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An overview of the evolution of overproduced esterases in the mosquito *Culex pipiens*

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Insecticide resistance genes have developed in a wide variety of insects in response to heavy chemical application. Few of these examples of adaptation in response to rapid environmental change have been studied both at the population level and at the gene level. One of these is the evolution of the overproduced esterases that are involved in resistance to organophosphate insecticides in the mosquito *Culex pipiens*. At the gene level, two genetic mechanisms are involved in esterase overproduction, namely gene amplification and gene regulation. At the population level, the co-occurrence of the same amplified allele in distinct geographic areas is best explained by the importance of passive transportation at the worldwide scale. The long-term monitoring of a population of mosquitoes in southern France has enabled a detailed study to be made of the evolution of resistance genes on a local scale, and has shown that a resistance gene with a lower cost has replaced a former resistance allele with a higher cost.

Keywords: insecticide resistance; selection; adaptation; fitness cost

1. INTRODUCTION

The mosquito *Culex pipiens*, common in temperate and tropical countries, is subjected to insecticide control in many places. Worldwide surveys of resistance to organophosphate (OP) insecticides have disclosed that only three loci have developed major resistance alleles (Pasteur *et al*. 1981; Wirth *et al*. 1990; Georghiou 1992; Poirié *et al*. 1992). The first two loci, *Est-2* (or *esterase B*) and *Est-3* (or *esterase A*), code for detoxifying carboxylester hydrolases, and resistant alleles correspond to an esterase overproduction (Fournier *et al*. 1987; Mouchès *et al*. 1987; Poirié *et al*. 1992). Six distinct overproduced allozymes have been described so far at the esterase B locus (B1, B2, B4, B5, B6 and B7) and four (A1, A2, A4 and A5) at the esterase A locus (Pasteur *et al*. 1981, 1984; Raymond *et al*. 1989; Georghiou 1992; Poirié *et al*. 1992; Xu *et al*. 1994; Vaughan & Hemingway 1995). The two esterase loci are closely linked, and are separated by an intergenic DNA fragment of 2–6 kilobases (Rooker *et al*. 1996; Guillemaud *et al*. 1997). The third locus, *Ace.1*, codes the acetylcholinesterase (insecticide target), and insensitive alleles have been reported in various locations (Raymond *et al*. 1986; Bourguet *et al*. 1996, 1997), although it is not known how many resistant *Ace.1* alleles have occurred independently.

2. DISSECTING MUTATIONS

The overproduction of esterase is the result of two non-exclusive mechanisms. The first mechanism involves gene amplification either of the esterase B locus or, in some situations, of both the esterase A and B loci (Mouchès *et al*. 1986; Raymond *et al*. 1989; Poirié *et al*. 1992; Guillemaud *et al*. 1997; Vaughan *et al*. 1997). The latter situation, the co-amplification of two esterase loci, explains the tight statistical association of some electromorphs, such as A2 and B2 (Rooker *et al*. 1996; Guillemaud *et al*. 1996). Although, strictly speaking, only A4, A2 and A1 are alleles of the A esterase locus, and only B2 and B4 are alleles at the B esterase locus, A1, A4–B4 and A2–B2 behave as alleles of a single supergene, which is the result of the complete linkage disequilibrium between the A and B esterase genes produced by the amplification.

The second mechanism, gene regulation, explains the overproduction of esterase A1 (Rooker *et al*. 1996). However, it might also contribute to the overproduction of other variants in addition to gene amplification.

The level of gene amplification varies between the various amplified alleles: for B1, it could reach 250 copies (Mouchès *et al*. 1986), whereas for B4 it has never been found above a few copies (Poirié *et al*. 1992; Guillemaud *et al*. 1997). It varies also within and between populations for a given amplified allele, as shown for example for the A2–B2 amplified allele (Callaghan *et al*. 1998).

