the Adamaoua region,[3] was resistant to DDT and pyrethroids in almost all localities studied. These results agree with those of Etang et al.[2] Because of the humid climate and fertility of the soil, agriculture is intensive in the western and coastal regions of Cameroon. Agro-industrial companies established in these regions apply several pesticides against herbivorous insect pests,[15] which probably contributes to the selection for resistance alleles in mosquitoes. Similarly, in some forest localities, wood exploitation requires significant amounts of pesticides because of xylophages. These chemicals sprayed on tree timbers may be driven by rain runoff into mosquito larval habitats, where selection occurs. Additionally, household use of pyrethroid-based personal protection, especially ITNs and LLINs, may increase insecticide pressure on mosquitoes. Indeed, Stump et al.[27] observed a rapid increase of kdr mutation frequencies in vector populations in western Kenya, where large-scale ITN programmes were taking place akin to what is now ongoing in Cameroon. Moreover, *A. arabiensis* and *A. gambiae* S form populations from Gounougou and Loum, respectively, respected the Hardy-Weinberg equilibrium, while those from Ndop and Djoum formed exclusively of the latter species had an excess of heterozygotes. This result contrasted with previous findings[9] that flagged up the abundance of homozygous individuals in an identical environment.

The East African kdr allele has previously been found in Cameroon at much lower frequencies than the West African allele.[7,9,10] When it does occur, as was the case in this study, it was restricted to the *A. gambiae* S form individuals and was often paired with the West African kdr allele. Reimer et al.[9] suggest that the East African allele provides greater protection against pyrethroids when paired with the West African allele. Thus, the spread of the East African kdr allele in Cameroon is a serious matter of concern and could increase in frequency under continued pyrethroid use. One susceptible *A. arabiensis* individual from Gounougou was found with both East and West African kdr alleles. Previous studies[2,24] in northern Cameroon did not find kdr alleles in this species, but some mutants were reported within the sympatric *A. gambiae* S form populations. It is likely that these mutations introgressed from the *A. gambiae* S form, as earlier observed in West Africa.[8]

In some populations from the northern (Gounougou), south-eastern (Soa and Djoum) and western (Santchou and Foumbot) regions, there was little or no cross-reactivity between type I (permethrin) and type II (deltamethrin and lambda-cyhalothrin) pyrethroids. Previous studies in West and East Africa[5,11,12,27] have demonstrated a strong connection between kdr allele and the resistant phenotype of mosquitoes. In this study the presence or absence of this allele at the genomic level did not correlate well with the susceptibility status of some mosquitoes from several villages (Gounougou, Djoum, Ndop and Loum). Similar findings have been reported by Reimer et al.[9] in mosquitoes from the western and eastern regions of Cameroon; they suggested the presence of alternative mechanisms of resistance. Brooke[28] is rather doubtful whether kdr mutation alone is sufficient to produce a measurable insecticide resistance phenotype in the absence of co-factors that could, and probably do, include detoxification enzyme systems.

Some populations along or close to the coast (Tiko, Mengong and Bonassama) were still susceptible to all pyrethroids tested despite originating from cropland, forest and urban areas under high pesticide pressure. All these populations were predominantly *A. gambiae* M molecular forms (mainly Forest-M). Lack of resistance in the M form in this study is consistent with reports from several West African countries, where low or no resistance to pyrethroids within *A. gambiae* M forms occurred even in locations where significant levels of resistance were found within sympatric S forms.[5,7] Those few coastal M form mosquitoes that did survive bioassays in this study had no West or East African kdr alleles present except for one (1/50), which was a heterozygote. As suggested by Etang et al.,[10] *A. gambiae* M form mosquitoes that are resistant are using alternative mechanisms of resistance. Earlier studies under similar settings reported no or low frequency of kdr alleles,[2] whereas levels of glutathione S-transferase and esterase activities were extremely high.[26]

None of the samples examined in this study showed resistance to bendiocarb (carbamate) and malathion (organophosphate); both compounds inhibit acetylcholinesterase activity in insects. Insensitivity to these compounds has been reported by Djogbénou et al.,[29] who recently identified a unique mutation (ace-1) in both M and S forms of *A. gambiae* s.s. in several West African populations. As with pyrethroids, carbamate and organophosphorous insecticides have been widely used in agriculture in Cameroon.[15,16] However, in contrast to pyrethroids, all the studies conducted in the country have until now never recorded a diminution of susceptibility to carbamates and organophosphates in anopheline populations.[22] These chemicals may therefore be good alternatives to pyrethroids for use in control operations. Reports from some West African settings characterized by high frequencies of kdr

