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# An Insensitive Acetylcholinesterase in *Culex pipiens* (Diptera: Culicidae) from Portugal

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**ABSTRACT** Resistance mechanisms of a strain (PRAIAS) of northern house mosquito, *Culex pipiens* L., collected in Portugal in 1993, and highly resistant to organophosphates and carbamates, were investigated by comparing the resistance characteristics to 3 organophosphorous (temephos, chlorpyrifos, malathion) and 1 carbamate (propoxur) insecticides in the presence or absence of synergists; and by determining the possible occurrence of overproduced esterases or insensitive acetylcholinesterase (AChE). The reference strain MSE from southern France, with an insensitive AChE, was included in all analyses for comparison. For organophosphorous insecticides, resistance in PRAIAS was caused by an insensitive AChE and an increase in oxidative metabolism, although the 2nd mechanism has only a marginal effect. For propoxur, the insensitive AChE was the only resistance mechanism detected. Biochemical properties of both the French and Portuguese insensitive AChEs were similar. We cannot exclude the possibility that PRAIAS and MSE strains possess exactly the same insensitive AChE allele.

**KEY WORDS** *Culex pipiens*, insensitive acetylcholinesterase, insecticide resistance

INSECTICIDE RESISTANCE in the northern house mosquito, *Culex pipiens* L., has been studied in various geographical areas of the world. The most complex geographical situation seems to be around the Mediterranean sea, because the number of resistance genes present in this region represents >50% of the known organophosphorous resistance genes described in this species (e.g., Poirié et al. 1992; Ben Cheikh and Pasteur 1993; Severini et al. 1993; Raymond and Marquine 1994; Chevillon et al. 1995a, b). Some of the resistance genes are probably derived from mutation events occurring in the region (e.g., the overproduced esterases A1 and A4-B4); others (e.g., the overproduced linked esterases A2-B2) were probably introduced through passive migration (see Raymond et al. 1992, Rivet et al. 1993, Raymond and Marquine 1994). Reconstructing the various historical events that have occurred requires a thorough knowledge of resistance genes in natural populations; this is a prerequisite for understanding the evolution of insecticide resistance.

Although population samplings in the western Mediterranean Sea have been intensive in southern France (Pasteur et al. 1981; Rivet et al. 1993; Chevillon et al. 1995b, 1996), Italy (Severini et al. 1993), Corsica (Raymond and Marquine 1994), Sardinia (Chevillon et al. 1995a), and Tunisia (Ben Cheikh and Pasteur 1993); the Iberian Peninsula has been studied only on its Catalan region (Rivet et al. 1993, Chevillon et al. 1995b). To complete population sampling in this geographic area, a large population survey was done in Portugal. Here we report on the selection performed on 1 sample from this survey to obtain an homogenous resistance strain in few generations and its study with bioassays and biochemical analysis.

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## Materials and Methods

**Insects.** The following 3 types of mosquitoes were used: (1) S-LAB, a susceptible reference strain isolated by Georghiou et al. (1966); (2) MSE, a resistant strain to organophosphorous and carbamate insecticides with an insensitive AChE (Raymond et al. 1986); and (3) Praias, a natural population collected from Praias do Sado (Portugal) in November 1993. This population was mass selected during 6 generations by exposing 4th instars to rates of propoxur that induced 60–90% mortality (each generation is designated as Praias G$x$ with $x$ being the generation number). Finally, 25 single-pair crosses were isolated and their offspring were raised separately. The strain PRAIAS is derived from 2 of these families in which both parents displayed no reduction of AChE activity in presence of a $9.09 \times 10^{-5}$ mg/liter of propoxur (see Raymond et al. 1985b). To obtain heterozygous individuals for the resistance genes, MSE or PRAIAS males were mass crossed with S-LAB females. Off-

spring were designated as PRAIAS-$F_1$ or MSE-$F_1$, depending on the strain used as male parent.

**Insecticide Bioassays.** Resistance characteristics of the 3 strains and the offspring of the different crosses were analyzed with bioassays done in plastic cups on 4th instars as described in Raymond and Marquine (1994). Four insecticides of analytical or technical grade were used. These were malathion (Interchim, Montluçon, France), chlorpyrifos (Interchim), temephos (American Cyanamid, Princeton, NJ) and propoxur (Bayer, Leverkusen, Germany). The action of 2 synergists, DEF (S, S, S, tributyl phosphorotrithioate, Interchim) and PB (piperonyl butoxide, FLUKA AG, St. Quentin, France), was investigated by exposing larvae to a standard concentration (0.008 mg/liter for DEF and 5 mg/liter for PB) 4 h before the addition of insecticide solution. At these concentrations, no mortality occurred with the synergist.

