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INSECTICIDE RESISTANCE IN INSECT VECTORS OF HUMAN DISEASE

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■ **Abstract** Insecticide resistance is an increasing problem in many insect vectors of disease. Our knowledge of the basic mechanisms underlying resistance to commonly used insecticides is well established. Molecular techniques have recently allowed us to start and dissect most of these mechanisms at the DNA level. The next major challenge will be to use this molecular understanding of resistance to develop novel strategies with which we can truly manage resistance. State-of-the-art information on resistance in insect vectors of disease is reviewed in this context.

INTRODUCTION

Insecticides play a central role in controlling major vectors of diseases such as mosquitoes, sandflies, fleas, lice, tsetse flies, and triatomid bugs. In 1955 the World Health Organization (WHO) assembly proposed the global eradication of the most prevalent vector-borne human disease, malaria, by the use of residual house-spraying of DDT. However, the insecticide euphoria soon ended and in 1976 WHO officially reverted from malaria eradication to malaria control. This marked shift from malaria eradication to primary health care was an emotive issue, eliciting a rapid and complete change of rhetoric from WHO (12). Several issues had prompted this switch, but a major cause of the change in policy was the appearance of DDT resistance in a broad range of the mosquito vectors. In 1975 WHO reported that 256 million people were living in areas where DDT and/or BHC resistance was undermining malaria control efforts. (This did not include the African region, where 90% of malaria occurs and where DDT resistance had already been noted in *Anopheles gambiae,* the major malaria vector.)

The resistance problems continued with the switch to newer insecticides such as the organophosphates, carbamates and pyrethroids. Operationally, many control programs have switched from blanket spraying of house interiors to focal use of insecticides on bednets. Focal spraying limits the insecticides of choice largely to pyrethroids due to the speed of kill required to protect the occupant of the

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bednet and the safety margin needed for insecticides used in such close contact with people. Today the major emphasis in resistance research is on the molecular mechanisms of resistance and rational resistance management, with a view to controlling the development and spread of resistant vector populations. In Africa, WHO and the World Bank have instigated major new initiatives with other major donors and the scientific community internationally to ''roll back'' malaria. One major problem these initiatives are tackling is the presence of two developing foci of pyrethroid resistance in the most important African malaria vector, *An. gambiae*.

SCALE OF THE PROBLEM

The amount of resistance in insect vector populations is dependent both on the volume and frequency of applications of insecticides used against them and the inherent characteristics of the insect species involved. Tsetse flies, for example, were controlled by wide-scale spraying of DDT for many years, but DDT resistance has never developed in this species. Another example of an insect vector exhibiting little or no resistance to insecticides is the triatomid bug. In both cases the major factor influencing insecticide resistance development is the life cycle of the insect pest, in particular the long life cycles for the bugs, and the production of very small numbers of young by the tsetses. In contrast, mosquitoes have all the characteristics suited to rapid resistance development, including short life cycles with abundant progeny.

Mosquito Resistance

The major mosquito vectors span the *Culex, Aedes,* and *Anopheles* genera. *Culex* are the major vectors of filariasis and Japanese encephalitis, *Aedes* of dengue and dengue hemorrhagic fever, and *Anopheles* of malaria. The range of many of these species is not static. For example, several *Aedes* species recently extended their range in Asia and Latin America, leading to an increased risk of dengue in these areas.

DDT was first introduced for mosquito control in 1946. In 1947 the first cases of DDT resistance occurred in *Aedes tritaeniorhynchus* and *Ae. solicitans* (15). Since then more than 100 mosquito species are reported as resistant to one or more insecticide, and more than 50 of these are anophelines (113). Insecticides used for malaria control have included -BHC, organophosphorus, carbamate, and pyrethroid insecticides, with the latter now taking increasing market share for both indoor residual spraying and large-scale insecticide-impregnated bednet programs. Other insecticide groups, such as the benzylphenyl ureas and Bti, have had limited use against mosquitoes. Resistance has tended to follow the switches of insecticides. Resistance to -BHC/dieldrin is widespread despite the lack of use of these insecticides for many years. Organophosphate (OP) resistance, either in

