THE MOLECULAR GENETICS OF RESISTANCE: RESISTANCE AS A RESPONSE TO STRESS

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ABSTRACT

In this overview of the molecular genetics of resistance, pesticides are regarded as one of the many environmental stresses against which insects must defend themselves to survive. Examined at the genetic level, pesticide resistance appears to be a preadapted response to stress and not due to novel mutations caused by pesticide exposure. The genetic mutations—gene amplification, altered gene regulation, structural alteration of a gene—which result in resistance are described and explained and a possible distribution mechanism of resistance genes is considered. Resistance mechanisms, their associated biological processes and the types of genetic mutations associated with each are detailed. Finally, the potential of molecular technology for the development of novel methods to detect and monitor for resistance is examined and compared to more traditional technology.

Key Words: Pesticide resistance, molecular, mutation, genetic, adaptation, stress, insect.

RESUMEN

En esta revisión de la genética molecular de la resistencia, los pesticidas son considerados como uno de los muchos estréses ambientales de los cuales los insectos deben defenderse para sobrevivir. Examinada a nivel genético, la resistencia a los pesticidas parece ser una respuesta preadaptada al estrés y no debida a nuevas mutaciones causadas por la exposición a los pesticidas. Son descritas y explicadas las mutaciones genéticas-amplificación genética, regulación génica alterada, alteración estructural de un gen-que producen la resistencia, y es considerado un posible mecanismo de distribución de los genes de resistencia. Son detallados los mecanismos de resistencia, los procesos biológicos y los tipos de mutaciones asociados con cada uno de ellos. Finalmente, es examinado y comparado a la tecnología más tradicional el potencial de la tecnología molecular para el desarrollo de nuevos métodos de deteccción y monitoreo de la resistencia.

When we discuss pesticide resistance, we generally refer to our ability to control a pest, not the pest's ability to defend itself. Yet resistance is really a form of self-defense. To an insect, exposure to a pesticide is just one of the myriad of dangers which must be avoided in order to survive. In this sense, pesticide exposure may be described as an environmental stress and resistance as the overt expression of an insect's natural response to that stress. Insects have been confronted with lethal and nonlethal stresses for as long as they have existed. And, they have evolved effective defense mechanisms to deal with these stresses. Their potential to adapt and develop resistance to stress becomes most apparent when examined at the molecular genetic level. It is the objective of this paper to examine how and why insects adapt to stress, particularly pesticide exposure at the molecular genetic level and to emphasize that resistance is a part of the normal response of insects to stress. In addition, the advantages and limitations of traditional and molecular technologies for monitoring for resistance are briefly compared. This paper is a general overview to introduce readers to concepts of molecular genetics and pesticide resistance. It is not a technical review of the molecular biology of specific resistance mechanisms.

STRESS, RESISTANCE AND TOLERANCE: DEFINITIONS AND INTERACTIONS

Before discussing the molecular genetics of resistance, some definition and discussion of the relationships of stress, resistance, and tolerance is required. Stress has been broadly defined as "any environmental change that acts to reduce the fitness of an organism" (Koehn & Bayne 1989). Stresses may have physical, biotic, and/or toxic components (Fig. 1) which, in turn, may be acute, chronic, and/or seasonal and affect insects at the community, population, and/or individual level. The deleterious effects of excesses in temperature or humidity are self-evident. Exposure to ultraviolet radiation can influence trophic-level interactions of entire communities to the advantage of one population at the expense of another (Bothwell et al. 1994). Predation, parasitism, disease, inter- and intraspecies competition all act to determine the success or failure of a population to occupy a particular ecological niche. Toxic components of the environment are also stresses that can affect populations and can be divided into three groups: pollutants, which may be natural or artificial, pesticides, and plant al-

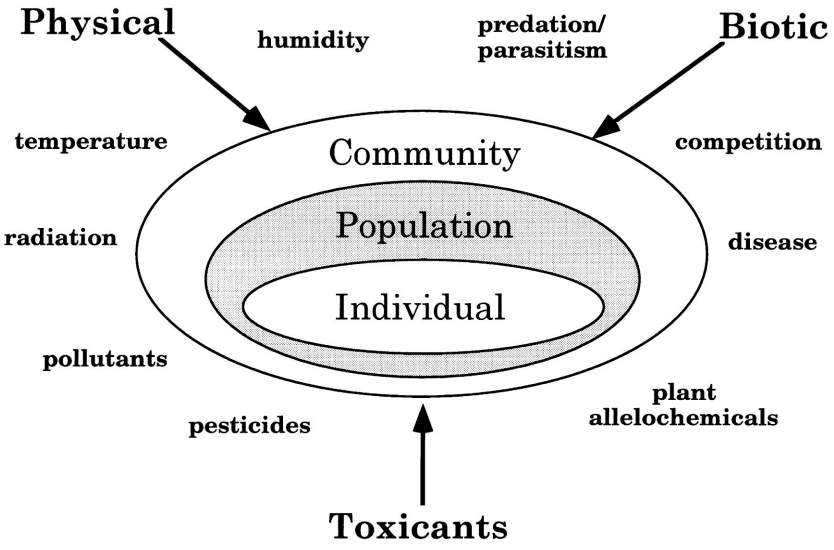

Figure 1. Examples of environmental stresses which can act on living organisms and force individuals, populations, and entire communities to continuously adapt to new and ever changing conditions in order to survive.

lelochemicals. Each of these stresses can affect insects differently, but all must be adequately dealt with for continued survival.

Resistance has been defined as the development of the ability in a strain of an organism to tolerate doses of a toxicant which would prove lethal to the majority of individuals in a normal (i.e. susceptible) population of the same species (Anonymous 1957). This definition is somewhat imprecise, because it infers that resistance can develop in an individual either before or after exposure to a toxin, two very different events. Resistance is the phenotypic expression throughout a population of a heritable trait that was already expressed in at least some of the individuals in the population *prior* to exposure to a toxicant. The development of a measurable shift in a population's susceptibility to a toxin is due to the specific selection of these pre-adapted individuals in the population, often over several generations, by exposure to amounts of toxicant which are sublethal to the pre-adapted individuals but may or may not be sublethal to others in the population. For many years this adaptive event was difficult to understand; however, we now know that the toxic components of some pesticides are similar to ones present in plants (e.g. pyrethrum) and that the detoxification systems that deal with these plant allelochemicals are the same systems that detoxify pesticides. Therefore insects which can detoxify certain plant allelochemicals well are pre-adapted to detoxify and develop resistance to pesticides which have the same mode of action as the allelochemicals even before the insects are ever exposed to the pesticides.

