Distribution of Organophosphate and Carbamate Resistance in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) in West Africa

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ABSTRACT The distribution of organophosphate and carbamate resistance was investigated in 33 samples of *Culex pipiens quinquefasciatus* Say from 25 cities in Côte d'Ivoire and Burkina Faso. Organophosphate resistance levels were higher in Côte d'Ivoire than in Burkina Faso. Chlorpyrifos resistance ratios at LC_{95} ranged from 4 to 30 times in Côte d'Ivoire and from 3 to 6 times in Burkina Faso. For temephos, ratios ranged from 3 to 18 and from 1 to 2, respectively. Of 27 samples from Côte d'Ivoire, 25 also displayed cross resistance to carbamates as shown by a mortality plateau in bioassays with propoxur and carbosulfan (similar to chlorpyrifos). Cross resistance to organophosphates and carbamates was caused by an insensitive acetylcholinesterase allele (Ace^R). This gene was absent from Burkina Faso, except in Niangoloko near the Côte d'Ivoire border. Organophosphate resistance also was associated with the presence of A2–B2 overproduced esterases which had higher frequencies in Côte d'Ivoire (75–100%) than in Burkina Faso (40–50%). Two other esterases with the same electrophoretic mobility as C2 from Puerto Rico and B1 from California were identified for the 1st time in West Africa. "C2" was widespread, whereas "B1" was present in only a few mosquitoes from Côte d'Ivoire. These differences in resistance patterns should be taken into consideration in planning urban mosquito control strategies within 2 countries.

KEY WORDS *Culex pipiens quinquefasciatus*, West Africa, resistance, organophosphate, carbamate

IN AFRICA, STRONG insecticide selection pressure by urban pest control in the 1950s was followed rapidly by the emergence of resistant populations of *Culex pipiens quinquefasciatus* Say in treated areas. In West Africa, resistance to organochlorine insecticides was recorded in 1958 in Côte d'Ivoire and Burkina Faso (Adam et al. 1958, Hamon et al. 1958) and subsequently in Mali (Hamon et al. 1961). In 1968, all *Cx. p. quinquefasciatus* samples from 7 West African countries were resistant to dieldrin and most to DDT (Mouchet et al. 1968). However, these populations were still susceptible to a wide range of organophosphorous compounds. Organophosphate resistance was described a few years after these insecticides had replaced DDT and dieldrin, but it initially was restricted to small foci. In 1963, diazinon and malathion resistance was reported in Freetown (Sierra Leone), but populations reverted to normal susceptibility within 3 mo after the end of treatments (Thomas and Mouchet *in* Hamon and Mouchet 1967). In 1968, larval susceptibility of 6 samples from Liberia, Côte d'Ivoire, and Benin was tested with 8 organophosphates; only 1 population (Parakou, Benin) showed resistance to diazinon but not to the other organophosphates, including temephos and chlorpyrifos (Subra et al. 1968). During the 1970s, cases of organophosphate resistance in *Cx. p. quinquefasciatus* populations rapidly increased throughout the world (e.g., Brown 1986). In strains from West Africa (Liberia), East Africa, and Asia, chlorpyrifos resistance was associated with the presence of 2 esterases with high activity (Curtis and Pasteur 1981, Villani et al. 1983). These esterases also were found in California populations and were designated as A2 and B2 (Raymond et al. 1987). A2–B2 esterases were responsible for a low level of organophosphate resistance in Ouagadougou (Burkina Faso) (Majori et al. 1986) and Bouaké (Côte d'Ivoire) (Magnin et al. 1988).

The current survey updates information on the status of organophosphate and carbamate resistance in *Cx. p. quinquefasciatus* from West Africa, thereby helping to plan urban mosquito control. Investigations were performed in major cities from 2 neighboring countries (Côte d'Ivoire and Burkina Faso), and covered a wide range of biogeographic areas to assess how resistance is distributed and to understand the modes of dispersal of resistance genes among populations.