the form of broad-spectrum OP resistance or malathion-specific resistance, occurs in the major vectors *An. culicifacies* (59), *An. stephensi* (30, 44), *An. albimanus* (3, 51), *An. arabiensis* (45) and *An. sacharovi* (54). *An. culicifacies* is recognized as a species complex (101): In Sri Lanka malathion resistance occurs in *An. culicifacies* species B, while in India resistance is in species B and C (59, 88). Species B in Sri Lanka is resistant to fenitrothion, which is independent of the malathion-specific resistance (58, 60), and is developing pyrethroid resistance (SHPP Karunaratne, personal communication). Organophosphorus insecticide resistance is widespread in all the major *Culex* vectors (53), and pyrethroid resistance occurs in *C. quinquefasciatus* (1, 7, 19). Pyrethroid resistance has been noted in *An. albimanus* (13), *An. stephensi* (107) and *An. gambiae* (18, 20, 112) among others, while carbamate resistance is present in *An. sacharovi* and *An. albimanus* (57). Pyrethroid resistance is widespread in *Ae. aegypti* (6, 48, 70) and cases of OP and carbamate resistance have also been recorded in this species (72, 76).

The development of pyrethroid resistance in *An. gambiae* is particularly important given the recent emphasis by the WHO and other organizations on the use of pyrethroid-impregnated bednets for malaria control. Two cases of pyrethroid resistance in *An. gambiae*, from the Ivory Coast and Kenya, are well documented (18, 112). The west African focus appears to be larger and has higher levels of resistance than that in east Africa.

Sandfly Resistance

The peridomestic vectors of *Leishmania*, *Plebotomus papatasi*, *Lutzomyia longipalpis*, and *L. intermedia* are controlled primarily by insecticides throughout their range. The control of these sandflies is often a by-product of anti-malarial house-spraying. The only insecticide resistance reported to date in sandflies is to DDT in Indian *P. papatasi* (29).

Head and Body Louse Resistance

The body louse *Pediculus humanus* has developed widespread resistance to organochlorines (16), is malathion resistant in parts of Africa (113), and has low-level resistance to pyrethroids in several regions (35). Resistance to organochlorine insecticides, such as DDT and lindane, has been recorded in the human head louse *Pediculus capitis* in Israel, Canada, Denmark, and Malaysia (16, 73, 113). Permethrin has been extensively used for head louse control since the early 1980s (77, 103). The first reports of control failure with this insecticide were in the early 1990s in Israel (77), the Czech Republic (97), and France (21).

Simulium Resistance

Some cytospecies of the *Simulium damnosum* complex are vectors of onchocerciasis. In 1974 the Onchocerciasis Control Programme in west Africa established a long-term insecticide-based control program for this vector. Temephos resis-

tance occurred initially, prompting a switch to chlorphoxim, but resistance to this insecticide occurred within a year (49). Resistance in this species is currently being managed by a rotation of temephos, Bti, and permethrin, the insecticide usage being determined by the rate at which water is flowing in rivers forming the major breeding sites of these vectors.

THE BIOCHEMISTRY OF RESISTANCE

Insecticide Metabolism

Three major enzyme groups are responsible for metabolically based resistance to organochlorines, organophosphates, carbamates, and pyrethroids. DDT-dehydrochlorinase was first recognized as a glutathione S-transferase in the house fly, *Musca domestica* (23). It has been shown to have this role commonly in anopheline and *Aedes* mosquitoes (40, 85). Esterases are often involved in organophosphate, carbamate, and to a lesser extent, pyrethroid resistance. Monooxygenases are involved in the metabolism of pyrethroids, the activation and/or detoxication of organophosphorus insecticides and, to a lesser extent, carbamate resistance.

Esterase-Based Resistance

The esterase-based resistance mechanisms have been studied most extensively at the biochemical and molecular level in *Culex* mosquitoes and the aphid *Myzus persicae*. Work is in progress on related and distinct esterase resistance mechanisms in a range of *Anopheles* and *Aedes* species. Broad-spectrum organophosphate resistance is conferred by the elevated esterases of *Culex*. All these esterases act by rapidly binding and slowly turning over the insecticide: They sequester rather than rapidly metabolize the pesticide (62).

Two common esterase loci, *estα* and *estβ,* are involved alone or in combination in this type of resistance in *Culex* (109). In *C. quinquefasciatus* the most common elevated esterase phenotype involves two enzymes, est$\alpha 2^1$ and est$\beta 2^1$ (A_2 and B_2 on an earlier classification) (110). The classification of these esterases is based on their preferences for α- or β-naphthyl acetate, their mobility on native polyacrylamide gels, and their nucleotide sequence (53). Smaller numbers of *C. quinquefasciatus* populations have elevated estβ1 alone, elevated estα1 alone or co-elevated estβ1 and estα3 (27, 53). When purified estα and estβ from the insecticide-susceptible PelSS strain were compared to various enzymes purified from resistant strains, up to 1000-fold differences among the inhibition-kinetic constants occurred for the oxon analogues of various OPs (63).

