African Journal of Biotechnology Vol. 8 (8), pp. 1386-1397, 20 April, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Review

Insecticide resistance and glutathione S-transferases in mosquitoes: A review

Azael Che-Mendoza[1], R. Patricia Penilla[2]* and D. Américo Rodríguez[2]

[1]Centro Regional de Control de Vectores "Panchimalco", Servicios de Salud de Morelos, Jojutla, Morelos, México.
[2]Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Chiapas, México.

Accepted 9 February, 2009

Mosquito glutathione S-transferases (GSTs) have received considerable attention in the last 20 years because of their role in insecticide metabolism producing resistance. Many different compounds, including toxic xenobiotics and reactive products of intracellular processes such as lipid peroxidation, act as GST substrates. Elevated levels of GST activity have been reported in organophosphate, organochlorine and pyrethroid resistant mosquitoes. Particulary GST-based resistance is considered the major mechanism of DDT resistance in anopheline species. To date different GST enzymes structurally conserved have been identified suggesting that they may have an important role on common pathways of compound detoxification. In this review we describe the major characteristics of this enzyme family and the principal studies that have contributed to a better knowledge of its role in mosquito insecticide resistance. Finally some aspects on insect GST-based resistance and their implications in traditional biochemical assays for detecting and monitoring GST activity are discussed.

Key words: Insecticide resistance, GST-based metabolic resistance, glutathione S-transferase.

INTRODUCTION

The glutathione S-transferases (GSTs) are members of a large family of multifunctional intracellular enzymes involved in the detoxification of endogenous and xenobiotic compounds via glutathione conjugation, dehydrochlorination, glutathione peroxidase (GPx) activity or passive/sacrificial binding (Hayes and Wolf, 1988; Mannervik et al., 1988; Pickett and Lu, 1989; Yang et al., 2001). GSTs can also serve as nonenzymatic binding proteins (known as ligandins) participating in the intracellular transport (Listowsky et al., 1988) and signalling processes (Adler et al., 1999, Cho et al., 2001). This diversity of enzymatic and nonenzymatic functions is related to the genetic capacity to encode different GST isoforms by most organisms.

Elevated levels of GST activity have been found to be associated to insecticide resistance in many insects. One or more GSTs have often been implicated in the resistance to organophosphates (OPs) in the house-fly, *Musca domestica* (Wei et al., 2001), organochlorine (OC) 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) in the fruit fly, *Drosophila melanogaster* (Tang and Tu, 1994) and more recently also reported in pyrethroid (PYR) resistance strains of planthopper, *Nilaparvata lugens* (Vontas et al., 2001, 2002). In mosquitoes, the metabolic resistance based on GST is the major mechanism of DDT-resistance (Hemingway and Ranson, 2000).

Some relevant aspects of the genetic organization and metabolic function of mosquito GSTs in insecticide resistance have been reviewed (Hemingway, 2000; Hemingway and Ranson, 2000; Hemingway et al., 2004; Enayati et al., 2005), and also a compendium about particular aspects of GST including a brief overview of GSTs in mosquitoes has been published (Ranson and Hemingway, 2005). Here we give a recent and detailed review of different aspect of the GST and its role in insecticide resistance in mosquitoes, focusing on the evidence generated mostly from the major African malaria vector, *Anopheles gambiae*, the Thai vector *Anopheles dirus* and the dengue vector, *Aedes aegypti*.

CLASIFICATION AND NOMENCLATURE OF GSTs

The GSTs (EC 2.5.1.18) are the family of enzymes more

*Corresponding author. E-mail: penilla@insp.mx.

abundant from the transferases superfamily and they are widely found in most aerobic eukaryotes and prokaryotes (Sheehan et al., 2001). There are three unrelated GST protein families known in eukaryotes: microsomals, members of the superfamily MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) (Jakobsson et al., 1999); cytosolics, occurring in the cytoplasma, referred also to as soluble GSTs; and mitochondrials, also known as class kappa (Pearson, 2005). The mitochondrial kappa family is found in mammalian mitochondria and peroxisomes (Pemble et al., 1996; Morel et al., 2004), nevertheless there is no evidence of sequences related to this family in diptera species (Ding et al., 2003). Little is known about the insect microsomal GSTs (MGSTs), and although they are very different in size and structure they have conjugation activities similar to those of the cytosolic GSTs (Pearson, 2005). Particularly for *Anopheles gambiae,* three different microsomal GSTs (MGSTs) have been identified (Ranson et al., 2002). However only the citosolic GSTs have been implicated in insecticide resistance (Hemingway et al., 2004; Enayati et al., 2005; Ranson and Hemingway, 2005), on which is the primary focus of this review.

Before the *A. gambiae* genome and its GST genetic map were published (Ranson et al., 2002; Holt et al., 2002), the major criteria for the assignment of GSTs to a particular class ("subfamily") was based on their amino acid sequence homology and immunological properties (Toung et al., 1990; Beall et al., 1992; Fournier et al., 1992). Nevertheless due to many individual GSTs displaying broad and overlapping substrate selectivities, the current criteria for GST classification include, to have an identity of over 40% of the amino acid sequence and other properties such as phylogenetic relationships, immunological properties, tertiary structure and their ability to form heterodimers and chromosomal location which are also employed (Ding et al., 2003; Hemingway et al., 2004; Ranson and Hemingway, 2005). At least six classes of cytosolic proteins with domains similar to the GST have been identified in dipteran and other insect species: delta, epsilon, omega, sigma, theta and zeta, being possibly the existence of novel GST classes (Ding et al., 2003; Tu and Akgul, 2005). Each of these GST classes are represented in *A. gambiae* and *A. aegypti*. The larger classes, Delta and Epsilon are specific to insects (Ranson et al., 2002; Ding et al., 2003). A group of citosolic GST in *An. gambiae* has been designed as unclassified, denoted by a 'u', *i. e.* GSTu (Ding et al., 2003). The identification of GSTu orthologs in *A. aegypti* and their absence in *D. melanogaster* suggests that these GSTs may be specific to mosquitoes (Lumjuan, 2005).

The delta, sigma and epsilon classes initially were referred as class I, II and III respectively, until the Greek letter-based nomenclatura was adopted in line with the mammalian system of GST nomenclature (Chelvanayagam et al., 2001) and supported by phylogenetic analysis between mammalian and insect GSTs (Ranson et al., 2001; Ding et al., 2003). Currently the insect GST nomenclature consist of three parts; the name of the specie from which the GST was isolated, the GST class and the part ending in a number which may specify the order of discovery or the genome organization. For example, AgGSTD5-5 is a member of the *A. gambiae* GST, where "D" refers to the delta class and the double number "5-5" indicates a homodimer enzyme. In reference to the gene encoding for a subunit of this enzyme it is termed AgGSTd5 (italicized), "d" refers to the delta class and the subunit number remains the same (Wongsantichon et al., 2003; Hemingway et al., 2004). From here we are going to be referring GST names using this nomenclature, and in some cases the old names are going to be expressed in parentheses.

CYTOSOLIC GST STRUCTURE AND MODE OF ACTION

The cytosolic GSTs are homo- or heterodimeric proteins, that is they are formed by two subunits or polypeptide chains of approximately 25 kDa in size each (Armstrong, 1991). Each subunit folds into two domains, the N-terminal (extreme 5´) and C-terminal (extreme 3´) joined by a variable linker region. The N-terminal domain (1 - 80 residues) adopts a similar conformation to the thioredoxin domain (arranged in a βαβαββα motifs) found in all GST structures (Sheehan et al., 2001). This domain comprises mostly of active or G-sites, which is the binding site of endogenous tripeptide GSH (γ-L-glutamyl-L-cysteinyl-glycine) also known as glutathione. The larger C-terminal domain consists of a variable number of alpha helices, and includes largely the residues of hydrophobic H-site or substrate binding site. The high level of diversity in this region confers in part the specificity of the GSTs for a broad range of electrophilic substrates (Mannervik and Danielson, 1988).

The active site residue tends to be highly conserved within GST classes, but differs between classes. In most mammalian GSTs, the active site residue responsible for the GSH thiol residue activation in catalysis appears to be a tyrosine (Sheehan et al., 2001), but in the delta and epsilon insect GST classes, this role is performed by a serine residue (Ranson and Hemingway, 2005; Udomsinprasert et al., 2005). Although each subunit has a kinetically independent active site, their quaternary structure is essential for their activity (Danielson and Mannervik, 1985). The subunits only hybridize with subunits of the same class (Armstrong, 1991). The formation of homo- and heterodimers increases the diversity of GSTs from a small number of genes (Sheehan et al., 2001).