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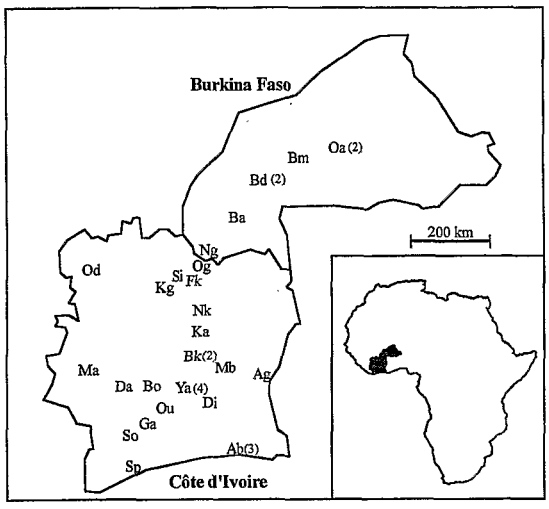

Fig. 1. Locations of the 25 towns surveyed. Numbers of multiple samples are indicated in parentheses. Abbreviations: Côte d'Ivoire: Ab, Abidjan; Ag, Abengourou; Bk, Bouaké; Bo, Bouaflé; Da, Daloa; Di, Dimbokro; Fk, Ferkessédougou; Ga, Gagnoa; Ka, Katiola; Kg, Korhogo; Ma, Man; Mb, M'bahiakro; Nk, Niakaramandougou; Od, Odienné; Og, Ouangolo; Ou, Oumé; Si, Sinématiali; So, Soubré; Sp, San Pédro; Ya, Yamoussoukro. Burkina Faso: Ba, Banfora; Bd, Bobo-Dioulasso; Bm, Boromo; Ng, Niangoloko; Oa, Ouagadougou.

Materials and Methods

Mosquitoes. To avoid mortality and possible changes in genetic composition, samples of *Cx. p. quinquefasciatus* were collected as egg rafts in breeding sites (such as polluted drains, septic tanks, and pit latrines) and transported to the laboratory on wet cotton in petri dishes maintained at 4–10°C. Egg rafts and hatched larvae were transferred to distilled water and supplied with baker's yeast. After 24 h, larvae were fed ad libitum with ground pet croquettes. Larval rearing was done at 26–31°C and a photoperiod of 24:00 (L:D) h, and density was kept at 150–200 larvae per liter of water. Part of each sample was reared to the adult stage and stored in liquid nitrogen for biochemical assays and electrophoretic studies.

Mosquitoes were collected along various transects, the main one followed the road which runs from Côte d'Ivoire to Burkina Faso (950 km from Abidjan to Ouagadougou). From March 1994 to June 1995, 33 samples were collected in 20 representative towns in Côte d'Ivoire and 5 in Burkina Faso (Fig. 1). In the largest towns, multiple collections were made to compare resistance pattern among districts.

Field samples were compared to S-Lab, a susceptible reference strain (Georghiou et al. 1966) reared and tested under the same conditions.

Larval Bioassays. Four insecticides of technical grade quality were used: 2 organophosphates, chlorpyrifos-ethyl (99.8%) and temephos (96.5%) [both provided by Agrevo, Berkhamsted, UK]; and two carbamates, propoxur (99.4%) [Bayer, Leverkusen, Germany] and carbosulfan (88.1%) [FMC, Philadelphia, PA]. Insecticide solutions were made in 95% ethanol and stored at 4°C for 2 mo.

Bioassays were performed on late 3rd and early 4th instars (usually 4 d after hatching). Sets of 20 larvae were assayed in 99 ml of distilled water, to which 1 ml of insecticide solution at the required concentration was added. Five replicates of 20 larvae per concentration, and 5–9 concentrations providing between 0 and 100% mortality were used for each bioassay. Mortality was recorded after a 24-h exposure. Controls were made with 1 ml of ethanol and mortality never exceeded 4%. Temperature was maintained at 27±1°C during bioassays. Samples were tested with chlorpyrifos, propoxur, and, when possible, with temephos and carbosulfan.

