Molecular Mechanisms of Metabolic Resistance to Synthetic and Natural Xenobiotics

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Abstract

Xenobiotic resistance in insects has evolved predominantly by increasing the metabolic capability of detoxificative systems and/or reducing xenobiotic target site sensitivity. In contrast to the limited range of nucleotide changes that lead to target site insensitivity, many molecular mechanisms lead to enhancements in xenobiotic metabolism. The genomic changes that lead to amplification, overexpression, and coding sequence variation in the three major groups of genes encoding metabolic enzymes, i.e., cytochrome P450 monooxygenases (P450s), esterases, and glutathione-*S*-transferases (GSTs), are the focus of this review. A substantial number of the adaptive genomic changes associated with insecticide resistance that have been characterized to date are transposon mediated. Several lines of evidence suggest that P450 genes involved in insecticide resistance, and perhaps insecticide detoxification genes in general, may share an evolutionary association with genes involved in allelochemical metabolism. Differences in the selective regime imposed by allelochemicals and insecticides may account for the relative importance of regulatory or structural mutations in conferring resistance.

INTRODUCTION

Resistance of insects to xenobiotics, which include naturally occurring plant allelochemicals and synthetic insecticides, is an ongoing challenge to pest management and at the same time an ideal system for studying the processes of microevolution and environmental adaptation. At the biochemical level, resistance to xenobiotics typically involves increases in the metabolic capabilities of detoxificative enzymes as well as decreases in target site sensitivity. In the last decade, dramatic progress has been made in identifying genes associated with xenobiotic resistance and structural changes affecting their functions. Insect proteins targeted by xenobiotics (target site proteins) generally become insensitive to xenobiotics through a few conserved point mutations, probably because their functions are so vital that only a few changes can decrease their xenobiotic sensitivity without disrupting their normal functions (37). In contrast, genes associated with metabolic resistance mediate environmental response functions (9) that have relatively fewer intrinsic constraints and tend to be more tolerant of genomic changes altering function, expression, or both. Sequence amplifications, transcriptional enhancements, and coding mutations in genes encoding esterases, cytochrome P450 monooxygenases (P450s), and glutathione-*S*-transferases (GSTs) have been identified as resistance mechanisms. With excellent reviews on these enzymes detailing their contributions to resistance (36, 55, 104), we emphasize the internal spontaneous genomic alterations leading to amplification, overexpression, and coding sequence alteration of these metabolic genes. Given the involvement of esterases, P450s, and GSTs in protecting insects against both plant allelochemicals and insecticides, we also review the biochemical/molecular mechanisms for allelochemical tolerance and examine evolutionary associations between insecticide resistance and allelochemical adaptation.

Metabolic resistance: class of xenobiotic resistance that involves biochemical transformation of a substance, ultimately reducing its capacity to interact with a target molecule

Esterases: a group of hydrolase enzymes capable of hydrolyzing compounds containing ester bonds

Cytochrome P450 monooxygenases (P450s): phase I metabolic enzymes capable of oxidizing endogenous and exogenous compounds by oxidation or other related reactions

Glutathione-S-transferases (GSTs): phase II metabolic enzymes capable of conjugating reduced glutathione to the electrophilic centers of exogenous and endogenous compounds

Gene amplification: a spontaneous genomic alteration event that increases the copy number of one or more genes in a genome

Upregulation: a genomic change that increases production of an enzyme or protein without increasing its genomic copy number

MOLECULAR MECHANISMS OF METABOLIC RESISTANCE TO INSECTICIDES

P450-Mediated Resistance

Because of their genetic diversity, broad substrate specificity, and catalytic versatility, P450s and their associated P450 reductases comprise the only metabolic system that can mediate resistance to all classes of insecticides (36). More than 660 insect P450 genes, distributed in *CYP4*, *CYP6*, *CYP9*, *CYP12*, *CYP15A*, *CYP18A*, *CYP28A*, *CYP29A*, *CYP48*, *CYP49*, *CYP301-CYP318*, *CYP319A*, *CYP321A*, *CYP324*, *CYP325*, *CYP329*, and *CYP332-CYP343* families and subfamilies, have been characterized in genome sequencing projects and smaller resistance research projects (23, 92, 116; http://drnelson.utmem.edu/cytochromeP450.html, http://p450.antibes.inra.fr/). Several approaches, including cross-strain comparisons of gene sequences, copy number, expression levels, and substrate binding preferences, have been used to determine the molecular mechanisms for P450-mediated resistance.

Resistance via upregulation. To date, no gene amplification events have been documented to account for P450-mediated resistance. Rather, 25 P450 genes, belonging to the families *CYP4*, *CYP6*, *CYP9*, and *CYP12*, are overproduced via upregulation in resistant insects. The genomic changes leading to P450 overexpression have been ascertained for only a few of the 25 P450 genes implicated in insecticide resistance, and these changes are discussed below.

Upregulation via mutations in* trans*-regulatory loci. Although overexpression could theoretically result from increases in transcription, mRNA stability, and/or protein translation, it appears that in most cases increased expression is achieved through mutations and insertions/deletions (indels) in *cis*-acting promoter sequences and/or

trans-acting regulatory loci. Several approaches, including genetic crosses, chromosome substitution, promoter sequence comparisons, and transgenic analyses, have been employed to identify changes responsible for hypertranscription of house fly (*M. domestica*) P450 *CYP6A1* and *CYP6D1*, and fruit fly (*Drosophila melanogaster*) P450 *CYP6A2*, *CYP6A8*, and *CYP6G1* genes.

In *M. domestica*, alleles of *CYP6A1* (on chromosome 5) and *CYP6D1* (on chromosome 1) in insecticide-resistant Rutgers and Learn pyrethroid-resistant (LPR) strains are downregulated by factors derived from chromosome 2 of susceptible strains, as evidenced by reduction of transcription of both resistance genes to the level in susceptible strains when both copies of chromosome 2 in crosses were from susceptible strains (16, 77). These observations suggest that overexpression of *CYP6A1* and *CYP6D1* alleles is due at least in part to loss-of-function mutations in negative regulatory loci on chromosome 2 of the Rutgers and LPR strains. For *CYP6D1*, a *Gfi-1*-like protein designated *MdGfi-1* may serve as a negative regulatory repressor (61). For *CYP6A1*, linkage analysis has indicated that its regulatory repressor locus is located close to *aristapedia* and *carnation eye*, two markers on chromosome 2, to which loci for DDT and diazinon resistance are mapped (36). An aliesterase found in this region, *Mdα*E7, has been proposed as the *trans*-repressor locus for *CYP6A1* because *CYP6A1* overproduction was associated with the absence of the wild-type Gly137 *Mdα*E7 allele in the strains tested (99). However, the subsequent discovery of the wild-type Gly137 *Mdα*E7 allele in the pyrethroid-resistant LPR strain, which overexpresses *CYP6A1*, argues against this hypothesis (105). More experiments utilizing *Mdα*E7 RNAi or *CYP6A1* promoter-*Mdα*E7 protein gel shift assays may clarify whether *Mdα*E7 aliesterase is simply a detoxification enzyme or whether it also functions as a negative repressor.

In *D. melanogaster*, *CYP6A2* and *CYP6A8* genes (on chromosome 2) are also downregulated by repressor loci present on chromosome 3, the homolog of the house fly chromosome 2 (31, 79, 80). As in the case of the house fly *CYP6A1* and *CYP6D1*, hypertranscription of *CYP6A2* and *CYP6A8* in the DDT- and malathion-resistant 91-R and MHIII-D23 strains is due at least in part to loss-of-function mutations in the repressor loci (79, 80). At present, the nature of these repressor loci remains unknown. Given that ecdysone response element (EcRE) and Broad-complex (BR-C) binding sites are present within its promoter (31) and that maintenance of the constitutive expression of *CYP6A2* depends on the synthesis of endogenous ecdysone in both susceptible and resistant strains (111), expression of *CYP6A2* may be regulated by ecdysone receptor (EcR) and BR-C.

Upregulation via indels or mutations in* cis*-acting elements. Cross-strain comparisons of the promoter sequences for the house fly genes *CYP6D1* (104, 106) and *CYP6D3* (63) as well as fruit fly genes *CYP6A2* (31), *CYP6A8* (80), *CYP12A4* (12), and *CYP6G1* (17, 25, 102) have been conducted to identify mutations and indels in *cis*-acting elements that might cause upregulation. Neither nucleotide substitutions nor indels are found in the 5′-promoter and 3′-untranslated region (UTR) sequences of *CYP12A4*, although lufenuron resistance maps to a 30-kb region at 91F3 where the overexpressed *CYP12A4* resides (12). In contrast, multiple mutations and/or short indels are detected in the promoter sequences of *CYP6D1* (43 mutations, one 15-bp insertion located downstream of a putative silencer element for the *Gfi-1*-like repressor *MdGfi-1*), *CYP6D3* (16 nucleotide substitutions), and *CYP6A8* (14 mutations, two short deletions of an AA dinucleotide and three short deletions of a TAC trinucleotide). Whether and how these changes contribute to the overexpression of the corresponding resistant P450 genes need to be resolved.

In the *CYP6A2* promoter region, there are six nucleotide substitutions between the DDT-resistant *CYP6A2 91R* allele and the

Indel: insertion and/or deletion

EcRE: ecdysone-response element

UTR: untranslated region

LTR: long terminal repeat

DDT-susceptible *CYP6A2 ry* allele (31). Transformation of the *CYP6A2 91R* allele with its upstream promoter sequence into DDT-susceptible ry506 flies resulted in a moderate level of constitutive expression of the *CYP6A2 91R* allele, whereas expression of the resident *CYP6A2 ry* allele was barely detectable. Such differential expression of the two *CYP6A2* alleles in the same genetic background suggests that the six nucleotide substitutions are partially responsible for the upregulation of the *CYP6A2 91R* allele in resistant strains.

Perhaps the most striking genomic changes in *cis* are found in the 5′-promoter region of the overexpressed *CYP6G1* alleles in *D. melanogaster* and its sister species *D. simulans*, which appear to contribute to resistance to DDT, imidacloprid and nitenpyram (two neonicotinoid nicotinic acetylcholine receptor agonists), and lufenuron (an insect growth regulator) (17, 25, 102). In *D. melanogaster*, a defective *Gypsy*-like long terminal repeat (LTR) retrotransposon known as *Accord* is inserted approximately 300 bp upstream from the transcription start site. Two worldwide surveys confirm a perfect correlation of the *Accord* insertion with DDT and imidacloprid resistance and with *CYP6G1* upregulation, although there is variation in *Accord* insertion size across lines (17, 25). In parallel, a full-length (4803-bp) *Doc* non-LTR retrotransposon is inserted 200 bp upstream of the putative transcription start site of the homologous *CYP6G1* gene in the California *D. simulans* populations (102). This *D. simulans Doc* insertion also leads to upregulation of *CYP6G1*, but its correlation with DDT resistance appears to be less significant than that of the *D. melanogaster Accord* insertion (102). The mechanisms by which these *Accord* and *Doc* insertions might cause *CYP6G1* upregulation encompass a wide range of options, from disrupting existing repressor elements, to introducing enhancer elements, to altering the physical distance between regulatory elements and the transcriptional start site. These mechanisms remain to be analyzed. Despite the consistency of this sequence in a large number of resistant strains, more recent studies have not been able to document a consistent association between *CYP6G1* mRNA and protein expression and DDT LC50 in susceptible and resistant strains, some of which are the same as those used in the earlier study (35). Expression analyses of a large number of other P450 genes have identified some, such as *CYP12D1*, whose transcripts overaccumulate in DDT-resistant strains (35, 87).