The superiority of insecticide binding in enzymes from the resistant strains suggests that there has been positive insecticide selection pressure to maintain elevation of favorable alleles of the esterases in insecticide-resistant insects. Although there are minor variations between the inhibition kinetics of the different elevated alleles, the reason why the est$\alpha 2^1$/est$\beta 2^1$ phenotype is so common

(in more than 90% of resistant populations) compared to the other elevated esterase phenotypes is not obvious. This advantage may be linked to a third gene, which is co-elevated with esterases estα2/estβ2 but not with the other esterase phenotypes (50).

Metabolic studies on *Culex* homogenates suggests that increased rates of esterase-mediated metabolism plays little or no role in resistance. One exception to this is *C. tarsalis,* where two resistance mechanisms co-exist: one involving elevated sequestering esterases, the other involving non-elevated metabolically active esterases (120). In contrast to the situation in *Culex,* a number of *Anopheles* species have a non-elevated esterase mechanism that confers resistance specifically to malathion through increased rates of metabolism (44–46) (11, 68). In *An. stephensi* three esterases with malathion carboxylesterase activity have been isolated and characterized (47, 52).

Glutathione S-Transferase-Based Resistance

Many studies have shown that insecticide-resistant insects have elevated levels of glutathione S-transferase activity in crude homogenates, which suggests a role for GSTs in resistance (37, 38). GSTs are dimeric multifunctional enzymes that play a role in detoxification of a large range of xenobiotics (86). The enzymes catalyze the nucleophilic attack of reduced glutathione (GSH) on the electrophilic centers of lipophilic compounds. Multiple forms of these enzymes have been reported for mosquitoes, house fly, *Drosophila*, sheep blow fly, and grass grub (22, 24, 105).

Two families of insect GST are recognized, and both appear to have a role in insecticide resistance in insects. In *Ae. aegypti* at least two GSTs are elevated in DDT-resistant insects (39, 41), while in *An. gambiae* a large number of different GSTs are elevated, some of which are class I GSTs (84, 85). The *Ae. aegypti* and *An. gambiae* GSTs in resistant insects are constitutively over-expressed. The GST-2 of *Ae. aegypti* is over-expressed in all tissues except the ovaries of resistant insects (39).

Monooxygenase-Based Resistance

The monooxygenases are a complex family of enzymes found in most organisms, including insects. These enzymes are involved in the metabolism of xenobiotics and have a role in endogenous metabolism. The P450 monooxygenase are generally the rate-limiting enzyme step in the chain. These enzymes are important in adaptation of insects to toxic chemicals in their host plants. P450 monooxygenases are involved in the metabolism of virtually all insecticides, leading to activation of the molecule in the case of organophosphorus insecticides, or more generally to detoxification. P450 enzymes bind molecular oxygen and receive electrons from NADPH to introduce an oxygen molecule into the substrate.

Elevated monooxygenase activity is associated with pyrethroid resistance in *An. stephensi, An. subpictus, An. gambiae* (14, 55, 112), and *C. quinquefasciatus*

(65). Currently this enzyme system is poorly studied in insect vectors of disease. The nomenclature of the P450 superfamily is based on amino acid sequence homologies, with all families having the CYP prefix followed by a numeral for the family, a letter for the subfamily, and a numeral for the individual gene. To date insect P450s have been assigned to six families: five are insect-specific and one, CYP4, has sequence homologies with families in other organisms (8).

Target-Site Resistance

The organophosphorus, carbamates, organochlorine, and pyrethroid insecticides all target the nervous system. Newer classes of insecticides are available for vector control, but the high cost of developing and registering new insecticides inevitably means that insecticides are developed initially for the agricultural market and then utilized for public health vector control, where their activities and safety profile are appropriate and where the market is sufficiently large to warrant the registration costs for public health use. Compounds targeting the nicotinic acetylcholine receptor have recently made this transition from agriculture into public health.