Biochemical Assays. *Overproduced Esterases.* Esterases of high activity were investigated on single adults using electrophoresis on cellulose acetate plates (HELENA system) as described in Abderrazak et al. (1993) using L buffer (Tris-maleate-EDTA, pH 7.4). Gels were soaked in dilute electrophoresis buffer (1/8) and run at 200 V for 23 min. Staining procedure was adapted from Pasteur et al. (1988) for starch gels. Gels were incubated (1–2 min.) with 10 mg of α- and β-naphthyl acetate in phosphate buffer (pH 6.5). Bands were stained with 10 mg of Fast Garnett GBC. Electrophoretic patterns of field mosquitoes were compared with reference strains with known esterases: Tem-R strain (Georghiou et al. 1980) for B1 esterase, Puerto for C2 (Yébakima et al. 1995), Barriol for A1 (Chevillon et al. 1995), SeLax for A2–B2 (Wirth et al. 1990), and Cyprus for A5–B5 (Poirié et al. 1992).

Acetylcholinesterase (AChE). AChE genotypes were investigated on single adults using a microplate assay (Raymond and Marquine 1994). Because insensitive AChE (Ace^R) causes high resistance to carbamates, genotypes were identified by the difference of AChE activity in the presence or absence of propoxur. The inhibition curves of AChE activities in the presence of propoxur first were established with reference strains from Côte d'Ivoire to determine the concentration providing the best discrimination between genotypes (unpublished data). The final concentration of propoxur was 0.02 mM.

Data Analysis. Mortality data were analyzed using log dose-probit mortality software developed by Raymond et al. (1993) based on Finney (1971). In addition to probit regression analysis, this program allows the comparison of probit lines by testing parallelism of slopes, and provides confidence limits of resistance ratios. In many bioassays, mortality data were not linear, but rather displayed a plateau. In this case, values for lethal concentrations were estimated graphically after plotting on log-probit paper. Frequencies of resistant individuals were compared using chi-squared contingency tables. When multiple tests were made, the level of signif-

Table 1. Resistance ratios observed at LC_{50} (RR_{50}) and LC_{95} (RR_{95}) of *Cx. p. quinquefasciatus* from Côte d'Ivoire and Burkina Faso with chlorpyrifos and propoxur

Towns[a]	Chlorpyrifos ethyl				Propoxur			
	RR_{50}[b]	CI_{95}[c]	RR_{95}[b]	CI_{95}[c]	RR_{50}[b]	CI_{95}[c]	RR_{95}[b]	CI_{95}[c]
				Côte d'Ivoire				
Kg	14*	—	30	—	4.0*	—	>40	—
Bo	12*	—	>23	—	3.3*	—	>400	—
Da	9.4*	—	>18	—	4.1*	—	>400	—
Ya1	14*	—	>23	—	>710*	—	>400	—
Ya2	5.7*	—	>18	—	2.9*	—	>400	—
Ya3	10*	—	>18	—	>710*	—	>400	—
Ya4	5.6*	—	>18	—	2.9*	—	>400	—
Ga	2.1*	—	21	—	2.4*	—	>400	—
So	2.5*	—	>23	—	1.9*	—	>400	—
Si	1.7*	—	23	—	1.4*	—	>40	—
Og	6.1*	—	>23	—	2.1*	—	>400	—
Ag	3.4*	—	>18	—	1.2*	—	>400	—
Sp	3.1*	—	>23	—	1.9*	—	>400	—
Nk	4.6*	—	>23	—	2.2*	—	>400	—
Ma	2.2*	—	9	—	<1.4*	—	>400	—
Ou	1.9*	—	16	—	<1.4*	—	>400	—
Fk	1.7*	—	11	—	1.9*	—	>40	—
Mb	2.1*	—	16	—	<1.4*	—	>400	—
Bk1	6.0*	—	12	—	2.1*	—	>400	—
Bk2	4.1*	—	14	—	2.7*	—	>400	—
Ab1	5.0*	—	8	—	2.1*	—	2.8	—
Ab2	7.5*	—	>27	—	2.3*	—	>400	—
Ab3	2.7*	—	4.5	—	2.0*	—	3.2	—
Ka	2.4*	—	4.0	—	1.4*	—	1.9	—
Di	2.0	1.5–2.7	4.0	2.4–5.6	1.2	1.0–1.6	1.3	0.8–2.1
Od	4.6	3.0–7.1	5.5	2.4–14.3	3.0	2.3–4.0	2.8	1.6–4.6
				Burkina Faso				
Ng	2.1*	—	4.5	—	1.5*	—	1.7	—
Ba	1.5	1.1–2.1	2.8	1.9–4.4	0.93	0.7–1.3	1.8	0.9–2.1
Bd1	1.8	1.3–2.4	3.3	2.0–4.6	1.3	0.9–1.8	1.6	1.0–2.4
Bd2	1.4	1.0–1.9	3.3	2.0–4.6	1.1	0.7–1.7	1.3	0.8–1.9
Bm	1.6	1.3–2.2	3.7	2.5–5.4	1.3	1.0–1.6	1.3	0.9–2.0
Oa1	1.5	1.2–2.1	3.4	2.2–5.2	1.5	1.2–1.9	1.4	0.9–2.1
Oa2	2.5	1.9–3.3	5.9	3.8–9.0	0.86	0.6–1.2	1.4	0.9–2.1