Five replicates of 5 concentrations (20 larvae per concentration), causing mortality between 0 and 100%, were done with each insecticide. To standardize the concentration of solvents, their final concentrations (acetone for malathion and 95% ethanol for the others) were adjusted to 1%. Mortality data were analyzed assuming the probit model (Raymond 1993). Regression lines were considered identical if the hypothesis of parallelism was not rejected at the 0.05 probability level (by using a chi-square test [Finney 1971, p. 107]) and if the 95% CI of the resistance ratio included 1.0 (Robertson and Priesler 1992). When data were not linear assuming the probit model, $LC_{50s}$ were read directly from graphs.

**Identification of Known Resistance Genes.** *Overproduced Esterase.* Overproduced esterases were investigated in single adult homogenates by using starch gel electrophoresis with TME 7.4 buffer systems (Tris, 0.1 M; malic anhydride, 0.1 M; EDTA, 0.01 M and $MgCl_2$, $6H_2O$, 0.01 M; pH 7.4 [Pasteur et al. 1988]). Mosquitoes with known overproduced esterases were run in each gel as controls; these included esterase A1 (strain BARRIOL [N. Pasteur and M. R., unpublished data]), esterases A4 and B4 (strain VIM; Poirié et al. 1992), and esterases A2 and B2 (strain SELAX; Wirth et al. 1990).

*Insensitive Acetylcholinesterase.* AChE activity was measured using acetylthiocholine as substrate as described by Ellman et al. (1961). For each strain or for both crosses, 5 mosquitoes were homogenized together in 500 ml of 0.1 M sodium phosphate buffer (pH 7, containing 1% Triton X-100, Sigma, St. Quentin, France) with a glass pestle. Homogenates were centrifuged at 10,000 × *g* for 5 min. A sufficient amount of AChE were obtained by pooling the supernatants of 20 extractions from each strain or $F_1$. Different concentrations of propoxur were added to 100 μl of mosquito homogenates, and the substrate–reagent solution (final concentrations: 5,5′-dithio*bis*-2-nitrobenzoic acid 1.7 mM; acetylthiocholine 7.4 mM) was added 15 min later. Activity was measured at 420 nm over a period of 3 min with a spectrophotometer (Kontron-Uvikon 930, Paris, France); we used quartz cuvettes. Test conditions were established to ensure that rates of reaction were linear during the recording period. Residual AChE activities were measured at different concentrations of propoxur in comparison with AChE activity in absence of insecticide. A Kolmogorov–Smirnov test (Sokal and Rohlf 1995) was used to compare residual AChE activities.

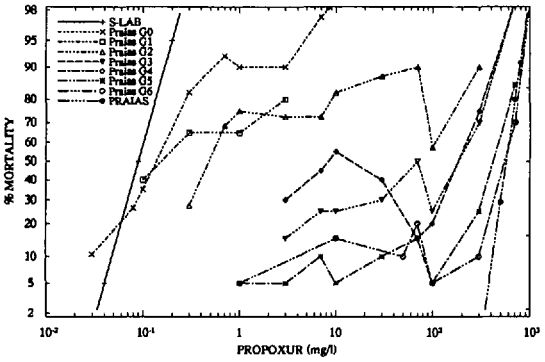


**Fig. 1.** Development of propoxur resistance in Praias. Each generation was selected by exposing larvae to propoxur concentrations that induced 60–90% mortality. Praias G0 was the natural population (i.e., before laboratory selection). PRAIAS corresponds to the current strain PRAIAS.

## Results

**Evolution of Resistance in Praias.** Fig. 1 shows the level of propoxur resistance observed in successive generations of selection. The $LC_{50}$ of the natural population Praias (Praias G0) was 0.15 ppm; it reached 0.45 ppm after 2 generations of selection and jumped to 200 ppm for Praias G3. Further propoxur selection increased the $LC_{50}$ up to 570 ppm. This discontinuous progression of $LC_{50}$s is the result of the progressive elimination of a plateau, indicating that susceptible genotypes were progressively eliminated (Fig. 1). This plateau further indicates that resistance is probably monogenic in PRAIAS. The PRAIAS strain was maintained in absence of insecticide selection, and its resistance has not changed after 10 generations (data not shown), indicating that this strain is probably homozygous for its resistance factor(s) (see Raymond 1993, Chevillon et al. 1996).

