Research Article

Received: 3 September 2011 Revised: 19 December 2011 Accepted article published: 1 February 2012 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.3297

Identification and expression profiles of nine glutathione *S*-transferase genes from the important rice phloem sap-sucker and virus vector *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae)

Wen-Wu Zhou,[a] Xi-Wang Li,[a] Yin-Hua Quan,[b] Jiaan Cheng,[a] Chuan-Xi Zhang,[a] Geoff Gurr[a,c] and Zeng-Rong Zhu[a]*

Abstract

BACKGROUND: Glutathione *S*-transferases (GSTs) have received considerable attention in insects for their roles in insecticide resistance. *Laodelphax striatellus* (Fallén) is a serious rice pest. *L. striatellus* outbreaks occur frequently throughout eastern Asia. A key problem in controlling this pest is its rapid adaptation to numerous insecticides. In this research, nine cDNAs encoding GSTs in *L. striatellus* were cloned and characterised.

RESULTS: The cloned GSTs of *L. striatellus* belonged to six cytosolic classes and a microsomal subgroup. Exposure to sublethal concentrations of each of the six insecticides, DDT, chlorpyrifos, fipronil, imidacloprid, buprofezin and beta-cypermethrin, quickly induced (6 h) up-expression of *LsGSTe1*. The expression of *LsGSTs2* was increased by chlorpyrifos, fipronil and beta-cypermethrin. Furthermore, exposure of *L. striatellus* to fipronil, imidacloprid, buprofezin and beta-cypermethrin increased the expression of the *LsGSTm* gene after 24 or 48 h.

CONCLUSION: This work is the first identification of GST genes from different GST groups in Auchenorrhyncha species and their induction characteristics with insecticide types and time. The elevated expression of GST genes induced by insecticides might be related to the enhanced tolerance of this insect to insecticides and xenobiotics.

Keywords: glutathione S-transferases; *Laodelphax striatellus*; gene expression; insecticide resistance

1 INTRODUCTION

Intracellular metabolism is of vital importance in chemical circulation and defence reactions of all living organisms. A diverse superfamily of multifunctional enzymes, glutathione *S*-transferases (GSTs, EC 2.5.1.18) play significant roles in the metabolism of intracellular compounds in multiple kingdoms and phyla. A wide range of endobiotic and xenobiotic compounds, such as insecticides, herbicides, plant secondary metabolites, organic pollutants and electrophilic drugs, are catalysed by these enzymes.[1] More specific examples of GST activity include the principal glutathione (GSH, γ-glutamyl-cysteinyl-glycine) conjugation, which renders cytotoxic and genotoxic chemicals more soluble and easier to excrete.[2] GSTs also exhibit GSH-dependent peroxidation (or isomerisation) activity and several other non-catalytic functions such as non-substrate ligand binding and intracellular transportation of drugs, hormones, fatty acids, hematin and bilirubin and stress signal processing.[3–5]

In general, GSTs can be classified into at least four subgroups according to their subcellular location and function: cytosolic, mitochondrial, microsomal and bacterial antibiotic resistance proteins.[6] In insects, most GSTs are soluble enzymes belonging to the cytosolic subgroup, and they have been grouped into eight classes (omega, sigma, theta, zeta, delta, epsilon, chi and iota) based on their sequence relatedness, genome organisations and functional characters.[7] The classes delta, epsilon, chi and iota are considered to be insect specific.[5] Most of the GST classes are encoded by multigene families, and the alternative