3. EVOLUTION AT THE WORLDWIDE SCALE

Overproduced allozymes that are electrophoretically identical are often found in distinct geographic areas. This is the case, for example, for the pair A2–B2, which is present in Africa, South and North America, Asia and Europe, or for B1, present in China and North America. This situation could be explained by a recurrent mutation

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process that generates each resistance allele. The other possibility is that each overproduced allele is the result of a non-recurrent mutation, and has subsequently spread within populations owing to its advantage in OP-treated areas, and between populations by migration (not excluding passive transportation). There is now considerable molecular evidence for the latter possibility.

First, restriction maps of the DNA within and around the esterase B structural gene can be built, in susceptible mosquitoes, with a single copy of the gene, as well as in mosquitoes with an amplified esterase gene (the amplicon is larger than the DNA area mapped). When such restriction maps are compared, large differences are observed. For example, two maps from susceptible mosquitoes from the same breeding site have only 40% of their restriction sites in common (Raymond *et al.* 1996). However, when strains with the B2 electromorph are compared, restriction maps are strictly identical, independently of the geographic origins of the insects considered (Raymond *et al.* 1991). A similar situation is found for the B1 electromorph: mosquitoes from various localities within the Americas and China possess the same restriction map (Qiao & Raymond 1995). The similarity of the restriction maps of all B1 (or all B2) haplotypes from diverse and distant geographic areas indicates that all B1 (or all B2) alleles are identical by descent. The same result has been obtained at the sequence level on the intron of the esterase A gene from various geographic origins: the polymorphism found in alleles of non-overproduced esterases is one of the largest thus far described, and all A2 alleles display exactly the same sequence (Guillemaud *et al.* 1996). All of these results could be explained only if a unique molecular event has generated the A2–B2 amplification, which has subsequently spread by migration worldwide. Another independent event is responsible for the B1 amplification, again followed by an extensive migration. It seems that there is a similar situation for the other variants with a large geographical distribution, such as A5–B5 and A4–B4 in the Mediterranean area (Raymond & Marquine 1994; Chevillon *et al.* 1995; Severini *et al.* 1997).

In addition, there is direct (Highton & Van Someren 1970) and indirect (Chevillon *et al.* 1995; Pasteur *et al.* 1995) evidence of large-scale migration of this mosquito by passive transportation by man, and the presence of one female with A2–B2 in an aircraft has been established (Curtis & White 1984). The local invasion of A2–B2 in southern France has been documented: A2–B2 was first found near the international Marseille airport and seaport, and has spread within a few years in all surrounding OP-treated areas (Rivet *et al.* 1993).

The number of independent amplification events at the esterase A and B loci cannot be estimated by just counting the number of overproduced electromorphs. This is due to the following reasons. First, A and B loci are amplified simultaneously, as are A2 and B2 (Rooker *et al.* 1996) and also the associated A4 and B4, and A5 and B5 (Guillemaud *et al.* 1997), so that only one amplification event is responsible for the presence of the two electromorphs. Second, an overproduction of esterase is not necessarily the result of gene amplification, as the overproduction of esterase A1 is due to gene regulation (Rooker *et al.* 1996). Third, the same electromorph could

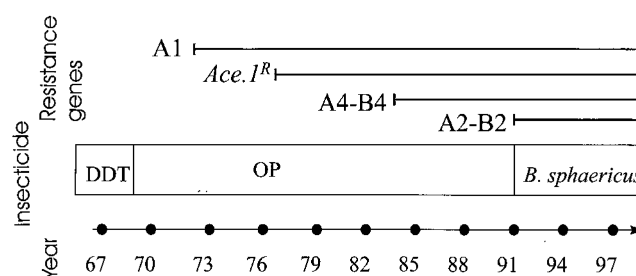

Figure 1. History of insecticide treatments in the Montpellier area, and the occurrence of OP resistance genes. See text for details.

correspond to distinct alleles, as exemplified by B4 and B5 (Poirié *et al.* 1992), and B1 and an unnamed electromorph (Vaughan *et al.* 1995).