Resistance via coding sequence changes. An increasing amount of evidence, including the incomplete loss of resistance when P450s are not overexpressed in *D. melanogaster*, suggests that point mutations may play a secondary role in P450-mediated resistance (36). Examination of the alleles present in different strains indicates that varying numbers of amino acid substitutions exist in the *CYP6X1*, *CYP6D1*, *CYP6D3*, and *CYP6A2* sequences of resistant strains (36, 63, 104, 135). More directly, molecular modeling and heterologous expression have indicated that the three amino acid mutations (R335S, L336V, and V476L) present in the DDT-resistant *CYP6A2* allele are in close proximity to the active site and are responsible for increasing its metabolism of DDT, 7-ethoxycoumarin, and 7-benzoyloxycoumarin (2, 36).

Defining catalytic activity. Attributing insecticide resistance to an overexpressed P450 necessitates determining its metabolic activity against that insecticide. Among the 25 overexpressed P450 genes, only house fly *CYP6D1* (104, 132), *CYP6A1* (4, 36, 99, 104), and *CYP12A1* (49) and *D. melanogaster CYP6A2* (2, 32, 100) and *CYP6A8* (52) have been catalytically defined to any extent. In vitro metabolism assays in the presence or absence of CYP6D1 antibody have demonstrated that CYP6D1 can catalyze hydroxylation of the pyrethroid insecticides permethrin, deltamethrin, and cypermethrin at the

4′ position of the pyrethroid phenoxybenzyl moiety in the presence of cytochrome b_5 (104, 132). Heterologous expression in *Escherichia coli* of CYP6A1 and CYP12A1, both of which are overexpressed in the diazinon-resistant Rutgers strain of the house fly, has demonstrated that they metabolize diazinon by desulfuration and oxidative ester cleavage (4, 49, 99). CYP6A1 also epoxidizes the cyclodiene insecticides aldrin and heptachlor as well as a variety of terpenoids, including farnesyl, geranyl, and neryl methyl esters, and juvenile hormones I and III (5, 36). Given that CYP6A1 mRNA levels are higher in the pyrethroid-resistant LPR strain than in the Rutgers strain, even though LPR has lower diazinon resistance (104), the possibility exists that CYP6A1 is also capable of metabolizing pyrethroids.

Heterologous *E. coli*–mediated expression of CYP6A2, which is overexpressed in the DDT- and malathion-resistant 91-R strain of *D. melanogaster*, has demonstrated that it metabolizes aldrin, heptachlor, diazinon, aflatoxin B1, and 7,12-dimethylbenz [a] anthracene (2, 36, 100). Heterologous yeast-mediated expression of CYP6A8 isolated from the susceptible Oregon-R-C strain has indicated that it is capable of metabolizing lauric acid but not DDT or heptachlor (52). Although CYP6G1 and CYP12A4 have not been directly tested for insecticide metabolism in any of these in vitro systems, transgenic *D. melanogaster* overexpressing CYP6G1 or CYP12A4 display moderate DDT resistance or enhanced lufenuron resistance, respectively, suggesting that the CYP6G1 and CYP12A4 proteins can metabolize DDT and lufenuron, respectively (12, 25).

GST-Mediated Resistance

Another important group of metabolic enzymes involved in insecticide resistance are GSTs. GSTs can mediate resistance to organophosphate (OP), organochlorines, and pyrethroids. To date, seven GST genes have been implicated in insecticide resistance by gene amplification or overexpression.

Diversity, nomenclature, and functions. GSTs were originally grouped into three classes (I, II, and III) in insects (18, 33, 96, 117). Mammalian GSTs, in contrast, were grouped into one microsomal and eight cytosolic classes (Alpha, Mu, Pi, Theta, Sigma, Zeta, Kappa, and Omega), with members in each class sharing 40% or higher amino acid identity (18). As insect genome projects revealed increased diversity of GSTs (23, 92, 96, 117), a unified nomenclature system has been proposed in which the insect GST classes are assigned Greek letters corresponding to the mammalian GST classes (18). According to the unified system (18), class I and III insect GSTs are designated Delta and Epsilon classes, respectively, and class II insect GSTs are included in several mammalian classes, with many belonging to the Sigma class. Accordingly, the name of each GST is composed of the initials of the species scientific name, followed by the acronym GST, a capital letter for the initial of the class name, and a numeral for the individual gene (e.g., *AgGSTD2 = AgGST1–2*) (18).

GSTs play a pivotal role in detoxification and cellular antioxidant defense against oxidative stress by conjugating reduced glutathione (GSH) to the electrophilic centers of natural and synthetic exogenous xenobiotics, including insecticides, allelochemicals, and endogenously activated compounds, such as unsaturated carbonyls, epoxides, organic hydroperoxides, lipid peroxidation products, and oxidized DNA bases (33, 78, 85). Some insect GSTs catalyze the dehydrochlorination of DDT to the noninsecticidal metabolite DDE (78, 85, 95, 113) by using GSH as a cofactor rather than as a conjugate. In addition to conjugation and dehydrochlorination, GSTs are also involved in intracellular and circulatory transport of endogenous lipophilic compounds, xenobiotic binding, and sequestration. Conjugation, dehydrochlorination, or xenobiotic binding and

OP: organophosphate
GSH: glutathione

sequestration may be responsible for resistance of insects to OP (59), organochlorines (95, 113), and pyrethroids (119, 121).

GST gene amplification. Among the six GST genes implicated in insecticide resistance, two of them appear to be amplified in resistant strains. These include *MdGSTD3* in OP-resistant *Musca domestica* and *NlGSTD1* in pyrethroid-resistant *Nilaparvata lugens*.

***GST genes and OP resistance in* Musca domestica.** On the basis of biochemical evidence showing elevated production of three GST isoforms in OP-resistant house fly strains, four house fly GST genes belonging to the Delta class were isolated (114, 124). Northern blot analysis revealed greater levels of transcripts of the four GST genes in the OP-resistant Cornell-R (resistant) strain compared with susceptible strains. When compared within the isogenic set of Cornell-R, -R* (revertant susceptible), and -HR (hyper-resistant) strains, however, only the abundance of *MdGSTD3* and *MdGSTD4* transcripts was correlated with the resistance level (114, 124). Screening of a Cornell-HR genomic DNA library with a 170-bp fragment specific for *MdGSTD3* and *MdGSTD4* identified 10 loci: 3 for *MdGSTD3*, 2 for *MdGSTD4*, 2 for *MdGSTD5*, and 3 fusion genes containing the 5′ end of *MdGSTD3* and the 3′ end of *MdGSTD4* or *MdGSTD5* (133). Further *MdGSTD3*-specific genomic PCR revealed 12 *MdGSTD3*-like loci in the OP-resistant Cornell-R strain but only 3 *MdGSTD3*-like loci in the susceptible strain, suggesting that amplification and postduplication divergence of the *MdGSTD3* and *MdGSTD4* genes may contribute to OP resistance in *M. domestica*. Heterologous expression and metabolism assays showed that MdGSTD3 and MdGSTE6A (MdGST-6A), but not MdGSTD4, can metabolize methyl-parathion, diazinon, and lindane (113, 125), indicating that MdGSTD3 is involved in OP resistance but MdGSTD4 is not. Cross-strain comparisons can be used to determine the involvement of *MdGSTE6A* in OP resistance.

***NlGSTD1 and pyrethroid resistance in* Nilaparvata lugens.** Elevated GST activities have recently been implicated in pyrethroid resistance in several insects (119). In contrast to DDT and OP resistance, GSTs are not involved in the direct metabolism of pyrethroids but rather are involved in the sequestration of pyrethroids and/or the detoxification of lipid peroxidation products induced by pyrethroids. In the brown planthopper, *Nilaparvata lugens*, partial protein purification demonstrated that pyrethroid resistance was associated with the GST fraction having peroxidase activity (119, 121). Subsequent cloning and heterologous expression of NlGSTD1 cDNA isolated from the resistant strain indicated more directly that it coded for a peroxidase (121). Northern blot analyses demonstrated higher levels of *NlGSTD1* transcripts in the resistant strain than in the susceptible strain. Southern analysis aimed at determining the copy number for this gene detected several more intense bands in the resistant strain, indicating that the *NlGSTD1* gene confers pyrethroid resistance via gene amplification in *N. lugens.*

GST overexpression. The remaining five GST genes implicated in insecticide resistance appear to be overexpressed in resistant insects. These include *PxGSTE1* in OP-resistant *Plutella xylostella*, *AgGSTE2* in DDT-resistant *Anopheles gambiae*, *DmGSTD1* in DDT-resistant *D. melanogaster*, and *AaGSTD1* and *AaGSTE2* in DDT-/pyrethroid-resistant *Aedes aegypti*.

***PxGSTE1 and OP and benzoylphenylureas resistance in* Plutella xylostella.** The diamondback moth, *Plutella xylostella*, a worldwide pest of brassicaceous crops, has displayed an extraordinary capacity to develop resistance to all classes of insecticides including OP and benzoylphenylureas. Four GST isoenzymes have been purified from *P. xylostella*,

two of which, GST3 and the closely related GST4, degrade the OP insecticides parathion, methylparathion, and paraoxon (20, 64). That both the methylparathion-resistant strain and the teflubenzuron-resistant strain overproduce these two OP-metabolizing isoenzymes allowed subsequent isolation of the GST3 gene, *PxGSTE1*, from the methylparathion-resistant caterpillars (59). The heterologously expressed PxGSTE1 metabolized the OP insecticides (parathion and methylparathion) and model substrates (CDNB and DCNB) at efficiencies comparable to that of the purified GST3 isoenzyme. Although Northern and Western blot analyses documented higher PxGSTE1 expression in the methylparathion-resistant strain, Southern blot analyses failed to provide any evidence of gene amplification, suggesting that overproduction of *PxGSTE1* results from upregulation in the methylparathion-resistant strain. Upregulation of *PxGSTE1* is also responsible for chlorfluazuron resistance in the chlorfluazuron-selected resistant strain (109).