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

in *A. gambiae* populations indicate that carbamate-treated curtains could have a significantly greater effect than those treated by pyrethroids in preventing house-entry by malaria vectors.[30]

Our data confirm that most populations of *A. gambiae* s.l. in Cameroon have developed resistance to pyrethroids and DDT. By contrast, no resistance to carbamates and organophosphates was detected. These two compounds could therefore be useful alternatives to pyrethroids for malaria vector-control interventions in Cameroon.

Authors' contributions: EF conceived the work; EF, FS, JE, CFBB, AC and GCL designed the study protocol; HNMN, SP, JA and EF performed field surveys and bioassays; HNMN, SP and LR performed molecular analyses; HNMN, CFBB and EF analysed and interpreted the data; HNMN drafted the manuscript, which was critically revised by EF, FS, AC and GCL. All authors read and approved the final manuscript. EF is the guarantor of the paper.

Acknowledgements: The authors are grateful to the Permanent Secretary of the National Malaria Control Programme and to all the Provincial Delegates of Public Health for support and assistance during field surveys. They thank Valerie Spanu for her help with Figure 1. They also thank anonymous reviewers for constructive comments on the manuscript.

Funding: Field surveys and molecular analyses were supported by WHO and the Global Funds against Aids, Tuberculosis, and Malaria. Support for kdr genotyping was provided by a grant from the US National Institutes of Health (AI40308) to GCL.

Conflicts of interest: None declared.

Ethical approval: Not required.