Taking into account the protein and DNA studies published to date, the number of independent initial amplification events at both esterase A and B loci is between five and ten. The imprecision arises from the fact that a thorough checking has not been performed yet for all known overproduced esterases. This number corresponds to amplification events that have spread geographically as a result of the advantage they give in insecticide-treated areas, and are therefore at high frequencies and easily detected. A more thorough sampling will probably detect additional events that either are still geographically restricted or are at a low frequency, so that the above estimates should be regarded as a minimum figure. Each known amplification event has spread geographically, sometimes across continents, such as A2–B2 (Raymond *et al.* 1991) and B1 (Qiao & Raymond 1995), and sometimes only in a restricted area, such as A4–B4 in the western Mediterranean (Poirié *et al.* 1992; Severini *et al.* 1997) and A5–B5 in the eastern Mediterranean (Poirié *et al.* 1992; Severini *et al.* 1994, 1997). This relatively low number of independent amplification events, recorded on a world-scale for a pest species with large population sizes, indicates that advantageous mutations (i.e. any molecular event generating a gene amplification at these loci) could be limiting. Once an amplification has occurred, it can apparently spread easily and invade. Clearly, migration cannot be ignored as a driving force in studies and monitoring of insecticide-treated populations of the mosquito *Culex pipiens*.

4. EVOLUTION AT THE LOCAL SCALE

There are very few places in the world where the local evolution of resistance genes has been studied through time. The best-documented place is the Montpellier area (southern France), where information on OP resistance genes has been collected regularly since the occurrence of OP resistance. Different pesticides (DDT, chlorpyrifos, temephos, fenitrothion and *Bacillus sphaericus* toxin) have been used to control *Culex pipiens* (figure 1). The occurrence of OP resistance and resistance genes in this area has already been described in detail elsewhere (Pasteur & Sinègre 1975; Pasteur *et al.* 1981; Raymond *et al.* 1986; Chevillon *et al.* 1995; Guillemaud *et al.* 1998). Briefly, the