***AgGSTE2 (AgGST3–2) and DDT resistance in* Anopheles gambiae.** Earlier biochemical studies revealed an association between elevated GST activities (eightfold) and DDT resistance in the African malaria mosquito *Anopheles gambiae* (91). Efforts to characterize these DDT-metabolizing GST isoenzymes led to the cloning of 10 Delta (*AgGSTD1* through *AgGSTD10*), 1 Sigma (*AgGSTS1*), and 2 Epsilon (*AgGSTE1* and *AgGSTE2*) GST genes from *A. gambiae* (94, 95). Another 18 GST genes have been identified by the *A. gambiae* genome project (92, 96).

Among the 31 GSTs, AgGSTD5, AgGSTD6, and AgGSTE2 metabolize DDT when heterologously expressed in *E. coli* (85, 94, 95). The DDT dehydrochlorinase (DDTase) activity of the AgGSTE2 exceeds the levels reported so far for any GST enzyme and is 350-fold higher than those of the AgGSTD5 and AgGSTD6 GSTs (85). Moreover, *AgGSTE2* is overexpressed at both mRNA and protein levels in the DDT-resistant ZAN/U strain and is physically located within the Epsilon GST cluster that coincides with *rtd1*, one of the two major DDT-resistant loci identified with microsatellite markers in the ZAN/U strain (27, 85, 93, 95). Collectively, these findings suggest that *AgGSTE2* is responsible for some level of DDT resistance in this strain.

DDTase: DDT dehydrochlorinase

***DmGSTD1 and DDT resistance in* Drosophila melanogaster.** Although increased GST activity has been reported in several resistant strains of *D. melanogaster*, direct molecular evidence for the involvement of GSTs has been demonstrated only in the DDT-resistant PSU-R strain selected from the susceptible Oregon-R strain (115). Of the two Delta GST sequences analyzed, the DmGSTD1 protein was overproduced in the resistant PSU-R strain and metabolized DDT. Thus, *DmGSTD1* overexpression is the major mechanism underlying DDT resistance in the PSU-R strain of *D. melanogaster*.

***AaGSTD1 and AaGSTE2 and DDT/pyrethroid resistance in* Aedes aegypti.** In the DDT-resistant GG strain of the yellow fever mosquito *Aedes aegypti*, two of three biochemically and immunologically distinct GST isoenzymes, *GST-1a* and *GST-2* (*AaGSTD1*), are overexpressed (47) either 2- to 5-fold (*GST-1a*) or 25- to 50-fold (*AaGSTD1*) depending on the age and sex of the individuals (47). Overexpression of *AaGSTD1* in the resistant GG strain is due largely to a loss-of-function mutation in an unidentified *trans*-acting repressor that acts to repress *AaGSTD1* mRNA transcription and/or decrease *AaGSTD1* mRNA stability in the susceptible strains (48). However, in the DDT- and permethrin-resistant PMD-R strain, *AaGSTD1* was not overexpressed (78); rather, *AaGSTE2*, the putative ortholog of *AgESTE2*, was overexpressed. Heterologous expression demonstrated that the recombinant AaGSTE2–2 enzyme exhibits both DDTase and glutathione peroxidase activity.

Esterase-Mediated Resistance

The third important group of metabolic enzymes involved in insecticide resistance are esterases. Esterases are frequently implicated in the resistance of insects to OP, carbamates, and pyrethroids through gene amplification, upregulation, coding sequence mutations, or a combination of these mechanisms.

Esterase mutations. Mutations in esterase-coding sequences have been documented in the OP-resistant dipterans *Culex tarsalis*, *Chrysomyia putoria*, *M. domestica*, and *L. cuprina*, in the hymenopteran *Anisopteromalus calandrae*, and in the lepidopteran *Plodia interpunctella* (15, 22, 127, 134). Staining of protein gels with general carboxylesterase substrates revealed that OP resistance in these species is associated with decreased or null carboxylesterase activity. Molecular data support the so-called mutant aliesterase hypothesis (84), which suggests that OP resistance results from amino acid substitutions that increase OP hydrolysis but reduce carboxylester hydrolysis.

OP resistance in *L. cuprina* and *M. domestica* maps to a pair of homologous esterase genes, *Lcα E7* and *Mdα E7*, that share 76% amino acid identity and identical intron positions (15, 22) and have acquired the same Gly137Asp mutation in diazinon-type-resistant strains (22, 83). Molecular modeling of the *Lcα E7* protein has indicated that Gly137, one of three residues comprising the oxyanion hole, is positioned just 4.6 Å away from the oxygen of catalytic residue Ser200 (15, 83). Molecular modeling also suggests that the Gly137Asp mutation alters the orientation of the Ser200-attacking water molecule to facilitate its attack on a phosphorylated serine but diminish reactivity with an acylated serine, leading to gains in the rates of OP hydrolysis and losses in carboxylester hydrolysis. Enzyme assays against OP insecticides and model substrates with heterologously expressed resistant *Lcα E7* and *Mdα E7* alleles as well as synthetic *Lcα E7* chimeras demonstrate that the Gly137Asp mutation enables dianizon-type OP hydrolysis while diminishing or disabling carboxylester hydrolysis.

Another mutation, Trp251Leu, is responsible for the malathion-type resistance in *L. cuprina* and is located in the acyl binding pocket, close to the catalytic Ser200 (4.3 Å) in the three-dimensional structure model (15). It has been proposed that the Trp251Leu mutation creates more space to accommodate substrates with bulky acid groups (e.g., malathion) and reduces the steric obstruction to the inversion that must occur around phosphorus during hydrolysis of OPs (51). Kinetic assays show that, unlike the Gly137Asp mutation, the Trp251Leu mutation has exceptionally high hydrolase activity against malathion, intermediate activity against dimethyl OP, and low activity against diethyl OP, consistent with the OP cross-resistance spectrum between the two OP resistance types (15, 51). Artificial substitutions of the Trp251 with other smaller residues show similar (Ser, Ala, Thr) or better (Gly) OP hydrolysis and malathion carboxylesterase rates than the natural Trp251Leu mutation. However, the effects of these mutations are not additive; a Gly137Asp/Trp251Leu double mutant is less effective at improving OP hydrolysis and malathion carboxylesterase activities than are two single mutants (51).

Esterase gene amplification. Amplifications of specific esterase genes have been documented in at least two orders, including Hemiptera (*Myzus persicae*, *Schizaphis graminum*, and *N. lugens*) and Diptera (*Culex pipiens* complex and *C. tritaeniorhynchus*) (41, 60, 97, 120). In *M. persicae*, overproduction of carboxylesterase *E4* or its paralog *FE4* protein via gene amplification is responsible for enhanced degradation and sequestration of a wide range of insecticides including OPs, carbamates, and pyrethroids (42). *E4* and *FE4* genes are products of a recent gene duplication that share 99% nucleotide identity in their cDNA sequence but differ in

their promoter region and 5′-UTR (41, 42). In susceptible wild-type aphids, the *E4* and *FE4* esterase genes are adjacent to each other in a head-to-tail arrangement, with the *E4* gene located 19 kb upstream of the *FE4* gene. In resistant aphids (R1, R2, or R3), one of the two paralogs is amplified to an extent that is comparable to their resistance levels, while the other gene is maintained as a single copy (40). The amplicon, or the repeated unit of amplification, is approximately 24 kb for the *E4* gene and approximately 20 kb for the *FE4* gene (41, 42). The *E4* and *FE4* amplicons are arranged as tandem arrays of head-to-tail direct repeats, with the *E4* or *FE4* gene situated at a position that is close to the 5′ end of the *E4* or *FE4* amplicon (41, 42).

The *E4* amplification is genetically linked to the autosomal 1,3 translocation event, whereas the *FE4* amplification involves multiple rearrangements, including intrachromosomal inversions and interchromosomal reciprocal interchanges, leading to its wide variation in chromosomal location, zygosity, and copy number (11, 39). But all *FE4*-based resistant aphid clones tested have an *FE4* amplicon cluster located near the subtelomeric repeat in autosome 1, indicating this is the original site of *FE4* gene amplification and, possibly, the location of the single-copy *E4* and *FE4* genes in susceptible aphids (11). The close proximity of this postulated original *E4* and *FE4* locus to the autosome 1 subtelomeric DNA consisting of numerous copies of a 169-bp MpR (*Myzus persicae* group repeat) (110) may predispose the *E4* and *FE4* genes to spontaneous amplification events, which are then selected for because they encode the ability to cope with plant allelochemicals and, more recently, with the widespread use of insecticides. That inverted repeat sequences exist within the *E4* amplicon and a non-LTR retrotransponson-like sequence is associated with the telomeric DNA (11) suggest the possible involvement of transposable elements.

Similarly, OP resistance in the *C. pipiens* complex is due to coamplification (e.g., allele pairs *A2-B2*, *A4-B4*, etc.), single amplification (*B1*), or upregulation (*A1* only) of two paralogous esterase loci on chromosome II, *Est-3* encoding A (or alpha) esterase and *Est-2* encoding B (or beta) esterase, which share 47% amino acid identity (29, 97, 118). The two paralogous genes are oriented in a head-to-head arrangement, with the *Est-3* gene (allele $Est\alpha3^1$) located 1759 bp upstream of the *Est-2* gene (allele $Est\beta1$) in the unamplified susceptible Pel-SS strain (50, 53, 54, 118). Immediately upstream of the *Est-3* locus is a full-length putative aldehyde oxidase (AO) gene oriented in a tail-to-tail arrangement with $Est\alpha3^1$ (53). When amplification occurs, the length of the intergenic spacer sequence containing their common promoter region often increases. In the resistant Pel-RR strain containing the $Est\alpha2^1$-$Est\beta2^1$ coamplicon, the intergenic spacer increases from 1759 bp to 2689 bp owing to two large insertions (428 bp and 512 bp, respectively) and one small insertion of 5 bp (50, 54). In the resistant Col strain containing the $Est\alpha3^1$-$Est\beta1$ (*A3-B1*) coamplicon, the >10 kb intergenic spacer contains multiple 5′-truncated AO genes around the presumed amplicon breakpoint (29, 53). In the resistant Tem-R strain containing the *B1* amplicon, the *B1* amplification unit covers at least 30 kb, including a constant and highly conserved core of 25 kb (82).

Amplicon: the repeated unit of gene amplification

Many other independent de novo amplification events in the two loci generate different allele pairs or alleles that vary in amplicon structure, gene copy number, tissue location, resistance level, fitness cost, and geographical distribution (53, 82). Detailed amplicon structures for these amplified alleles remain largely unknown, but it seems that all amplicons are about 30 to 60 kb long (14, 82) and cluster as a DNA puff in a single extended chromosomal region on chromosome 2L. These amplified alleles (or allele pairs) might replace each other (97) or coexist in the same resistant individuals by forming a composite recombinant allele or allele pair (e.g., *A2-B2/A4-B4*) (10). For any amplified allele or allele pair,

the amplification level is variable and insecticide exposures lead to higher copy number (14).