References

1. Kelly-Hope L, Ranson H, Hemingway J. Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. *Lancet Online* 2008, doi:10.1016/S1473-3099(08)0045-8.
2. Etang J, Manga L, Chandre F, Guillet P, Fondjo E, Mimpfoundi R, et al. Insecticide susceptibility status of *Anopheles gambiae* s.l. (Diptera: Culicidae) in the Republic of Cameroon. *J Med Entomol* 2003;**40**:491—7.
3. Wondji C, Simard F, Petrarca V, Etang J, Santolamazza F, Della Torre A, et al. Species and populations of the *Anopheles gambiae* complex with special emphasis on chromosomal and molecular forms of *Anopheles gambiae* s.s. *J Med Entomol* 2005;**42**:998—1005.
4. Slotman MA, Tripet F, Cornel AJ, Meneses CR, Lee Y, Reimer LJ, et al. Evidence for subdivision within the M molecular form of *Anopheles gambiae*. *Mol Ecol* 2007;**16**:639—49.
5. Diabaté A, Baldet T, Chandre F, Akogbeto M, Guiguemdé TR, Darriet F, et al. The role of agricultural insecticides in *Anopheles gambiae* s.l. resistance to pyrethroids in Burkina Faso, West Africa. *Am J Trop Med Hyg* 2002;**67**:617—22.
6. Della TA, Tu Z, Petrarca V. On the distribution and genetic differentiation of *Anopheles gambiae* s.s. molecular forms. *Insect Biochem Mol Biol* 2005;**35**:755—69.
7. Santolamazza F, Calzetta M, Etang J, Barrese E, Dia I, Caccone A, et al. Distribution of knockdown resistance mutations in *Anopheles gambiae* molecular forms in West and West-Central Africa. *Malar J* 2008;**7**:74.
8. Diabaté A, Brengues C, Baldet T, Dabiré KR, Hougard JM, Akogbeto M, et al. The spread of the Leu-Phe kdr mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and de novo phenomena. *Trop Med Int Health* 2004;**9**:1267—73.
9. Reimer L, Fondjo E, Patchoké S, Diallo B, Lee Y, Arash NG, et al. Relationship between kdr mutation and resistance to pyrethroid and DDT insecticides in natural populations of *Anopheles gambiae*. *J Med Entomol* 2008;**45**:260—6.
10. Etang J, Fondjo E, Chandre F, Morlais I, Brengues C, Nwane P, et al. First report of knock-down mutations in the malaria vector *Anopheles gambiae* from Cameroon. *Am J Trop Med Hyg* 2006;**74**:795—7.
11. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et al. Molecular characterisation of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. *Insect Mol Biol* 1998;**7**:179—84.
12. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Mol Biol* 2000;**9**:491—7.
13. Moreno M, Vicente JL, Cano J, Berzosa PJ, de Lucio A, Nzambo S, et al. Knockdown resistance mutations (kdr) and insecticide susceptibility to DDT and pyrethroids in *Anopheles gambiae* from Equatorial Guinea. *Trop Med Int Health* 2008;**13**:430—3.
14. Amou'ou J, Melingui A, Mounkam J, Tchepannou A. *Géographie — Le Cameroun*. CLE ed. Paris: Armand Colin; 1985.
15. UPAC. *Listes des pesticides agricoles homologués ou bénéficiant d'une autorisation provisoire de vente (APV) au Cameroun au 11 janvier 1999*. Douala: Rapport de l'Union Phytosanitaire Afrique Centrale; 1999.
16. Gaudard L. Mise en place et déroulement des traitements insecticides, campagne 2002/2003. Paris: CIRAD; 2002, Note N°196/02/DPA/LG/SF SODECOTON.
17. WHO. *Tests procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces. Report of a WHO informal consultation*. Geneva: World Health Organization; 1998.
18. Giner M, Vassal C, Kouaik Z, Chiroleu F, Vassal JM. Win DL version 2.0. Paris: CIRAD-CA, U.R.B.I/M.A.B.I.S.; 1999.
19. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925;**18**:265—7.
20. Gillies MT, Coetzee M. A supplement to the anopheline of Africa south of the Sahara. *S Afr Ins Med Res* 1987;**2**:55.
21. Fanello C, Santolamazza F, Della Torre A. Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Med Vet Entomol* 2002;**16**:461—4.
22. Tripet F, Wright J, Lanzaro G. A new high performance PCR diagnostic for the detection of pyrethroid knockdown resistance kdr in *Anopheles gambiae*. *Am J Trop Med Hyg* 2006;**74**:658—62.
23. Raymond M, Rousset F. GENEPOP, version 1.2. A population genetics software for exact tests and ecumenicism. *J Hered* 1995;**86**:248—9.
24. Chouaïbou M, Etang J, Brévault T, Nwane P, Hinzoumbé CK, Mimpfoundi R, et al. Dynamics of insecticide resistance in the malaria vector *Anopheles gambiae* s.l. from an area of extensive cotton cultivation in Northern Cameroon. *Trop Med Int Health* 2008;**13**:1—11.

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018

25. Müller P, Chouaïbou M, Pignatelli P, Etang J, Walker ED, Donnelly MJ, et al. Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in *Anopheles arabiensis* samples from an area of cotton fields in northern Cameroon. *Mol Ecol* 2008;**17**:1145—55.
26. Etang J, Manga L, Toto JC, Guillet P, Fondjo E, Chandre F. Spectrum of metabolic-based resistance to DDT and pyrethroids in *Anopheles gambiae* s.l. populations from Cameroon. *J Vect Ecol* 2007;**32**:123—33.
27. Stump AD, Atieli FK, Vulule JM, Besansky NJ. Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of *Anopheles gambiae* in response to insecticide-treated bed net trials. *Am J Trop Med Hyg* 2004;**70**:591—6.
28. Brooke BD. Kdr: can a single mutation produce an entire insecticide resistance phenotype? *Trans R Soc Trop Med Hyg* 2008;**102**:524—5.
29. Djogbénou L, Chandre F, Berthomieu A, Dabiré R, Koffi A, Alout H, et al. Evidence of introgression of the ace-1^R mutation and of the ace-1 duplication in West Africa *Anopheles gambiae* s.s. *Plos ONE* 2008;**3**:e2172.
30. Fanello C, Carneiro I, Ilboudo-Sanogo E, Cuzin-Ouattara N, Badolo A, Curtis CF. Comparative evaluation of carbosulfan- and Permethrin-impregnated curtains for preventing house-entry by the malaria vector *Anopheles gambiae* in Burkina Faso. *Med Vet Entomol* 2003;**17**:333—8.

Please cite this article in press as: Ndjemaï HNM, et al. The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Trans R Soc Trop Med Hyg* (2009), doi:10.1016/j.trstmh.2008.11.018