Acetylcholinesterase

The organophosphates and carbamates target acetylcholinesterase (AChE). AChE hydrolyzes the excitatory neurotransmitter acetylcholine on the post-synaptic nerve membrane. Insect AChE has a substrate specificity intermediate between vertebrate AChE and butyrylcholinesterase. The predominant molecular form in insects is a globular amphiphilic dimer which is membrane-bound via a glycolipid anchor. Alterations in AChE in organophosphate-and carbamate-resistant insects result in a decreased sensitivity to inhibition of the enzyme by these insecticides (5, 51). The organophosphorus insecticides are converted to their oxon analogues via the action of monooxygenases before acting as AChE inhibitors. In *C. pipiens,* AChE1 and AChE2 differ in their substrate specificity, inhibitor sensitivity, and electrophoretic migration pattern (69). Only AChE1 appears to be involved in conferring insecticide resistance.

GABA Receptors

Resistance to dieldrin was recorded in the 1950s, but the involvement of the GABA receptors in this resistance was not elucidated until the 1990s. The GABA receptor in insects is a heteromultimeric gated chloride-ion channel, a widespread inhibitory neurotransmission channel in the insect's central nervous system and in neuromuscular junctions (9). The insect GABA receptor is implicated as a site of action for pyrethroids and avermectins as well as cyclodienes. Studies showing that cyclodiene-resistant insects are resistant to picrotoxin and phenylpyrazole insecticides, and that the effect of ivermectin on cultured neurons can be reversed by picrotoxin pretreatment, suggest that these insecticides exert their effect by

interacting with the chloride ionophore associated with the insect GABA receptor (10, 62).

Sodium Channels

The pharmacological effect of DDT and pyrethroids is to cause persistent activation of the sodium channels by delaying the normal voltage-dependent mechanism of inactivation (100). Insensitivity of the sodium channels to insecticide inhibition was first recorded in *Musca domestica* (32). In mosquitoes there have been many reports of suspected ''kdr''-like resistance inferred from cross resistance between DDT and pyrethroids, which act on the same site within the sodium channel. These reports have been validated by electrophysiological measurements in *Ae.* aegypti and *An. stephensi* (48, 107).

THE MOLECULAR BIOLOGY OF RESISTANCE

Metabolic Mechanisms

Over-expression of enzymes capable of detoxifying insecticides or amino acid substitutions within these enzymes, which alter the affinity of the enzyme for the insecticide, can result in high levels of insecticide resistance. Increased expression of the genes encoding the major xenobiotic metabolizing enzymes are the most common cause of insecticide resistance in mosquitoes. These large enzyme families may contain multiple enzymes with broad overlapping substrate specificities, and there is a high probability that at least one member of the family will be capable of metabolizing one or more insecticides. Increased production of these enzymes may have a lower associated fitness cost than those associated with alterations in the structural genes because the primary function of the enzyme is not disrupted.

Mutations in Structural Genes

In many cases of resistance caused by increased metabolism of the insecticide the exact genetic mechanism is not known. As yet no validated reports exist of mutations within detoxifying enzymes leading to resistance to insecticides in disease vectors. Two examples have been reported in non-vector species: because both of these mechanisms may be present in disease vectors, we describe them here.

Resistance to the organophosphate insecticide malathion is caused by a single amino acid substitution (Trp^{251}-Leu) within the E3 esterase of the sheep blow fly, *Lucilia cuprina* (17). Malathion-resistant strains of *L. cuprina* have very low levels of activity with aliphatic esters that are conventionally used as stains for esterase activity (80). A similar phenotype has been observed in malathion-resistant strains of *An. stephensi*, *An. arabiensis*, and *An. culicifacies*. At least three enzymes are able to metabolize malathion in *An. stephensi* but it is not yet

known whether a point mutation similar to that described in *Lucilia* is responsible for malathion resistance in *Anopheles* (51).

A second distinct amino acid substitution (Gly^{137}-Asp) within the active site of the *Lucilia* E3 isozyme confers broad cross resistance to many organophosphorus insecticides but not to malathion (79). This same mutation is present in OP-resistant strains of house fly.

Gene Amplification

The metabolic resistance mechanism studied in most detail in insect disease vectors is the elevated esterase-based system in *Culex* (110, 111). In the three *Culex* species studied to date at the molecular level the homologous *estβ* gene is amplified in resistant insects (64, 111, 114, 115). Insecticide resistance via amplification of genes involved in their detoxification is common in several insects. The most common amplified esterase-based mechanism in *Culex* involves the co-amplification of two esterases, *estα2*[1] and *estβ2*[1] in *C. quinquefasciatus* and other members of the *C. pipiens* complex worldwide (109). Other strains of *C. quinquefasciatus* have *estα3* and *estβ1* co-amplified (27), while the TEM-R strain has amplified *estβ1* alone (75). Similarly, *C. tritaeniorhynchus* has a single amplified *est*β, *Ctrest*β*1* (64).