**Resistance Characteristics of the Strains.** Concentration–mortality curves obtained with chlorpyrifos, temephos, and malathion and propoxur were linear for all strains ($P > 0.1$) as shown for chlorpyrifos (Fig. 2), indicating homogeneity of the tolerance in the 3 strains. MSE displayed the same resistance level for chlorpyrifos, temephos, and propoxur as determined in 1985 (Raymond et al. 1986). PRAIAS displayed the same resistance

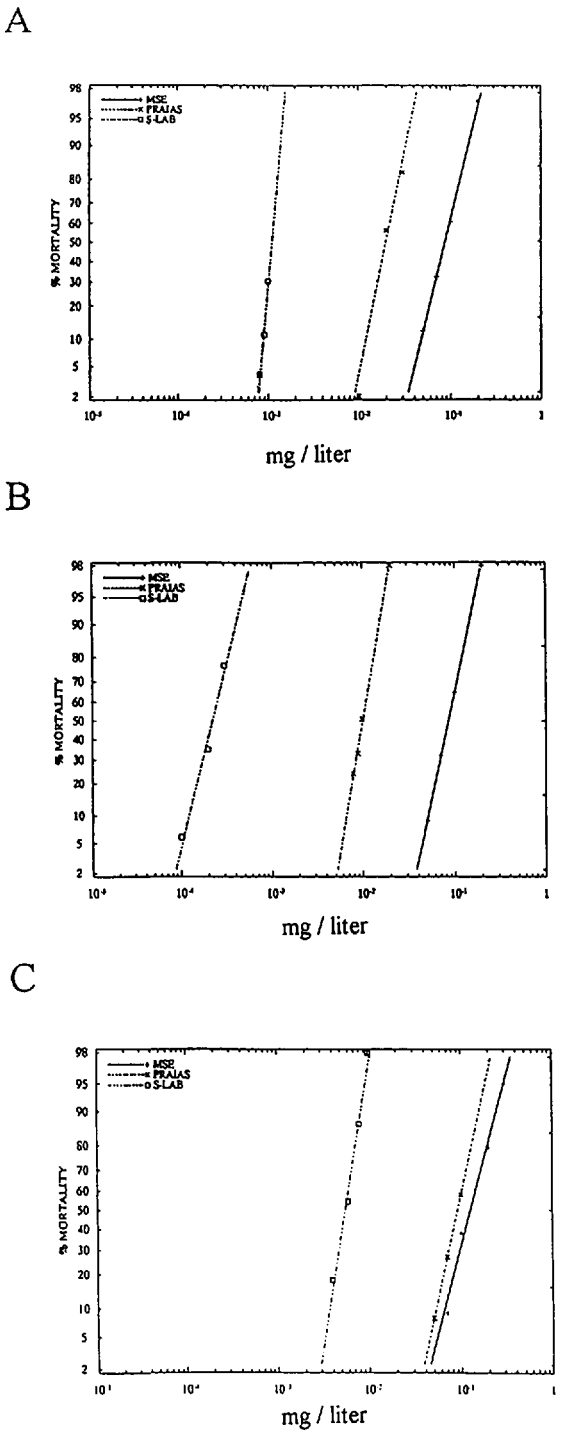


**Fig. 2.** Concentration–mortality lines obtained in bioassays of chlorpyrifos with the susceptible reference strain S-LAB and the 2 resistant strains MSE and PRAIAS. The bioassays were done: (A) in absence of synergists, (B) in presence of DEF, and (C) in presence of PB.

level as MSE for malathion (150-fold) and propoxur (1,600-fold): for both insecticides, the hypothesis of parallelism of PRAIAS and MSE mortality lines was not rejected ($\chi^2 = 5.65$, df = 4, $P > 0.2$, for malathion, and $\chi^2 = 5.65$, df = 4; $P > 0.2$, for propoxur) and the 95% CI of the resistance ratio included 1.0. For temephos, lines were not parallel ($P < 0.05$), but the resistance ratio (computed as described by Finney [1971], pp. 109–117) at the $LC_{50}$ included the value 1. For chlorpyrifos, parallelism was not rejected ($\chi^2 = 1.22$, df = 3, $P > 0.7$), but the resistance of both strains compared with S-LAB was significantly ($P < 0.001$) different. MSE displayed a 4.3-fold resistance to chlorpyrifos compared with PRAIAS (Table 1).