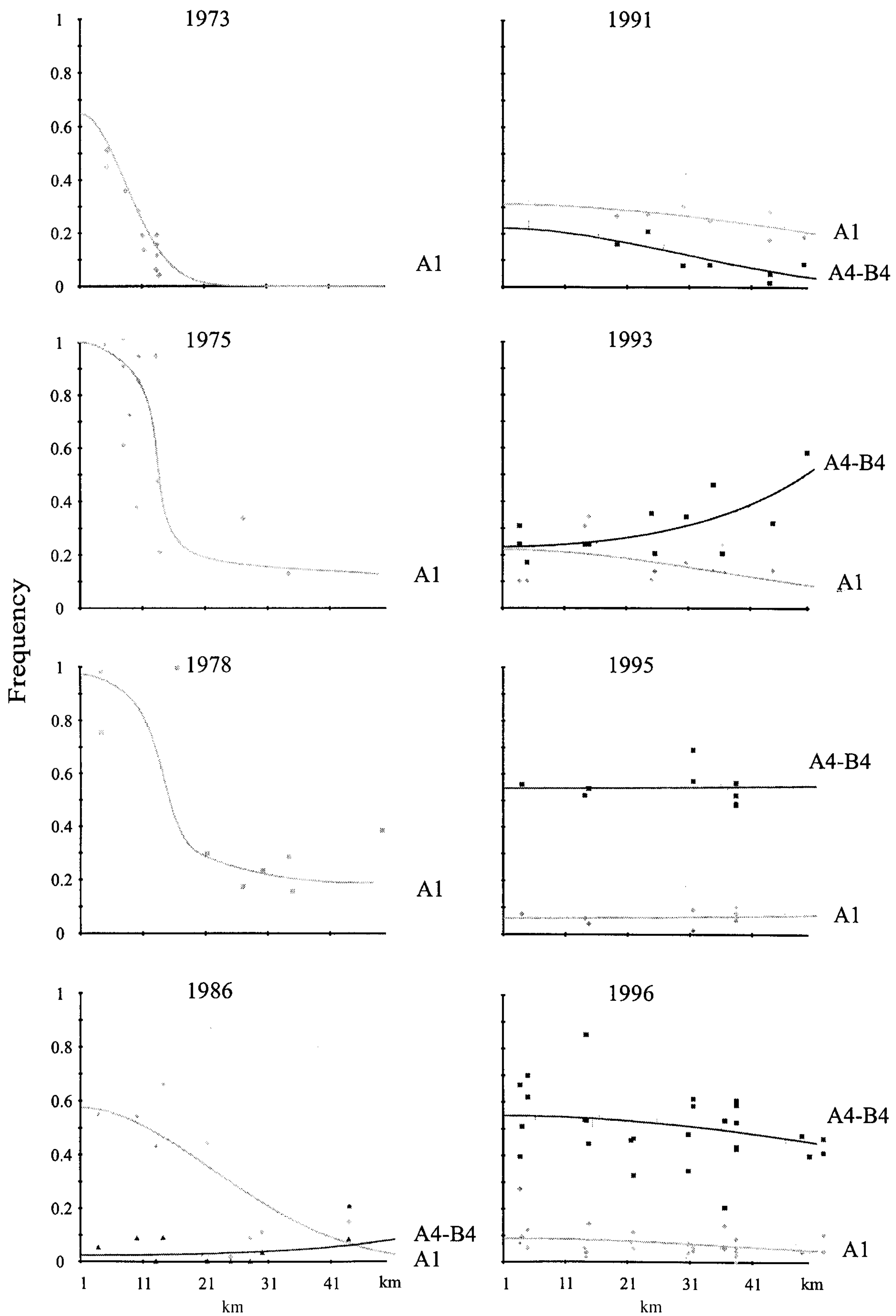

Figure 2. Frequency of overproduced A1 (grey) and A4–B4 (black) along the same transect in the Montpellier area. The frequency of A2–B2 is too low to be represented. The transect starts in the treated area near the sea, crosses the boundary of the treated/non-treated area, and ends 50 km from the sea. Each point represents an independent sample. Eight sampling years are indicated. See Guillemaud *et al.* (1998) for details.

first resistance gene (overproduced A1 esterase that was the result of gene regulation) occurred in 1972, only four years after control began with chlorpyrifos (an OP insecticide). It was followed by the occurrence of a modified target (an insensitive acetylcholinesterase *Ace.1*^{R}) in 1977, and by two pairs of overproduced esterase A and B allozymes (both the result of gene amplification): A4–B4 in 1984 and A2–B2 in 1991 (figure 1). One particularly convenient feature of *Culex pipiens* in the south of France is that its control has been limited to the populations along the Mediterranean coast. Thus, it is possible to identify, in a linear transect orthogonal to the coastline, an insecticide-treated area (close to the sea) and a non-treated one (further northwest) (figure 2). On this transect, the evolution of overproduced esterases is apparent through time (Guillemaud *et al*. 1998). First, A1 increased in frequency, until 1978. It displayed a steep and stable cline, indicating that this allele is associated with a cost, i.e. it is selected against in the non-treated area. At this time, the insensitive *Ace.1*^{R} allele occurred, with the global effect of decreasing the frequency of overproduced esterase A1 in the treated area. In 1986, a new overproduced esterase allele occurred: A4–B4. Since its first detection, and during its continuous spread, this allele failed to display a typical cline pattern, with a higher frequency in the treated area. This indicates that it is associated with a lower cost than its competing allele A1. It is apparent that an allele replacement has occurred, the first resistance allele A1 being replaced over a ten-year period (compare 1986 and 1996 in figure 2) by the less costly A4–B4 allele (Guillemaud *et al*. 1998).