The *estα* and *estβ* genes have arisen as the result of a gene duplication event, which must have occurred prior to speciation within the *Culex* genus. The genes occur in a head-to-head arrangement approximately 1.7 Kb apart in susceptible insects (109). In resistant insects with *est*α2^1/*est*β2^1 the amplified genes are 2.7 Kb apart, the difference being accounted for by expansion with three indels in the intergenic spacer (109). The indels may have introduced further regulatory elements to the amplicon intergenic spacer (52).

The identical RFLP patterns of the *estα2*[1] and *estβ2*[1] loci in resistant *C. pipiens* complex populations worldwide suggest that the amplification of these alleles occurred once and has since spread by migration (92). Other alleles of the esterase A and B loci are amplified in some *Culex* strains. For example, a strain of *C. pipiens* from Cyprus has an estimated 40 to 60 copies of the *estα5*[1] and *estβ5*[1] genes (42) whereas in the TEM-R strain from California amplification is found only at the B locus. The chromosomal region containing these esterase genes presumably represents an amplification hot spot, a theory supported by the amplification of the homologous esterase B gene in a distinct *Culex* species, *C. tritaeniorhynchus* (64).

An extensive study examining the spread and fitness of insects containing different esterase amplicons has been undertaken in southern France (93).

Transcriptional Regulation Esterases

Elevated levels of esterases may not always be the result of gene amplification. The expression of estα1 in the Barriol strain of *C. pipiens* from southern France is thought to be increased due to changes in an unidentified regulatory element

rather than underlying amplification of the estα gene (42). Amplified esterases can also be expressed at different levels. For example, there is fourfold more estβ than estα in resistant *C. quinquefasciatus,* although the genes are present in a 1:1 ratio. This difference in expression is reflected at the protein and mRNA level (63, 81a).

Glutathione S-Transferases

The primary role of GSTs in mosquito insecticide resistance is in the metabolism of DDT to nontoxic products, although they also have a secondary role in organophosphate resistance (54). GST-based DDT resistance is common in a number of anopheline species, reflecting the heavy use of this insecticide for malaria control over several decades. Molecular characterization of GSTs is most developed in *An. gambiae,* although work on *An. dirus* from Thailand suggests that a similar arrangement of GSTs occurs. However, there is evidence for more limited allelic diversity in this species (52, 86, 87).

Two classes of insect GSTs have been recognized and members of both classes are important in the metabolism of insecticides in mosquitoes and other insects. The sequence of a single class II *An. gambiae* GST has been published (94), and we have cloned and sequenced the major class II GST from *Ae. aegypti* (D Grant, M Wajidi, H Ranson, and J Hemingway, unpublished data). This *Aedes* GST, GST-2, is over-expressed in the DDT resistant GG strain of *Ae. aegypti*. In this species the resistance mutation is thought to lead to disruption of a transacting repressor. The mutation prevents the normal function of the repressor leading to elevated levels of GST-2 enzyme in resistant mosquitoes (39).

The insect class I GSTs are encoded by a large gene family in *An. gambiae*, *M. domestica*, and *D. melanogaster.* The genomic organization of this GST class in these three insect species is strikingly different (89). In *D. melanogaster* eight divergent intronless genes are found within a 14 Kb DNA segment (106). In *An. gambiae* multiple class I GST genes are also clustered in a single location. At least one member of this family is intronless (91) but other class I GSTs in *An. gambiae* contain one or more introns (Figure 1). One of these genes, aggst1α, is alternatively spliced to produce four distinct mRNA transcripts, each of which shares a common 5′ exon with a different 3′ exon (89). The products of these spliced genes differ in their ability to metabolize DDT (91) and some of these metabolically active GSTs are upregulated in resistant mosquitoes (90).

The organization of this class I GST gene family in insecticide-resistant and insecticide-susceptible *An. gambiae* is very similar. Hence the actual GST-based resistance mechanism is probably caused by a *trans*-acting regulator. The development of a fine-scale microsatellite map (119) and bacterial artificial chromosome (BAC) library for *An. gambiae* have now made this species amenable to a positional cloning approach. Such an approach is being used to define the regulator responsible for this GST resistance.