**Detoxification by Nonoxidative Enzymes.** Nonoxidative degradation of organophosphates or carbamates may occur by the action of esterases, glutathione *S*-transferases, or both. Esterases are inhibited by DEF (Lewis 1969). Addition of DEF in bioassays with the 3 organophosphates caused a systematic decrease in the $LC_{50}$ of the susceptible S-LAB for all insecticides. With MSE and PRAIAS (Table 1), we detected either a slight decrease or none at all. We conclude that DEF did not decrease resistance ratios for organophosphates when the other strains were compared with S-LAB. For propoxur, the $LC_{50}$ was not affected by the addition of DEF in S-LAB, and slightly decreased in MSE and PRAIAS. However, these decreases were not significant ($P > 0.05$ for both strains). These results indicate that DEF does not inhibit a resistance mechanism for organophosphorous or the carbamate in MSE or PRAIAS. None of the 30 PRAIAS mosquitoes analyzed by starch gel electrophoresis had an overproduced esterase (results not shown); the same result occurred in MSE (Raymond et al. 1986).

**Detoxification by P450-Dependent Mixed Function Oxidases.** P450-dependent mixed function oxidases that can metabolize insecticides are present in all living organisms. PB inhibits mixed function oxidases and subsequently reduces the quantity of molecules available to degrade or activate, or both, the insecticide. All strains were affected by the addition of PB (Table 1). $LC_{50}$s increased when the insecticide was first naturally activated by mixed function oxidases (chlorpyrifos, temephos, and malathion) or decreased when such activation does not take place (propoxur). The resistance ratio of MSE or PRAIAS was always lower in the presence of PB than in its absence for each of the 3 organophosphorous insecticides, indicating that mixed function oxidases explain part of the organophosphate resistance of these strains. However, because all resistance ratios were significantly >1.0, another resistance mechanism was probably present. For propoxur, the resistance ratio of MSE and PRAIAS increased with the presence of PB, indicating that mixed function oxidases have no detectable effect on propoxur resistance for these strains. Similar results were found for MSE by