* *Correspondence to: Zeng-Rong Zhu, State Key Laboratory of Rice Biology, Key Laboratory of Agricultural Entomology, Ministry of Agriculture, and Institute of Insect Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China. E-mail: zrzhu@zju.edu.cn*

a *State Key Laboratory of Rice Biology, Key Laboratory of Agricultural Entomology, Ministry of Agriculture, and Institute of Insect Sciences, Zhejiang University, Hangzhou, Zhejiang, China*

b *Department of Entomology, College of Plant Protection, Nanjing Agricultural University, Nanjing, Jiangsu, China*

c *EH Graham Centre for Agricultural Innovation, Charles Sturt University, Orange, Australia*

splicing also increases the heterogeneity, causing an extremely high diversity both at the transcript and the functional level.[8] The available genome and expressed sequence tag (EST) datasets have allowed a more extensive identification of GST genes in many insects. Within Diptera, 37 GSTs have been identified in *Drosophila melanogaster*,[9] 28 in *Anopheles gambiae*[10] and 27 in *Aedes aegypti*.[11] In orthopterans, ten GSTs have been found in the oriental migratory locust *Locusta migratoria manilensis*.[12] The coleopteran *Tribolium castaneum*[13] has about 35 GSTs, and the lepidopteran *Bombyx mori* has 23,[14] while the hymenopteran *Apis mellifera*[15] and *Nasonia vitripennis*[16] have eight and 19 respectively. In hemipterans, two Aphididae members *Acyrthosiphon pisum* and *Myzus persicae* have 20 and 21 GSTs respectively,[17] but no information is available on the GSTs of other Hemiptera, including the important pest family Delphacidae.

The small brown planthopper (SBPH), *Laodelphax striatellus* (Fallén), is widely distributed from the Philippines to Siberia and into Europe, mainly in the temperate zone.[18] In Asian countries, SBPH is one of the most economically important pests. As an oligophagous plant-feeder, SBPH causes great damage to many crops, including rice, wheat and maize, by direct feeding and as a plant virus vector.[19] The extensive spraying of insecticides to control this pest has resulted in insecticide resistance in SBPH in various geographical areas.[20] Many insecticides, such as imidacloprid and fipronil, have become ineffective even at relatively high doses. The detoxification enzyme proteins extracted from resistant SBPH show modified metabolic activities compared with those from susceptible SBPH. It is apparent that metabolic pathways play important roles in insecticide resistance in SBPH.[20] Until now, little has been known about the actual response of these enzyme families in rice planthoppers, as each of them has tens of genes with diverse functions. From the three whole-body transcriptome databases of *L. striatellus*,[21] a total of nine GST genes were identified, and a comprehensive analysis of them was carried out, including the gene characteristics and expression patterns when exposed to insecticides. To the authors' knowledge, the present study provides the first insights into the *L. striatellus* GSTs at the molecular level and has cloned GST genes (although not the whole family) of Auchenorrhyncha insects for the first time. It is believed that the findings of this study will be helpful for studying GST-related insecticide resistance in this and related species.

2 MATERIALS AND METHODS

2.1 Insect

The strain of *L. striatellus* that was used in all experiments originated from a field population collected in Hangzhou in eastern China. The insects were reared on susceptible rice seedlings cv. Taichung Native 1 (TN1) (susceptible to almost all herbivores of rice) at $25 \pm 1\,^{\circ}C$ and 80% relative humidity under a 16 : 8 h light : dark regime for at least 30 generations.

2.2 Transcriptome searching of *L. striatellus* GST cDNAs

Previously, two *L. striatellus* transcriptome datasets were published by Qian *et al.*[21] The datasets were elucidated using a mixture of whole *L. striatellus* bodies at all developmental stages. A third *L. striatellus* transcriptome dataset was recently constructed in the present authors' laboratory (unpublished data), also using a mixture of whole bodies at all developmental stages. GST genes from *L. striatellus* were identified by searching the sequences in these transcriptome databases for keywords (GST, glutathione transferase and glutathione *S*-transferase) or by using the basic local alignment search tool (BLAST) algorithm to search for other known insect GST genes. To confirm the identity of a GST gene, further searches of putative GST cDNAs were conducted using BLASTX to compare the sequence against the non-redundant database at NCBI (http://www.ncbi.nlm.nih.gov/). If sequences did not have a complete open reading frame (ORF), they were again compared with the local *L. striatellus* cDNA database.