Resistance and tolerance are often used interchangeably in the literature and to define one another; however, tolerance is also used to describe shifts in susceptibility that occur within a single generation *after* exposure to stress which is not expressed by succeeding generations until *after* exposure to a similar stress. This phenomenon is different from the one previously described. For example 6-day-old bollworms, *He-*

liothis zea (Boddie), only detoxify relatively small amounts of methyl parathion, but at 12 days, they can detoxify 30-fold more methyl parathion. If gossypol, a toxic allelochemical present in cotton, is added to the diet, then 12-day-old larvae can detoxify not 30-fold but 75-fold more methyl parathion. The presence of gossypol in the diet induced the larvae to produce more detoxifying enzymes (Muehleisen et al. 1989). Yet, the 6-day-old progeny of these individuals do not retain either the 30- or 75-fold increase in tolerance of their parents but must develop it over time and after exposure to gossypol. The ability to metabolize methyl parathion is inherent; the increase in tolerance to it is not. The difference between resistance and tolerance can become blurred when a population is subjected to a strong selection pressure, such as chronic pesticide exposure, and the mechanism that is induced by the pesticide exposure to yield tolerance is also the mechanism that is specifically selected to yield resistance. Resistance, as it will be discussed here, refers to a decrease in susceptibility which is heritable and does not need to be induced before it can be expressed; however, exposure to a stress, such as an insecticide, may result in an increase in the expression of the resistance gene(s) which may or may not be heritable.

Two other terms which are often used and may be confused with one another are cross-resistance and multi-resistance. Cross-resistance is resistance to two or more classes of pesticides which occurs because the pesticides have the same, or very similar, modes of action. Organophosphate and carbamate pesticides intoxicate by similar modes of action, and resistance to one usually results in resistance to the other. Multi-resistance refers to resistance to two or more classes of pesticides because of the coexistence of two or more different resistance mechanisms. For example, a resistant insect may have both metabolic resistance to organophosphates and target-site resistance to pyrethroids.

The Ways and Means of Altering Genetic Material

It should be apparent that pre-exposure to plant allelochemicals can only help explain those cases of resistance where the pesticide is rendered inactive by the same detoxification mechanism. It does not explain resistance mechanisms which do not appear to be selected for by plant allelochemicals or seem to arise spontaneously or increase in amplitude after exposure to a pesticide. How can exposure of the parental generation to stress result in their progeny being more resistant to that stress? The answer is that exposure to a stress causes the genetic material (i.e. the DNA) to be altered.

There appear to be three general types of alterations, i.e. mutations, that can occur and result in resistance (Fig. 2). A gene may be *amplified* so that instead of only having one copy of the gene, there are now many copies present in the DNA. If an insect has ten copies of a gene, then it can make ten times as much product as an insect with only one copy of the gene. If the amplified gene encodes for a detoxifying enzyme, then that insect can detoxify 10-fold as much toxicant as the insect with only one gene.

The expression of a gene may also be altered to yield resistance. In this case, there is only one copy of the gene present in the mutated insect but that gene's regulation is altered so that it produces more (or less) product compared to a susceptible individual. For example, in a susceptible insect the gene to gene-product ratio may be 1:1 but in a resistant insect that ratio may be changed. The gene may be *up-regulated* to produce more product, that is, the gene to gene-product ratio is now 1:10 or *down-regulated* to make less product (the ratio is now 1:0.1). When a pesticide is applied in its toxic form, up-regulation of a detoxifying enzyme will increase resistance. When a pro-insecticide, i.e. the material must be metabolized first in order to become toxic, is applied, down-regulation of the metabolizing enzyme will increase resistance.

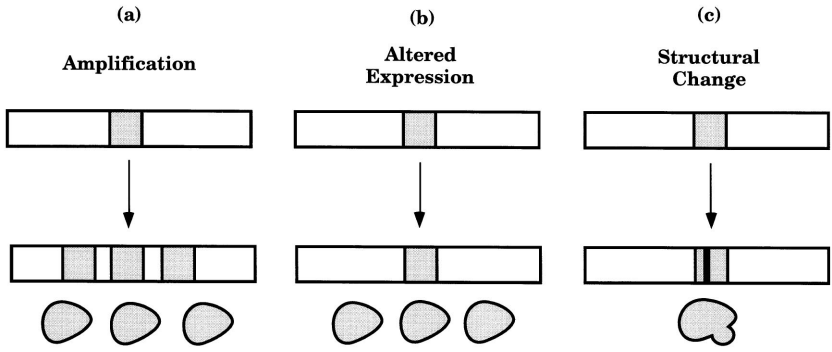

Figure 2. Graphic representation of the types of genetic mutations which occur and cause resistance. (a) A gene is amplified to increase its number of copies in the genome and consequently increase the amount of gene product made (b) the regulatory expression of a gene is altered to increase the amount of gene product made (note that gene expression may also be altered to decrease the amount of product made) (c) the genetic code is rewritten to produce a structurally different product.

The third type of mutation that can result in resistance is a structural change in a gene which yields a corresponding structural change in its product. A single *point mutation*, i.e. one nucleotide in the gene's coding region is substituted with a different nucleotide so that a different amino acid is encoded for at a specific position and this change causes the gene product to have a different three-dimensional structure, can result in resistance in several ways. It may decrease the ability of the insecticide to physically bind to its site of action, or increase or decrease the gene product's ability to metabolize the insecticide. A structural change does not alter the quantity of the product made but alters the quality of the product made.

It is important to recognize that these alterations to an insect's DNA do not create new genes. They only affect pre-existing genes. The idea that exposure to a pesticide causes resistance genes to be "created" has been debated periodically; however, no substantiating data have ever been proffered. It is much more likely that resistance genes already exist in the pesticide-naive population at low frequency prior to selection by a pesticide.