Esterase gene upregulation. The upregulation of esterase genes has been implicated in the resistance mechanisms of species in the orders Hemiptera (the tarnished plant bug, *Lygus lineolaris*, and the green peach aphid, *M. persicae*), Hymenoptera (the parasitoid wasp *Habrobracon hebetor)*, Lepidoptera (the Asiatic rice borer, *Chilo suppressalis*), and Diptera [the horn fly, *Haematobia irritans* (L.), and *C. pipiens* complex] (42, 38, 50, 54). In the *E4*-amplified resistant *M. persicae*, cytosines of the CpG doublets within the amplified *E4* gene are methylated (creating 5-methylcytosine) or demethylated by an unknown mechanism (38, 40). Methylation leads to expression of the amplified *E4* genes and demethylation leads to underexpression and gene silencing (38, 39). In the absence of insecticide selection, aphid clones with the R3 level of resistance and 80 methylated *E4* genes sometimes spontaneously demethylate their genes and become revertant clones displaying the R1 level of resistance. Because of their potential for reversal of this process, these revertant clones can spontaneously produce offspring that display the R3 level of resistance (40).

In the *A1*-based resistant *C. pipiens* complex, overproduction of the *A1* allele is achieved through upregulation rather than gene amplification (97). In other resistant strains involving amplification of the *Est-3* and *Est-2* loci, upregulation achieved by expansion of their common promoter region also contributes to the overproduction of both transcripts (28, 50, 54). In fact, transient luciferase expression assays have shown that the intergenic sequence of the $Est\alpha 2^1$-$Est\beta 2^1$ amplicon isolated from the resistant Pel-RR strain is transcriptionally more active in directing luciferase expression than the wild-type intergenic spacer sequence isolated from the susceptible Pel-SS strain (50, 54).

BIOCHEMICAL/MOLECULAR MECHANISMS UNDERLYING ALLELOCHEMICAL TOLERANCE

Although much of the information on xenobiotic metabolism in insects derives from studies of pesticide resistance due to the enormous economic implications of the phenomenon, the relationship between detoxifications of pesticides and allelochemicals is gaining increased recognition (46). The exact nature of this relationship, however, has defied efforts at characterization until the advent of molecular toxicological studies.

Selective agents on insect detoxification systems, allelochemicals, or plant defense chemicals are, in general, fundamentally different from insecticides (7, 8). Whereas insecticides tend to be applied as pure compounds, occasionally with synergists, allelochemicals almost invariably occur as complex mixtures of structurally related compounds. In addition, insecticides are designed to effect rapid mortality; many of the most successful and widely used are fast-acting neurotoxins. In contrast, allelochemicals can function effectively to protect plants from herbivory through antixenosis (nonpreference) or growth reduction (8). Owing to the idiosyncratic distribution of allelochemical biosynthesis among plant families, polyphagous insects likely encounter a broad diversity of chemical structures; in contrast, insecticides are often applied in a broadcast fashion and are more likely to be encountered irrespective of host plant identity. As a result, whereas the acquisition of metabolic resistance to insecticides within a species can be associated with an increase in tolerance of 10,000-fold or greater, intraspecific variation in metabolic resistance to allelochemicals tends to be of much smaller magnitude.

Esterase and Allelochemical Tolerance

Esterase involvement in allelochemical metabolism and tolerance has been

documented only at the biochemical level in a few cases. Two closely related tiger swallowtails, *Papilio glaucus* and *P. canadensis* (described originally as two subspecies of *P. glaucus*), are differentially tolerant of phenolic glycosides in their salicaceous host plants (75). Synergism bioassay with coadministration of the phenolic glycosides salicortin or tremulacin and various inhibitors for detoxification enzymes indicated that esterase is responsible for higher glycoside resistance in *P. canadensis*. Subsequent enzymatic assays demonstrated that soluble esterase activities were threefold higher in *P. canadensis* and inducible by phenolic glycosides (75). In the tobacco cutworm *Spodoptera litura*, sublethal doses of the widely occurring plant glycoside rutin resulted in a significant increase in midgut carboxylesterase activity, even though it is not metabolized (45). A third case involves the gypsy moth, *Lymantria dispar*, in which the survival rate of first instars feeding on artificial diets containing phenolic glycosides was positively correlated with esterase activity, suggesting that esterase may be responsible for glycoside metabolism (76).

GST and Allelochemical Tolerance

Participation of insect GSTs in allelochemical tolerance has also been documented primarily at the biochemical level. GST isoenzymes isolated from the polyphagous fall armyworm, *Spodoptera frugiperda*, metabolize organothiocyanates (allyl thiocyanate, benzyl thiocyanate, and 2-phenylethyl isothiocyanate) and α,β-unsaturated carbonyls (*trans*-2-octenal, *trans*-2-nonenal, 2, 4-hexadienal, *trans*, *trans*-2, 4-heptadienal, *trans*, *trans*-2, 4-nonadienal, and *trans*, *trans*-2, 4-decadienal) (122, 123, 130). In contrast, GSTs isolated from the less polyphagous *Trichoplusia ni* can metabolize only two organothiocyanates (allyl thiocyanate and benzyl thiocyanate), and the more specialized *Anticarsia gemmatalis* can metabolize only one organothiocyanate (benzyl thiocyanate), consistent with their host range difference (123).

Biochemical assays have also demonstrated the inducibility of GSTs by allelochemicals and host plant foliage. Among the most potent inducers are host plants in the Apiaceae (including parsley, *Petroselinum crispum*, and parsnip, *Pastinaca sativa*) and Brassicaceae (crucifers), and among the active allelochemical inducers are furanocoumarins (xanthotoxin), indoles (indole-3-carbinol, indole-3-acetonitrile), flavonoids (flavone), isothiocyanates (allyl and benzyl isothiocyanate), α,β-unsaturated carbonyls (*trans*-cinnamaldehyde, *trans*-2-hexenal, *trans*, *trans*-2, 4-decadienal, and benzaldehyde), and glucosinolates (sinigrin and β-phenylethylisothiocyanate) (122, 123). Some allelochemicals, however, act as potent GST inhibitors (quercetin, ellagic acid, juglone, apigenin, and tannic acid) (65, 129) or transcription repressors (quercetin) (1). The presence of effective GST inhibitors or repressors in plant tissues may represent counteradaptations evolved to circumvent GST-mediated metabolism of co-occurring allelochemicals by insect herbivores.

Several cDNA sequences encoding GSTs that may be involved in allelochemical metabolism have been cloned from the spruce budworm, *Choristoneura fumiferana* (34), the tobacco hornworm, *Manduca sexta* (108), and the Hessian fly, *Mayetiola destructor* (128). Although *CfGSTS1* (*CfGST*) from *C. fumiferana* is expressed mainly in fat body and is associated with larval diapause, its inducibility at both mRNA and protein levels by balsam fir foliage and the insecticides (e.g., Bt delta-endotoxin, tebufenozide, and permethrin) suggests that it may also be involved in detoxification of both allelochemicals and insecticides (34). *MdesGSTD1* (*mdesgst1–1*) from the avirulent GP biotype Hessian fly is expressed in midgut, fat body, and salivary glands of larvae. In siRNA experiments, injection of double-stranded *MdesGSTD1* RNA into embryos resulted in significantly reduced (50% decrease) survival of first instars on

wheat plants and delayed development in the surviving larvae, implicating *MdesGSTD1* in the adaptation of Hessian fly larvae to wheat defense compounds (128).

Among the three *M. sexta* GST cDNA sequences, *MsGSTD1* (*MsGST1*) and *MsGSTS1* (*MsGST2*) were isolated from larval midguts (108), and *MsGSTD2* (*GST-msolf1*) was isolated from adult antennae (98). Of these, *MsGSTD1* is inducible not only by the drug phenobarbital, as are many P450s, but also by 2-undecanone, a host plant trichome constituent (108). *MsGSTD2* is antennae specific and highly enriched in male antennae (98). Enzymatic analyses have demonstrated that the endogenous GSTs from antennae are capable of metabolizing *trans*-2-hexenal, a volatile green leaf aldehyde that stimulates the olfactory system of *M. sexta*. These data, and that the sex pheromone of *M. sexta* consists of a complex mixture of aldehydes, suggest that *MsGSTD2* may play a dual role of detoxifying volatile allelochemicals that interfere with sex pheromone detection and inactivating the aldehyde sex pheromone signal itself (98).

P450 and Allelochemical Tolerance

Insect P450s play a paramount role in allelochemical metabolism and tolerance. P450s metabolize all classes of plant allelochemicals because of their catalytic versatility and broad substrate specificity. Participation of insect P450s in allelochemical metabolism and tolerance has been defined at both the biochemical and molecular levels.

P450 genes involved in allelochemical tolerance. Biochemical evidence for P450 involvement in allelochemical tolerance is compelling. Insect P450s can metabolize a wide range of plant allelochemicals, including furanocoumarins, terpenoids, indoles, glucosinolates, flavonoids, cardenolides, phenylpropenes, ketohydrocarbons, alkaloids, lignans, pyrethrins, and the isoflavonoid rotenone (36, 43, 103); both pyrethrins and rotenone have long been used as botanical insecticides. Moreover, many intact host plants, including corn, parsnip, parsley, cowpeas, cotton, peanuts, soybean, and hairy indigo, and isolated allelochemicals, including monoterpenes, indoles, furanocoumarins, and flavones, induce expression of particular P450s (103). The molecular genetics of these metabolic reactions and induction responses, however, have been defined in only a few systems.

Perhaps best characterized are the P450s involved in furanocoumarin metabolism within the genus *Papilio* (24, 56–58, 68, 71, 103). The first two of these P450 cDNAs, *CYP6B1v1* and *CYP6B3v1*, were isolated from the black swallowtail, *P. polyxenes*, a specialist restricted to feeding on furanocoumarin-containing plants in Apiaceae and Rutaceae. *CYP6B1v1* and *CYP6B3v1* are inducible by the linear furanocoumarin xanthotoxin and, to a lesser extent, by the less frequently encountered angular furanocoumarin angelicin (56, 57, 89, 90, 103). Subsequently, cDNAs representing 16 *CYP6B* genes were isolated from *P. glaucus* and *P. canadensis*, two more polyphagous papilionids; transcripts of all these P450s are inducible by furanocoumarins at ecologically relevant concentrations (68). That these *Papilio* cDNAs encode furanocoumarin-metabolizing P450 enzymes was demonstrated by baculovirus-mediated expression of their cDNAs in Sf9 and Tn5 cells (6, 19, 71, 86, 103, 126).

Additional strong evidence for P450 participation in plant allelochemical metabolism exists for two other types of interactions between plants and oligophagous insects. Within the cactophilic *Drosophila*, three *CYP28A* genes have been isolated from *D. mettleri* and *D. nigrospiracula* (43), which develop in necrotic tissues of or soils underlying the alkaloid-containing columnar cacti saguaro and cardon (26). These P450 transcripts are inducible in response to alkaloids, with *CYP28A1* in *D. mettleri* induced by senita alkaloids, *CYP28A2* in *D. mettleri* induced by senita and saguaro alkaloids, and *CYP28A3* in *D. nigrospiracula* induced by saguaro alkaloids

(26). In *M. sexta*, a specialist on Solanaceae, at least five *CYP4* and three *CYP9A* genes are expressed in the larvae (107, 112). Among these, *CYP4M1* and *CYP4M3* transcripts are induced by the solanaceous allelochemicals nicotine, 2-undecanone, and 2-tridecanone, as well as by the synthetic substance clofibrate. *CYP9A2*, *CYP9A4*, and *CYP9A5* transcripts are induced by 2-undecanone, 2-tridecanone, indole-3-carbinol, xanthotoxin, and clofibrate, but not by nicotine (112), despite its presence in some host plants. Substrate specificities for these P450s have not yet been defined.