5. CONCLUSION

A handful of overproduced alleles have occurred in *Culex* mosquitoes in response to OP selection throughout the world. Owing to the advantage they provide in OP-treated areas, they have subsequently spread within populations, and then between populations. The latter phenomenon is considerably facilitated by the fact that most OP-treated areas are connected by plane or by other transportation systems that are suitable for passive migration by mosquitoes. As a result, the various overproduced esterase alleles, occurring independently in distinct geographic areas, have come into contact in the same populations, and have competed. Depending of the resistance they provide, the associated cost they possess and various other genetic characteristics (e.g. dominance relationships), one particular allele might eventually increase in frequency and replace the other resistance genes at the same locus. This is illustrated at the local-scale by the replacement of A1 by A4–B4 in the Montpellier area, and by the invasion of A2–B2 on a worldwide basis. Which allele will remain (and at which amplification level) is still uncertain, although it seems apparent that selection is sorting alleles with a minimum fitness cost and with a low or intermediate OP resistance.

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REFERENCES

Bourguet, D., Capela, R. & Raymond, M. 1996 An insensitive acetylcholinesterase in *Culex pipiens* L. mosquitoes from Portugal. *J. Econ. Entomol*. **89**, 1060–1066.

Bourguet, D., Lenormand, T., Guillemaud, T., Marcel, V. & Raymond, M. 1997 Variation of dominance of newly arisen adaptive genes. *Genetics* **147**, 1225–1234.

Callaghan, A., Guillemaud, T., Makate, N. & Raymond, M. 1998 Polymorphism and fluctuations in copy number of amplified esterase genes in *Culex pipiens* mosquitoes. *Insect Molec. Biol*. **7**, 295–300.

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

Curtis, C. F. & White, G. B. 1984 *Plasmodium falciparum* transmission in England: entomological data relative to cases in 1983. *J. Trop. Med. Hyg*. **87**, 101–194.

Fournier, D., Bride, J.-M., Mouchès, C., Raymond, M., Magnin, M., Bergé, J.-B., Pasteur, N. & Georghiou, G. P. 1987 Biochemical characterization of the esterase A1 and B1 associated with organophosphate resistance in the *Culex pipiens* L. complex. *Pestic. Biochem. Physiol*. **27**, 211–217.

Georghiou, G. P. 1992 World distribution of esterases involved in organophosphate insecticide resistance in *Culex* mosquitoes, and methods for detection. In *Insecticides: mechanism of action and resistance* (ed. D. Otto & B. Weber), pp. 407–408. Andover: Intercept.

Guillemaud, T., Rooker, S., Pasteur, N. & Raymond, M. 1996 Testing the unique amplification event and the worldwide migration hypothesis of insecticide resistance genes with sequence data. *Heredity* **77**, 535–543.

Guillemaud, T., Makate, N., Raymond, M., Hirst, B. & Callaghan, A. 1997 Esterase gene amplification in *Culex pipiens*. *Insect Molec. Biol*. **6**, 319–327.

Guillemaud, T., Lenormand, T., Bourguet, D., Chevillon, C., Pasteur, N. & Raymond, M. 1998 Evolution of resistance in *Culex pipiens*: allele replacement and changing environment. *Evolution* **52**, 430–440.

Highton, R. B. & Van Someren, C. C. 1970 The transportation of mosquitoes between international airports. *Bull. Wld Hlth Org*. **42**, 334–335.

Mouchès, C., Pasteur, N., Bergé, J. B., Hyrien, O., Raymond, M., Robert de Saint Vincent, B., De Silvestri, M. & Georghiou, G. P. 1986 Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. *Science* **233**, 778–780.

Mouchès, C., Magnin, M., Bergé, J.-B., De Silvestri, M., Beyssat, V., Pasteur, N. & Georghiou, G. P. 1987 Overproduction of detoxifying esterases in organophosphate-resistant *Culex* mosquitoes and their presence in other insects. *Proc. Natn. Acad. Sci. USA* **84**, 2113–2116.