LC_{50} and LC_{95} in mg/liter for the susceptible reference strain S-Lab were 0.0016 and 0.0022 with chlorpyrifos and 0.14 and 0.25 with propoxur, respectively. *, Rejection of linearity of dose-mortality response ($P < 0.05$).

[a] See Fig. 1 for explanation of these symbols.

[b] Resistance ratios ($LC_{50(95)}$ sample tested/$LC_{50(95)}$ of S-Lab).

[c] 95% CI of RR_{50} and RR_{95}.

icance of each test was adjusted to take into account other simultaneous tests (Rice 1989).

Results

Côte d'Ivoire. All samples tested were resistant to organophosphates when compared with the susceptible reference strain S-Lab (Tables 1 and 2). Resistance ratios (RRs) were higher with chlorpyrifos than with temephos. At LC_{50}, RRs ranged from 1.7 to 14 times for chlorpyrifos and from 2.2 to 5.7 times for temephos. At LC_{95}, RRs were higher and ranged from 4 to 30 and 3.3 to 18 times, respectively. Most populations also were highly resistant to carbamates, with RR_{95} >400 times for propoxur and >145 times for carbosulfan. Most log dose-probit mortality relationships with chlorpyrifos, propoxur, and carbosulfan deviated significantly from linearity (Tables 1 and 2). These curves usually displayed a plateau of mortality, implying polymorphism for a major gene affecting resistance. The frequency of the resistant phenotypes was estimated from the mortality observed at the plateau (Fig. 2). In all except 4 samples, the level of the plateau did not differ significantly for chlorpyrifos, propoxur, and carbosulfan. These results strongly indicated that the same mechanism was involved in cross-resistance among the 3 insecticides and that this mechanism was most likely an insensitive AChE. In only 2 samples (Di, Od) were mortality curves with chlorpyrifos and propoxur well fitted by a straight line. These samples appeared homogeneous, and their low resistance ratios to propoxur indicated that the resistance mechanism(s) to both insecticides was absent or at very low frequency.

All log dose-probit mortality relationships with temephos were consistent with straight lines (Table 2), although a plateau seemed to be present in a few populations.