**Table 1. Responses of S-LAB, MSE, and PRAIAS strains of *Cx. pipiens* to insecticides with and without synergists**

| Tested compounds[a] | | S-LAB | | MSE | | PRAIAS | | RR (95% CI) MSE/S-LAB | | RR (95% CI) PRAIAS/S-LAB | | RR (95% CI) MSE/PRAIAS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No synergist | | | | | | | | | | | | |
| Chlorpyrifos | $LC_{50}$[b] (range) | 1.1 | (1.04–1.24) | 87.1 | (81.4–93.6) | 20.0 | (18.6–21.4) | 79.2 | (50.6–124) | 18.2 | (11.5–28.8) | 4.3 (3.1 –6.7) |
| | Slope (SE) | 13.3 | (2.71) | 50. | (0.44) | 6.0 | (0.58) | | | | | |
| Temephos | $LC_{50}$ (range) | 1.51 | (1.42–1.62) | 8.62 | (8.16–9.14) | 7.54 | (7.26–7.83) | 5.5 | (2.3–27.9) | 5.1 | (3.5–8) | 1.15 (0.9 –1.4) |
| | Slope (SE) | 7.1 | (0.54) | 5.6 | (0.51) | 9.3 | (0.89) | | | | | |
| Malathion | $LC_{50}$ (range) | 43.8 | (42.2–45.3) | 7,114 | (6,674–7,596) | 6,033 | (5,701–6,434) | 162.5 | (132–200) | 136.7 | (127–192) | 1.15 (0.85–1.2) |
| | Slope (SE) | 9.9 | (0.82) | 5.2 | (0.42) | 7.16 | (0.85) | | | | | |
| Propoxur | $LC_{50}$ (range) | 359 | (346–370) | 555,000 | (525,000–581,000) | 572,000 | (543,000–596,000) | 1,547 | (1,180–2,028) | 1,626 | (271–17,245) | 0.97 (0.9 –1) |
| | Slope (SE) | 13.1 | (1.23) | 9.0 | (0.99) | 9.37 | (0.99) | | | | | |
| With DEF | | | | | | | | | | | | |
| Chlorpyrifos | $LC_{50}$ (range) | 0.22 | (0.21–0.24) | 85.5 | (80.2–91.3) | 10.1 | (9.7–10.7) | 389.9 | (93–2,521) | 45.4 | (36.2–56.9) | 8.32 (4.9 –16.5) |
| | Slope (SE) | 4.88 | (0.53) | 5.6 | (0.5) | 7.1 | (0.81) | | | | | |
| | SR[d] | 4.9 | — | 1.01 | — | 1.97 | — | | | | | |
| Temephos | $LC_{50}$ (range) | 0.56 | (0.53–0.58) | 8.6 | (8.2–9.1) | 4.45 | (4.21–4.72) | 15.0 | (12.4–18.3) | 7.85 | (2.5–102.6) | 1.88 (1.6 –2.3) |
| | Slope (SE) | 7.8 | (0.58) | 5.6 | (0.51) | 5.35 | (0.48) | | | | | |
| | SR | 2.7 | — | 1.03 | — | 1.69 | — | | | | | |
| Malathion | $LC_{50}$ (range) | 2.21 | (2.04–2.38) | 4,953 | (4,686–5,212) | 3,703 | (3,521–3,887) | 2,240 | (1,755–2,859) | 1,675 | (1,370–2,046) | 1.32 (1.2 –1.5) |
| | Slope (SE) | 4.58 | (0.36) | 8.5 | (0.87) | 6.2 | (0.48) | | | | | |
| | SR | 20 | — | 1.44 | — | 1.64 | — | | | | | |
| Propoxur | $LC^{50}$ (range) | 359 | (346–371) | 516,000 | (469,000–551,000) | 565,000 | (540,000–588,000) | 1,438 | (1,078–1,918) | 1,585 | (279–15,338) | 0.91 (0.7 –1.2) |
| | Slope (SE) | 12.2 | (1.14) | 6.8 | (0.95) | 10.74 | (1.06) | | | | | |
| | SR | 1.0 | — | 1.03 | — | 1.02 | — | | | | | |
| With PB | | | | | | | | | | | | |
| Chlorpyrifos | $LC_{50}$ (range) | 5.6 | (5.32–5.87) | 127.3 | (117.5–137.8) | 91.5 | (84.9–101.4) | 22.8 | (18.3–28.3) | 15.8 | (3.4–546.3) | 1.35 (1.2 –1.6) |
| | Slope (SE) | 7.3 | (0.54) | 0.99 | (0.35) | 5.3 | (0.71) | | | | | |
| | SR | 0.22 | — | 0.69 | — | 0.22 | — | | | | | |
| Temephos | $LC_{50}$ (range) | 12.0 | (11.4–12.7) | 31.6 | (27.9–35.5) | 27.6 | (24.8–30.3) | 2.6 | (2–3.5) | 2.3 | (1.7–3.1) | 1.15 (0.91–1.4) |
| | Slope (SE) | 9.37 | (0.87) | 2.8 | (0.27) | 4.1 | (0.34) | | | | | |
| | SR | 0.12 | — | 0.27 | — | 0.27 | — | | | | | |
| Malathion | $LC_{50}$ (range) | 116 | (109–124) | 3,021 | (2,856–3,198) | 4,279 | (3,884–4,645) | 26.2 | (12.7–65.4) | 36.8 | (29–46.7) | 0.71 (0.6 –0.9) |
| | Slope (SE) | 6.6 | (0.54) | 75. | (0.69) | 4.1 | (0.40) | | | | | |
| | SR | 0.4 | — | 2.35 | — | 1.41 | — | | | | | |
| Propoxur | $LC_{50}$ (range) | 37.5 | (34.7–41.1) | 248,000 | (231,000–263,000) | 464,000 | (439,000–489,000) | 6,570 | (1,716–12,496) | 12,372 | (9,642–15,875) | 0.55 (0.4 –0.6) |
| | Slope (SE) | 4.8 | (0.53) | 6.1 | (0.57) | 7.89 | (0.72) | | | | | |
| | SR | 9.6 | — | 2.12 | — | 1.22 | — | | | | | |

RR, resistance ratio at $LC_{50}$; SR, synergism ratio at $LC_{50}$.

[a] Each regression was estimated with a total sample of 500 mosquitoes.

[b] $LC_{50}$ expressed in μg/liter.

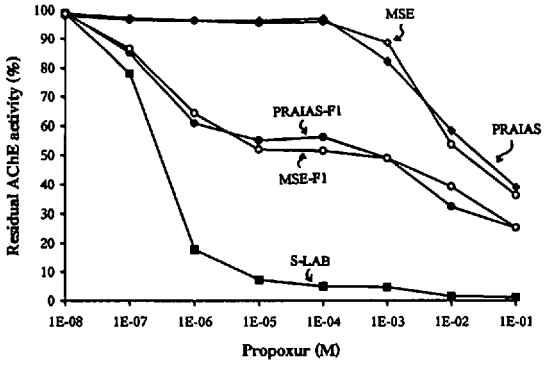


**Fig. 3.** Residual AChE activity of mosquito homogenates with $Ace^{SS}$ (S-LAB), 2 $Ace^{RR}$ (MSE and PRAIAS), and $Ace^{RS}$ (MSE-F1 and PRAIAS-F1) genotypes in the presence of increasing concentrations of propoxur.