Specialist/generalist counterdefense strategies: CYP6Bs as a paradigm. Specialization and generalization represent ends of the spectrum for the utilization of host plants by herbivorous insects. Whereas specialists typically encounter high levels of a narrow and predictable range of dietary allelochemicals, generalists have to cope with a tremendous diversity of allelochemicals idiosyncratically distributed among potential host plants. To overcome their unique toxicological challenges, in theory, specialists should have a highly efficient and specialized detoxification system, whereas generalists require an all-purpose detoxification system capable of degrading a broad range of plant toxins present in their host plants as well as complex regulatory machinery capable of inducing a subset of enzymes when encountering a particular allelochemical.

Long-term studies focusing on P450-mediated allelochemical metabolism in two specialists (*P. polyxenes* and the parsnip webworm, *Depressaria pastinacella*) and three generalists (*P. glaucus*, *P. canadensis*, and the corn earworm, *Helicoverpa zea*) provide a paradigm for dissecting the evolutionary and molecular mechanisms that facilitate polyphagy and specialization. These five lepidopteran species are able to feed on furanocoumarin-containing plants, but their host ranges and the rate at which they encounter furanocoumarins differ significantly. The two specialists feed exclusively on furanocoumarin-containing plants. *P. glaucus* feeds occasionally on furanocoumarin-containing host plants; *H. zea* feeds rarely on furanocoumarin-containing host plants; and *P. canadensis*, in contrast, normally does not encounter furanocoumarins in any of its host plants. From these five species, two *CYP6B* cDNAs/genes have been characterized from each of the two specialists, nine have been characterized from each of the *Papilio* generalists, and four have been characterized from *H. zea* (24, 56, 58, 69, 71, 74, 103). Cloning of an additional highly divergent *CYP321A1* sequence from *H. zea* demonstrated that sequences outside the *CYP6B* subfamily [but phylogenetically in the same clade (71)] also metabolize furanocoumarins (101). Given the similar cloning efforts directed at these species, the differential recovery rates of *CYP6B* sequences favor the hypothesis that generalists may have more allelochemical-metabolizing genes to cope with the diversity of allelochemicals they encounter.

SRS: substrate recognition site

Molecular modeling and mutagenesis studies have demonstrated that CYP6B1 from the specialist *P. polyxenes* has an aromatic resonant network consisting of Phe116 and His117 of SRS1 (substrate recognition site 1), Phe371 of SRS5, and Phe484 of SRS6, as well as a polar cage that surrounds the aromatic network in its hydrophobic catalytic pocket (6, 19, 66). This structural arrangement stabilizes the CYP6B1 catalytic pocket in a configuration optimal for interacting with its principal substrates but compromises its ability to accommodate structurally different allelochemicals. Neither of these networks exists in the catalytic pockets of the generalist *H. zea* CYP6B8 and *P. glaucus* CYP6B4 (71, 66). As a result, the catalytic pocket predicted in generalist CYP6B proteins appears to be larger and more flexible, allowing for metabolism of a wide range of allelochemicals but at the cost of lower catalytic efficiency.

Metabolic data support these structural predictions, with the generalist CYP6B8 efficiently metabolizing six diverse plant

ARE: antioxidant-response element

XRE: xenobiotic-response element

allelochemicals (xanthotoxin, quercetin, flavone, chlorogenic acid, indole-3-carbinol, and rutin) and the specialist CYP6B1 efficiently metabolizing linear and angular furanocoumarins (xanthotoxin and angelicin) and less efficiently metabolizing flavone and α-napthoflavone (66, 126). The catalytic efficiencies with which CYP6B8 metabolizes the six allelochemicals are 7.1- to 42.2-fold lower than the levels achieved by CYP6B1 in metabolizing its principal furanocoumarin substrate and are comparable to the rates at which CYP6B1 metabolizes flavone and α-napthoflavone. CYP6B1 also displays higher activities against linear furanocoumarins than the generalist P450s from the sibling species *P. glaucus* and *P. canadensis* (71). The notably inverse relationship between catalytic efficiencies and substrate ranges of CYP6Bs suggests a trade-off for host plant use strategies, which may partially explain why most herbivorous insects are oligophagous.

Metabolism analyses using larval midguts show that P450-mediated constitutive metabolism of furanocoumarins is significantly higher in the two specialists than in the three generalists (73, 131). The inducibility of P450-mediated furanocoumarin metabolism, however, is generally higher in the three generalists than in the two specialists. Consistent with these larval metabolism assays, transcripts of the specialist *P. polyxenes CYP6B1* are constitutively detectable and 2.9- to 3.7-fold inducible by xanthotoxin (89), whereas transcripts of the generalist *P. glaucus CYP6B4* are barely detectable and 312-fold inducible by xanthotoxin (68). Moreover, when transfected in Sf9 cells, the −146/+22 CYP6B1:CAT promoter construct displays 20- to 30-fold-higher basal activities and 17- to 20-fold-higher xanthotoxin-inducible activities than the −506/+29 CYP6B4:CAT promoter construct (81). The *CYP6B4* promoter, however, displays higher inducibility than the *CYP6B1* promoter (sixfold versus twofold) by xanthotoxin. Mutational analyses suggest that the juxtaposition of the EcRE/ARE (antioxidant-response element)/XRE (xenobiotic-response element)-xan element, C/EBP binding site, and Inr element near the transcription site and TGAC core sequence repeats are responsible for the high level of constitutive transcription of the *CYP6B1v3* promoter (13, 88). Variations in the overlapping EcRE/ARE/XRE-xan element and the presence of additional EcRE and Oct-1 elements in the CYP6B4 promoter may account for its lower constitutive expression and higher inducibility (13, 81, 88). All of these findings are consistent with the notion that the regulatory machineries for this detoxification system are on at all times and adjustable within a narrow range in specialists. In contrast, the regulatory machinery in generalists is constitutively off and highly induced by allelochemicals.

Evolutionary associations between insecticide- and allelochemical-metabolizing P450s. For almost four decades, it has been hypothesized that the ability to cope with a toxin-rich diet serves as a preadaptation for the acquisition of insecticide resistance (46). Because many synthetic insecticides resemble plant allelochemicals or are, in the case of the pyrethroids, derived from them, the possibility exists that the P450s responsible for insecticide detoxification have evolved from the P450s responsible for allelochemical detoxification. Recent studies on *H. zea* (66, 67, 69, 70, 72, 73, 101) support this notion. In this species, exposure to xanthotoxin enhanced resistance to the insecticide cypermethrin (73), with at least two P450s capable of metabolizing both substrates (66, 73, 101).

The cloning of seven P450s distributed in the *CYP6B*, *CYP4M*, and *CYP321A* subfamilies from this species (67, 69, 101) and induction analyses have demonstrated that *CYP321A1* transcripts are highly inducible by xanthotoxin (101), and that four *CYP6B* transcripts are inducible to varying degrees by a range of allelochemicals naturally encountered in host plants (xanthotoxin, indole-3-carbinol, chlorogenic acid, and flavone) as

well as synthetic chemicals not naturally encountered (cypermethrin and phenobarbital) (70). The *CYP6B* transcripts are also inducible to some extent by the plant defense signaling molecules jasmonate and salicylate, allowing this species to eavesdrop on plant defense signals and activate their detoxification systems in advance of induced biosynthesis of host plant toxins (72). Baculovirus-mediated expression of the CYP6B8 and CYP321A1 proteins has directly demonstrated that the proteins are capable of metabolizing a range of allelochemicals (xanthotoxin, chlorogenic acid, quercetin, and flavone) and insecticides (diazinon, cypermethrin, and aldrin) (66, 101). These data provided compelling genetic and molecular evidence for an evolutionary association between resistance to insecticides and resistance to plant allelochemicals.

RESISTANCE EVOLUTION AND TRANSPOSABLE ELEMENTS

Resistance, in nature, is an expansion and fixation of adaptive genomic alterations driven by allelochemical and/or insecticide selection. Because most host plant allelochemicals and insecticides act as selection agents rather than mutagens, adaptive genomic changes should arise primarily from internal spontaneous alteration events. Transposable elements (TEs) not only comprise a significant fraction of eukaryotic genomes but also can have a dramatic impact on the variability of eukaryotic genomes in the forms of insertion and ectopic recombination (30). Thus many of the genomic changes such as gene amplification, overexpression, and coding sequence mutation associated with resistance may be transposon mediated.

That transposons have been found frequently within or in close proximity to resistance genes provides indirect evidence that transposons are involved in generating resistance-related adaptive genomic changes. In *D. melanogaster*, a LTR TE 17.6 and a 1.7-kb *Bari-1* TE are present in the 3′-flanking DNA of the DDT-resistant *CYP6A2* (36) and the lufenuron-resistant *CYP12A4* (12), respectively, although there appears to be no correlation between TE insertion and resistance. In *M. domestica*, *mariner*-like TEs exist in the 5′-flanking DNA of the pyrethroid-resistant *CYP6D1* and *CYP6D3* paralogs (62). Inverted repeat sequences exist within the E4 amplicon in *M. persicae* (11) and two repetitive sequence elements exist within the B1 amplicon in the OP-resistant *Culex pipiens* Tem-R strain (82). Even the two amino acid mutations in the OP-resistant *M. domestica MdEα7* could be mediated by the transposon *maT*, because two defective genomic copies of *maT* with 85% identity to each other were found in the first intron and in the 3′-flanking sequence of *MdEα7*, respectively (21).

Several recent studies provide direct evidence for a definite link between resistance and transposons. In the putative Bt-toxin receptor gene cadherin, insertion of a 2.3-kb LTR retrotransposon Hel-1 leads to 3′-truncated nonfunctional cadherin protein and Bt resistance in a laboratory-selected *Heliothis virescens* strain (44). Parallel insertions of *Accord* LTR or *Doc* non-LTR retrotransposon into the 5′-regulatory region of *CYP6G1* in *D. melanogaster* or *D. simulans* are associated with *CYP6G1* upregulation and DDT resistance (17, 102). Also in *D. melanogaster*, insertion of *Doc1420* retrotransposon into the second exon of CG10618 (CHKov1) generates two sets of altered transcripts and a novel protein that confers OP resistance (3).