2.3 RNA isolation, cDNA synthesis and full-length cDNA clone

RNA was extracted from individuals of *L. striatellus* using TRIzol reagent (Invitrogen, CA) according to the manufacturer's protocol. The RNA was treated with DNase (Takara, Japan) to remove any contaminating genomic DNA. The RNA was then reverse transcribed to produce cDNA using PrimeScript first-strand cDNA synthesis kit (Takara, Japan). Based on the DNA sequence data obtained from transcriptome searching, nine pairs of gene-specific primers were designed and synthesised for the GST cDNAs with complete ORFs (Table 1). The PCR conditions were determined empirically for amplification of each GST cDNA. Electrophoresis was then carried out using the PCR products. DNA bands of the expected size were excised from the agarose gel and purified using DNA gel extraction kit (Axygen, USA). These PCR products were cloned into pMD18-T vector (Takara, Japan), and then at least three independent clones were sequenced from each cDNA (GenScript Biotech., Nanjing, China).

2.4 Phylogenetic analysis

Deduced amino acids of GSTs from different insects were aligned using ClustalW (v.1.83).[22] The percentages of the amino acid identity of different GSTs were determined using DNASTAR software. Phylogenetic trees were determined by the neighbour-joining method, with 1000 bootstrap resampling statistics implemented in MEGA 4.0.[23]

2.5 Insecticide treatments

Responses of the GST genes to insecticides were investigated by exposing nymphs to six synthesised insecticides for 6–72 h by the rice stem dipping method.[24] Sublethal concentrations of the insecticides (LC_{50} in 24 h) were used for each insecticide treatment. Third-instar nymphs (1 day after moulting from the second-instar nymph stage) were used to avoid any bias from pupation during insecticide exposure. At each time point, 30 insects were collected from each insecticide treatment, and every treatment was repeated 3 times. The nymphs were exposed to dichlorodiphenyltrichloroethane (DDT) (Sigma, St Louis, MO), chlorpyrifos (organophosphate insecticide), fipronil (phenyl pyrazole-derived insecticide), imidacloprid (neonicotinoid insecticide), buprofezin (insect growth regulator) and beta-cypermethrin (synthetic pyrethroid pesticide) (Xinnong Chemical Industrial Group Co. Ltd, Taizhou, Zhejiang, China).

RNA from each sample was extracted using TRIzol reagent (Invitrogen, CA) and treated with DNAse I (Invitrogen, CA) according to the manufacturer's protocol. The concentration of each RNA sample was adjusted to 1 $\mu g\,\mu L^{-1}$ with nuclease-free water, and the RNA was reverse transcribed in a 20 µL reaction system using the AMV RNA PCR kit (TaKaRa, Japan). The sequences of the specific primer sets for qRT-PCR are listed in Table 1. The actin gene of SBPH was used as an internal gene, and the primers designed for qRT-PCR were 5′-AAACTGGGACGACATGGAGAA-3′ (as the forward primer) and 5′-GCGACTCGCAACTCGTTGTA-3′ (as

Table 1. Primers for gene clone and RT-PCR of GST genes of *Laodelphax striatellus*