Raymond et al. (1986). When PRAIAS was compared with MSE, the resistance ratio decreased for all insecticides (except for temephos) with the addition of PB (Table 1), indicating that mixed function oxidases are more important in the former strain than in the latter.

**AChE Activity.** Residual AChE activity in presence of increasing concentrations of propoxur was measured in the 3 strains and 2 $F_1$ crosses (Fig. 3). AChE activity in S-LAB was almost completely inhibited by $10^{-4}$ M propoxur, whereas activity in the MSE and PRAIAS strains was unaffected. This result confirms previous studies on the existence of a propoxur insensitive AChE in MSE (Raymond et al. 1985a, 1986) and indicates that a similar resistance phenomenon also exits in the Portuguese strain PRAIAS. Residual AChE activities in the PRAIAS strain were not different from those of MSE ($\chi^2 = 0.549$, df = 7, $P > 0.9$), indicating that inhibition properties of insensitive AChE are similar in the 2 strains. This similarity was further confirmed by analysis of the distribution of residual AChE activities in the $F_1$ offspring of PRAIAS and MSE with S-LAB, which were not significantly different ($\chi^2 = 1.03$, df = 7, $P > 0.9$).

## Discussion

Our investigation has revealed that mixed function oxidases and insensitive AChE are involved in resistance to 3 organophosphates and propoxur in Portugal. The resistance ratio of propoxur resistance increased in both resistant strains when PB was used, indicating that the protection level provided by mixed function oxidases is reduced when another resistance mechanism is present. Such a situation occurs when 2 resistant mechanisms have an additive interaction, which is the case when a detoxification enzyme and an insensitive target site are both present (Raymond et al. 1989). The latter resistant gene is probably autosomally inherited in PRAIAS as it is the case in MSE (Raymond et al. 1987). To our knowledege, this is the 1st report of mixed function oxidases and insensitive AChE in Portuguese *Cx. pipiens*.

Since its first discovery in mites by Smissaert in 1964, insensitive acetylcholinesterase has been reported in a growing number of insects and acarine species (see Fournier and Mutero [1994] for a recent review). Only one locus codes for acetylcholinesterase in insects (Greenspan et al. 1980, Fournier et al. 1988, Fournier and Mutéro 1994), but several resistant alleles can occur in natural populations. This situation occurs in cattle tick *Boophilus microplus* (Can) (Nolan and Schnitzerling 1975a, b); house fly, *Musca domestica* L. (Oppenoorth 1982); sweet potato white fly, *Bemisia tabaci* (Gennadius) (Byrne and Devonshire 1993); Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Wierenga and Hollingworth 1993); and *Drosophila melanogaster* (L.) (Pralavorio and Fournier 1992). In *Cx. pipiens* complex, insensitive AChE was first found in southern France (Raymond et al. 1985a, 1986) and has been subsequently reported in various places: central France (Rivet et al. 1993), Corsica (Raymond and Marquine 1994), Italy (Bonning and Hemingway 1991, Severini et al. 1993), Burma (Tang et al. 1990), Cuba (Bisset et al. 1990), Tanzania (Khayrandish and Wood 1993a, b), Tunisia (Ben Cheikh and Pasteur 1993), Spain (Chevillon et al. 1995b), and Portugal (our study).

Based on the information available about *Cx. pipiens*, >2 insensitive AChE alleles likely exist. For example, the $Ace^R$ allele from Rangoon, Burma, is only insensitive to fenthion and not to other insecticides (Tang et al. 1990); this is not the case in the other situations. However, a precise comparison of the different studies is difficult because different techniques were used so that estimation of the exact number of insensitive alleles present in the strains or samples analyzed is not possible when differences are small. Results of our study indicate that alleles from both the French and the Portuguese strains have similar biochemical properties: they provide the same resistance to organophosphorous insecticides in presence of PB (which removes physiologically the effect of resistance mechanisms mediated by mixed function oxidases) and to propoxur. In addition, they show the same residual activity for various concentrations of propoxur. Therefore, we cannot exclude the presence of same allele for insensitive acetylcholinesterase in the 2 countries until further research clarifies this question. The insensitive AChE present in PRAIAS had a relatively high frequency in field samples (the Praias sample displayed a small plateau at 90% mortality [Fig. 1]; therefore, at least 10% of individuals were heterozygous for the resistance gene), indicating that *Cx. pipiens* is exposed to high rates of insecticides (organophosphorous, carbamate, or both) under natural conditions, despite the absence of organized mosquito control in Portugal. These insecticides could come from private use or agriculture practices

(see, e.g., Failloux et al. 1994). The geographical distribution of this resistance mechanism in Portuguese populations remains to be determined.