The frequency with which transposon-mediated genetic change is associated with insecticide resistance contrasts with the essential absence of transposon involvement in allelic variation in known allelochemical resistance. Transposon-mediated changes in regulation can lead to massive, rapid changes in expression, responses that are potentially highly adaptive in the face of rapid introduction of a major mortality agent in the environment (i.e., an insecticide). Mutational changes in plant allelochemicals are unlikely to bring about orders-of-magnitude changes in mode of action or in toxicity. Accordingly,

Transposons: DNA sequences that copy and move themselves in eukaryotic genomes

mutational change associated with allelochemical resistance may be acquired more slowly as a result of the accumulation of small changes in structural genes.

CONCLUSIONS

Understanding the molecular mechanisms underlying insecticide resistance and elucidating the evolutionary processes by which resistance is acquired remain urgent priorities. New challenges presented by the massive influx of genome sequencing information include developing methods for determining the functions of large inventories of detoxification proteins and characterizing regulatory networks that coordinate responses across gene families. A continuing challenge, however, is to use what is known of molecular mechanisms of metabolic resistance in the design and implementation of environmentally sustainable pest management programs. Irrespective of their structures, modes of action, or methods of application, it is a virtual certainty that the chemical pesticides in use at present, in development, or yet to be conceived will ultimately select for resistance in target insect populations and that such resistance will at least in some cases involve metabolic enzyme systems.

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LITERATURE CITED

1. Ahmad S, Pardini S. 1990. Antioxidant defense of the cabbage looper, *Trichoplusia ni*: enzymatic responses to the superoxide-generating flavonoid, quercetin and photodynamic furanocoumarin, xantotoxin. *Photochem. Photobiol.* 51:305–12
2. Amichot M, Tarès S, Brun-Barale A, Arthaud L, Bride JM, Bergé JB. 2004. Point mutations associated with insecticide resistance in the *Drosophila* cytochrome P450 *Cyp6a2* enable DDT metabolism. *Eur. J. Biochem.* 271:1250–57
3. **Aminetzach YT, Macpherson JM, Dmitri A, Petrov DA. 2005. Pesticide resistance via transposition-mediated adaptive gene truncation in *Drosophila*. *Science* 309:764–67**
4. Andersen JF, Utermohlen JG, Feyereisen R. 1994. Expression of housefly CYP6A1 and NADPH-cytochrome P450 reductase in *Escherichia coli* and reconstitution of an insecticide-metabolizing P450 system. *Biochemistry* 33:2171–77
5. Andersen JF, Walding JK, Evans PH, Bowers WS, Feyereisen R. 1997. Substrate specificity for the epoxidation of terpenoids and active site topology of house fly cytochrome P450 6A1. *Chem. Res. Toxicol.* 10:156–64
6. Baudry J, Li W, Pan L, Berenbaum MR, Schuler MA. 2003. Molecular docking of substrates and inhibitors in the catalytic site of CYP6B1, an insect cytochrome P450 monooxygenase. *Protein Eng.* 16:577–87
7. Berenbaum MR. 1990. Plant consumers and plant secondary metabolites: past, present, and future. *Oxford Rev. Evol. Biol.* 7:285–307
8. Berenbaum MR. 1995. Chemical defense: theory and practice. *Proc. Natl. Acad. Sci. USA* 92:2–8
9. Berenbaum MR. 2002. Postgenomic chemical ecology: from genetic code to ecological interactions. *J. Chem. Ecol.* 28:873–96

3. Showed that transposon insertion into one gene could generate a new functional protein that confers insecticide resistance.

10. Berticat C, Marquine M, Raymond M, Chevillon C. 2001. Recombination between two amplified esterase alleles in *Culex pipiens*. *J. Hered.* 92:349–51
11. Blackman RL, Spence JM, Field LM, Devonshire AL. 1999. Variation in the chromosomal distribution of amplified esterase (FE4) genes in Greek field populations of *Myzus persicae* (Sulzer). *Heredity* 82:180–86
12. Bogwitz MR, Chung H, Magoc L, Rigby S, Wong W, et al. 2005. Cyp12a4 confers lufenuron resistance in a natural population of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 102:12807–12
13. Brown RP, Berenbaum MR, Schuler MA. 2004. Transcription of a lepidoteran cytochrome P450 promoter is modulated by multiple elements in its 5' UTR and repressed by 20-hydroxyecdysone. *Insect Mol. Biol.* 13:337–47
14. Callaghan A, Guillemaud T, Makate N, Raymond M. 1998. Polymorphism and fluctuations in copy number of amplified esterase genes in *Culex pipiens* mosquitoes. *Insect Mol. Biol.* 7:295–300
15. Campbell PM, Newcomb RD, Russell RJ, Oakeshott JG. 1998. Two different amino acid substitutions in the ali-esterase, E3, confer alternative types of organophosphorus insecticide resistance in the sheep blowfly, *Lucilia cuprina*. *Insect Biochem. Mol. Biol.* 28:139–50
16. Cariño FA, Koener JF, Plapp FW, Feyereisen R. 1994. Constitutive overexpression of the cytochrome P450 gene *CYP6A1* in a house fly strain with metabolic resistance to insecticides. *Insect Biochem. Mol. Biol.* 24:411–18
17. **Catania F, Kauer MO, Daborn PJ, Yen JL, ffrench-Constant RH, et al. 2004. World-wide survey of an Accord insertion and its association with DDT resistance in *Drosophila melanogaster*. *Mol. Ecol.* 13:2491–504**
18. Chelvanayagam G, Parker MW, Board PG. 2001. Fly fishing for GSTs: a unified nomenclature for mammalian and insect glutathione transferases. *Chem. Biol. Interact.* 133:256–60
19. **Chen JS, Berenbaum MR, Schuler MA. 2002. Amino acids in SRS1 and SRS6 are critical for furanocoumarin metabolism by CYP6B1v1, a cytochrome P450 monooxygenase. *Insect Mol. Biol.* 11:175–86**
20. Chiang FM, Sun CN. 1993. Glutathione transferase isozymes of diamondback moth larvae and their role in the degradation of some organophosphorus insecticides. *Pestic. Biochem. Physiol.* 45:7–14
21. Claudianos C, Brownlie J, Russell R, Oakeshott J, Whyard S. 2002. maT-A clade of transposons intermediate between mariner and Tc1. *Mol. Biol. Evol.* 19(12):2101–9
22. Claudianos C, Russell RJ, Oakeshott JG. 1999. The same amino acid substitution in orthologous esterases confers organophosphate resistance on the house fly and a blowfly. *Insect Biochem. Mol. Biol.* 29:675–86
23. Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, et al. 2006. A deficit of metabolic enzymes: pesticide sensitivity and environmental response in the honey bee. *Insect Mol. Biol.* In press
24. Cohen MB, Schuler MA, Berenbaum MR. 1992. A host-inducible cytochrome P450 from a host-specific caterpillar: molecular cloning and evolution. *Proc. Natl. Acad. Sci. USA* 89:10920–24
25. Daborn PJ, Yen JL, Bogwitz MR, Goff G, Le Feil E, et al. 2002. A single P450 allele associated with insecticide resistance in *Drosophila*. *Science* 297:2253–56
26. Danielson PB, MacIntyre RJ, Fogleman JC. 1997. Molecular cloning of a family of xenobiotic-inducible drosophilid cytochrome P450s: evidence for involvement in host-plant allelochemical resistance. *Proc. Natl. Acad. Sci. USA* 94:10797–802

17. Conducted a worldwide survey of *D. melanogaster* populations and demonstrated complete concordance among *Accord* insertion in the *CYP6G1* promoter region, overexpression, and insecticide resistance.

19. Established that an aromatic network holds the catalytic pocket of the specialist cytochrome P450 CYP6B1 in an optimal configuration for its principal furanocoumarin substrates.

27. David J, Strode C, Vontas J, Nikou D, Vaughan A, et al. 2005. The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. *Proc. Natl. Acad. Sci. USA* 102:4080–84
28. De Silva D, Hemingway J. 2002. Structural organization of the Est$\alpha 3^1$ gene in a Colombian strain of *Culex quinquefasciatus* differs from that in Cuba. *Med. Vet. Entomol.* 16:99–105
29. De Silva D, Hemingway J, Ranson H, Vaughan A. 1997. Resistance to insecticides in insect vectors of disease: Est-alpha-3, a novel amplified esterase associated with Est-beta-1 from insecticide resistant strains of the mosquito *Culex quinquefasciatus*. *Exp. Parasitol.* 87:253–59
30. Dewannieux M, Heidmann T. 2005. LINEs, SINEs and processed pseudogenes: parasitic strategies for genome modeling. *Cytogenet. Genome Res.* 110:35–48
31. Dombrowski SM, Krishnan R, Witte M, Maitra S, Diesing C, et al. 1998. Constitutive and barbital-induced expression of the *CYP6A2* allele of a high producer strain of *CYP6A2* in the genetic background of a low producer strain. *Gene* 221:69–77
32. Dunkov BC, Guzov VM, Mocelin G, Shotkoski F, Brun A, et al. 1997. The *Drosophila* cytochrome P450 gene *Cyp6a2*: structure, localization, heterologous expression, and induction by phenobarbital. *DNA Cell Biol.* 16:1345–56
33. Enayati AA, Ranson H, Hemingway J. 2005. Insect glutathione transferases and insecticide resistance. *Insect Mol. Biol.* 14:3–8
34. Feng QL, Davey KG, Pang ASD, Ladd TR, Retnakaran A, et al. 2001. Developmental expression and stress induction of glutathione S-transferase in the spruce budworm, *Choristoneura fumiferana*. *J. Insect Physiol.* 47:1–10
35. Festucci-Buselli RA, Carvalho-Dias AS, de Oliveira-Andrade M, Caixeta-Nunes C, Li H-M, et al. 2005. Expression of *Cyp6g1* and *Cyp12d1* in DDT resistant and susceptible strains of *Drosophila melanogaster*. *Insect Mol. Biol.* 14:69–77
36. Feyereisen R. 2005. Insect cytochrome P450. In *Comprehensive Molecular Insect Science*, Vol. 4, ed. LI Gilbert, K Latrou, SS Gill, pp. 1–77. Oxford, UK: Elsevier
37. Ffrench-Constant RH, Pittendrigh B, Vaughan A, Anthony N. 1998. Why are there so few resistance-associated mutations in insecticide target genes? *Philos. Trans. R. Soc. London B* 353:1685–93

38. Demonstrated that methylation leads to transcription of amplified esterase genes and resistance and demeythlation leads to gene silencing.