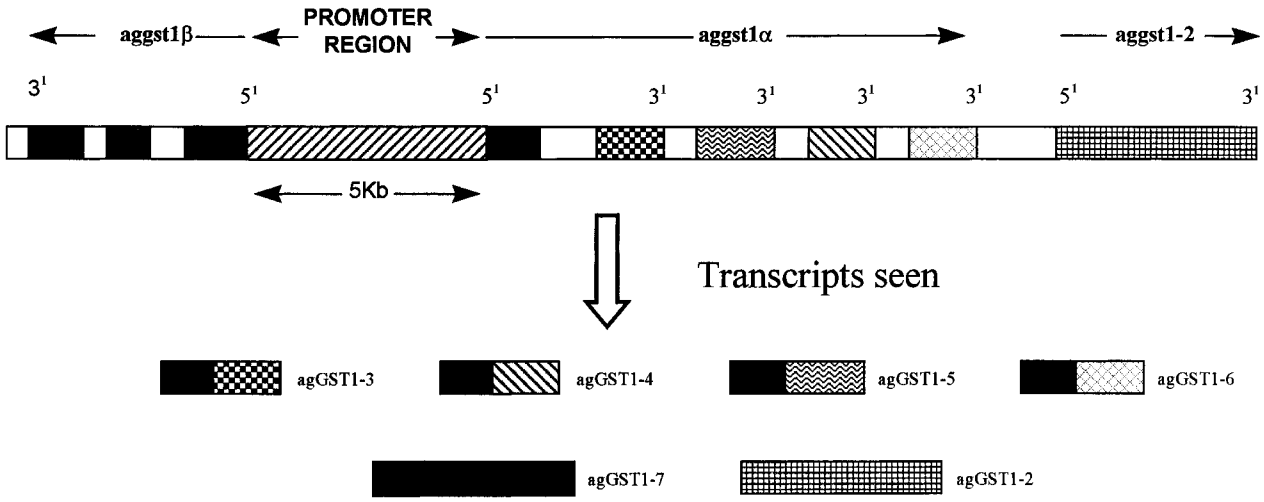

Figure 1 The class I glutathione S-transferase family of *Anopheles gambiae*, which includes an intronless gene *aggst1-2*, an alternatively spliced gene *aggst1*α with four distinct viable transcripts, and the *aggst1*β gene with two introns.

Monooxygenases

Insect microsomal P450 monooxygenases belong to six families. Increased transcription of genes belonging to the CYP4, CYP6, and CYP9 families has been observed in insecticide-resistant strains in different insect species. As yet, it is not known which enzymes are responsible for insecticide metabolism in mosquitoes. Seventeen partial cDNAs encoding CYP4 P450s have been identified in *An. albimanus* (98) and a similar level of diversity is present in *An. gambiae* (H Ranson & F Collins, unpublished) but the role, if any, that this family plays in resistance in the mosquito is not known. Studies on the Australian cotton bollworm, *Helicoverpa armigera,* show that the resistance-associated P450s can vary between different strains; CYP6B2 is over-expressed in one pyrethroid-resistant strain (118) whereas in another, CYP4G8 is over-expressed (83). Increased expression of CYP9A1, a member of a third family, is found in the related species *Heliothis virescens* (95).

There is strong evidence to suggest that P450 monooxygenase-based resistance in *M. domestica* and *D. melanogaster* is mediated by mutations in *trans*-acting regulatory genes. CYP6A8 is highly expressed in the DDT-resistant 91-R strain of *D. melanogaster* but not detectable in the uninduced 91-C susceptible strain (28, 67). Hybrids between the two strains show low levels of expression, suggesting that the 91-C strain carries a repressor that suppresses transcription of CYP6A8. A mutation in this repressor is thought to be responsible for the high level of expression of CYP6A8 in 91-R. In the house fly, CYP6D1-mediated metabolic resistance to pyrethroids is controlled by a nearly completely dominant *cis*-factor on autosome 1 and an incompletely recessive *trans*-factor on autosome 2 (66).

Target-Site Resistance

Non-silent point mutations within structural genes are the most common cause of target-site resistance. For selection of the mutations to occur, the resultant amino acid change must reduce the binding of the insecticide without causing a loss of primary function of the target site. Therefore the number of possible amino acid substitutions is very limited. Hence, identical resistance-associated mutations are commonly found across highly diverged taxa. The degree to which function is impaired by the resistance mutation is reflected in the fitness of resistant individuals in the absence of insecticide selection. This fitness cost has important implications for the persistence of resistance in the field.

The main sodium and GABA channel genes in insects have been cloned and their sequences compared in resistant and susceptible insects. Acetylcholinesterase-based resistance has been well characterized in *Drosophila* (36), but the elucidation of this mechanism at the molecular level in mosquitoes has proved more difficult.