How resistance genes are spread throughout a population is not completely certain. It is clear that pesticide exposure plays a key role. However, other factors may also be important in the spread of resistance genes. One hypothesis which has received considerable attention is that *transposable* or *mobile elements* play a significant role in some cases of gene amplification. Transposable or mobile elements (TEs) are discrete sections of DNA that can move to new chromosomal locations and proliferate at a higher frequency relative to other genomic sequences (i.e. more and more copies of the TE are inserted into the genome) after they have moved. TEs can also move genes that were previously not mobile and whose functions are not related to the transposition. The genes that are moved with the TEs are also replicated at a higher frequency (Berg 1989). Gene amplifications may be initially distributed throughout a population by TEs or a gene may be transposed to a new location where its expression is altered to yield resistance. For example, it has been indirectly demonstrated in the laboratory that the transposition of *alleles*, alternative forms, of the gene *Met* results in insecticide resistance in *Drosophila* (Wilson & Turner 1992). But

so far there is absolutely no evidence that such an event has occurred in a field population.

Resistance Mechanisms and Associated Types of Mutations

Interestingly, only specific types of genetic mutations appear to be associated with specific resistance mechanisms (Table 1). The two types of mechanisms that cause high levels of resistance are generally referred to as metabolic resistance and target-site insensitivity, respectively. Each of these consists of several biological mechanisms. Metabolic resistance can be divided into three principle enzyme systems: cytochrome P450 monooxygenases (P450s), nonspecific esterases, and glutathione S-transferases (GSTs). Components of each of these enzyme systems may be mutated to alter the detoxification of a pesticide.

Cytochrome P450s catalyze a variety of detoxification reactions in insects, including the hydroxylation of DDT, the epoxidation of cyclodienes, the aromatic hydroxylation of the carbamates carbaryl and propoxur, and oxidation of phosphorothioates (Feyereisen et al. 1990). Given the variety of reactions stimulated by these enzymes, it is likely that several different P450 enzymes are present in any one insect and that several alleles of each gene may exist. Such is the case for the mosquito *Anopheles albimanus* in which seventeen P450 genes were discovered (Scott et al. 1994) and for the termites *Mastotermes darwiniensis* and *Coptotermes acinaciformis* in which multiple isoenzymes of cytochrome P450s were detected biochemically (Haritos et al. 1994). There is no evidence to suggest that P450 genes are amplified or structurally altered to yield insecticide resistance. But there are numerous examples of their expression being altered by various substances (Rose et al. 1991, Jeong et al. 1992, Waxman & Azaroff 1992, Snyder et al. 1993). By definition, if the expression of a P450 is

Table 1. The Genetic Mutations Associated with Enzymes and Receptors That Result in Different Types of Resistance.

Types of Resistance	Associated Genetic Mutations		
	Gene Amplification	Altered Expression	Structural Change
Metabolic			
P450 oxidases	ND[1]	+	ND
Esterases	+	ND	+
GSTs	ND	+	?
Target site insensitivity			
Acetylcholinesterase	ND	ND	+
GABA receptor	ND	ND	+
Sodium channel	ND	?	?
JH receptor	ND	?	?
Other			
Reduced penetration	—	—	—
Behavioral change	—	—	—

[1]ND = not detected; + = confirmed or strongly indicated; ? = implied but not confirmed; — = no data available.

altered to yield resistance, then susceptible and resistant strains will have quantitatively different amounts of that P450. Three P450s have been demonstrated to be over-expressed by resistant insect strains: P450Lpr (Wheelock & Scott 1992), CYP6A1 (Cariño et al. 1994), and CYP6A2 (Waters et al. 1992), respectively. P450Lpr has been directly implicated as the major enzyme causing pyrethroid resistance in one strain of house fly (Wheelock & Scott 1992, Hatano & Scott 1993). CYP6A1 appears to be a major cyclodiene-metabolizing enzyme in the house fly (Andersen et al. 1994). The primary function of CYP6A2 has not been reported. A major handicap to determining which P450s are involved in resistance is our inability to distinguish the activities of individual uncharacterized P450s from each other with a high degree of accuracy. The activity of a specific P450 towards an insecticide must be determined because its over-expression does not necessarily prove that it is the enzyme responsible for resistance. The over-expression of CYP6A1 by the house fly is a case in point. CYP6A1 is over-expressed in a house fly strain that is resistant to DDT, organophosphates, and carbamates but does not have significant resistance to cyclodienes. To determine which P450s are actually responsible for resistance, more specific substrates are needed.

Esterases are a large group of enzymes which metabolize a wide variety of substrates. All esterases are able to hydrolyze ester bonds in the presence of water. Since many insecticides, especially organophosphates and carbamates, contain ester bonds, it is not surprising that the mechanism of resistance in many cases is caused by elevated levels of esterases (Fournier et al. 1987, Field et al. 1988, Carlini et al. 1991, Kettermen et al. 1992, Chen & Sun 1994). Esterase levels can be elevated by either gene amplification or altered gene expression. So far, no data have indicated that the expression of esterase genes is altered to yield resistance, but the molecular characterization of esterases is limited to a very few insect species and this type of mutation cannot be discounted.

Considerable data show that certain non-specific esterase genes are amplified to yield resistance. The esterases which cause resistance in *Myzus persicae* Sulz. and *Culex* mosquitoes have been particularly well-studied. In these insects the resistant esterase genes are highly amplified and up to 250 copies of the same gene may be found in a single individual (Mouchès et al. 1986, Poirié et al. 1992). The more the esterase genes are amplified, the greater the level of resistance that they provide (Field et al. 1988, Poirié et al.1992). This increase in resistance appears to be because the esterases interact with the insecticides more readily than the insecticides' own target. When the esterases are present in approximately an equal molar ratio to the insecticides, they are able to effectively sequester the insecticides and then slowly hydrolyze the insecticides (Devonshire & Moore 1982, Ketterman et al. 1992, Karunaratne et al. 1993).