Pasteur, N. & Sinègre, G. 1975 Esterase polymorphism and sensitivity to Dursban organophosphate insecticide in *Culex pipiens pipiens* populations. *Biochem. Genet*. **13**, 789–803.

Pasteur, N., Iseki, A. & Georghiou, G. P. 1981 Genetic and biochemical studies of the highly active esterases A′ and B associated with organophosphate resistance in mosquitoes of the *Culex pipiens* complex. *Biochem. Genet*. **19**, 909–919.

Pasteur, N., Georghiou, G. P. & Iseki, A. 1984 Variation in organophosphate resistance and esterase activity in *Culex quinquefasciatus* Say from California. *Génét. Sélect. Evol*. **16**, 271–284.

Pasteur, N., Marquine, M., Rousset, F., Failloux, A.-B., Chevillon, C. & Raymond, M. 1995 The role of passive migration in the dispersal of resistance genes in *Culex pipiens quinquefasciatus* within French Polynesia. *Genet. Res.* **66**, 139–146.

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

Qiao, C.-L. & Raymond, M. 1995 The same esterase B1 haplotype is amplified in insecticide resistant mosquitoes of the *Culex pipiens* complex from the Americas and China. *Heredity* **74**, 339–345.

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

Raymond, M., Fournier, D., Bride, J.-M., Cuany, A., Bergé, J., Magnin, M. & Pasteur, N. 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., Beyssat-Arnaouty, V., Sivasubramanian, N., Mouchès, C., Georghiou, G. P. & Pasteur, N. 1989 Amplification of various esterase B's responsible for organophosphate resistance in *Culex* mosquitoes. *Biochem. Genet.* **27**, 417–423.

Raymond, M., Callaghan, A., Fort, P. & Pasteur, N. 1991 Worldwide migration of amplified insecticide resistance genes in mosquitoes. *Nature* **350**, 151–153.

Raymond, M., Qiao, C. L. & Callaghan, A. 1996 Esterase polymorphism in insecticide susceptible populations of the mosquito *Culex pipiens*. *Genet. Res.* **67**, 19–26.

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

Rooker, S., Guillemaud, T., Bergé, J., Pasteur, N. & Raymond, M. 1996 Coamplification of esterase A and B genes as a single unit in the mosquito *Culex pipiens*. *Heredity* **77**, 555–561.

Severini, C., Marinucci, M. & Raymond, M. 1994 Insecticide resistance genes in *Culex pipiens* (Diptera: Culicidae) from Italy: esterase B locus at the DNA level. *J. Med. Entomol.* **31**, 496–499.

Severini, C., Romi, R., Marinucci, M., Guillemaud, T. & Raymond, M. 1997 First record of A5-B5 esterases in an organophosphate-resistant field population of *Culex pipiens* from Italy. *Med. Vet. Entomol.* **11**, 123–126.

Vaughan, A. & Hemingway, J. 1995 Mosquito carboxylesterase Esta21 (A2). *J. Biol. Chem.* **270**, 17 044–17 049.

Vaughan, A., Rodriguez, M. & Hemingway, J. 1995 The independent gene amplification of electrophoretically indistinguishable B esterases from the insecticide-resistant mosquito *Culex quinquefasciatus*. *Biochem. J.* **305**, 651–658.

Vaughan, A., Hawkes, N. & Hemingway, J. 1997 Co-amplification explains linkage disequilibrium of two mosquito esterase genes in insecticide-resistant *Culex quinquefasciatus*. *Biochem. J.* **325**, 359–365.

Wirth, M., Marquine, M., Georghiou, G. P. & Pasteur, N. 1990 Esterase A2 and B2 in *Culex quiquefasciatus* (Diptera: Culicidae): role in organophosphate resistance and linkage. *J. Econ. Entomol.* **27**, 202–206.

Xu, J., Qu, F. & Liu, W. 1994 Diversity of amplified esterase B genes responsible for organophosphate resistance in *Culex quinquefasciatus* from China. *J. Med. Coll. People's Liberation Army* **9**, 20–23.