The GST-based detoxification of both endogenous and xenobiotic compounds can be in a direct way (phase I metabolism) or by the catalysis of reactive products formed by other enzymatic detoxification systems (phase II metabolism) (Yu, 1996; Sheehan et al., 2001). In a reaction of conjugation, the active site residue interacts

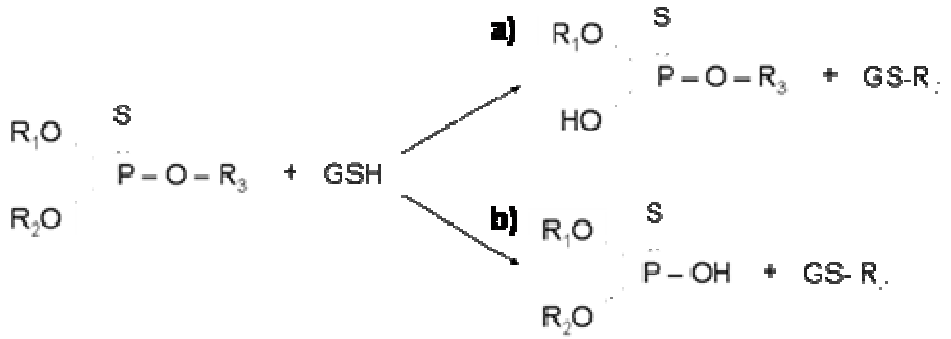

Figure 1. GST-mediated detoxification of organophosphate insecticides. The GST achieves two conjugation reactions acting as enzymes from the phase I of detoxification: a) O-dealkylation and b) O-dearylation conjugation. R1 and R2 represent the alkyl (either ethyl or methyl) portion; and R3 is a substituted aryl or alkyl group; GSH, glutathione; GS-R, thiolate anion conjugated with Rn portion (Dauterman, 1983; Hayes and Wolf, 1988).

with the GSH sulphydryl group (-SH), to generate the catalytically active thiolate anion (GS^-). This nucleophilic thiolate anion is then capable of attacking the electrophilic centre of any lipophilic compound to form the corresponding GS-conjugate (Jakoby and Ziegler, 1990; Armstrong, 1991). The conjugation neutralizes the electrophilic sites of the substrate, leading to its detoxification by the elimination of highly reactive electrophiles or rendering the product more water soluble and therefore more readily excretable from the cell (Habig et al., 1974; Hayes and Wolf, 1988). These conjugates are eliminated from the cell via the glutathione S-conjugate export pump (phase III detoxification system) (Sheehan et al., 2001).

In other reactions of detoxification the GSTs are also able to dehydrochlorinate insecticides such as DDT, in a reaction where GSH acts as a co-factor rather than a conjugate (Clark and Shamaan, 1984). The detoxification also can be done by passive binding to insecticides or mainly by removal of reactive oxygen species (ROS) and detoxification of lipid peroxidation (LPO) products (both of which are oxidative stress products).

INSECTICIDE DETOXIFICATION

Organophosphates

Detoxification occurs by the conjugation of GSH to OP insecticides via two distinct patways: an O-dealkylation or O-dearylation conjugation (Figure 1). In O-dealkylation the GSH is conjugated with the alkyl portion of the insecticide, while in the O-dearylation the GSH reacts with the leaving group. The reactions have been reported in housefly, *M. domestica* (Oppenoorth et al., 1979; Ugaki et al., 1985) and in diamondback moth, *Plutella xylostella* (Chiang and Sun, 1993) and verified by the use of recombinant GST enzymes in both species (Huang et al., 1998).

The GSTs often act as a secondary resistance mechanism in conjunction with a P^{450}- or esterase- based resistance mechanism (Hemingway et al., 1991). Most OP insecticides are usually applied in the non-insecticidal phosphorothionate form and are activated to the insecticidal organophosphate form (oxon analogue) by the action of cytochrome P^{450}s within the insect. These oxons are more neurotoxic (potent acetylcholinesterase inhibitors) than their thionate analogues. Detoxification of the oxon analogues of fenitrothion has been reported in *Anopheles subpictus* (Hemingway et al., 1991). This cooperative enzyme system of detoxification would be more rapid and efficient than independent mechanisms and it is therefore important in insecticide resistance (Bogwitz, 2005).

Organochlorines

The GSTs catalyse two detoxification reactions of halogenated hydrocarbons: dehydrochlorination and GSH conjugation (Tang and Tu, 1994) (Figure 2). The DDT-dehydrochlorination is the major route of detoxification for this insecticide (Hayes and Wolf, 1988) and probably the most common DDT resistance mechanism in mosquitoes (Brown, 1986; Hemingway, 2000). In the glutathione-dependent DDT dehydrochlorination, the GS^- generated in the active site acts as a general base and removes hydrogen from DDT resulting in the elimination of chlorine to generate the non-toxic DDE (1,1-dichloro-2,2-bis-[*p*-chlorophenyl]ethane). In this reaction the GSH levels do not change at the end of the reaction (Lipke and Chalkley, 1962) due to the GSH regenerated acting as a cofactor rather than a conjugate (Clark and Shamaan, 1984) (Figure 2.1). An increased rate of glutathione-dependent dehydrochlorination confers resistance to DDT in *A. aegypti* (Grant et al., 1991; Lumjuan et al., 2005), *A. dirus* (Prapanthadara et al., 1996, 2000b) and *A. gambiae* (Prapanthadara et al., 1993, 1995; Ranson et al., 2001;

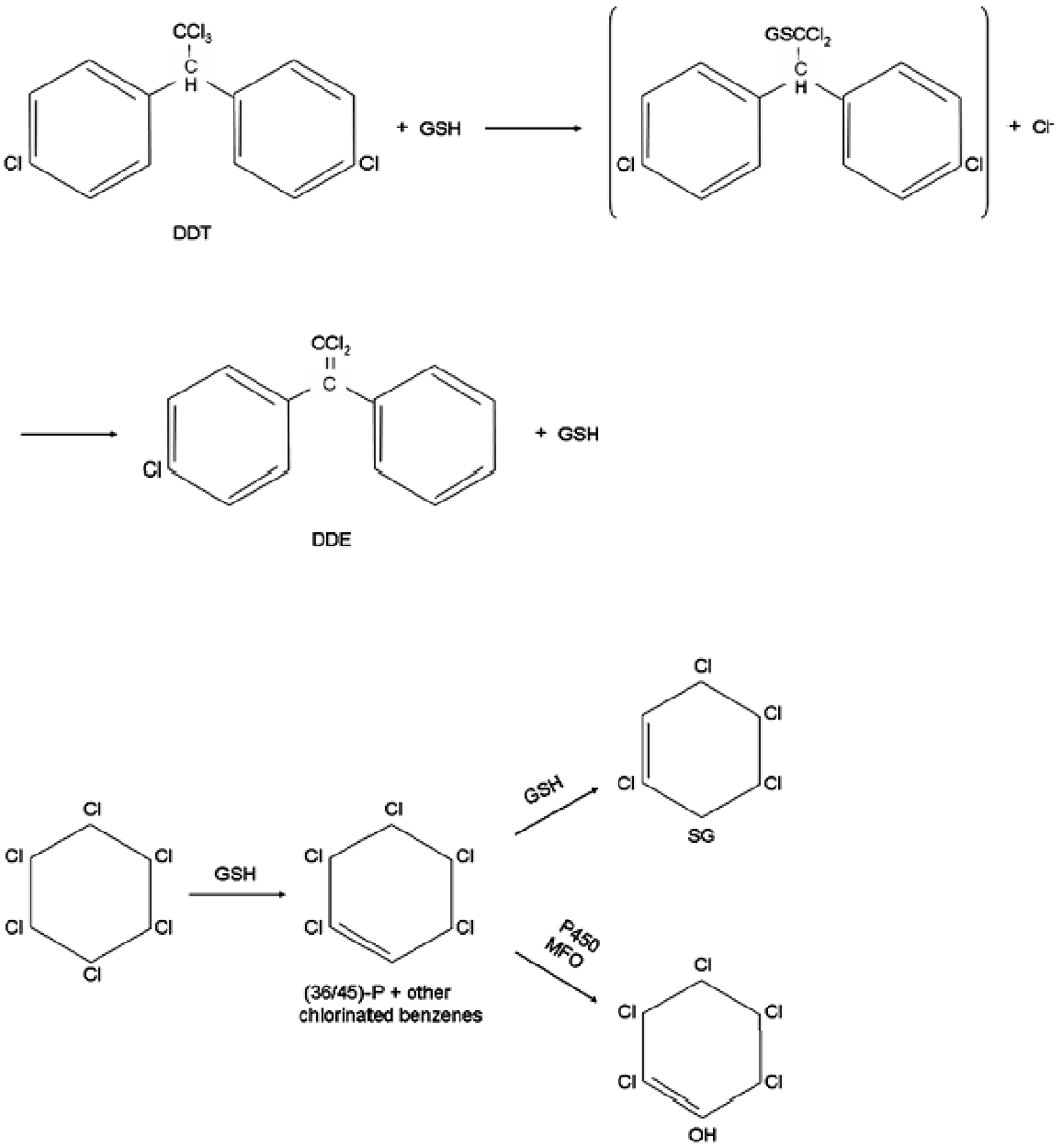

Figure 2. GST-mediated detoxification of organochlorine insecticides. 1. Dehydrochlorination of DDT (Matsumura, 1985). 2. Metabolism of lindane: (a) GSH-dependent dehydrochlorination; (b) GSH conjugation, both reactions catalized by GST (Tanaka et al., 1981); c) P^{450} MFO-mediated hydroxylation (Bloomquist, 1998). GS-, thiolate anion conjugated; GSH, glutathione; (36/45)-P, (36/45)-pentachlorocyclohexene; MFO, mixed function oxidases.

