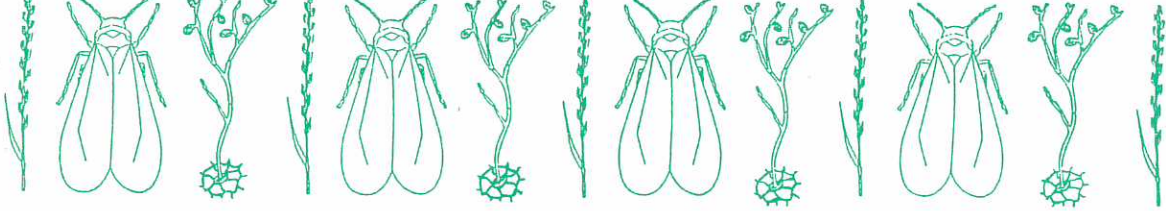

RESISTANCE 91

ACHIEVEMENTS AND DEVELOPMENTS IN COMBATING PESTICIDE RESISTANCE

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ROLE OF MUTATION AND MIGRATION IN THE EVOLUTION OF INSECTICIDE RESISTANCE IN THE MOSQUITO *CULEX PIPIENS*

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ABSTRACT

The well documented history of organophosphorous (OP) insecticide resistance in two heavily controlled areas, southern France and Corsica, indicates that mutation is a rare event and a limiting step for generating new resistance alleles in a given area. In contrast, the role of migration in the evolution of insecticide resistance has been underestimated, as illustrated by resistant overproduced esterases A1 or A2-B2 which have a unique origin and are, respectively, distributed over the whole Mediterranean area or Africa, North America and Asia.

INTRODUCTION

The evolution of insecticide resistance in insect populations is dependent on the existence and the incidence of resistance genes. Two forces -mutation and migration- are responsible for the occurrence and distribution of resistance genes within and between pest populations. Mutation, mediated through events such as nucleotide substitution, insertion, deletion, gene duplication or amplification etc., is ultimately responsible for generating resistant variants within a species. In any particular population, however, the presence of resistance genes can result not only from mutation *in situ*, but also from the immigration of individuals possessing these genes, either through active dispersal or by the passive transport of insects, usually by human agency.

In this paper we explore the relative contributions of mutation and migration to

the evolution of organophosphorus (OP) resistance in the *Culex pipiens* complex. On the basis of monitoring data from many parts of the world we argue that mutations causing resistance are very rare events that can severely limit the capacity of populations to respond to insecticide selection. In contrast, there is now evidence that migration, in some cases at least, has been a powerful force promoting the geographical spread of resistance genes.

MUTATION IS A RARE EVENT

Evidence for mutation being a rate-limiting step in the evolution of resistance comes from two cases with a carefully documented history of insecticide treatment and a continuous record of resistance monitoring based on toxicological and biochemical assays.

Resistance to chlorpyrifos in southern France

In 1969, a control program involving only chlorpyrifos was initiated along the French Mediterranean coast with an organisation capable of treating up to 20,000 larval breeding sites of *C. pipiens*. Resistance to chlorpyrifos was first detected by routine monitoring three years later in the Montpellier area (1). This *ca.* 10-fold resistance was initially restricted to a single village, but subsequently spread (2, 3) and was present throughout the treated area in 1978 (4). This general and low level of resistance did not greatly reduce control efficacy, and no increase in dose or application frequency was apparently needed (1).

In 1974, the putative biochemical mechanism responsible for this resistance was identified as an overproduced esterase first termed A' (5, 6) and then A1 (7), coded by the *Est-3*^{A} allele. Susceptible mosquitoes possessed this esterase or another allozyme in an amount undetectable by starch gel electrophoresis, while resistant insects overproduced A1 at a level of up to 3% of the total body protein (4, 8, 9). The strict correlation between resistance and the presence of A1 in various natural populations in 1974 and 1975 implied that this esterase was the sole significant resistance mechanism in France during this period (2, 4, 10).

In 1978, chlorpyrifos resistance increased to a level of 100-fold, an event that was subsequently attributed to the appearance of a second resistance mechanism: target-site insensitivity mediated through altered acetylcholinesterase (11-13). The allele responsible (termed *Ace*^{R}) was initially localised on the coast of Hérault department in 1978 (4) but subsequently increased in frequency and geographical range, impairing the efficacy of chlorpyrifos to the extent that other insecticides were

needed to regain adequate control. Consequently, temephos and fenitrothion were introduced into the control program from 1981 onwards.

Since Ace^R confers considerably higher resistance to chlorpyrifos than A1 (100 fold for Ace^R *vs* 3-10 fold for A1, (12)), its presence prior to 1978 should have been detectable through resistance monitoring or from localised control difficulties. The obvious conclusion is that the appearance of Ace^R was delayed well beyond the period in which it could potentially have been selected in field populations. Whether its eventual appearance resulted from mutation within the treated area or by migration of insects from other localities is not known, but in either case it seems that the rarity of the mutation needed to generate Ace^R from its wildtype homologue (Ace^S) was a primary constraint on the selection of this more powerful mechanism of chlorpyrifos resistance.