Burkina Faso. Most samples showed lower resistance ratios to organophosphates and carbamates than in Côte d'Ivoire (Tables 1 and 2). A mortality

Table 2. Resistance ratios observed at LC_{50} (RR_{50}) and LC_{95} (RR_{95}) of *Cx. p. quinquefasciatus* from Côte d'Ivoire and Burkina Faso with temephos and carbosulfan

Towns	Temephos				Carbosulfan			
	RR_{50}	CI_{95}	RR_{95}	CI_{95}	RR_{50}	CI_{95}	RR_{95}	CI_{95}
				Côte d'Ivoire				
Kg	4.0	3.3–5.0	10.9	7.4–16.7	>310*	—	>145	—
Bo	5.7	4.8–7.1	18.3	11.5–28.7	2.5*	—	>145	—
Da	5.3	4.4–6.6	8.7	5.5–13.3	2.3*	—	>145	—
Ga	3.1	2.6–3.9	12.2	7.2–20.4	—	—	—	—
Sp	3.1	2.5–3.9	6.1	4.2–9.3	1.7*	—	>145	—
Ma	2.9	2.3–3.7	4.3	3.0–6.6	1.6*	—	>145	—
Bk1	3.3	2.8–4.1	6.1	4.0–8.9	2.0*	—	>145	—
Bk2	3.3	2.7–4.1	5.2	3.6–7.6	2.1*	—	>145	—
Ab1	3.1	1.5–6.5	4.2	1.7–10.8	2.0*	—	>145	—
Ab2	2.2	1.8–2.8	3.4	2.4–4.9	2.1*	—	>145	—
Ab3	4.1	3.4–5.1	5.7	3.9–8.3	1.0*	—	1.3	—
Ka	2.3	1.9–3.0	3.3	2.2–4.9	1.3*	—	1.2	—
Od	3.3	2.7–4.2	4.0	2.7–5.9	—	—	—	—
				Burkina Faso				
Bd1	1.5	1.3 –1.9	2.0	1.3–3.1	0.91	0.65–1.27	0.82	0.36–1.87
Oa1	0.73	0.57–0.94	1.0	0.7–1.5	0.77	0.55–1.09	0.70	0.30–1.61

LC_{50} and LC_{95} in mg/liter for the susceptible reference strain S-Lab were 0.0015 and 0.0023 with temephos and 0.0032 and 0.0069 with carbosulfan, respectively. See Table 1 for footnotes.

plateau was observed with chlorpyrifos and propoxur at a level 96% in a single sample (Ng), collected close to the Côte d'Ivoire border (Fig. 1). All other samples were homogeneous in their response to organophosphates and carbamates. The hypothesis of parallelism with S-Lab was always rejected for both organophosphates, and most RRs at LC_{95} were significantly >1, ranging from 3.3 to 5.9 times for chlorpyrifos and from 1 to 2 times for temephos. Therefore, populations were slightly resistant to organophosphates, and all but Ng populations were fully susceptible to carbamates (Probit lines parallel to S-Lab and resistance ratios close to 1).

Comparison of Resistance Levels Among Different Populations Within Towns. Multiple samples were collected in 3 towns in Côte d'Ivoire (Ab, Bk,