How recently these esterases have been amplified in *Culex* populations has been a matter of some debate. One group has suggested that two esterases associated with resistance, A2 and B2, were amplified in a single event within the past forty years, i.e. since the use of organophosphate insecticides became widespread, and that A2 and B2 have been distributed across three continents by migration since that event (Raymond et al. 1991). If this hypothesis is correct then all A2 and B2 genes should be identical. But data show that they are not. Kinetic studies of the insecticidal interaction of purified A2 and B2 esterases found that different forms of each enzyme were present in a number of resistant strains (Ketterman at al. 1993), and three amino acid differences have been found between the two B2 genes that have been sequenced (Vaughan et al. 1995). Therefore, not all of the A2 and B2 genes are identical and either these genes were amplified in at least two separate events or they were amplified

once long ago and have since diverged. Examination of other amplified esterase genes from different *Culex* strains clearly reveals numerous differences at the molecular level and strongly suggests that multiple amplification events have occurred with them (Vaughan et al. 1995).

In addition to amplification, esterases may be mutated to produce structurally different enzymes which are able to metabolize insecticides more efficiently. In the Australian sheep blow fly *Lucilia cuprina* and the mosquito *C. tarsalis*, a carboxylesterase appears to be structurally altered in resistant populations to produce high levels of resistance to malathion (Whyard et al. 1994a, 1994b). In neither species is more of the enzyme produced. The difference between susceptible and resistant populations is strictly a qualitative difference in the enzyme produced. Whether or not the carboxylesterases from the blow fly and mosquito are homologous to each other will only be known through further molecular and biochemical analysis.

The final group of enzymes which may provide metabolic resistance are the GSTs. Both organophosphate and cyclodiene pesticides can be detoxified by GST pathways. These enzymes have been somewhat less studied at the biochemical and molecular level in insects than the P450s and esterases. Elevated GST levels are found in many resistant insect strains (Motoyama & Dauterman 1975, Ottea & Plapp 1984, Ahammad-Sahib et al. 1994, Hoffman & Fisher 1994) and increased GST activity is clearly the underlying resistance mechanism in some cases (Kao & Sun 1991, Wang et al. 1991, Prapanthadara et al. 1993). But in other resistant populations the increased GST activity does not cause resistance (Bush et al. 1993, Hemingway et al. 1993, Argentine et al. 1994). Both insecticides and plant allelochemicals induce increased GST production (Yu 1992a, Lagadic et al. 1993, Leszczynski et al. 1994), and generalist plant feeders seem to rely on GST pathways more heavily than do specialist feeders to metabolize plant allelochemicals (Yu 1992b). Because GSTs can metabolize a wide variety of substances, increased GST activity may be part of a generalized compensatory change due to exposure to an environmental stress. How GST activity is increased has only been examined in a few Diptera. In dipterans, increased GST activity does not appear to be the result of gene amplification. In both the house fly and *Drosophila*, several GSTs contribute to resistance and their expression appears to have been increased through a regulatory change (Wang et al. 1991, Cochrane et al. 1992, Fournier et al. 1992). In addition, at least one resistance gene is structurally altered in *Drosophila* (Cochrane et al. 1992). Whether or not this structural change affects the resistance level in the flies remains to be determined. As numerous GSTs have been found in several insects, each of which appears to be encoded by a different gene (Cochrane et al. 1992, Fournier et al. 1992, Baker et al. 1994), it is likely that resistance caused by GSTs is due to altered gene expression and/or structural changes and is not due to gene amplification in most, if not all, cases.

The second major resistance mechanism is target-site insensitivity, which refers to an alteration of the molecule(s) that directly interacts with the pesticide to reduce toxicity. Both acetylcholinesterase (AChE) and the gamma-aminobutyric acid (GABA) receptor are known targets of insecticides, and resistant alleles of each have been found. Voltage-gated sodium channels and the juvenile hormone (JH) receptor are putative targets of insecticides. Their direct interaction with insecticides has not been confirmed, but it is clearly evident that they play a key role in the intoxication process.

Acetylcholinesterase is the target site of both organophosphates and carbamates. These pesticides bind to AChE and prevent the enzyme from stopping the action of the neurotransmitter acetylcholine. Multiple forms of AChE that confer varying degrees of resistance have been found in a variety of arthropods (Nolan et al. 1972, Devonshire & Moore 1984, Pralavorio & Fournier 1992). In each case examined so far, the affinity

of AChE for the pesticide has been reduced. Neither gene amplification nor altered expression of the gene encoding AChE has been detected. Instead, point mutations have occurred to structurally change the enzyme. Recently, Mutero et al. (1994) identified five point mutations in *D. melanogaster* which are associated with reduced sensitivity. Several strains of resistant flies were found to have a combination of mutations. Individually, the mutations gave only low levels of resistance, but when several of them were combined, high levels of resistance resulted. Mutero et al. (1994) hypothesize that decreased sensitivity by AChE is the result of a combination of several mutations, each of which provides a little resistance instead of the appearance of a single mutation which yields strong resistance.

GABA receptors are the primary target of cyclodiene insecticides. In vertebrates, these receptors group together to form a complete chloride ion channel. It is inferred that invertebrates have similar ion channels. Most cases of cyclodiene resistance appear to be due to decreased sensitivity of the GABA subtype A receptor (ffrench-Constant et al. 1991), an integral part of the chloride ion channel. Like AChE, decreased sensitivity by GABA receptors is due to a structural change of the protein. Neither amplification nor altered expression of the GABA receptor gene has been detected. Unlike AChE, only a single point mutation which causes one specific amino acid to be substituted with another results in high levels of resistance to cyclodienes. Other point mutations have been detected, but no others appear to cause resistance or are consistently associated with resistance (ffrench-Constant et al. 1993, Thompson et al 1993).