Ortelli et al., 2003).

Another organochlorine insecticide like lindane is suggested to be detoxified initially by a dehydrochlorination reaction and subsequently by conjugation to glutathione (Tanaka et al., 1981) both reactions being catalysed by GST (Clark et al., 1986; Bloomquist, 1998; Wei et al., 2001). However, the major routes of metabolism of lindane include dehydrochlorination by GST giving various chlorobenzenes, along with subsequent P^{450}-mediated hydroxylation to yield several chlorophenols (Bloomquist, 1998) (Figure 2.2).

Pyrethroids

GST's role in the detoxification of PYRs has been basically attributed to its capacity to reduce the peroxidative damage induced by PYRs, mainly by detoxifying lipid peroxidation products (Vontas et al., 2001). This evidence was suggested for a delta class GST from a PYR resis-

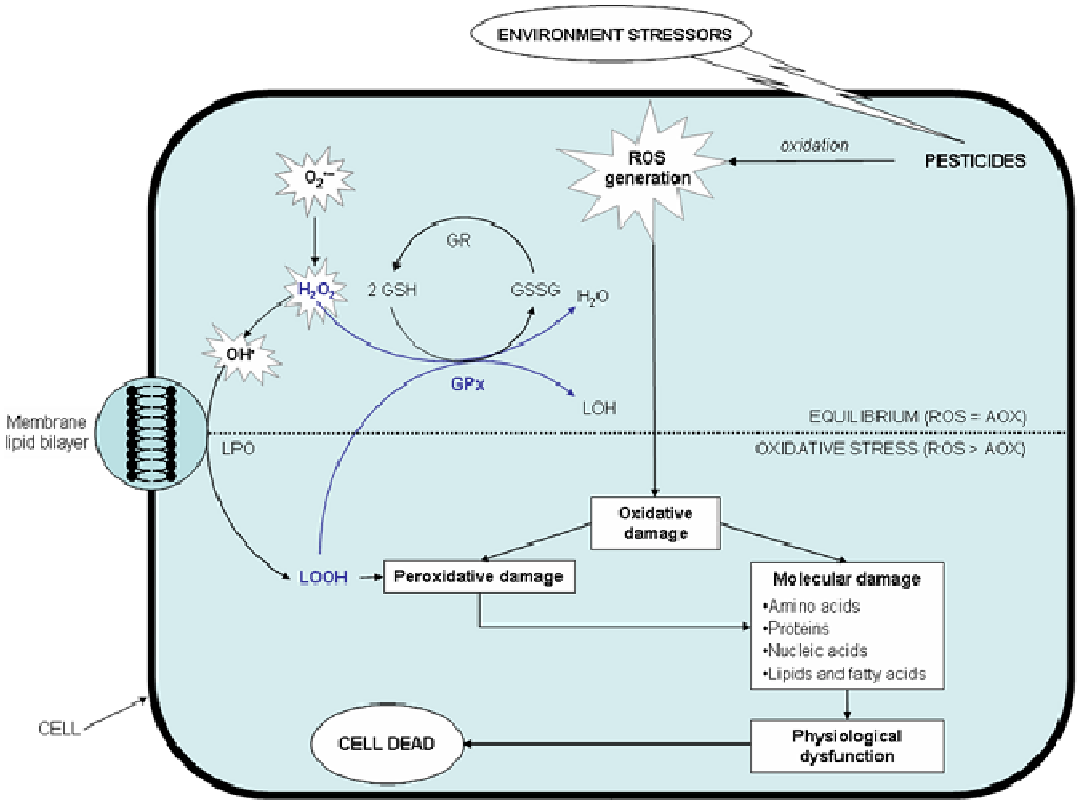

Figure 3. GST antioxidant defence against insecticide. Together with other AOX enzymes (not shown) the GSTs protect the cells from oxidative stress generated by environmental chemicals such as pesticides. Oxidative stress occurs when the generation of ROS exceeds the cell's ability to neutralize and eliminate them (ROS>AOX). LPO is iniated by ROS to generate LOOH, which can propagate the autocatalytic chain of LPO by continually generating free radicals. The GSTs prevent LPO inactivating the H_2O_2 (primary defense) and acting as a second line of defence reducing LOOHs to corresponding LOHs, and conjugating HNE to GSH (secondary defense). O_2^-, superoxide anion; H_2O_2, hydrogen peroxide; OH^-, hydroxyl radical; GPx, gluthatione peroxidase; GSH, glutathione; GSSG, glutathione disulfide often improperly called oxidized glutathione; GR, glutathione reductase; LPO, lipid peroxidation; LOOH, lipid hydroperoxide: LOH monohydroxylated lipid; ROS, reactive oxygen species; AOX, antioxidants.

tant strain of rice brown planthopper, *Nilaparvata lugens* whose recombinants showed high peroxidase activity (Vontas et al., 2002) and recently reported for an epsilon class GST in *A. aegypti* mosquitoes (Lumjuan et al., 2005). Several GSTs which accepted an LPO product as substrate have also been reported in *Drosophila melanogaster* (Singh et al., 2001; Sawicki et al., 2003).

It is suggested that GSTs may also protect against PYR toxicity in insects through a passive sequestration process (Kostaropoulos et al., 2001). The evidence of some GSTs binding to various PYRs has been reported in *A. dirus* (Prapanthadara et al., 1998, 2000b; Jirajaroenrat et al., 2001; Udomsinprasert and Ketterman, 2002). The use of GST inhibitors (e.g diethyl maleate) in PYR resistant *Culex* strains suggests that GST-mediated metabolism has a relative contribution in PYR resistance (Xu et al., 2005). While in the field, *Anopheles albimanus* populations and slight increases of GST activities under continuous PYR selection were also detected (Penilla et al., 2006).

Oxidative stress induced by insecticide

Exposure to insecticides induces oxidative stress (Abdollahi et al., 2004) and insect GSTs may contribute to antioxidant defence by direct GPx activity preventing and repairing the damage of secondary products generated by ROS and by direct conjugation of trans-4-hydroxy-2-nonenal (HNE), one of the major end products of LPO (Parkes et al., 1993; Singh et al., 2001; Vontas et al., 2001; Sawicki et al., 2003; Ding et al., 2005) (Figure 3).

The GPx activity has been detected in insect GST´s from the delta, epsilon and sigma classes (Tang and Tu, 1994; Ranson et al., 1997b; Prapanthadara et al., 1998; Singh et al., 2001; Vontas et al., 2001; Ortelli et al., 2003; Sawicki et al., 2003; Ding et al., 2005; Lumjuan et al., 2005). Recently, in epsilon and delta GST gene promoters from anophelines, putative binding sites and regulatory/reponse elements involved in the induction of GST expression in response to oxidative stress have been found supporting the antioxidant physiological role

Table 1. Substrate specifity of some cytosolic GSTs characterized in anopheline species.