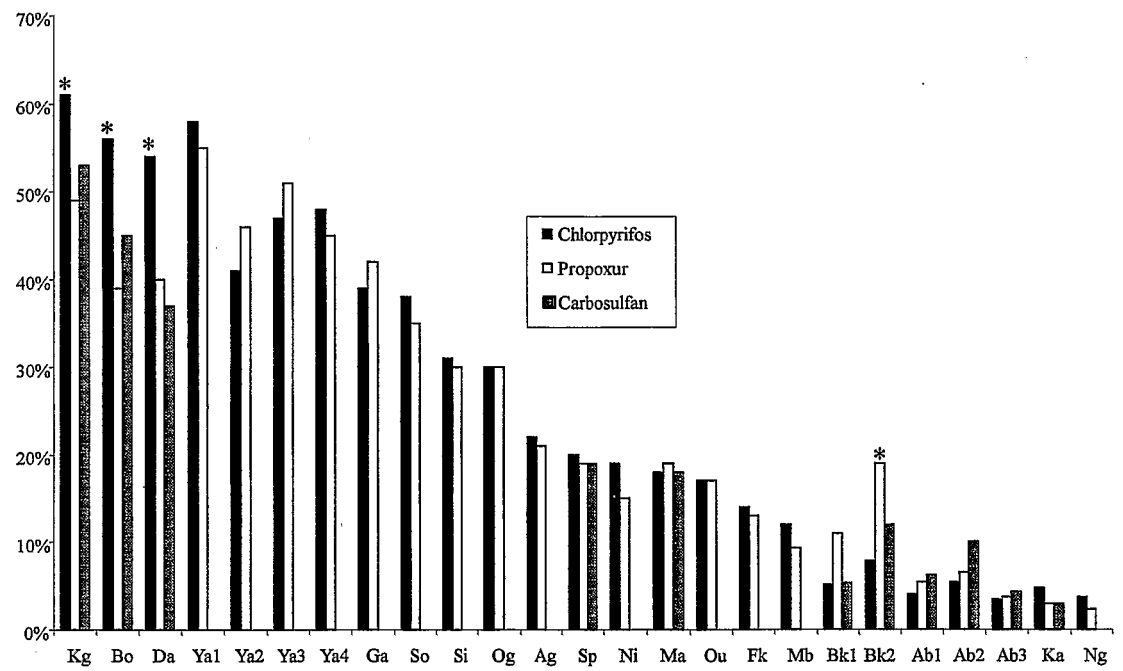

Fig. 2. Percentages of resistant phenotypes estimated from plateau mortality, on probit mortality–log dose graphs for chlorpyrifos, propoxur, and carbosulfan: evidence for a cross-resistance mechanism to organophosphates and carbamates. *, samples with significant differences observed among insecticides.

Table 3. Prevalence of organophosphate and carbamate resistance mechanisms in *Cx. p. quinquefasciatus* from Côte d'Ivoire and Burkina Faso

Towns[a]	Overproduced Esterases				Insensitive AChE			
	A2–B2	B1	C2	*n*	Ace^{SS}	Ace^{RS}	Ace^{RR}	*n*
				Côte d'Ivoire				
Kg	81.3	—	54.2	48	42.1	49.1	8.8	57
Bo	74.5	—	8.5	47	50.0	41.4	8.6	58
Da	83.0	—	46.8	47	60.7	33.9	5.4	56
Og	95.8	10.4	37.5	48	56.3	35.4	8.3	48
Sp	73.3	—	55.6	45	82.2	17.8	—	45
Ma	95.8	6.3	41.7	48	78.7	19.1	2.1	47
Bk	100	—	41.7	48	87.5	12.5	—	48
Ab	93.7	10.6	37.7	159	93.1	3.4	3.4	58
Ka	83.9	—	11.8	93	96.6	3.4	—	58
				Burkina Faso				
Oa	39.6	—	12.5	48	100	—	—	58
Bd	55.3	—	25.5	47	100	—	—	48

Frequencies of insects with a particular esterase and frequencies of AChE genotypes are given as percentages. *n*, Number of mosquitoes assayed.

[a] See Fig. 1 for explanation of these symbols.

Ya) and 2 towns in Burkina Faso (Bd, Oa) to compare resistance levels of *Cx. p. quinquefasciatus* among districts. Because dose–mortality relationships with chlorpyrifos and carbamates displayed a plateau in the 3 towns in Côte d'Ivoire, we tested the difference between frequencies of the resistant phenotype larvae among districts. These frequencies were not significantly different, except in 2 cases (Fig. 2)— for propoxur, the frequency of the resistant phenotype was higher in Bk2 than in Bk1, and for chlorpyrifos and propoxur it was higher in Ya1 than in Ya2, Ya3, and Ya4. These differences may be related to small temporal variations because in these 2 cases, breeding sites were sampled at intervals of 1 mo. Parallelism of probit lines from samples from different districts was not rejected with temephos at the 5% level. RR at LC_{50} did not vary significantly among districts except in Ab, but the difference did not exceed 1.8 times.

In Burkina Faso, 2 districts were tested in Bd and Oa, and a significant difference was observed only for chlorpyrifos in Oa. This difference was low and did not exceed 1.8 times.