Voltage-gated sodium channels play an integral role in the transmission of neural impulses. Pyrethroids disrupt neural transmissions by interrupting the normal functioning of voltage-gated sodium channels. Target-site insensitivity to pyrethroids, a phenotypic response commonly referred to as knockdown resistance (kdr), results in these sodium channels becoming less sensitive to intoxication. Although it is clear that sodium channels are adversely affected by pyrethroids, there is disagreement as to whether or not kdr is the result of a mutation to the sodium channels or to some other molecule which is integral to the functioning of the sodium channels. A point mutation to a sodium channel, which could structurally alter it, was detected in a resistant insect strain (Amichot et al. 1992) but so far this mutation has not been shown to cause resistance. Other reports indicate that the mutation which causes kdr is closely linked to (physically close to or a part of) the gene encoding one type of sodium channel (Knipple et al. 1994, Dong & Scott 1994) and this linkage has been interpreted as strong evidence that a mutation(s) to the sodium channel gene is associated with kdr. On the other hand, a mutation to a regulatory protein or receptor could also result in kdr. There is some indirect evidence to support this alternative hypothesis (Rossignol 1991, Osborne & Pepper 1992). A third hypothesis that has been proposed and for which there is limited electrophysiological evidence is that kdr is caused by changes to two closely linked genes. One involves an altered sodium channel and the other may be associated with calcium-activated phosphorylation of a protein(s) involved with neurotransmitter release (Pepper & Osborne 1993). At this time, it can only be stated that there is no indication that a gene amplification event is associated with kdr. It is most likely that either the expression of a gene associated with sodium channel function has been altered and/or a structural mutation to sodium channels results in kdr.

Juvenile hormone analogs such as methoprene compete with the natural hormone for the JH receptor. Most resistance to JH analogs is either metabolic and/or a reduction in penetration of the insecticide through the cuticle. The only reports of target site insensitivity to JH analogs are in mutants isolated from laboratory colonies of

Drosophila (Shemshedini & Wilson 1990, Wilson & Turner 1992). The resistance gene *Met* that has been isolated from these colonies is associated with a less sensitive cystolic JH binding protein. Transposition of alleles of *Met* by a TE can induce resistance (Turner 1993); however, transposition is not required for the resistance gene to be expressed. Therefore, it seems most likely that the insensitive JH binding protein is either the result of a mutation that structurally changes the protein or the expression of the insect growth cycle has been altered. Gene amplification of JH receptors has not been implicated.

Two other types of resistance that have been described are the reduced penetration of a pesticide and altered behavior to avoid a pesticide. It is presumed that the cuticular structure is somehow altered to reduce the rate of penetration of a pesticide. Avoidance behavior appears to be stimulated by brief contact, either through tactile or olfactory receptors, with a pesticide. Alone, neither of these mechanisms cause high levels of resistance, but they are often found in combination with other types of resistance and can make a significant contribution to the overall resistance displayed by an insect. For example, Raymond et al. (1989) calculated that reduced penetration interacts with any other resistance mechanism multiplicatively. Experimental data support this conclusion (Hoyer & Plapp 1968, Plapp & Hoyer 1968). The underlying physiological, genetic and molecular mechanisms that cause these types of resistance can only be speculated and the genetic mutations which cause them cannot be inferred at this time.

Detecting and Monitoring for Resistance

Resistance is a widespread phenomenon and resistant populations of nearly all economically important pests can now be found (Georghiou 1994, Leibee & Capinera this issue). Where control failures have occurred, the history of pesticide application usually indicates that chronic pesticide exposure resulted in high levels of resistance which caused the failures. Does the development of a resistant population then mean that a control failure is inevitable? Certainly the continuous selection of the same resistance mechanism(s) over and over will result in resistance levels that are high enough to cause a control failure. But if the selection pressure (i.e. the pesticide) for each resistance mechanism is removed prior to significant resistance developing, then a control failure may be avoided (for an alternative view on this subject, see Hoy this issue). Keys to the successful avoidance of a control failure are the detection of resistance at low level(s), the routine monitoring for changes in the level(s) and type(s) of resistance present in the pest population and the implementation of, and strict adherence to, a multi-tactic pest management program. In many cases, the two former elements have not been fully utilized to allow for the design of an effective pest management program. This lack of implementation is due in part to the unavailability of easy to use resistance detection methods.

The ideal detection method is fast, inexpensive, easy to use, diagnostic for all types of resistance and able to detect resistance at frequencies as low as 1%. Numerous methods to detect resistance are currently available but all fall short of being the ideal technique. In fact, it is very unlikely that such an ideal technique will ever be developed. Instead, we must rely upon traditional methods, principally bioassays and a limited number of biochemical assays, and novel molecular techniques to detect and monitor for resistance. Certain general features are shared by the detection assays within each of these two technological groupings, i.e. traditional and molecular; and consequently, they have similar advantages and disadvantages which are summarized in Table 2.

In general, detection methods which utilize molecular technology are better able to distinguish between the different resistance genotypes, i.e. heterozygotes (SR), homozygous susceptible (SS) and homozygous resistant (RR), than traditional detection assays. Because they detect only genetic differences, molecular assays can eliminate the environmental components which often increase the variability in bioassay and biochemical results. Direct comparison of a molecular assay and a bioassay (Aronstein et al. 1994) indicates that molecular assays better approximate the perfectly diagnostic assay described by Roush & Miller (1986) than traditional bioassays and, therefore, can require up to 5-fold fewer insects to yield the same information. And unlike traditional assays, molecular techniques can use material from a single insect to perform several different assays so that the resistance levels to a wide variety of pesticides can be determined from the same individuals. However, molecular assays are limited to the detection of known resistance genes and a separate assay must be done for each gene. Traditional bioassays are better able to detect the overall level of resistance present in a population in a single test. In fact, molecular assays cannot detect many types of resistance at this time since with few exceptions we do not know which specific genes cause resistance. In time, molecular assays will be developed to detect more resistance genes; however, it is unlikely that molecular assays will completely replace the traditional ones. In addition, molecular assays are more costly in both material and equipment and require greater technical training than simple bioassays. They cannot be used in the field and usually take significantly longer to complete.

Although many resistance genes have not been isolated, it is clear from the molecular and genetic data that it is highly unlikely that resistance genes are the result of novel mutations which create new genes. Instead resistance, a phenotypic response, is the result of the selection by pesticides of alleles of pre-existing genes, i.e. specific genotypes, that regulate or enhance particular defensive mechanisms. How many

TABLE 2. COMPARISON OF THE ADVANTAGES AND DISADVANTAGES OF DETECTION METHODS THAT ARE BASED ON EITHER MOLECULAR OR TRADITIONAL TECHNOLOGY.