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## References Cited


**Ben Cheikh, H., and N. Pasteur. 1993.** Resistance to temephos an organophosphorous insecticide, in *Culex pipiens* from Tunisia, North Africa. J. Am. Mosq. Control. Assoc. 9: 335–337.

**Bisset, J., M. M. Rodriguez, C. Diaz, E. Ortiz, M. C. Marquetti, and J. Hemingway. 1990.** The mechanisms of organophosphate and carbamate resistance in *Culex quinquefasciatus* (Diptera: Culicidae) from Cuba. Bull. Entomol. Res. 80: 245–250.

**Bonning, B. C. and J. Hemingway. 1991.** The efficacy of acetylcholinesterase in organophosphorus and carbamate resistance in *Culex pipiens* L. from Italy. Pestic. Biochem. Physiol. 40: 143–148.

**Byrne, F. J., and A. L. Devonshire. 1993.** Insensitive acetylcholinesterase and esterase polymorphism in susceptible and resistant populations of the tobacco whitefly, *Bemisia tabaci* (Genn.). Pestic. Biochem. Physiol. 45: 34–42.

**Chevillon, C., G. Addis, M. Raymond, and A. Marchi. 1995a.** Population structure in Mediterranean islands and risk of genetic invasion in *Culex pipiens*. Biol. J. Linn. Soc. 55: 329–343.

**Chevillon, C., N. Pasteur, M. Marquine, D. Heyse, and M. Raymond. 1995b.** Population structure and dynamics of selected genes in the mosquito *Culex pipiens*. Evolution 49: 997–1007.

**Ellman, G. L., D. Courtney, V. Andres, and R. M. Featherstone. 1961.** A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88–95.

**Failloux, A. B., A. Ung, M. Raymond, and N. Pasteur. 1994.** Insecticide susceptibility in mosquitoes from Polynesia. J. Med. Entomol. 31: 639–644.

**Finney, D. J. 1971.** Probit analysis. Cambridge University Press, Cambridge, U.K.

**Fournier, D., and A. Mutéro. 1994.** Modification of acetylcholinesterase as a mechanism of resistance to insecticides. Comp. Biochem. Physiol. 108C: 19–31.

**Fournier, D., J. M. Bride, F. Karch, and J.-B. Bergé. 1988.** Acetylcholinesterase from *Drosophila melanogaster*, identification of two subunits encoded by the same gene. FEBS Lett. 238: 333–337.

**Georghiou, G. P., R. L. Metcalf, and F. E. Gidden. 1966.** Carbamate resistance in mosquitoes: selection of *Culex pipiens fatigans* Wied. for resistance to Baygon. Bull. W.H.O. 35: 691–708.

**Greenspan, R. J., J. A. Finn, and J. C. Hall. 1980.** Acetylcholinesterase mutants in *Drosophila* and their effects on the structure and function of the central nervous system. J. Comp. Neurol. 189: 741–774.

**Khayrandish, A., and R. J. Wood. 1993a.** Organophosphorus insecticide resistance in a new strain of *Culex quinquefasciatus* (Diptera: Culicidae) From Tanga, Tanzania. Bull. Entomol. Res. 83: 67–74.

**1993b.** A multiple basis for insecticide resistance in a strain of *Culex quinquefasciatus* (Diptera: Culicidae) from Muheza, Tanzania, studied as resistance declined. Bull. Entomol. Res. 83: 75–86.

**Lewis, J. B. 1969.** Detoxification of diazinon by subcellular fractions of diazinon-resistant and susceptible houseflies. Nature (Lond.) 224: 917–918.

**Nolan, J., and H. J. Schnitzerling. 1975a.** Characterization of acetylcholinesterases of acaricide resistant and susceptible strains of the cattle tick *Boophilus microplus* (Can.). I. Extraction of the critical component and comparison with enzyme from other sources. Pestic. Biochem. Physiol. 5: 178–188.

**1975b.** Characterization of acetylcholinesterases of acaricide resistant and susceptible strains of the cattle tick *Boophilus microplus* (Can.). II. The substrate specificity and catalytic efficiency of the critical enzyme component. Pestic. Biochem. Physiol. 6: 142–147.

**Oppenoorth, F. J. 1982.** Two paraoxon-resistant acetylcholinesterase mutants in the house fly. Pestic. Biochem. Physiol. 18: 26–27.