In contrast, the rapid selection of A1 resistance, within three years of the start of chlorpyrifos treatments, implies either that this mechanism was already present at low frequencies or arose through mutation or migration very early during the control program. Again, there is evidence that the mutation leading to overproduction of A1 was a rare, possibly unique, event and that its subsequent spread was a consequence of gene flow rather than several homologous mutations. Wherever it has been studied, the A1 mechanism is in strong linkage disequilibrium with an unamplified allele ($Est\text{-}2^{0.64}$) at another esterase locus (*Est-2*, later named esterase B) that is seemingly unconnected with OP resistance (14). The close association of A1 and a particular allele at the highly polymorphic *Est-2* locus is only readily explicable if the mutation causing A1 overproduction occurred in a single locality (possibly in the Montpellier area, see above), the association A1 - $Est\text{-}2^{0.64}$ being subsequently enhanced by a hitch-hiking effect (15).

Resistance to temephos in Corsica

A control program was initiated in Corsica in 1973, involving weekly applications of temephos during the mosquito breeding season. Yet despite this selection pressure being sustained over 15 years, the maximum level of resistance documented by a survey in 1988 was only *ca.* 14-fold, and this did not significantly impair the effectiveness of the control treatments.

The relatively low resistance to temephos, apparently involving the increased production of several esterases (including A1, see below), contrasts with that documented elsewhere in the world, particularly in California where a very powerful mechanism conferring ca. 800-fold resistance to temephos occurred in 1974 (16). If present in Corsica, the Californian mechanism (the overproduction of a different esterase termed B1 (8, 9)), would be expected to have given a substantial selective

advantage in populations intensively exposed to temephos. Once again, the non-appearance of B1 resistance in Corsica between 1973 and 1988 indicates that neither mutation nor migration occurred to generate this mechanism *de novo* or to transport it into Corsican populations.

MIGRATION CAN BE A FREQUENT EVENT

Discussions of the impact of migration on resistance development have focussed on the role of immigration of susceptible insects as a factor retarding selection of resistance in treated populations (*e.g.* 17, 18). In comparison, the importance of gene flow for transferring alleles for resistance into susceptible populations has received little attention (but see 19). For *C. pipiens*, however, there appear to be at least two good examples of migration not merely promoting the evolution of resistance but being responsible for the spread of particular alleles between adjacent countries and even between continents.

Esterase A1 in the Mediterranean

Following 15 years of temephos usage in Corsica, several putative resistance genes have been detected at high frequencies in *C. pipiens* populations, including the overproduced esterase A1 (20). Two lines of evidence suggest that the presence in Corsica of A1, at least, is a consequence of one or more migration events. Firstly, overproduction of A1 was initially recognised as a resistance mechanism in the Montpellier area in 1972, and was already spreading through southern France when temephos treatment began in Corsica. Secondly, A1 resistance in Corsica, as in southern France, is in strong linkage disequilibrium with the *Est-2*$^{0.64}$ allele (20). Both these observations support a hypothesis that A1 resistance arose only once and was subsequently introduced into Corsica by mosquitoes migrating from mainland France.

A1 resistance is now widely distributed in a contiguous region bordering the Mediterranean (Fig. 1), having been reported from Spain, France, Italy, Greece, Egypt and Tunisia (4,5, 21-23, and unpublished data). It is still unknown outside this region (e.g. tropical Africa, Asia, North America) despite extensive monitoring and widespread use of chemicals capable of selecting this mechanism to detectable frequencies. The apparent absence of A1 elsewhere in the world reinforces our previous conclusions regarding the rarity of mutations leading to overproduction of esterases. It is far more likely that the migration events already implicated to explain the introduction of A1 resistance into Corsica have also caused its appearance in

other Mediterranean countries and will, if unchecked, promote its spread outside this restricted geographical area.

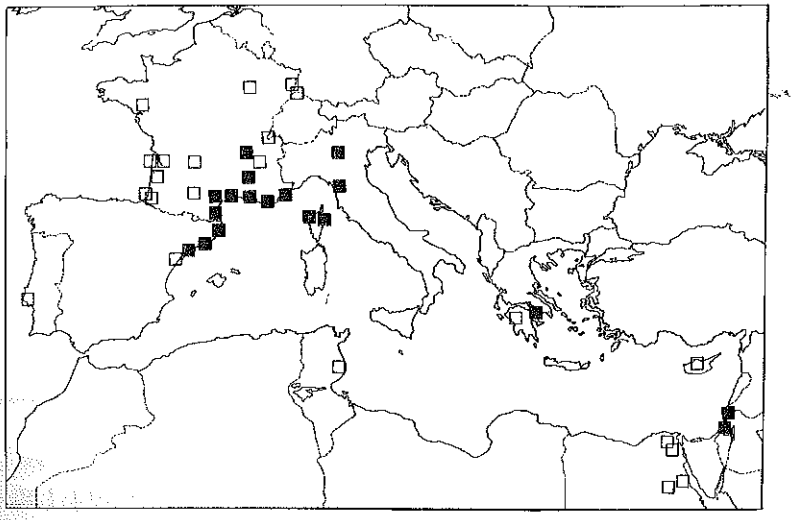

Figure 1. Distribution of A1 esterase in Mediterranean. Open squares indicate the absence of A1, and black ones indicate its presence. Data are from (4, 21, 23, 32, 33).