Molecular Detection Assay	vs.	Traditional Detection Assay
can distinguish resistant genotypes even at low levels		less sensitive; only detects resistant phenotypes, not genotypes
distinguishes SS, SR, and PR genotypes reliably even when phenotypes are hard to distinguish		cannot distinguish SR and RR from each other unless the phenotypes are distinct from one another
generally greater accuracy and less variability because environmental components are eliminated		less accurate and more variable because environmental components cannot be separated from genetic ones
fewer insects can yield more information because material from one insect can be used for several assays		requires more insects to get the same data because the same individuals cannot be used in several bioassays
currently not adapted for field use		many are easily used in the field
generally less rapid, it may take days to complete a test		very rapid, often only requiring a couple of hours to do
material and equipment often costly; more technical expertise often needed		generally inexpensive and simple to prepare and execute
limited to detection of known resistance genes		can detect any type of resistance, even if the resistance gene is not known

genes can cause resistance is not known. Data on various esterases and cytochrome P450s suggests that multiple genes may yield the same type of resistance. Only further analyses will determine if this is true. How resistant genes are distributed within and between populations is also not clear and requires further study. Understanding the genetic flow of resistance genes and the biological costs of resistance are of particular importance because without this information it will be difficult, if not impossible, to design successful control programs for many pests. Hopefully, the elucidation of the molecular genetics of resistance genes will help in the design of effective control programs and suggest novel control methods.

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REFERENCES CITED

AHAMMAD-SAHIB, K. I., R. M. HOLLINGWORTH, M. E. WHALON, P. M. IOANNIDIS AND E. J. GRAFIUS. 1994. Polysubstrate monooxygenases and other xenobiotic-metabolizing enzymes in susceptible and resistant Colorado potato beetle. Pestic. Biochem. Physiol. 49:1-12.

AMICHOT, M., C. CASTELLA, A. CUANNY, J. B. BERGÉ AND D. PAURON. 1992. Target modification as a molecular mechanism of pyrethroid resistance in *Drosophila melanogaster*. Pestic. Biochem. Physiol. 44:183-190.

ANDERSEN, J. F., J. G. UTERMOHLEN AND R. FEYEREISEN. 1994. Expression of house fly CYP6A1 and NADPH-cytochrome P450 reductase in *Escherichia coli* and reconstitution of an insecticide-metabolizing P450 system. Biochemistry 33:2171-2177.

ANONYMOUS. 1957. World Health Expert Committee on Insecticides 7th Report. WHO Technical Report Series No. 125.

ARGENTINE, J. A., K. Y. ZHU, S. H. LEE AND J. M. CLARK. 1994. Biochemical mechanisms of azinophosmethyl resistance in isogenic strains of Colorado potato beetle. Pestic. Biochem. Physiol. 48:63-68.

ARONSTEIN, K., P. ODE AND R. J. FFRENCH-CONSTANT. 1994. Direct comparison of PCR-based monitoring for cyclodiene resistance in *Drosophila* populations with insecticide bioassay. Pestic. Biochem. Physiol. 48:229-233.

BAKER, W. L., A. G. CLARK, G. FAULDS AND J. S. NIELSEN. 1994. Multiple glutathione S-transferases in *Galleria mellonella*; their detection with fluorigenic substrates. Insect Biochem. Molec. Biol. 24:301-307.

BERG, D. E. 1989. Transposable elements in prokaryotes, pp. 99-137 *in* S. B. Levy and R. V. Miller [eds.] Gene Transfer in the Environment. McGraw-Hill, New York, New York.

BOTHWELL, M. L., D. M. J. SHERBOT AND C. M. POLLOCK. 1994. Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interations. Science 265-97-100.

BUSH, M. R., Y. A. I. ABDEL-AAL, K. SAITO AND G. C. ROCK. 1993. Azinophosmethyl resistance in the tufted apple bud moth (Lepidoptera: Torticidae): reversion, diagnostic concentrations, associated esterases, and glutathione transferases. J. Econ. Entomol. 86:213-225.

CARIÑO, F. A., J. F. KOENER, F. W. PLAPP AND R. FEYEREISEN. 1994. Constitutive over-expression of the cytochrome P450 gene CYP6A1 in a house fly strain with metabolic resistance to insecticides. Insect Biochem. Molec. Biol. 24:411-418.

CARLINI, E. J., B. A. MCPHERON, C. M. FELLAND AND L. A. HULL. 1991. Elevated esterase activity in resistant tufted apple bud moth, *Platynota idaeusalis* (Walker) (Lepidoptera: Tortricidae). Comp. Biochem. Physiol. 99C:375-377.

CHEN, W-L. AND C. N. SUN. 1994. Purification and characterization of carboxylesterases of a rice brown planthopper, *Nilaparvata lugens* Ståal. Insect. Biochem. Molec. Biol. 24:347-355.

COCHRANE, B. J., M. HARGIS, P. CROCQUET-DE-BELLIGNY, F. HOLTSBERG, AND J. CORONELLA. 1992. Evolution of glutathione S-transferases associated with insecticide resistance in *Drosophila*. ACS Symp. Ser. American Chem. Soc. Washington, D.C.: The Society 505:53-70.

DEVONSHIRE, A. L. AND G. D. MOORE. 1982. A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). Pestic. Biochem. Physiol. 18:235-246.

DEVONSHIRE, A. L. AND G. D. MOORE. 1984. Different forms of insensitive acetylcholinesterase in insecticide-resistant house flies (*Musca domestica*). Pestic. Biochem. Physiol. 21:336-340.

DONG, K. AND J. G. SCOTT. 1994. Linkage of *kdr*-type resistance and the *para*-homologous sodium channel gene in German cockroaches (*Blatella germanica*). Insect Biochem. Molec. Biol. 24:647-654.

FEYEREISEN, R., J. F. KOENER, F. A. CARIÑO AND A. S. DAGGETT. 1990. Biochemistry and molecular biology of insect cytochrome P450., pp. 263-272 *in* H. H. Hagedorn, J. G. Hildebrand, M. G. Kidwell, and J. H. Law [eds.] Molecular Insect Science, Plenum, New York.