Gene name	Old name	No. of transcripts	DDTase activity[1]	CDNB activity[2]	DCNB activity[2]	CHP activity[2]	References[3]
A. gambiae							
GSTd1	GST1-α	GSTd1-3					1, 4, 7, 9
		GSTd1-4					7, 9
		GSTd1-5	4.8±0.09	56.44±8.7	0.33±0.03	<0.13±0.0	6, 7, 9
		GSTd1-6	7.7±0.72	195±11.9	0.64±0.03	0.98±0.06	6, 7, 9
GSTd2	GST1-2	1		0.4			5, 9
GSTe1	GST3-1z	1	n.d.	3.9±0.18	8.61±0.82	0.001±0.0	1, 2, 8, 9
	GST3-1k			30.8±4.3	4.48±0.05	0.175±0.0	
GSTe2	GST3-2	1	2,770	2.88±0.8	5.74±2.70	n.d.	1, 2, 8, 9
				13.1±0.40	5.8±0.24		
GSTe4		1	n.d.	16.3±1.59	0.07±0.01	n.d.	1, 2, 9
GSTe8		1	n.d.	7.9±0.52	0.1±0.01	n.d.	1, 2, 9
A. dirus							
Purified enzyme	GST4a		4.4-15.80	s.u.	0.60-0.80	0.012	4, 5, 7
Purified enzyme	GST4c		1,308.74	s.u.	n.d.	n.d.	7
GST1AS1	GST1-1	GSTd1	27.4±0.01	174.3±4.86	0.28±0.01	0.65±0.06	1, 2, 3, 6, 9
	GST1-2	GSTd2	1.87±0.82	39.7-43.3	0.08±0.01	n.d.	
	GST1-3	GSTd3	2.66±0.29	59.7- 64.6	0.16±0.01	n.d.	
	GST1-4	GSTd4	7.50±1.68	29.1- 38.3	0.03±0.04	0.05±0.01	
GSTd5	GST1-5	1	78.8±13.2	192		0.3±0.004	9
GSTd6	AdGST1-6		1.28±0.22	1.37			9

[1] nmol DDE formed/mg protein; [2]μmol/min/mg protein; [3] *A. gambie*: 1. Ding et al., 2003; 2. Ortelli et al., 2003; 3. Ranson et al., 1997a; 4. Ranson et al., 1997b; 5. Ranson et al., 1998; 6. Ranson et al., 2001; 7. Ranson et al., 2002. [3] A. dirus: 1. Jirajaroenrat et al.2001; 2. Oakley et al., 2001; 3. Pongjaroenkit et al., 2001; 4. Prapanthadara et al., 1995; 5. Prapanthadara et al., 1996; 6. Prapanthadara et al., 1998; 7. Prapanthadara et al., 2000b; 8. Udomsinprasert et al., 2005 ; n.d. enzyme activity no detected
s.u. substrate used by the enzyme, but the data is not specificated or they were measured using different units, that is inhibition %.

of some GSTs (Ding et al., 2005; Udomsinprasert et al., 2005).

GST-BASED RESISTANCE AND HISTORICAL EVIDENCE IN MOSQUITOES

From DDTase enzyme to GST enzyme purification

In 1974, evidence of DDT being metabolized to DDE by a glutathione-dependent dehydrochlorinase (DDTase) was shown in *M. domestica* and was demonstrated that this enzyme was identical to a GST (Clark and Shamaan, 1984; Clark et al., 1986). These authors were the first to notice that the high activity of GST enzymes with DCNB (1, 2-dichloro-4-nitrobenzene) correlated with the increased DDTase activity in DDT resistance fly strains. This new evidence suggested the existence of this mechanism in mosquitoes (Brown, 1986).

Ten years later Prapanthadara and colleagues started using fractions of crude homogenates from fourth-instar larvae of *A. gambiae* and *A. dirus*, through sequential column chromatography and showed that three GST subgroups, all containing multiple GST isoenzymes, were able to detoxify the DDT in both species (Prapanthadara et al., 1993, 1995, 1996, 2000b). The GSTs with the highest activity DDTase were found in elevated amounts in DDT resistant *A. gambiae* (Prapanthadara et al., 1995). Like in *M. domestica*, the DDTase activity was found to be correlated with the GST activities toward DCNB (Prapanthadara et al., 1993, 1995).

Although several isoenzymes of *A. dirus* have been at least partially purified (Prapanthadara et al., 1995, 1996, 2000b), only two of them, the GST4a and GST4c, were successfully purified and characterized (Table 1). The GST4c had 83-fold greater DDTase specific activity than the GST4a, but only the GST4a showed activity with dichloronitrobenzene (DCNB), 7.4-fold greater than with chlorodinitrobenzene (CDNB) conjugation activity (Prapanthadara et al., 1995, 1996, 2000a).

These studies demonstrated that the GSTs, when they are present in increased amounts (quantitative differences), have an important role in insecticide resistance, but also showed that not all of the GST enzymes are associated with resistance (qualitative differences).

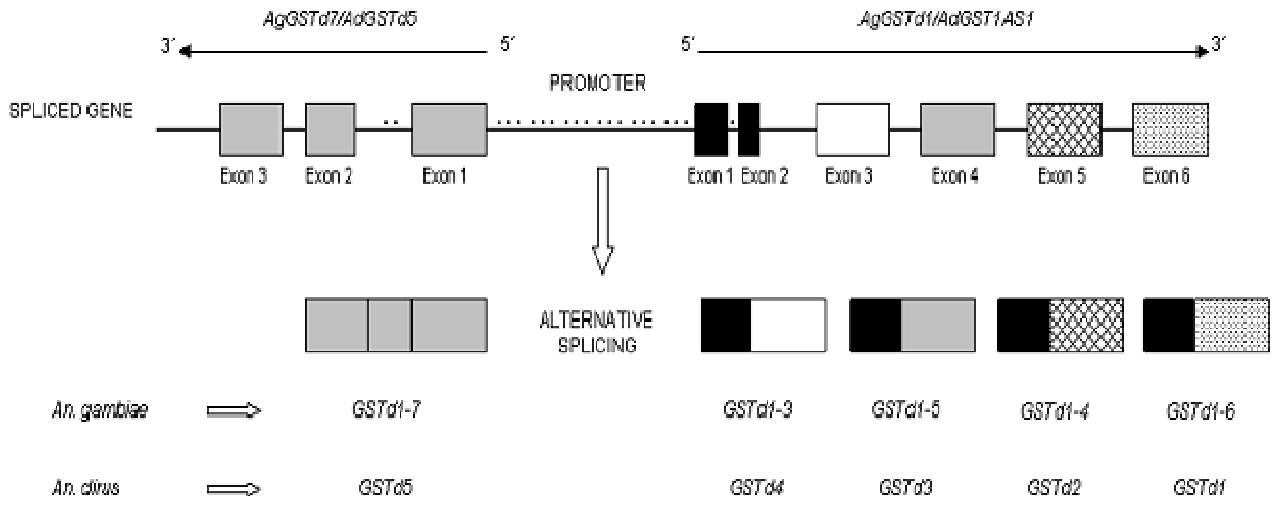

Figure 4. General model of genomic organization of two GSTd orthologs in mosquitoes. Both *An. gambiae* and *An. dirus*, have two GSTd gene arranged in divergent orientations. One gene contains three exons alternatively spliced to produce one mature transcript. A second gene produces four diferent transcripts each sharing exon 1 and exon 2 (black solid boxes), that encode for a 5´UTR region and a common N-terminal, and one of the other exons which encode for different C-terminal domains. Thus, the N-terminal domain of the GSTs derives from the same exon, whereas the C-terminal domain arises from different exons. The horizontal line denotes the introns and the dashes on the line the putative promoter regions. The distances between exons are not in the real scales (Pongjaroenkit et al., 2001; Ranson et al., 1998; Udomsinprasert et al., 2005).

Isolation of GST genes

The first studies demonstrating the capacity of individual GSTs metabolising DDT were carried out by Ranson et al. (1997a, b). They were able to isolate and clone three genes from *A. gambiae* larvae, AgGSTd1-2 (AgGST1-2), AgGSTd1-5 (AgGST1-5) and AgGSTd1-6 (AgGST1-6). Using recombinant enzymes, the AgGSTd1-5 and AgGSTd1-6 showed higher CDNB and DDT activities (Ranson et al., 1997b) (Table 1). A primer encompassing the conserved N-terminal region of these GSTs was used to amplify and sequence a GST gene from *A. dirus*, the AdGSTd1 (AdGST1-1) (Prapanthadara et al., 1998). The amino acid sequence of this gene had 91% identity to the AgGSTd1-6, but it showed a 4-fold difference in the rates of DDT metabolism (Prapanthadara et al., 1998) (Table 1). In general the GST delta class of *A. dirus* showed lower levels of CDNB and DCNB activities than their orthologs in *A. gambiae* (Table 1).

Alternatively spliced GST genes

Using primers designed on selected sequences of genomic libraries, five sequences in addition to the intron-less gene AgGSTd1-2 (AgGST1-2) were found. These sequences were exons of two GST genes sequentially arranged in divergent orientations, AgGSTd1 (AgGST1α) and AgGSTd7 (AgGST1β) (Ranson et al., 1998). AgGSTd1 contains five coding exons alternatively spliced to produce four mature transcripts, two of them belonging to genes AgGSTd1-5 and AgGSTd1-6 previously described, and two sequences termed AgGSTd1-3 (AgGST1-3) and AgGSTd1-4 (AgGST1-4) (Figure 4). All these GST genes were expressed in high levels in both larvae and adults (Ranson et al., 1998). However they accounted for only 6% of the total DDT metabolism in resistant strains (Ranson et al., 1997b).