38. Field LM. 2000. Methylation and expression of amplified esterase genes in the aphid *Myzus persicae* (Sulzer). *Biochem. J.* 349:863–68

39. Field LM, Blackman RL. 2003. Insecticide resistance in the aphid *Myzus persicae* (Sulzer): chromosome location and epigenetic effects on esterase gene expression in clonal lineages. *Biol. J. Linn. Soc.* 79:107–13
40. Field LM, Blackman RL, Tyler-Smith C, Devonshire AL. 1999. Relationship between amount of esterase and gene copy number in insecticide-resistant *Myzus persicae* (Sulzer). *Biochem. J.* 339:737–42
41. Field LM, Devonshire AL. 1997. Structure and organization of amplicons containing the E4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae* (Sulzer). *Biochem. J.* 322:867–71
42. Field LM, Devonshire AL. 1998. Evidence that the E4 and FE4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae* (Sulzer) are part of a gene family. *Biochem. J.* 330:169–73
43. Fogleman JC, Danielson PB, Macintyre RJ. 1998. The molecular basis of adaptation in *Drosophila*: the role of cytochrome P450s. *Evol. Biol.* 30:15–77
44. Gahan LJ, Gould F, Heckel DG. 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens*. *Science* 293:857–60

45. Ghumare SS, Mukherjee SN, Sharma RN. 1989. Effects of rutin on the neonate sensitivity, dietary utilization and midgut carboxylesterase activity of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Proc. Indian Acad. Sci. Anim. Sci*. 98:399–404
46. Gordon HT. 1961. Nutritional factors in insect resistance to chemicals. *Annu. Rev. Entomol*. 6:27–54
47. Grant GF, Dietze EC, Hammock BD. 1991. Glutathione S-transferase isozymes in *Aedes aegypti*: purification, characterization, and isozyme-specific regulation. *Insect Biochem*. 21:421–33
48. Grant DF, Hammock BD. 1992. Genetic and molecular evidence for a *trans*-acting regulatory locus controlling glutathione S-transferase-2 expression in *Aedes aegypti*. *Mol. Gen. Genet*. 234:169–76
49. Guzov VM, Unnithan GC, Chernogolov AA, Feyereisen R. 1998. *CYP12A1*, a mitochondrial cytochrome P450 from the housefly. *Arch. Biochem. Biophys*. 359:231–40
50. Hawkes NJ, Hemingway J. 2002. Analysis of the promoters for the β-esterase genes associated with insecticide resistance in the mosquito *Culex quinquefasciatus*. *Biochim. Biophys. Acta* 1574:51–62
51. Heidari R, Devonshire AL, Campbell BE, Bell KL, Dorrian SJ, et al. 2004. Hydrolysis of organophosphorus insecticides by in vitro modified carboxylesterase E3 from *Lucilia cuprina*. *Insect Biochem. Mol. Biol.* 34:353–63
52. Helvig C, Tijet N, Feyereisen R, Walker FA, Restifo LL. 2004. *Drosophila melanogaster* CYP6A8, an insect P450 that catalyzes lauric acid (omega-1)-hydroxylation. *Biochem. Biophys. Res. Commun*. 325:1495–502
53. Hemingway J, Coleman M, Paton M, Vaughan MA, De Silva D. 2000. Aldehyde oxidase is coamplified with the world's most common *Culex* mosquito insecticide resistance-associated esterases. *Insect Mol. Biol*. 9:93–99
54. Hemingway J, Hawkes N, Prapanthadara L, Jayawardenal KGI, Ranson H. 1998. The role of gene splicing, gene amplification and regulation in mosquito insecticide resistance. *Philos. Trans. R. Soc. London B* 353:1695–99
55. Hemingway J, Hawkes NJ, McCarroll L, Ranson H. 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem. Mol. Biol*. 34:653–65
56. Hung C-F, Harrison TL, Berenbaum MR, Schuler MA. 1995. CYP6B3: a second furanocoumarin-inducible cytochrome P450 expressed in *Papilio polyxenes*. *Insect Mol. Biol*. 4:149–60
57. Hung C-F, Prapaipong H, Berenbaum MR, Schuler MA. 1995. Differential induction of cytochrome P450 transcripts in *Papilio polyxenes* by linear and angular furanocoumarins. *Insect Biochem. Mol. Biol*. 25:89–99
58. Hung C-F, Berenbaum MR, Schuler MA. 1997. Isolation and characterization of CYP6B4, a furanocoumarin-inducible cytochrome P450 from a polyphagous caterpillar (Lepidoptera: Papilionidae). *Insect Biochem. Mol. Biol*. 27:377–85
59. Huang HS, Hu NT, Yao YE, Wu CY, Chiang SW, et al. 1998. Molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the diamondback moth, *Plutella xylostella*. *Insect Biochem. Mol. Biol*. 28:651–58
60. Karunaratne SHPP, Vaughan A, Paton MG, Hemingway J. 1998. Amplification of a serine esterase gene is involved in insecticide resistance in Sri Lankan *Culex tritaeniorhynchus*. *Insect Mol. Biol*. 7:307–15
61. Kasai S, Scott JG. 2001. A house fly gene homologous to the zinc finger proto-oncogene *Gfi-1*. *Biochem. Biophys. Res. Commun*. 283:644–47
62. Kasai S, Scott JG. 2001. Cytochrome P450s *CYP6D3* and *CYP6D1* are part of a P450 gene cluster on autosome 1 in the house fly. *Insect Mol. Biol*. 10:191–96

48. Provided genetic and molecular evidence for a *trans*-acting regulatory locus controlling the upregulation of a resistance-conferring GST gene.

51. Revealed why the two amino acid mutations in the OP-resistant *LcαE7* alleles effect OP hydrolysis but reduce or eliminate carboxylester hydrolysis.

54. Reviewed the roles of gene amplification, splicing, and upregulation in insecticide resistance in mosquitoes.

63. Kasai S, Scott JG. 2001. Expression and regulation of *CYP6D3* in the house fly, *Musca domestica* (L.). *Insect Biochem. Mol. Biol.* 32:1–8
64. Ku CC, Chiang FM, Hsin CY, Yao YE, Sun CN. 1994. Glutathione transferase isozymes involved in insecticide resistance of diamondback moth larvae. *Pestic. Biochem. Physiol.* 50:191–97
65. Lee K. 1991. Glutathione S-transferase activities in phytophagous insects: induction and inhibition by plant phototoxins and phenols. *Insect Biochem.* 21:353–62
66. Li X, Baudry J, Berenbaum MR, Schuler MA. 2004. Structural and functional evolution of insect CYP6B proteins: from specialist to generalist P450. *Proc. Natl. Acad. Sci. USA* 101:2939–44
67. Li X, Berenbaum MR, Schuler MA. 2000. Molecular cloning and expression of CYP6B8: a xanthotoxin-inducible cytochrome P450 cDNA from *Helicoverpa zea*. *Insect Biochem. Mol. Biol.* 30:75–84
68. Li W, Berenbaum MR, Schuler MA. 2001. Molecular analysis of multiple *CYP6B* genes from polyphagous *Papilio* species. *Insect Biochem. Mol. Biol.* 31:999–1011
69. Li X, Berenbaum MR, Schuler MA. 2002. Cytochrome P450 and actin genes expressed in *Helicoverpa zea* and *Helicoverpa armigera*: ortholog/paralog identification, gene conversion, and evolution. *Insect Biochem. Mol. Biol.* 32:311–20
70. Li X, Berenbaum MR, Schuler MA. 2002. Plant allelochemicals differentially regulate *Helicoverpa zea* cytochrome P450 genes. *Insect Mol. Biol.* 11:343–52
71. Li W, Berenbaum MR, Schuler MA. 2003. Diversification of furanocoumarin-metabolizing cytochrome P450s in two papilionids: specificity and substrate encounter rate. *Proc. Natl. Acad. Sci. USA* 100:14593–98
72. Li X, Schuler MA, Berenbaum MR. 2002. Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature* 419:712–15
73. Li X, Zangerl AR, Schuler MA, Berenbaum MR. 2000. Cross-resistance between xanthotoxin and α-cypermethrin in *Helicoverpa zea*. *J. Econ. Entomol.* 93:18–25
74. Li W, Zangerl AR, Schuler MA, Berenbaum MR. 2004. Characterization and evolution of furanocoumarin inducible cytochrome P450s in the parsnip webworm, *Depressaria pastinacella*. *Insect Mol. Biol.* 13:603–13
75. Lindroth RL. 1989. Host plant alteration of detoxification activity in *Papilio glaucus glaucus*. *Entomol. Exp. Appl.* 50:29–36
76. Lindroth RL, Weisbrod AV. 1991. Genetic variation in response of the gypsy moth to aspen phenolic glycosides. *Biochem. Syst. Ecol.* 19:97–103
77. Liu N, Scott JG. 1996. Genetic analysis of factors controlling elevated cytochrome P450, *CYP6D1*, cytochrome b5, P450 reductase and monooxygenase activities in LPR house flies, *Musca domestica*. *Biochem. Genet.* 34:133–48
78. Lumjuan N, McCarroll L, Prapanthadara L, Hemingway J, Ranson H. 2005. Elevated activity of an Epsilon class glutathione transferase confers DDT resistance in the dengue vector, *Aedes aegypti*. *Insect Biochem. Mol. Biol.* 35:861–71
79. Maitra S, Dombrowski SM, Basu M, Raustol O, Waters LC, et al. 2000. Factors on the third chromosome affect the level of *CYP6A2* and *CYP6A8* expression in *Drosophila melanogaster*. *Gene* 248:147–56
80. Maitra S, Price C, Ganguly R. 2002. *CYP6A8* of *Drosophila melanogaster*: gene structure, and sequence and functional analysis of the upstream DNA. *Insect Biochem. Mol. Biol.* 32:859–70
81. McDonnell CM, Brown RP, Berenbaum MR, Schuler MA. 2004. Conserved regulatory elements in the promoters of two allelochemical-inducible cytochrome P450 genes differentially regulate transcription. *Insect Biochem. Mol. Biol.* 34:1129–39

66. Showed that the generalist P450 CYP6B8 is structurally more flexible and functionally more diverse than the related specialist P450 CYP6B1.

72. Demonstrated that *H. zea* can upregulate P450 detoxification genes in response to ingestion of plant defense signaling molecules jasmonate and salicylate.

79. Demonstrated that loss-of-function mutations in *trans*-regulatory loci lead to upregulation of P450 genes and insecticide resistance.