Identification of Resistance Mechanisms to Organophosphates and Carbamates. *Overproduced Esterases.* Single individuals displayed several esterases of high activity (Table 3). Two esterases, always associated and identified as A2–B2, were found in all samples tested. In Côte d'Ivoire, A2–B2 were at higher frequencies (73–100%) than in Burkina Faso (40–50%). Two other esterases of high activity, also present in these populations, were found for the first time in West Africa. The 1st one, which preferentially hydrolyzed α-naphthyl acetate and has the same electrophoretic mobility as C2 from Puerto Rico, was found at varying frequencies in all samples. The 2nd one, which preferentially hydrolysed β-naphthyl acetate and has the same electrophoretic mobility as B1 from California but with a much lower activity, was present at low frequency (10%) in 3 populations in Côte d'Ivoire (Ab, Og, Ma). The role of these 2 esterases in organophosphate resistance is unknown at present.

Insensitive Acetylcholinesterase. Mosquitoes with an insensitive AChE were identified in all populations displaying a mortality plateau for chlorpyrifos, propoxur, and carbosulfan (Table 3). In each sample, the frequency of mosquitoes with Ace^{SS} genotype was not significantly different from the mortality observed at the plateau in bioassays with chlorpyrifos, propoxur, or carbosulfan. This result indicated clearly that insensitive AChE was the main mechanism of cross-resistance to the 3 insecticides. It also showed effective dominance of the Ace^{R} allele.

Discussion

In West Africa, *Cx. p. quinquefasciatus* displayed large variation in resistance to organophosphates and carbamates. Clear differences were observed among populations within and between the 2 countries investigated that will effect control strategies.

Populations were slightly resistant to organophosphates, and at least 2 known mechanisms were involved: overproduced esterases and insensitive AChE. A2–B2 esterases were found in all samples. These 2 esterases, first observed in West Africa by Villani et al. (1983), previously were shown to confer a low resistance level to organophosphates in Ouagadougou (Burkina Faso) and Bouaké (Côte d'Ivoire) (Majori et al. 1986, Magnin et al. 1988). The frequencies of A2–B2 was higher in Côte d'Ivoire than in Burkina Faso, indicating that the former populations were submitted to a stronger selection pressure by organophosphates. This conclusion was supported by the greater increase in A2–B2 frequency in Bouaké (Bk) (from 60 to 98%) than in Ouagadougou (Oa) (from 30 to 40%) since 1986.

Two A and B type esterases (i.e., "C2" and "B1") also were identified for the first time in West Africa.

However, further studies are needed to assess their role in organophosphate resistance and to determine whether they are identical to C2 from Puerto Rico and B1 from California.

Biochemical assays and bioassays revealed that an insensitive AChE was present in 24 of the 26 samples collected in Côte d'Ivoire. The agreement between the proportion of Ace^R carriers and the mortality plateau observed in bioassays with chlorpyrifos confirmed that this insensitive target conferred a significantly higher level of resistance to chlorpyrifos than did esterases. In contrast, Ace^R conferred only low resistance to temephos, because the dose–mortality curves were always linear. A similar observation was made with *Cx. p. pipiens* (L.) from France, where an insensitive Ace^R allele produced a 12 times lower resistance to temephos than to chlorpyrifos (Raymond et al. 1986).