**Pasteur, N., G. Sinègre, and A. Gabinaud. 1981.** *Est-2* and *Est-3* polymorphisms in *Culex pipiens* L. from southern France in relation to organophosphate resistance. Biochem. Genet. 19: 499–508.

**Pasteur, N., G. Pasteur, F. Bonhomme, J. Catalan, and J. Britton-Davidian. 1988.** Practical isozyme genetics. Ellis Horwood, Chichester, U.K.

**Poirié, M., M. Raymond, and N. Pasteur. 1992.** Identification of two distinct amplifications of the esterase B locus in *Culex pipiens* L. mosquitoes from Mediterranean countries. Biochem. Genet. 30: 13–26.

**Pralavorio, M., and D. Fournier. 1992.** *Drosophila* acetylcholinesterase: characterization of different mutants resistant to insecticides. Biochem. Genet. 30: 77–83.

**Raymond, M. 1993.** PROBIT CNRS-UMII. Licence L93019. Avenix, G. Prato and A. Ratsira, 34680 St. George d'Orques, France.

**Raymond, M., and M. Marquine. 1994.** Evolution of insecticide resistance in *Culex pipiens* populations: the corsican paradox. J. Evol. Biol. 7: 315–337.

**Raymond, M., N. Pasteur, D. Fournier, A. Cuany, J.-B. Bergé, and M. Magnin. 1985a.** Le gène d'une acétylcholinestérase insensible au propoxur détermine la résistance de *Culex pipiens* L. à cet insecticide. C.R. Acad. Sci. Paris. Ser. III 300: 509–512.

**Raymond, M., D. Fournier, J.-B. Bergé, A. Cuany, J.-M. Bride, and N. Pasteur. 1985b.** Single mosquito test to determine genotypes with an acetylcholinesterase insensitive to inhibition to propoxur insecticide. J. Am. Mosq. Control. Assoc. 1: 425–427.

**Raymond, M., D. Fournier, J.-M. Bride, A. Cuany, J.-B. Bergé, M. Magnin, and N. Pasteur. 1986.** Identification of resistance mechanisms in *Culex pipiens* (Diptera: Culicidae) from southern France: insensitive acetylcholinesterase and detoxifying oxidases. J. Econ. Entomol. 79: 1452–1458.

**Raymond, M., N. Pasteur, and G. P. Georghiou. 1987.** Inheritance of chlorpyrifos resistance in *Culex*

*pipiens* L. (Diptera: Culicidae) and estimation of the number of genes involved. Heredity 58: 351–356.

**Raymond, M., D. G. Heckel, and J. G. Scott. 1989.** Interactions between pesticide genes: model and experiment. Genetics 123: 543–551.

**Raymond, M., M. Marquine, and N. Pasteur. 1992.** Role of mutation and migration in the evolution of insecticide resistance in the mosquito *Culex pipiens*, pp. 19–27. *In* I. Denholm, A. L. Devonshire, and D. W. Hollomon [eds.], Resistance '91: achievements and developments in combating pesticide resistance. Elsevier, London.

**Rivet, Y., M. Marquine, and M. Raymond. 1993.** French mosquito populations invaded by A2-B2 esterases causing insecticide resistance. Biol. J. Linn. Soc. 49: 249–255.

**Robertson, J. L., and H. K. Priesler. 1992.** Pesticide bioassays with arthropodss. CRC, Boca Raton, FL.

**Severini, C., R. Romi, M. Marinucci, and M. Raymond. 1993.** Mechanisms of insecticide resistance in field populations of *Culex pipiens* from Italy. J. Am. Mosq. Control. Assoc. 9: 164–168.

**Smissaert, H. R. 1964.** Cholinesterase inhibition in spider mites susceptible and resistant to organophosphate. Science (Washington, DC) 143: 129–131.

**Sokal, R. R., and F. J. Rohlf. 1995.** Biometry: the principles and practice of statistics in biological research. Freeman, New York.

**Tang, Z.-H., R. J. Wood, and S. L. Cammack. 1990.** Acetylcholinesterase activity in organophosphorus and carbamate resistant and susceptible strains of the *Culex pipiens* complex. Pestic. Biochem. Physiol. 37: 192–199.

**Wierenga, J. M., and R. Hollingworth. 1993.** Inhibition of altered acetylcholinesterases from insecticide-resistant Colorado potato beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 86: 673–679.

**Wirth, M. C., M. Marquine, G. P. Georghiou, and N. Pasteur. 1990.** Esterases A2 and B2 in *Culex quinquefasciatus* (Diptera: Culicidae): role in organophosphate resistance and linkage studies. J. Med. Entomol. 27: 202–206.