82. Mouches C, Pauplin Y, Agarwal M, Lemieux L, Herzog M, et al. 1990. Characterization of amplification core and esterase B1 gene responsible for insecticide resistance in *Culex*. *Proc. Natl. Acad. Sci. USA* 87:2574–78
83. Newcomb RD, Campbell PM, Ollis DL, Cheah E, Russell RJ, et al. 1997. A single amino acid substitution converts a carboxylesterase to an organophosphorus hydrolase and confers insecticide resistance on a blowfly. *Proc. Natl. Acad. Sci. USA* 94:7464–68
84. Oppenoorth FJ, Van Asperen K. 1960. Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. *Science* 132:298–99
85. Ortelli F, Rossiter LC, Vontas J, Ranson H, Hemingway J. 2003. Heterologous expression of four glutathione transferase genes genetically linked to a major insecticide-resistance locus from the malaria vector *Anopheles gambiae*. *Biochem. J.* 373:957–63
86. Pan L, Wen Z, Baudry J, Berenbaum MR, Schuler MA. 2004. Identification of variable amino acids in the SRS1 region of CYP6B1 modulating furanocoumarin metabolism. *Arch. Biochem. Biophys.* 422:31–41
87. Pedra JHF, McIntyre LM, Scharf ME, Pittendrigh BR. 2004. Genome-wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. *Proc. Natl. Acad. Sci. USA* 101:7034–39
88. Petersen RA, Niamsup H, Berenbaum MR, Schuler MA. 2003. Transcriptional response elements in the promoter of CYP6B1, an insect P450 gene regulated by plant chemicals. *Biochim. Biophys. Acta* 1619:269–82
89. Petersen RA, Zangerl AR, Berenbaum MR, Schuler MA. 2001. Expression of *CYP6B1* and *CYP6B3* cytochrome P450 monooxygenases and furanocoumarin metabolism in different tissues of *Papilio polyxenes* (Lepidoptera: Papilionidae). *Insect Biochem. Mol. Biol.* 31:679–90
90. Prapaipong H, Berenbaum MR, Schuler MA. 1994. Transcriptional regulation of the *Papilio polyxenes CYP6B1* gene. *Nucleic Acids Res.* 22:3210–17
91. Prapanthadara L, Hemingway J, Ketterman AJ. 1995. DDT-resistance in *Anopheles gambiae* Giles from Zanzibar, Tanzania, based on increased DDT-dehydrochlorinase activity of glutathione S-transferase. *Bull. Entomol. Res.* 85:267–74
92. Ranson H, Claudianos C, Ortelli F, Abgrall C, Hemingway J, et al. 2002. Evolution of supergene families associated with insecticide resistance. *Science* 298:179–81
93. Ranson H, Jeensen B, Wang X, Prapanthadara L, Hemingway J, et al. 2000. Genetic mapping of two loci affecting DDT resistance in the malaria vector *Anopheles gambiae*. *Insect Mol. Biol.* 9:499–507
94. Ranson H, Prapanthadara L, Hemingway J. 1997. Cloning and characterization of two glutathione S-transferases from a DDT-resistant train of *Anopheles gambiae*. *Biochem. J.* 324:97–102
95. Ranson H, Rossiter L, Ortelli F, Jesen B, Wang X, et al. 2001. Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. *Biochem. J.* 359:295–304
96. Ranson H, Hemingway J. 2005. Mosquito glutathione transferases. *Methods Enzymol.* 401:226–41
97. Raymond M, Chevillon C, Guillemaud T, Lenormand T, Pasteur N. 1998. An overview of the evolution of overproduced esterases in the mosquito *Culex pipiens*. *Philos. Trans. R. Soc. London B* 353:1–5
98. Rogers ME, Jani MK, Vogt RG. 1999. An olfactory-specific glutathione-S-transferase in the sphinx moth *Manduca sexta*. *J. Exp. Biol.* 202:1625–37
99. Sabourault C, Guzov VM, Koener JF, Claudianos C, Plapp FW, et al. 2001. Overproduction of a P450 that metabolizes diazinon is linked to a loss-of-function in the chromosome 2 ali-esterase (*Mdα E7*) gene in resistant house flies. *Insect Mol. Biol.* 10:609–18

100. Saner C, Weibel B, Wurgler FE, Sengstag C. 1996. Metabolism of promutagens catalyzed by *Drosophila melanogaster CYP6A2* enzyme in *Saccharomyces cerevisiae*. *Environ. Mol. Mutagen*. 27:46–58
101. Sasabe M, Wen Z, Berenbaum MR, Schuler MA. 2004. Molecular analysis of CYP321A1, a novel cytochrome P450 involved in metabolism of plant allelochemicals (fumanocoumarins) and insecticides (cypermethrin) in *Helicoverpa zea*. *Gene* 388:163–75
102. Schlenke TA, Begun DJ. 2004. Strong selective sweep associated with a transposon insertion in *Drosophila simulans*. *Proc. Natl. Acad. Sci. USA* 101:1626–31
103. Schuler MA. 1996. The role of cytochrome P450 monooxygenases in plant-insect interactions. *Plant Physiol.* 112:1411–19
104. Scott JG. 1999. Cytochromes P450 and insecticide resistance. *Insect Biochem. Mol. Biol.* 29:757–77
105. Scott JG, Zhang L. 2003. The house fly aliesterase gene (*MdaE7*) is not associated with insecticide resistance or P450 expression in three strains of house fly. *Insect Biochem. Mol. Biol.* 33:139–44
106. Seifert J, Scott JG. 2002. The *CYP6D1v1* allele is associated with pyrethroid resistance in the house fly, *Musca domestica*. *Pestic. Biochem. Physiol.* 72:40–44
107. Snyder MJ, Stevens JL, Andersen JF, Feyereisen R. 1995. Expression of cytochrome P450 genes of the CYP4 family in midgut and fatbody of the tobacco hornworm, *Manduca sexta*. *Arch. Biochem. Biophys.* 321:13–20
108. Snyder MJ, Walding JK, Feyereisen R. 1995. Glutathione S-transferases from larval *Manduca sexta* midgut: sequence of two cDNAs and enzyme induction. *Insect Biochem. Mol. Biol.* 25:455–65
109. Sonoda S, Tsumuki H. 2005. Studies on glutathione *S*-transferase gene involved in chlorfluazuron resistance of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) *Pestic. Biochem. Physiol.* 82:94–101
110. Spence JM, Blackman RL, Testa JM, Ready PD. 1998. A 169 bp tandem-repeat DNA marker for subtelomeric heterochromatin and chromosomal rearrangements in aphids of the *Myzus persicae* group. *Chromosom. Res.* 6:167–75
111. Spiegelman VS, Fuchs SY, Belitsky GA. 1997. The expression of insecticide resistance-related cytochrome P450 forms is regulated by molting hormone in *Drosophila melanogaster*. *Biochem. Biophys. Res. Commun.* 232:304–7
112. Stevens JL, Snyder MJ, Koener JF, Feyereisen R. 2000. Inducible P450s of the *CYP9* family from larval *Manduca sexta* midgut. *Insect Biochem. Mol. Biol.* 30:559–68
113. Syvanen M, Zhou Z, Wharton J, Goldsbury C, Clark A. 1996. Heterogeneity of the glutathione transferase genes encoding enzymes responsible for insecticide degradation in the housefly. *J. Mol. Evol.* 43:236–40
114. Syvanen M, Zhou ZH, Wang JY. 1994. Glutathione transferase gene family from the *Musca domestica*. *Mol. Gen. Genet.* 245P:25–31
115. Tang AH, Tu CP. 1994. Biochemical characterization of *Drosophila* glutathione S-transferases D1 and D21. *J. Biol. Chem.* 269:27876–84
116. Tijet N, Helvig C, Feyereisen R. 2001. The cytochrome P450 gene superfamily in *Drosophila melanogaster*: annotation, intron-exon organization and phylogeny. *Gene* 262:189–98
117. Tu CP, Akgul B. 2005. *Drosophila* glutathione-*S*-transferases. *Methods Enzymol.* 401:204–26
118. Vaughan A, Hawkes N, Hemingway J. 1997. Co-amplification explains linkage disequilibrium of two mosquito esterase genes in insecticide-resistant *Culex quinquefasciatus*. *Biochem. J.* 325:359–65

119. Vontas J, Small JG, Hemingway J. 2001. Glutathione-S-transferases as antioxidant defense agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochem. J.* 357:65–72
120. Vontas JD, Small GJ, Hemingway J. 2000. Comparison of esterase gene amplification, gene expression and esterase activity in insecticide susceptible and resistant strains of the brown planthopper, *Nilaparvata lugens* (Stal). *Insect Mol. Biol.* 9:655–60
121. Vontas JG, Small GJ, Nikou DC, Ranson H, Hemingway J. 2002. Purification, molecular cloning and heterologous expression of a glutathione-S-transferase involved in insecticide resistance from the rice brown planthopper, *Nilaparvata lugens*. *Biochem. J.* 362:329–37
122. Wadleigh RW, Yu SJ. 1987. Glutathione transferase activity of fall armyworm larvae toward alpha-, beta-unsaturated carbonyl allelochemicals and its induction by allelochemicals. *Insect Biochem.* 17:759–64
123. Wadleigh RW, Yu SJ. 1988. Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. *J. Chem. Ecol.* 14:1279–88
124. Wang J, McCommas S, Syvanen M. 1991. Molecular cloning of a glutathione S-transferase overproduced in an insecticide-resistant strain of the housefly (*Musca domestica*). *Mol. Gen. Genet.* 227:260–66
125. Wei SH, Clark AG, Syvanen M. 2001. Identification and cloning of a key insecticide-metabolizing glutathione S-transferase (MdGST-6A) from a hyper insecticide-resistant strain of the housefly *Musca domestica*. *Insect Biochem. Mol. Biol.* 31:1145–53
126. Wen Z, Pan L, Berenbaum MR, Schuler MA. 2003. Metabolism of linear and angular furanocoumarins by *Papilio polyxenes* CYP6B1 coexpressed with NADPH cytochrome P450 reductase. *Insect Biochem. Mol. Biol.* 33:937–47
127. Whyard S, Downe AER, Walker VK. 1995. Characterization of a novel esterase conferring insecticide resistance in the mosquito *Culex tarsalis*. *Arch. Insect Biochem. Physiol.* 29:329–42
128. Yoshiyama M, Shukle RH. 2004. Molecular cloning and characterization of a glutathione *S*-transferase gene from Hessian fly (Diptera: Cecidomyiidae). *Ann. Entomol. Soc. Am.* 97:1285–93
129. Yu SJ. 2002. Substrate specificity of glutathione-S-transferases from the fall armyworm. *Pestic. Biochem. Physiol.* 74:41–51
130. Yu SJ, Abo-Elghar OE. 2000. Allelochemicals as inhibitors of glutathione S-transferases in the fall armyworm. *Pestic. Biochem. Physiol.* 68:173–83
131. Zangerl AR, Berenbaum MR. 1993. Plant chemistry, insect adaptations to plant chemistry, and host plant utilization patterns. *Ecology* 74:47–54
132. Zhang M, Scott JG. 1996. Cytochrome b5 is essential for cytochrome P450 6D1-mediated cypermethrin resistance in LPR house flies. *Pestic. Biochem. Physiol.* 55:150–56
133. Zhou ZH, Syvanen M. 1997. A complex glutathione transferase gene family in the housefly *Musca domestica*. *Mol. Gen. Genet.* 256:187–94
134. Zhu YC, Dowdy AK, Baker JE. 1999. Detection of single-base substitution in an esterase gene and its linkage to malathion resistance in the parasitoid (Hymenoptera: Pteromalidae). *Pestic. Sci.* 55:398–404
135. Zhu YC, Snodgrass GL. 2003. Cytochrome P450 *CYP6X1* cDNAs and mRNA expression levels in three strains of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) having different susceptibilities to pyrethroid insecticide. *Insect Mol. Biol.* 12:39–49