A homologous genetic structure to AgGSTd1 was found in *A. dirus* (Pongjaroenkit et al., 2001). The gene AdGST1AS1 (for *A. dirus* GST class I alternatively spliced gene I) share a 78 - 93% nucleotide identity in the coding region with the AgGSTd. Similar to AgGSTd1, four mature transcripts result from AdGST1AS, being one of the previously reported by Prapanthadara et al. (1998) (Figure 4). The deduced amino acid sequence of transcripts between AdGST1AS1 and AgGST1α is highly conserved in each ortholog gene (ranging from 85 - 93% identity) (Jirajaroenrat et al., 2001; Pongjaroenkit et al., 2001). A similar pattern of alternative splicing has been observed also in *A. aegypti* GSTd1, but in contrast with *Anopheles* only three transcript (orthologs to GSTd1-5, GSTd1-4 and GSTd1-6 from *A. gambiae*) are found in AeGSTd1 with an amino acid sequence identity reportedly ranging from 75 - 85% (Lumjuan, 2005).

Indeed, an AgGSTd7 ortholog was identified in *A. dirus*, the AdGSTd5 (AdGSTd1-*5*) (Pongjaroenkit et al., 2001; Udomsinprasert et al., 2005). Both AdGSTd5 and AgGSTd7 are interrupted by introns and amplified for a product, sharing a 95% identity and 98% similarity in the

amino acid sequence (Udomsinprasert et al., 2005). AdGSTd5 is located upstream and is in reverse orientation to AdGST1AS1 in a similar form as AgGSTd7 and AgGSTd1 (Pongjaroenkit et al., 2001) (Figure 4). AdGSTd5 was only expressed in adult females and despite having displayed little activity with the classical GST substrate CDNB, it possessed the greatest DDT activity observed for *A. dirus* GST delta class (Udomsinprasert et al., 2005) (Table 1).

Other studies demonstrated that the AgGSTs1 is also the product of an alternatively spliced gene that produces two transcripts (Ranson et al., 2002; Ding et al., 2003), and it was reported to have a probable ortholog in *A. aegypti* (Lumjuan, 2005).

Although the size and sequence of the introns vary among orthologs, the coding region sequences and the intron positions are highly conserved. All the translation products of the transcripts from these genes share a common N-terminal domain, but are highly variable at the C-terminal. This characteristic confers the property to generate efficiently different substrate specificity with a minimal increase in gene duplication and length (Jirajaroenrat et al., 2001; Oakley et al., 2001).

The major GST responsible for DDT resistance

A first important discovery on DDT resistance in *A. gambiae* was the identification of two quantitative trait loci (QTL) associated to DDT-resistance rtd1 (resistance to DDT1) and rtd2 (Ranson et al., 2000). The second important finding was the identificacion of one gene associated to the rtd1 region and its overexpression in DDT resistant strain (ZAN/U), 5-fold greater than the susceptible strain (Kisumu). Recombinants of this gene, termed as AgGSTe2 (AgGST3-*2*), showed DDTase activity, representing up to 92% of DDT metabolism (Ranson et al., 2001).

The following analysis of *A. gambiae* genome located a cluster of eight GST genes epsilon class sequencially arranged within the genomic region rtd1, and quantitative PCR analysis demonstrated that five of theses genes were significantly expressed in high levels in DDT resistant strains compared with the susceptible strain (Ding et al., 2003). However, DDTase activity has been confirmed only in AgGSTE2-2 recombinants (Ranson et al., 2001; Ortelli et al., 2003).

Later, an ortholog of AgGSTe2 in a DDT and permethrin resistant *A. aegypti* strain (PMD strain) termed AaGSTe2 (Lumjuan *et al.*, 2005) has been identified, of which recombinants showed a DDTase activity (4.16 ± 0.28 nmol of DDE/µg), 1.5 fold greater than that reported for *A. gambiae* (Table 1). Unlike its *Anopheles* counterpart, the AaGSTE2-2 exhibited levels of GPx activity comparable to other insects GST like *D. melanogaster* (Singh et al., 2001; Sawicki et al., 2003) and *N. lugens* (Vontas et al., 2001), suggesting that high AaGSTe2 levels may confer some resistance to the secondary effects of exposure to PYRs (Lumjuan et al., 2005). Although microarray analyses in *A. gambiae* indicated that GSTe2 was elevated in two PYR resistance strains (ZAN_U and RSP strains), its specific role needs to be investigated (David et al., 2005).

In *A. dirus* the major GST in the DDT metabolism may be carried out by AdGST4c which has had higher levels of DDT activity among all the GST´s identified in this species. Nevertheless no reports of a GSTe2 ortholog in *A. dirus* have been formerly published, although partial sequences of orthologs had been deposited in the Genbank database for this species and others such as *Anopheles culicifacies* (http://www.ncbi.nlm.nih.gov/).

GST EXPRESSION AND REGULATORY FACTORS OF THE GST-BASED RESISTANCE

GST activity differences in development

With a few exception (Ding et al., 2003; Udomsinprasert et al., 2005), most of the individual GST´s identified in mosquitoes are expressed in both males and females and in all development stages. Nevertheless the pattern of expression of individual GST enzymes can be different during mosquito development (Grant and Matsumura, 1988, 1989). In general, the levels of GST activity increase through larval development and pupal stage and reach a maximum in the newly emerged adult, with GST activity declining with age (Hazelton and Lang, 1983). Such evidence could be related in part with the lack of DDT tolerance with age in adult mosquitoes (Lines and Nassor, 1991). Blood ingestion had been reported to increase the tolerance to DDT and PYRs (Halliday and Feyereisen, 1987) and GSTs could be involved since several GSTs genes are upregulated after blood ingestion in mosquitoes (Marinotti et al., 2005).

Molecular mechanism of the GST expression

The molecular mechanism responsible for elevated GST activity is mostly due to regulatory changes that increases its transcriptional rate (Hemingway et al., 2004; Enayati et al., 2005). Nevertheless, elevation of GST activity due to enhanced mRNA stability has been reported in *D. melanogaster* (Tang and Tu, 1995), while gene amplification has been observed in both *M. domestica* (Wang et al., 1991) and *N. lugens* (Vontas et al., 2002).

In mosquitoes, GST-based insecticide resistance appears to be caused at a transcriptional level. Various mutation types can lead to changes in gene expression, and these can occur in cis (for instance disruption or deletion of an upstream regulatory element of the gene, whatever the element function is enhancing or repressing gene expression) or in trans (for instance disruption of a gene coding for a protein that binds to the above mention cis element) (Feyereisen, 1995). In *A. aegypti* it is sug-

gested that mutation disrupts a trans-acting repressor allowing the overexpression of GST (Grant and Hammock, 1992). Mutations in a cis-acting regulatory factor may be involved, al least in part, in the GSTe2 overexpression in DDT-resistant strain of *A. gambiae* (Ranson et al., 2001; Ding et al., 2005). Particularly the deletion of two adenosine residues in the promoter region seems to increase the GST transcription activity in the mosquito (Ding et al., 2005).

It is suggested that, although the basal expression in the singleston GSTe genes may be controlled by core promoters located at the immediate upstream, their general expression may be under control by a common regulator element. For example, by examination of the GST gene expression under oxidative stress conditions in *A. gambiae*, it was observed that GSTe3 is induced in both DDT resistant and susceptible strains, with a higher response in the resistant strain. In addition, two other GSTe (GSTe2 and GSTe1) were detected only in the resistant strain. This differential response may be due to changes in regulatory proteins rather than in the promoters themselves (Ding et al., 2005).

In alternatively spliced genes, it is hypothized that GST gene promoters have multiple regulatory elements or binding sites that respond differently to specific or more general stress-related signals affecting the choice of spliced sites (Ranson et al., 1998; Pongjaroenkit et al., 2001). Particularly in the promoter regions of alternatively spliced GST genes from *A. dirus* two promoter regions have been identified, a proximal promoter to the coding region and a distal promoter located upstream to the former one (Pongjaroenkit et al., 2001; Udomsinprasert and Ketterman, 2002). Such as occurring in insects, probably each promoter is associated with stage and/or tissue-specific gene expression (Hoy, 1994; Harshman and James, 1998). In *A. dirus* it is speculated that the distal promoter would be acting as an enhancer/repressor to regulate GST expression (Udomsinprasert and Ketterman, 2002). For example, AdGST1AS1 could be involved in oxidative stress response and expressed as a housekeeping gene (gene expressed in all cells in order to maintain fundamental activities). But, during development increased oxidative stress in the cell is required to increase GST expression (Pongjaroenkit et al., 2001).

Some introns apparently are important in gene regulation and in the determination of the moment and the kind of tissue the gene will be transcribed to (Hoy, 1994). For example a promoter region in AdGSTd5 containing several binding sites for factors related in embryo or tissue development, has been located within an intron (Udomsinprasert et al., 2005) (Figure 4). This suggests that GSTs are differentially regulated by multiple mechanisms in response to xenobiotic modulation and/or in a tissue- or developmental-specific manner. The knowledge of regulatory elements involved in the induction of GST´s, will provide a better understanding of the molecular basis in the GST-based insecticide-resistance mechanism essential for the design of sensitive monitoring methods and then for an effective insecticide resistance management.