The wide distribution of Ace^R in Côte d'Ivoire theoretically could result either from the spread of a single mutation or from the independent occurrence of the same or different mutations in many localities. However, in other countries the unique origin and subsequent worldwide spread of A2–B2 resistance genes has been well documented (Raymond et al. 1991, Guillemaud et al. 1996). The presence of A2–B2 esterases in all our samples from Côte d'Ivoire and Burkina Faso indicated that a certain amount of gene flow is occurring or has occurred recently among populations. The variability in Ace^R frequencies among Côte d'Ivoire towns could be attributed to differences in insecticide selection pressure or to differences in time since Ace^R was introduced. Fitness cost associated with an insensitive AChE gene has been demonstrated for *Cx. p. pipiens* under laboratory conditions as well as in natural environments (Raymond et al. 1985, Chevillon et al. 1995). The absence (or very low frequency) of Ace^R in the main towns of Burkina Faso probably was caused by a lower insecticide selection pressure in this country than in Côte d'Ivoire, as was indicated by lower A2–B2 frequencies. Alternatively, Ace^R may have appeared initially in Côte d'Ivoire and has not had time to spread to Burkina Faso. At present, the border between the 2 countries seems to serve as a barrier for the spread of Ace^R. This allele was present at the border town Niangoloko (Ng), but was absent at Banfora (Ba) only 40 km farther north. In West Africa, roads are the main means of transportation for people and goods. Niangoloko is crossed by the only road connecting the 2 countries, and a customs post is located a few hundred meters south of the town. Most vehicles coming from Côte d'Ivoire are stopped there for several hours (sometimes several days) for customs formalities before being allowed to proceed. Most adult mosquitoes, passively transported in vehicles coming from Côte d'Ivoire, probably either seek resting sites in Niangoloko or die in vehicles after several hours of exposure to the sun. Therefore, most vehicles from Côte d'Ivoire are probably free of mosquitoes when leaving Niangoloko for Burkina Faso, which may delay the spread of Ace^R into *Cx. p. quinquefasciatus* populations in this country. Further genetic studies will be performed on mosquito samples to investigate gene flow between Côte d'Ivoire, Niangoloko, and other towns in Burkina Faso.

In populations of the *Cx. pipiens* complex, insensitive acetylcholinesterase alleles usually appear several years after intensive selection pressure with organophosphates and when overproduced esterases already are distributed widely. Then, Ace^R spreads among populations depending on the intensity of selection pressure, as was observed in France (Raymond et al. 1985, Chevillon,et al. 1995), Italy (Villani and Hemingway 1987, Severini et al. 1993), and Cuba (Bisset et al. 1990). However, in Spain, Ace^R frequencies in *Cx. p. pipiens* were not correlated with organophosphate treatments (Chevillon et al. 1995), and in Tanzania the Ace^R allele was reported from areas where there is no organized mosquito control (Khayrandish and Wood 1993). In Côte d'Ivoire, only sporadic mosquito control campaigns have been carried out during the last 20 yr and attempts to relate resistance levels to the agricultural use of insecticides were not conclusive. In Africa, *Cx. p. quinquefasciatus* is associated closely with urban environments and it is likely that the main selection pressure exerted is by the domestic use of insecticides. This conclusion seems in agreement with the absence of, or small variations in, insecticide resistance within towns in Côte d'Ivoire and Burkina Faso, where the overall insecticide pressure may be more or less similar among districts. Alternatively, gene flow between districts may be high as indicated by preliminary investigations on the genetic structure of *Cx. p. quinquefasciatus* in some Côte d'Ivoire towns (unpublished data).

In conclusion, despite the coexistence of several resistance genes in West Africa, the level of organophosphate resistance has remained relatively low, possibly because selection pressure from domestic use of insecticides is low compared with that resulting from systematic and organized mosquito control programs. However, resistance genes able to confer high resistance levels to certain insecticides are present and must be taken into account when planning organized mosquito control. The different resistance patterns observed in Côte d'Ivoire and Burkina Faso also should be considered. In Côte d'Ivoire, organophosphates still may be efficient for *Culex* control because resistance levels are low. However, increased use rapidly will select an increase in resistance because A2–B2 and Ace^R are present in all cities. Ace^R confers lower resistance to temephos than to chlorpyrifos, indicating that some organophosphate compounds may remain active in spite of its presence, as was observed for pirimiphos-methyl in Cuba (Bisset et al. 1991). In addition, a rotational scheme with organophosphates and other insecticides such as *Bacillus sphaericus* may decrease the intensity of selection for organophos-

phate resistance genes. In Burkina Faso, both organophosphates and carbamates still are effective for mosquito control. An increase of insecticide selection pressure will increase the frequency of A2–B2 esterases, and the risk exists that Ace^R will spread from Côte d'Ivoire. At an operational level, it is important that susceptibility level and evolution of resistance genes in target populations are monitored routinely.

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