FFRENCH-CONSTANT, R. H., D. P. MORTLOCK, C. D. SHAFFER, R. J. MACINTYRE AND R. T. ROUSH. 1991. Molecular cloning and transformation of cyclodiene resistance in *Drosophila*: an invertebrate γ-aminobutyric acid subtype A receptor locus. Proc. Natl. Acad. Sci. USA 88:7209-7213.

FFRENCH-CONSTANT, R. H., J. C. STEICHEN, T. A. ROCHELEAU, K. ARONSTEIN AND R. T. ROUSH. 1993. A single-amino acid substitution in a γ-aminobutyric acid subtype A receptor locus is associated with cyclodiene insecticide resistance in *Drosophila* populations. Proc. Natl. Acad. Sci. USA 90:1957-1961.

FIELD, L. M., A. L. DEVONSHIRE AND B. G. FORDE. 1988. Molecular evidence that insecticide resistance in peach-potato aphids (*Myzus persicae* Sulz.) results from amplification of an esterase gene. Biochem. J. 251:309-312.

FOURNIER, D., J. M. BRIDE, C. MOUCHÈS, M. RAYMOND, M. MAGNIN, J-B. BERGÉ, N. PASTEUR AND G. P. GEORGHIOU. 1987. Biochemical characterization of the esterases A1 and B1 associated with organophosphate resistance in *Culex pipiens* L. complex. Pestic. Biochem. Physiol. 27:211-217.

FOURNIER, D., J. M. BRIDE, M. POIRIÉ, J-B. BERGÉ AND F. W. PLAPP. 1992. Insect glutathione S-transferases: biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides. J. Biol. Chem. 267:1840-1845.

GEORGHIOU, G. P. 1994. Principles of insecticide resistance management. Phytoprotection 75 (suppl.): 51-59.

HARITOS, V. S., J. R. J. FRENCH AND J. T. AHOKAS. 1994. Cytochrome P450 monooxygenase and glutathione S-transferase activity of two Australian termites: *Mastotermes darwiniensis* and *Coptotermes acinaciformis*. Insect Biochem. Molec. Biol. 24:929-935.

HATANO, R. AND J. G. SCOTT. 1993. Anti-P450Lpr antiserum inhibits the activation of chlorpyrifos to chlorpyrifos oxon in house fly microsomes. Pestic. Biochem. Physiol. 45:228-233.

HEMINGWAY, J., G. J. SMALL AND A. G. MONRO. 1993. Possible mechanisms of organophosphorus and carbamate insecticide resistance in german cockroaches (Dictyoptera: Blattelidae) from different geographical areas. J. Econ. Entomol. 86:1623-1630.

HOFFMAN, E. R. AND S. W. FISHER. 1994. Comparison of a field and laboratory-derived population of *Chironomus riparius* (Diptera: Chironomidae): biochemical and fitness evidence for population divergence. J. Econ. Entomol. 87:318-325.

HOY, M. A. 1995. Multitactic resistance management: An approach that is long overdue? Florida Entomol. 78:443-451.

HOYER, R. F. AND F. W. PLAPP. 1968. Insecticide resistance in the house fly: identification of a gene that confers resistance to organotin insecticides and acts as an intensifier of parathion resistance. J. Econ. Entomol. 61:1269-1276.

JEONG, T. C., H. G. JEONG AND K-H. YANG. 1992. Induction of cytochrome P-450 by dimethyl sulfoxide in primary cultures of adult rate hepatocytes. Toxicol. Letters 61:275-281.

KAO, C. H. AND C. N. SUN. 1991. *In vitro* degradation of some organophosphorus insecticides by susceptible and resistant diamondback moth. Pestic. Biochem. Physiol. 41:132-141.

KARUNARATNE, S. H. P. P., K. G. I. JAYAWARDENA, J. HEMINGWAY AND A. J. KETTERMAN. 1993. Characterization of a B-type esterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. Biochem. J. 294:575-579.

KETTERMAN, A. J., K. G. I. JAYAWARDENA AND J. HEMINGWAY. 1992. Purification and characterization of a carboxylesterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. Biochem. J. 287:355-360.

KETTERMAN, A. J., S. H. P. P. KARUNARATNE, K. G. JAYAWARDENA AND J. HEMINGWAY. 1993. Qualitative differences between populations of *Culex quinquefasciatus* in both the esterases A2 and B2 which are involved in insecticide resistance. Pestic. Biochem. Physiol. 47:142-148.

KNIPPLE, D. C., K. E. DOYLE, P. A. MARSELLA-HERRICK AND D. M. SODERLUND. 1994. Tight genetic linkage between the *kdr* insecticide resistance trait and a voltage-sensitive sodium channel gene in the house fly. Proc. Natl. Acad. Sci. USA 91:2483-2487.

KOEHN, R. K. AND B. L. BAYNE. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. Biological Journal of the Linnean Society 37:157-171.

LAGADIC, L., A. CUANY, J. B. BERGÉ AND M. ECHAUBARD. 1993. Purification and partial characterization of glutathione S-tranferases from insecticide-resistant and lindane-induced susceptible *Spodoptera littoralis* (Boisd.) larvae. Insect Biochem. Mol. Biol. 23:467-474.

LEIBEE, G. L. AND CAPINERA, J. L. 1995. Pesticide resistance in Florida insects limits management options. Florida Entomol. 78:386-399.

LESZCZYNSKI, B., M. MATOK AND A. F. G. DIXON. 1994. Detoxification of cereal plant allelochemicals by aphids: activity and molecular weights of glutathione S-transferase in three species of cereal aphids. J. Chem. Ecol. 20:387-394.

MOTOYAMA, N. AND W. C. DAUTERMAN. 1975. Interstrain comparison of glutathione-dependent reactions in susceptible and resistant houseflies. Pestic. Biochem. Physiol. 5:489-495.

MOUCHÈS, C., N. PASTEUR, J. B. BERGÉ, O. HYRIEN, M. RAYMOND, B. R. DE SAINT VINCENT, M. DE SILVESTRI G. P. GEORGHIOU. 1990. Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. Science 233:778-780.