CONCLUSION

Despite great advances during the last decade on mosquito GST knowledge, some relevant aspects like specific substrates, structural determination, functions, location and regulation remain unresolved mainly for the cytosolic GSTs. For example, although the fourth crystal structure of alternatively spliced GST is known (Pongjaroenkit et al., 2001; Chen et al., 2003; Udomsinprasert et al., 2005) the alternatively spliced *Aedes* GST isoforms remain biochemically uncharacterized, as well as the majority of the cytosolic GSTs identified in mosquitoes. Even more the GST diversity in other species is unknown, and it is expected to become higher in mosquitoes such as *Culex quinquefasciatus* whose breeding sites are characterizated to be highly polluted (Lipke and Chalkley, 1964; Ranson and Hemingway, 2005). Whether this diversity is related to insecticide resistance in different mosquito species should be investigated. In some anopheline populations such as the Mexican malaria vector *Anopheles albimanus* (Penilla et al., 2006) and the African malaria vector *A. gambiae* (Prapanthadara et al., 1995), the GST-based mechanism only confers resistance to DDT, probably as a result of DDT selection pressure on these populations. Interestingly in the Sri Lankan malaria vector *Anopheles subpictus*, the organophosphorus insecticide pressure could have maintained or reselected DDT resistance still observed (Hemingway et al., 1991). A similar phenomenon may be conferring DDT resistance in *Culex quinquefasciatus* from Thailand, where populations had never been exposed to DDT, but have been exposed to multiple toxic chemicals in water (Prapanthadara et al., 2000a). The hypothesis whether cytocrome P^{450} monooxigenases could be involved in DDT resistance in *A. aegypti* (Prapanthadara et al., 2002) has been recently supported by microarray experiments in *A. gambiae* (David et al., 2005; Vontas et al., 2005). Although these findings remain to be demonstrated with functional studies, these experiments suggested that DDT resistance may be the result of overexpression and down-regulation of several genes, including genes not formerly associated with insecticide resistance (Vontas et al., 2005). It is noteworthy that the factors responsible for GST regulation involved in resistance seem to be capable to regulate other GST expresions that may not be involves in resistance (Ding et al., 2005).

In many cases, GSTs with GPx capacity are associated to PYR resistance. However we must be cautious in interpreting such assumptions, as was explained in previous paragraphs. In addition to their direct role in insecticide detoxification, some GSTs may act as a second line of defense against the toxic effects of insecticides. It is preferable to say that elevated GST expression with GPx capacity increases tolerance to

PYRs rather than confer resistance to this insecticide.

In the same way, some authors have speculated how the presence of GST-based insecticide resistance affects the mosquito pathogen's survival and its vectorial capacity (McCarroll and Hemingway, 2002; Ranson and Hemingway, 2005). This question emerged from the evidence of the ROS generation involved in the defense mechanism against pathogen infections in insects (Ha et al., 2005a, b) including mosquitoes (Dimopoulos et al., 2002; Kumar et al., 2003). Further studies have demonstrated that some GSTs are upregulated with aging (Zou et al., 2000), and their overexpression could cause a life span extension in flies, as some researchers are trying to demonstrate(http://www.uams.edu/biochem/Hbenes.asp). Although the implications of these findings for vector-borne disease control still needs to be demonstrated, it should be considered that GSTs are only one part of this complicated mechanism of antioxidant defense where other antioxidant enzymes are involved.

To date, different GST orthologs (understood as genes in different species that evolved from a common ancestral gene by speciation) have been identified in mosquitoes. These orthologs are structurally conserved with high levels of amino acid identity. Although functionally catalytic differences are observed between orthologs, the identification of same GSTe2 and its implication in DDT metabolism in different mosquito species suggests that this GST may have an important role on common metabolism pathways of this insecticide. Nevertheless, it is suggested also that different routes of GST may catalyse DDT metabolism in different *A. aegypti* strains from Thailand (Lumjuan et al., 2005). These hypotheses are interesting themes for future researches.

Biochemical assays have been the traditional method applied for detecting and monitoring GST activity levels. These assays measure GST activity using model substrates, like CDNB and DCNB and a correlation between levels of GST activity and DDT resistance phenotype suggests a GST-based resistance mechanism. However the average of GST activity using CDNB is generally lower in GST epsilon class than in GST delta class (Table 1). Field studies reported that levels of GST activity in resistant anopheline strains were slightly higher than those of the susceptible strain; in fact it was not always possible to correlate DDT resistance with GST activity levels (Penilla et al., 2006). Additional evidence showed that the DDTase activity does not necessarily correlate with GST activity in anopheline mosquitoes (Prapanthadara et al., 2000a). Facing these limitations, the identification of ortholog enzymes and specific GSTs involved in insecticide resistance offers many possibilities for the development of new rapid, sensitive and viable methods for GST-based metabolic resistance mechanism detection.

REFERENCES

Abdollahi M, Ranjbar A, Shadnia S, Shekoufeh N, Rezaie A (2004). Pesticide and oxidative stress: a review. Med. Sci. Monit. 10:RA141-147.

Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, *et al* (1999). Regulation of JNK signaling by GSTp. Embo J. 18: 1321-1334.

Armstrong RN (1991). Glutathione S-transferases: reaction mechanism, structure, and function. Chem. Res. Toxicol. 4:131–140.

Beall C, Fyrberg C, Song S, Fyrberg E (1992). Isolation of a *Drosophila* gene encoding glutathione S-transferase. Biochem. Genet. 30: 515-527.

Bloomquist JR (1998). Chemistry and toxicology of the chlorinated cyclodienes and lindane. Rev. Pestic. Toxicol. 2: 333-355.

Bogwitz M (2005). The genetics resistance to lufenuron in *Drosophila melanogaster*. Ph.D. Thesis, University of Melbourne, Melbourne, Australia .

Brown AWA (1986). Insecticide resistance in mosquitoes: a pragmatic review. J. Am. Mosq. Control Assoc. 2: 123-140.

Chelvanayagam G, Parker MW, Board PG (2001). Fly fishing for GSTs: a unified nomenclature for mammalian and insect glutathione transferases. Chem. Biol. Interact. 133: 256-260.

Chen L, Hall PR, Zhou XE, Ranson H, Hemingway J, Meehan EJ (2003). Structure of an insect delta-class glutathione S-transferase from a DDT-resistant strain of the malaria vector *Anopheles gambiae*. Acta Cryst. D59: 2211-2217.

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.

Cho SG, Lee YH, Park HS, Ryoo K, Kang KW, Park J, Eom SJ, Kim MJ, Chang TS, Choi SY, Shim J, Kim Y, Dong MS, Lee MJ, Kim SG, Ichijo H, Choi EJ (2001). Glutathione S-transferase Mu modulates the stress-activated signals by suppressing apoptosis signal-regulating Kinase 1. Biol. Chem. 276:12749–12755.

Clark AG, Shamaan NA (1984). Evidence that DDT-dehydrochlorinase from the house fly is a glutathione S-transferase. Pestic. Biochem. Physiol. 22: 249-261.

Clark AG, Shamaan NA, Sinclair MD, Dauterman WC (1986). Insecticide metabolism by multiple glutathione S-transferases in two strains of the house fly, *Musca domestica* (L.). Pestic. Biochem. Physiol. 25: 169-175.

Danielson UH, Mannervik B (1985). Kinetic independence of the subunits of cytosolic glutathione transferase from the rat. Biochem. J. 231: 263-267.

David J-P, Strode C, Vontas J, Nikou D, Vaughan A, Pignatelli P, Christos L, Janet H, Hilary R (2005). The *Anopheles gambiae* detoxification chip: A highly specific microarray to study metabolic based insecticide resistance in malaria vectors. Proc. Natl. Acad. Sci. 102: 4080-4084.

Dimopoulos G, Christophides GK, Meister S, Schultz J, White KP, Barillas-Mury C, Kafatos FC (2002). Genome expression analysis of *Anopheles gambiae*: responses to injury, bacterial challenge, and malaria infection. Proc. Natl. Acad. Sci. 99: 8814-8819.

Ding Y, Ortelli F, Rossiter LC, Hemingway J, Ranson H (2003). The *Anopheles gambiae* glutathione transferase supergene family: annotation, phylogeny and expression profiles. BMC Genomics 4: 35.

Ding Y, Hawkes N, Meredith J, Eggleston P, Hemingway J, Ranson H (2005). Characterisation of the promoters of Epsilon glutathione transferases in the mosquito *Anopheles gambiae* and their response to oxidative stress. Biochem. J. 387: 879-888.