Esterases A2-B2 throughout the world

Proof that migration can disperse resistance genes over large geographical areas comes from work on two overproduced esterases (A2 and B2), generally associated, and implicated in OP resistance. This A2-B2 association has now been reported from at least four continents (Fig. 2), and may have appeared only within the last ten years in at least three localities: California, France and Italy (7, 21, 23).

Despite this wide distribution, recent mapping of the usually highly polymorphic esterase B region for six A2-B2 strains collected in Asia (Pakistan), Africa (Egypt, Congo, Ivory-coast) and North America (California, Texas), using 13 restriction enzymes, produced identical banding patterns (24). This finding indicates strongly that B2 (and A2) overproduction had a single origin and has subsequently been transported throughout the world. The source of the original mutation has been tentatively assigned to Africa or Asia, where the A2-B2 mechanism was first detected (25). This worldwide migration, almost certainly mediated through passive transport of mosquitoes on planes and boats (*e.g.* 26), has probably occurred within the last 30 years, *i.e.* subsequent to the widespread use of OP insecticides.

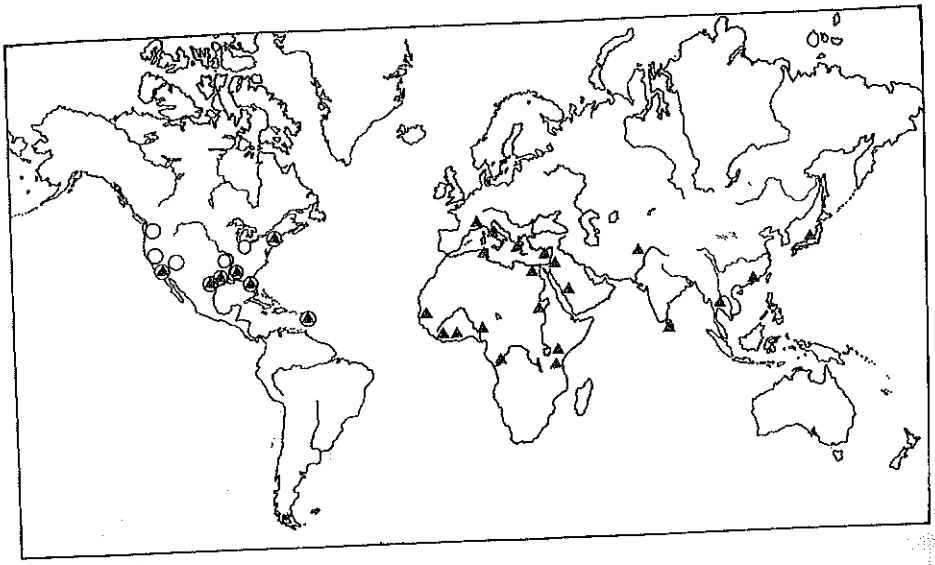

Figure 2. Known geographic distribution of overproduced esterase B1 ( ) and A2-B2 ( ) in the world, from (24).

CONCLUSIONS

The fact that mutation is a rare event is of no surprise, but that only a handful of efficient resistance genes have occurred in *Culex pipiens* since OP were used (*ca.* 30 years) indicates a limitation in the ability of this species to find adequate answers to the sudden presence of OP in its environment. This might be due to the nature of mutations leading to overproduce esterases through constitutive gene amplification (27- 29). If several independent steps, each with a low probability of occurrence, are required to generate an amplification, the combined probability, or overall mutation rate, could be vanishingly low.

Evidence of extensive migration within or between continents has been popularized among population geneticists with the now classical example of *Drosophila melanogaster* with transposable elements (*e.g.* P or I) which have invaded several continents from a single origin within the last few decades (*e.g.* 30), or with the repetitive north-south cline of the Adh^F gene (*e.g.* 31). It seems that OP insecticide resistance genes in *Culex pipiens* mosquitoes is another dramatic example of such extensive migration.

The occurrence of a new resistance gene in a treated population through a migration event has long been neglected as a significant phenomenon by people working on insecticide resistance. This was probably due to 1) an overestimate of mutation rate, leading to the conclusion that, as treated population sizes are generally large, resistant mutants will be produced within a few generations, so that migration has no dramatic effect on the evolution of resistance, and 2) an underestimate of migration rate, particularly long range passive migration by human activity.

In *Culex pipiens*, the evolution of OP resistance is more likely to be directed by migration events rather than by *de novo* mutations. It now remains to establish if this is the case for other pest species.

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