MUEHLEISEN, D. P., J. H. BENEDICT, F. W. PLAPP, JR. AND F. A. CARIÑO. 1989. Effects of cotton allelochemicals on toxicity of insecticides and induction of detoxifying enzymes in bollworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82:1554-1558.

MUTERO, A., M. PRALAVORIO, J-M. BRIDE AND D. FOURNIER. 1994. Resistance-associated point mutations in insecticide-insensitive acetylcholinesterase. Proc. Natl. Acad. Sci. USA 91:5922-5926.

NOLAN, J., H. J. SCHNITZERLING AND C. A. SCHUNTNER. 1972. Multiple forms of acetylcholinesterase from resistant and susceptible strains of the cattle tick, *Boophilus microplus* (Can.). Pestic. Biochem. Physiol. 2:85-94.

OSBORNE, M. P. AND D. R. PEPPER. 1992. Mechanisms of kdr and super-kdr resistance. ACS Symp. Ser. American Chem. Soc. 505:71-89.

OTTEA, J. A. AND F. W. PLAPP. 1984. Glutathione-S-transferase in the house fly: biochemical and genetic changes associated with induction and insecticide resistance. Pestic. Biochem. Physiol. 22:203-208.

PEPPER, D. R. AND M. P. OSBORNE. 1993. Electrophysiological identification of site-insensitive mechanisms in knockdown-resistant strains (kdr, super-kdr) of the housefly larva (*Musca domestica*). Pestic. Sci. 39:279-286.

PLAPP, F. W. AND R. F. HOYER. 1968. Insecticide resistance in the house fly: decreased rate of absorption as the mechanism of action of a gene that acts as an intensifier of resistance. J. Econ. Entomol. 61:1298-1303.

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.

PRAPANTHADARA, L. A., J. HEMINGWAY AND A. J. KETTERMAN. 1993. Partial purification and characterization of glutathione S-transferases involved in DDT resistance from the mosquito *Anopheles gambiae*. Pestic. Biochem. Physiol. 47:119-133.

RAYMOND, M., D. G. HECKEL AND J. G. SCOTT. 1989. Interactions between pesticide genes: model and experiment. Genetics 123:543-551.

RAYMOND, M., A. CALLAGHAN, P. FORT AND N. PASTEUR. 1991. Worldwide migration of amplified insecticide resistance genes in mosquitoes. Nature 350:151-153.

ROSE, R. L., F. GOULD, P. E. LEVI AND E. HODGSON. 1991. Differences in cytochrome P450 activities in tobacco budworm larvae as influenced by resistance to host plant allelochemicals and induction. Comp. Biochem. Physiol. 99B:535-540.

ROSSIGNOL, D. P. 1991. Analysis of pyrethroid binding by use of a photoreactive analogue: possible role for GTP-binding proteins in pyrethroid activity. Pestic. Biochem. Physiol. 41:103-120.

ROUSH, R. T. AND G. L. MILLER. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79:293-298.

SCOTT, J. A., F. H. COLLINS AND R. FEYEREISEN. 1994. Diversity of cytochrome P450 genes in the mosquito, *Anopheles albimanus*. Biochem. Biophys. Res. Comm. 205:1452-1459.

SHEMSHEDINI, L. AND T. G. WILSON. 1990. Resistance to juvenile hormone and an insect growth regulator in *Drosophila* is associated with an altered cytosolic juvenile hormone-binding protein. Proc. Natl. Acad. Sci. USA 87:2072-2076.

SNYDER, M. J., E-L. HSU AND R. FEYEREISEN. 1993. Induction of cytochrome P-450 activities by nicotine in the tobacco hornworm, *Manduca sexta*. J. Chem. Ecol. 19:2903-2916.

THOMPSON, M., J. C. STEICHEN AND R. H. FFRENCH-CONSTANT. 1993. Conservation of cyclodiene insecticide resistance-associated mutations in insects. Insect Molec. Biol. 2:149-154.

VAUGHAN, A., M. RODRIGUEZ AND J. HEMINGWAY. 1995. The independent gene amplification of indistinguishable esterase B electromorphs in insecticide-resistant *Culex quinquefasciatus*. Biochem. J. 305:651-658.

WANG, J-Y., S. MCCOMMAS AND M. SYVANEN. 1991. Molecular cloning of a glutathione S-transferase overproduced in an insecticide-resistant strain of the housefly (*Musca domestica*). Mol. Gen. Genet. 227:260-266.

WATERS, L. C., A. C. ZELHOF, B. J. SHAW AND L-Y. CHANG. 1992. Possible involvement of the long terminal repeat of transposable element *17.6* in regulating expression of an insecticide resistance-associated P450 gene in *Drosophila*. Proc. Natl. Acad. Sci. USA 89:4855-4859.

WAXMAN, D. J. AND L. AZAROFF. 1992. Phenobarbital induction of cytochrome P-450 gene expression. Biochem. J. 281:577-592.

WHEELOCK, G. D. AND J. G. SCOTT. 1992. The role of cytochrome P450 Lpr in deltamethrin metabolism by pyrethroid-resistant and susceptible strains of house flies. Pestic. Biochem. Physiol. 43:67-77.

WHYARD, S., R. J. RUSSELL AND V. K. WALKER. 1994a. Insecticide resistance and malathion carboxylesterase in the sheep blowfly, *Lucilia cuprina*. Biochem. Genet. 32:9-22.

WHYARD, S., A. E. DOWNE AND V. K. WALKER. 1994b. Isolation of an esterase conferring insecticide resistance in the mosquito *Culex tarsalis*. Insect Biochem. Molec. Biol. 24:819-827.

WILSON, T. G. AND C. TURNER. 1992. Molecular analysis of *Methoprene-tolerant*, a gene in *Drosophila* involved in resistance to juvenile hormone analog insect growth regulators, *in* C. Mullin and J. G. Scott [eds.], Molecular mechanisms of insecticide resistance. American Chemical Society Symp. Series 505:99-112.

YU, S. J. 1992a. Plant-allelochemical-adapted glutathione transferases in Lepidoptera. ACS Symp. Ser. American Chem. Soc. 505:174-190.

YU, S. J. 1992b. Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 85:675-682.