Enayati AA, Ranson H, Hemingway J (2005). Mini Review: Insect glutathione transferases and insecticide resistance. Insect. Mol. Biol. 14:3-8.

Feyereisen R (1995). Molecular biology of insecticide resistance. Toxicol. Lett. 82-83: 83-90.

Fournier D, Bride J-M, Poirie´ M, Berge´ J-B, Plapp FW (1992). Insect glutathione S-transferases. Biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides. J. Biol. Chem. 267: 1840–1845.

Grant DF, Matsumura F (1988). Glutathione S-transferase-1 in *Aedes aegypti* larvae. Purification and properties. Insect. Biochem. 18: 615-622.

Grant DF, Matsumura F (1989). Glutathione S-transferase 1 and 2 in susceptible and insecticide resistant *Aedes aegypti*. Pestic. Biochem. Physiol. 33: 132-143.

Grant DF, Dietze EC, Hammock BD (1991). Glutathione S-transferase isozymes in *Aedes aegypti:* purification, characterization, and isozyme specific regulation. Insect. Biochem. 4: 421-433.
Grant DF, Hammock BD (1992). Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathiones transferase-2 expression in *Aedes aegypti*. Mol. Gen. Genet. 234: 169-176.
Ha EM, Oh CT, Bae YS, Lee WJ (2005a). A direct role for dual oxidase in *Drosophila* gut immunity. Science 310: 847-850.
Ha EM, Oh CT, Ryu JH, Bae YS, Kang SW, Jang IH, Brey PT, Lee WJ (2005b). An antioxidant system required for host protection against gut infection in *Drosophila*. Dev. Cell. 8: 125-132.
Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249(22): 7130-7139.
Halliday WR, Feyereisen R (1987). Why does DDT toxicity change after a blood meal in adult female *Culex pipiens*? Pestic. Biochem. Physiol. 28: 172-181.
Harshman LG, James AA (1998). Differential gene expression in insects: transcriptional control. Annu. Rev. Entomol. 43: 671-700.
Hayes JD, Wolf CR (1988). Role of glutathione transferase in drug resistance. In: Sies H, Ketterer B (eds) Glutathione conjugation: Mechanisms and Biological Significance. Academic Press, London, pp. 315-355.
Hazelton GA, Lang CA (1983). Glutathione S-transferase activities in the yellow-fever mosquito [*Aedes aegypti* (Louisville)] during growth and aging. Biochem. J. 210: 281-287.
Hemingway J, Miyamoto J, Herath PRJ (1991). A possible novel link between organophosphorus and DDT insecticide resistance genes in *Anopheles*: supporting evidence from fenitrothion metabolism studies. Pestic. Biochem. Physiol. 39: 49-56.
Hemingway J (2000). Mini review: The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. Insect. Biochem. Mol. Biol. 30: 1009-1015.
Hemingway J, Ranson H (2000). Insecticida resistance in insect vectors of human disease. Annu. Rev. Entomol. 45: 371-391.
Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004). The molecular basis of insecticide resistance in mosquitoes. Insect. Biochem. Mol. Biol. 34: 653-665.
Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, Wincker P, Clark AG, Ribeiro JM, Wides R, Salzberg SL, Loftus B, Yandell M, Majoros WH, Rusch DB, Lai Z, Kraft CL, Abril JF, Anthouard V, Arensburger P, Atkinson PW, Baden H, de Berardinis V, Baldwin D, Benes V, Biedler J, Blass C, Bolanos R, Boscus D, Barnstead M, Cai S, Center A, Chaturverdi K, Christophides GK, Chrystal MA, Clamp M, Cravchik A, Curwen V, Dana A, Delcher A, Dew I, Evans CA, Flanigan M, Grundschober-Freimoser A, Friedli L, Gu Z, Guan P, Guigo R, Hillenmeyer ME, Hladun SL, Hogan JR, Hong YS, Hoover J, Jaillon O, Ke Z, Kodira C, Kokoza E, Koutsos A, Letunic I, Levitsky A, Liang Y, Lin JJ, Lobo NF, Lopez JR, Malek JA, McIntosh TC, Meister S, Miller J, Mobarry C, Mongin E, Murphy SD, O'Brochta DA, Pfannkoch C, Qi R, Regier MA, Remington K, Shao H, Sharakhova MV, Sitter CD, Shetty J, Smith TJ, Strong R, Sun J, Thomasova D, Ton LQ, Topalis P, Tu Z, Unger MF, Walenz B, Wang A, Wang J, Wang M, Wang X, Woodford KJ, Wortman JR, Wu M, Yao A, Zdobnov EM, Zhang H, Zhao Q, Zhao S, Zhu SC, Zhimulev I, Coluzzi M, della Torre A, Roth CW, Louis C, Kalush F, Mural RJ, Myers EW, Adams MD, Smith HO, Broder S, Gardner MJ, Fraser CM, Birney E, Bork P, Brey PT, Venter JC, Weissenbach J, Kafatos FC, Collins FH, Hoffman SL (2002). The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298(5591): 129-149.
Hooper GH (1967). The effect of larval density on mortality of *Culex pipiens* fatigans due to DDT and Dieldrin. Bull WHO 36: 179-80.
Hoy MA (1994). Comp. Insect molecular genetics: An introduction to principles and applications. Gainesville; Academic Press. http://www.uams.edu/biochem/Hbenes.asp
Huang HS, Hu NT, Yao YE, Wu CY, Chiang SW, Sun CN (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: 651-658.
Jakobsson PJ, Morgenstern R, Mancini J, Ford-Hutchinson A, Persson B (1999). Common structural features of MAPEG -- a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. Protein Sci. 8: 689-692.
Jakoby WB, Ziegler DM (1990). The enzymes of detoxication. Minireview. J. Biol. Chem. 265: 20715-20718.
Jirajaroenrat K, Pongjaroenkit S, Krittanai C, Prapanthadara L, Ketterman AJ (2001). Heterologous expression and characterization of alternatively spliced glutathione S-transferases from a single *Anopheles* gene. Insect Biochem. Mol. Biol. 31: 867-875.
Kostaropoulos I, Papadopoulos AI, Metaxakis A, Boukouvala E, Papadopoulou-Mourkidou E (2001). Glutathione S-transferase in the defence against pyrethroids in insects. Insect Biochem. Mol. Biol. 31: 313-319.
Kumar S, Christophides GK, Cantera R, Charles B, Han YS, Meister S, Dimopoulos G, Kafatos FC, Barillas-Mury C (2003). The role of reactive oxygen species on *Plasmodium* melanotic encapsulation in *Anopheles gambiae*. Proc. Natl. Acad. Sci. 100(24): 14139-14144.
Lines JD, Nassor NS (1991). DDT resistance in *Anopheles gambiae* declines with mosquito age. Med. Vet. Entomol. 5: 261-265.
Lipke H, Chalkley J (1962). Glutathione, oxidized and reduced, in some dipterans treated with 1,1,1-trichloro-2,2-di-(*p*-chlorophenyl)ethane. Biochem. J. 85: 104-109.
Lipke H, Chalkley J (1964). The conversión of DDT to DDE by some Anophelines. Bull. WHO 30: 57-56.
Listowsky I, Abramovitz M, Homma H, Niitsu Y (1988). Intracellular binding and transport of hormones and xenobiotics by glutathione S-transferase. Drug Metab. Rev. 19: 305-318.
Lumjuan N (2005). Characterisation of *Aedes aegypti* glutathione S-transferase enzyme family. Ph.D. Thesis, Liverpool School of Tropical Medicine, Liverpool, UK.
Lumjuan N, McCarrol 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: 861-871.
Mannervik B, Danielson UH (1988). Glutathione transferases--structure and catalytic activity. CRC Crit. Rev. Biochem. 23:283-337.
Marinotti O, Nguyen Q K, Calvo E, James AA, Ribeiro JMC (2005). Microarray analysis of genes showing variable expression following a bloodmeal in *Anopheles gambiae*. Insect. Mol. Biol. 14: 365-374.
McCarroll L, Hemingway J (2002). Can insecticide resistance status affect parasite transmision in mosquitoes? Insect Biochem. Mol. Biol. 35: 1345-1351.
Morel F, Rauch C, Petit E, Piton A, Theret N, Coles B, Guillouzo A (2004). Gene and protein characterization of the human glutathione S-transferase kappa and evidence for a peroxisomal localization. J. Biol. Chem. 279: 16246-16253.
Oakley AJ, Harnnoi T, Udomsinprasert R, Jirajaroenrat K, Ketterman AJ, Wilce MCJ (2001). The crystal structures of glutathione S-transferases isozymes 1-3 and 1-4 from *Anopheles dirus* species B. Protein Sci. 10: 2176-2185.
Oppenoorth FJ, Van der Pas LJT, Houx NWH (1979). Glutathione S-transferases and hydrolytic activity in a tetrachlorvinphos-resistant strain of housefly and their influence on resistance. Pestic. Biochem. Physiol. 11: 176-188.
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: 957-963.
Parkes TL, Hilliker AJ, Phillips JP (1993). Genetic and biochemical analysis of glutathione S-transferases in the oxygen defence system of *Drosophila melanogaster*. Genome 36: 1007-1014.
Pearson WR (2005). Phylogenies of glutathione transferase families. Methods Enzymol. 401: 186-204.
Pemble SE, Wardle AF, Taylor JB (1996). Glutathione S-transferase class Kappa: characterization by the cloning of rat mitochondrial GST and identification of a human homologue. Biochem. J. 319: 749-754.
Penilla RP, Rodríguez AD, Hemingway J, Torres JL, Solis F. Rodríguez MH (2006). Changes in glutathione S-transferase levels in DDT resistant natural Mexican populations of *Anopheles albimanus* under different insecticide resistance management strategies. Pestic. Biochem. Physiol. 86: 63-71.
Pickett CB, Lu AY (1989). Glutathione S-transferases: gene structure,