GABA Receptor Changes

The GABA receptors belong to a superfamily of neurotransmitter receptors that also includes the nicotinic acetylcholine receptors. These receptors are formed by the oligomerization of five subunits around a central transmitter-gated ion channel. Five different subunits have been cloned from vertebrates. To date only three subunits have been cloned from *Drosophila melanogaster*, but these do not fit readily into the vertebrate GABA subunit classification (61).

An alanine-to-serine substitution in the putative channel-lining domain of the GABA receptor confers resistance to cyclodienes such as dieldrin (γ- HCH) (34). The mutation was first identified in *Drosophila* but has since been shown to occur in a broad range of dieldrin-resistant insects, including *Ae. aegypti* (104). The only variation in resistant insects is that glycine rather than serine can sometimes be the substituted amino acid residue. Despite the widespread switch away from the use of cyclodiene insecticides for agricultural and public health use the resistance allele is still found at relatively high frequencies in insect field populations (4).

Sodium Channels

A reduction in the sensitivity of the insect's voltage-gated sodium channels to the binding of insecticides causes the resistance phenotype known as ''kdr.'' Changes associated with pyrethroid/DDT resistance in the sodium channels of insects are more variable than those seen in the GABA receptors but still appear to be limited to a small number of regions on this large channel protein.

The para sodium channel of houseflies contains 2108 amino acids, which fold into 4 hydrophobic repeat domains (I-IV) separated by hydrophilic linkers. The first mutation to be characterized in *kdr* insects was a leucine to phenylalanine point mutation in the S6 transmembrane segment of domain II in the sodium channel sequence of *M. domestica* (116, 117) which produces 10- to 20-fold resistance to DDT and pyrethroids. In ''*super-kdr*'' houseflies, this mutation also occurs with a second methionine to threonine substitution further upstream in the same domain, resulting in more than 500-fold resistance (117). Analysis of the domain region of the *para*-sodium channel gene in pyrethroid-resistant *An. gambiae* from the Ivory Coast showed an identical Leu to Phe mutation in this species (71).

A PCR-based diagnostic test discriminates between homozygous-susceptible, homozygous-resistant, and heterozygous individuals with the Leu to Phe mutation (71). Because *kdr* is semi-recessive or fully recessive (81), the ability to detect heterozygotes is of paramount importance in the early detection and management of resistance in the field.

The limited number of changes associated with kdr-type resistance may be constrained by the number of modifications that can influence pyrethroid/DDT binding to the sodium channels. However, a note of caution should be added:

There is already a tendency to investigate pyrethroid-resistant insects with a PCR approach confined to regions where a *kdr* mutation has already been seen. Hence consistent resistance-associated changes in other parts of the sodium-channel gene could be missed. A different approach to isolating kdr-type mutants has been used in *Drosophila*, utilizing the relative ease with which large numbers of mutants in the para sodium channel gene can be isolated based on their temperature sensitivity. Two classes of these mutations are in positions equivalent to the *kdr* and *super-kdr* mutations in different domains. The third class is in a novel position (Figure 2) (34).

Acetylcholinesterase

Vertebrates have two cholinesterases: acetylcholinesterase and butyrylcholinesterase. In *D. melanogaster* only a single cholinesterase gene, *Ace*, coding for acetycholinesterase has been cloned, based on a knowledge of its location via