regulation, and biological function. Annu. Rev. Biochem. 58:743-764.
Pongjaroenkit S, Jirajaroenrat K, Boonchauy C, Chanama U, Leetachewa S, Prapanthadara L, Ketterman AJ (2001). Genomic organization and putative promoters of highly conserved glutathione S-transferases originating by alternative splicing in *Anopheles dirus*. Insect Biochem. Mol. Biol. 31:75-85.
Prapanthadara L, Hemingway J, Ketterman AJ (1993). Partial purification and characterization of glutathione S-transferases involved in DDT resistance from the mosquito *Anopheles gambiae*. Pestic. Biochem. Physiol. 47: 119-133.
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 transferases. Bull. Entomol. Res. 85: 267-274.
Prapanthadara L, Koottathep S, Promtet N, Hemingway J, Ketterman AJ (1996). Purification and characterization of a major glutathione S-tranferase from the mosquito *Anopheles dirus* (species B*)*. Insect Biochem. Mol. Biol. 26: 277-285.
Prapanthadara L, Ranson H, Somboon P, Hemingway J (1998). Cloning, expression and characterization of an insect class I glutathione S-transferase from *Anopheles dirus* species B. Insect Biochem. Mol. Biol. 28: 321-329.
Prapanthadara L, Koottathep S, Promtet N, Suwonkerd W, Ketterman AJ, Somboon P (2000a). Correlation of glutathione S transferase and DDT dehydrochlorinase activities with DDT susceptibility in *Anopheles* and *Culex* mosquitos from northern Thailand. Southeast Asian J. Trop. Med. Public. Health. 31: 111-118.
Prapanthadara L, Promtet N, Koottathep S, Somboon P, Ketterman AJ (2000b). Isoenzymes of glutathione S-transferase from the mosquito *Anopheles dirus* species B: the purification, partial characterization and interaction with various insecticides. Insect Biochem. Mol. Biol. 30: 395-403.
Prapanthadara L, Promtet N, Koottathep S, Somboon P, Suwonkerd W, McCarroll L, Hemingway J (2002). Mechanisms of DDT and permethrin resistance in *Aedes aegypti* from Chiang Mai, Thailand. Dengue Bulletin 26: 185-189.
Ranson H, Cornel AJ, Fournier D, Vaughan A, Collins FH, Hemingway J (1997a). Cloning and localization of a glutathione S-transferase class I gene from *Anopheles gambiae*. J. Biol. Chem. 272: 5464-568.
Ranson H, Prapanthadara L, Hemingway J (1997b). Cloning and characterisation of two glutathione S-transferases from a DDT resistant strain of *Anopheles gambiae*. Biochem. J. 324: 97–102.
Ranson H, Collins FH, Hemingway J (1998). The role of alternative mRNA splicing in generating heterogeneity within the *Anopheles gambiae* class I glutathione S-transferase family. Proc. Natl. Acad. Sci. USA 95: 14284-14289.
Ranson H, Jensen B, Wang X, Hemingway J, Collins FH (2000). Genetic mapping of two loci affecting DDT resistance in the malaria vector *Anopheles gambiae*. Insect. Mol. Biol. 9: 499-507.
Ranson H, Rossiter L, Ortelli F, Jensen B, Wang X, Roth CW, Collins FH, Hemingway J (2001). Identification of a novel class of insect glutathione S-transferases involved in DDT resistance in the malaria vector, *Anopheles gambiae*. Biochem. J. 359: 295-304.
Ranson H, Claudianos C, Ortelli F, Abgrall C, Hemingway J, Sharakhova MV, Unger MF, Collins FH, Feyereisen R (2002). Evolution of supergene families associated with insecticide resistance. Science 298: 179-181.
Ranson H, Hemingway J (2005). Mosquito glutathione transferases. Review. Methods Enzymol. 401: 226-241.
Sawicki R, Singh SP, Mondal AK, Benes H, Zimniak P (2003). Cloning, expression and biochemical characterization of one Epsilon-class (GST-3) and ten Delta-class (GST-1) glutathione S-transferases from *Drosophila melanogaster*, and identification of additional nine members of the Epsilon class. Biochem. J. 370: 661-669.
Sheehan D, Meade G, Foley VM, Dowd CA (2001). Review article, Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J. 360: 1-16.
Singh SP, Coronella JA, Benes H, Cochrane BJ, Zimniak P (2001). Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. Eur. J. Biochem. 268: 2912-2923.
Tanaka K, Nakajima M, Kurihara N. (1981). The mechanism of resistance to lindane and hexadeuterated lindane in the third yumenoshima strain of house fly. Pestic. Biochem. Physiol. 16: 149-157.
Tang AH, Tu CP (1994). Biochemical caracterization of *Drossophila* glutathione S-transferase D1 and D2. J. Biol. Chem. 269: 27876-27884.
Tang AH, Tu CP (1995). Pentobarbital-induced changes in *Drosophila* glutathione S-transferase D21 mRNA stability. J. Biol. Chem. 270: 13819-13825.
Toung Y-PS, Hsieh T-S, Tu C-PD (1990). Drosophila glutathione S-transferase 1-1 shares a region of sequence homology with the maize glutathione S-transferase III. Proc. Natl. Acad. Sci. USA 87: 31-35.
Tu C-PD, Akgul B (2005). *Drosophila* glutathione S-transferases. Methods Enzymol. 401: 204-226.
Udomsinprasert R, Ketterman AJ (2002). Expression and characterization of a novel class of glutathione S-transferase from *Anopheles dirus*. Insect Biochem. Mol. Biol. 32: 425-433.
Udomsinprasert R, Pongjaroenkit S, Wongsantichon J, Oakley AJ, Prapanthadara L, Wilce MCJ, Ketterman AJ (2005). Identification, characterization and structure of a new Delta class glutathione transferase isoenzyme. Biochem. J. 388: 763-771.
Ugaki M, Shono T, Fukami J (1985). Metabolism of fenitrothion by organophosphorus-resistant and susceptible house flies, *Musca domestica* L. Pestic. Biochem. Physiol. 23: 33-40.
Vontas JG, Small GJ, Hemingway J (2001). Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in *Nilaparvata lugens*. Biochem. J. 357: 65-72.
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: 329-337.
Vontas J, Blass C, Koutsos AC, David JP, Kafatos FC, Louis C, Hemingway J, Christophides GK, Ranson H (2005). Gene expression in insecticide resistant and susceptible *Anopheles gambiae* strains constitutively or after insecticide exposure Insect Mol. Biol. 14(5): 509-521.
Wang J, McCommas S, Syvanen M (1991). Molecular cloning of a glutathione S-transferase overproduced in an insecticide resistant strain of the house fly (*Musca domestica*). Mol. Gen Genet. 227: 260-266.
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 house-fly *Musca domestica*. Insect Biochem. Mol. Biol. 31: 1145-1153.
Wongsantichon J, Harnnoi T, Ketterman AJ (2003). A sensitive core region in the structure of glutathione S-tranferase. Biochem. J. 373:759-765.
Xu Q, Liu H, Zhang L, Liu N (2005). Resistance in the mosquito, *Culex quinquefasciatus*, and possible mechanisms for resistance. Pest. Manag. Sci. 61(11): 1096-1102.
Yang Y, Cheng J-Z, Singhal SS, Saini M, Pandya U, Awasthi S (2001). Role of glutathione S-transferases in protection against lipid peroxidation. J. Biol. Chem. 276(22): 19220-19230.
Yu SJ (1996). Insect glutathione S-transferases. Zool. Stud. 35(1): 9-19.
Zou S, Meadows S, Sharp L, Jan LY, Jan YN (2000). Genome-wide study of aging and oxidative stress response in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. 97: 13726-13731.