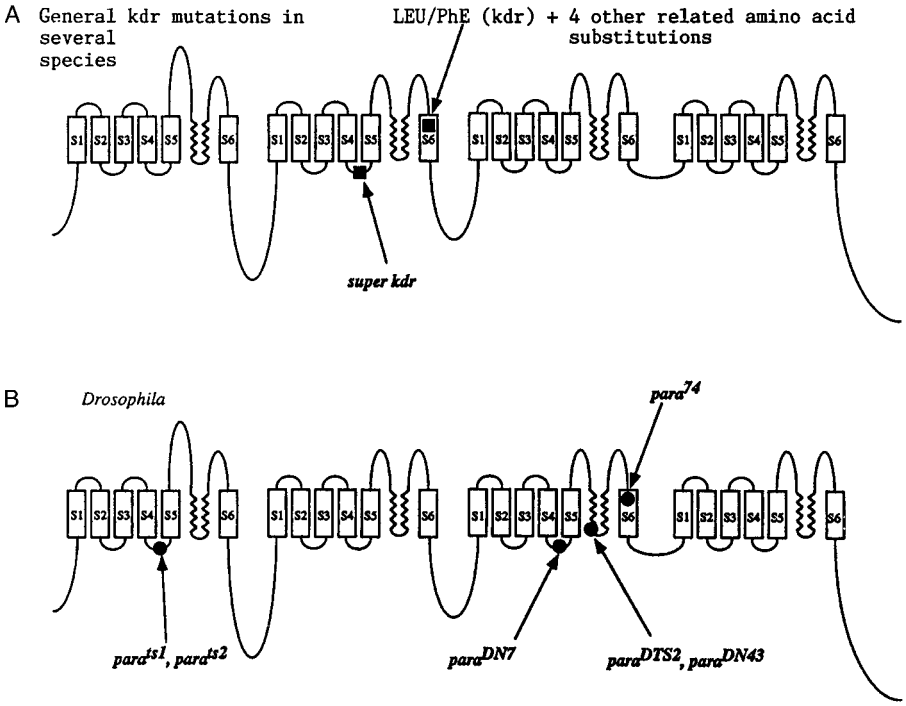

Figure 2 The location of amino acid substitutions occurring in the para-sodium channel gene of resistant strains of various insects. Mutations that have been documented in field strains of medical vectors are shown in *A*. *B* shows replacements in *Drosophila* isolated on the basis of temperature sensitivity paralytic phenotypes which confer DDT/pyrethroid resistance (adapted from 34).

isolation of a range of different mutants (43). A range of different amino acid substitutions in the *Ace* genes of *Drosophila* and the house fly *M. domestica* putatively cause resistance (reviewed in 33). Many of these resistance-associated residues are predicted to lie close to or within the acetylcholinesterase active site gorge (78). In *C. pipiens* at least two cholinesterase genes occur, both with acetylcholinesterase-like activity (69). A similar organization occurs in *Amphioxus*. To date the only acetylcholinesterase to have been cloned in *C. pipiens* is AChE2, which is not involved in insecticide resistance (69). The AChE2 gene is sex linked, unlike the resistance-conferring AChE1, which is autosomal. AChE genes have been cloned from the mosquitoes *Ae. aegypti* and *An. stephensi*, but both of these genes are also sex linked (2, 99).

As yet, there is no recorded AChE-based resistance mechanism in *An. stephensi*, but to date none of the acetylcholinesterase resistances recorded in insects have been sex linked, suggesting that these genes do not represent the insecticidal target site. However, detailed analysis of inhibition profiles for acetylcholinesterase from *Ae. aegypti* suggests that there is only one AChE locus in this species. If such is the case, altered acetylcholinesterase-based resistance in this species should be sex linked. At least one case of altered AChE has been reported in *Ae. aegypti* from Trinidad (108).

Five point mutations associated with resistance to organophosphorus and carbamate insecticides have been identified within the *D. melanogaster* acetylcholinesterase gene (78) and site-directed mutagenesis of the sex-linked AChE from *Ae. aegypti* has demonstrated that these same mutations also confer resistance in the mosquito enzyme (74). However, none of these mutations have been identified in field-collected or laboratory-selected strains of mosquitoes.

Insensitive AChE has a severe fitness cost in *C. pipiens* populations in southern France (31), which is probably caused by a reduction in the AChE activity of the mutated enzyme compared to the wild type.

MANAGEMENT OF INSECTICIDE-RESISTANT VECTOR POPULATIONS

The practice of using an insecticide until resistance becomes a limiting factor is rapidly eroding the number of suitable insecticides for vector control. Rotations, mosaics, and mixtures have all been proposed as resistance management tools (25, 26, 96). Numerous mathematical models have been produced to estimate how these tools should be optimally used (102). However, these models have rarely been tested under field conditions for insect vectors, due to the practical difficulties in estimating changes in resistance gene frequencies in large samples of insects (56). With the advent of different biochemical and molecular techniques for resistance-gene frequency estimation, field trials of resistance management strategies have become more feasible.

A large-scale trial of the use of rotations or mosaics of insecticides compared to single use of DDT or a pyrethroid is currently underway in Mexico (56, 82). Changes in resistance gene frequencies in *An. albimanus* are being monitored over a four-year period (82). Information resulting from such large-scale trials may allow us to establish rational strategies for long-term insecticide use in disease control programs.

As our ability to manipulate the insect genome improves and our understanding of the regulation of insecticide resistance mechanisms increase, new strategies should be devised for incorporating this new knowledge into these control programs.

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