GenBank	Gene	Primers	Sequence (5′-3′)	cDNA (bp)
JN628440	*LsGSTs1*	F	GCAGCTACTTGCTCTGTCA	717
		R	CGGTACAATCAATTGTTCTCGAT	
		RT-F	TTTTCGCTTATGCTGGTGTTGA	
		RT-R	AAAACAGGCAGCATCCCAAA	
JN628441	*LsGSTe1*	F	TCCAGTTCGACATTATAGAGTCTA	841
		R	CTTGCAAATAGCTTGCTCCAA	
		RT-F	ACAAATCTTTCCAGGCGTTGA	
		RT-R	TCCTTGTTGATTGGCCGAAT	
JN628442	*LsGSTz1*	F	CCATAGTTGAAGCCGTATTACT	764
		R	CGCTGTGACATTGTCCGTTT	
		RT-F	GGGAGATTTGCGAAGTAATTGC	
		RT-R	TGCTGTGCCCATTCTTTTTTC	
JN628443	*LsGSTs2*	F	CGTGCGACGATATACAACTT	729
		R	GTAGCACCAACGGTGTTAGTT	
		RT-F	ACCCCATTCGGCCAAGTT	
		RT-R	CAGCGATCCCAGCTTTTTTG	
JN628444	*LsGSTs3*	F	GCTTCAGGCCTAGATTGAA	692
		R	CAACAACTGACTCTTGAACCAT	
		RT-F	ACCGCTCTGGGAGAACCAA	
		RT-R	TTGGTTTGATTGATGGCCATT	
JN628445	*LsGSTt1*	F	CAAGTTTTAATAAATCATGAGTACT	770
		R	CTCTGCACCGGAAGATGAA	
		RT-F	TATCCTTGGAGCCTGCGAAA	
		RT-R	TGTTTTTATGCGATTCATCCATTC	
JN628446	*LsGSTd1*	F	CTGCTCCTTGAACACAAGAA	720
		R	GATTCGACAATGGAATTGTA	
		RT-F	TTCCCCTACAGCGCTCCAT	
		RT-R	GTTGTTTCCCCTCGAAGAGGTT	
JN628447	*LsGSTm*	F	CCAGTGAACGCGTTTTAGAT	564
		R	GCTCAGTCGATGATGGATGAA	
		RT-F	TGGCCGCCCTGCTGTA	
		RT-R	TTCAGCGCCAACCAATAGG	
JN628448	*LsGSTo1*	F	GGTTATATTATTCCGAAGTTGAA	739
		R	TTTCATGGCGCTCATCCAT	
		RT-F	TCCATTGAGAAAGGCCAAAGA	
		RT-R	CGTTTCTGTATCGATGTCGTTGA	

the reverse primer). The qRT-PCR was performed using the SYBR Premix Ex Taq kit (TaKaRa, Japan) according to the manufacturer's protocol. A prerun test was carried out to confirm the constant expression of actin gene in different samples. After the qRT-PCR assay, the results (threshold cycle value) were normalised to the expression level of the constitutive actin gene. A no-template control sample (nuclease-free water) was included in the experiment to detect contamination and to determine the degree of dimer formation. A relative quantitative method ($\Delta\Delta C_t$) was used to evaluate the quantitative variation.[25] Data were converted by SPSS v.19.0 software, and the Mann–Whitney test with a *P*-value of <0.05 was applied for analysing the fold change of gene expression and its significance.

2.6 Quantitative PCR of *L. striatellus* GSTs in different ages and organs

GST transcription profiles were first investigated at ten different life stage, sex and wing-form permutations: eggs, first-instar nymphs, second-instar nymphs, third-instar nymphs, fourth-instar nymphs, fifth-instar nymphs, macropterous adult females, macropterous adult males, brachypterous adult females and brachypterous adult males. For each biological replicate, 30 fresh individuals of each life stage were collected and the RNA immediately extracted.

Transcription profiles were then investigated in different larval tissues obtained by dissecting third-instar nymphs. The different nymph tissues studied were: head (A), midgut (M), hemolymph (H), fat body (F) and malpighian tubule (MT). Tissues were dissected from more than 300 fresh nymphs in ice-cold RNAlater (Ambion, Austin, TX) and stored in RNAlater at −80 °C until RNA extractions were carried out. Specifically, for the hemolymph collection, 40 insects were punctured in the thorax with a fine tungsten needle and placed head down into four 0.6 mL tubes whose bases had been punctured with a 25 gauge needle and plugged with cotton. The tubes were set into 1.5 mL Eppendorf tubes and centrifuged at 4 °C for 5 min at 5500 rpm. Hemolymph was then

Table 2. Percentage identities of amino acid residues among the eight cytosolic L. striatellus GSTs (italics denote sigma-class GSTs)

	LsGSTd1	*LsGSTe1*	*LsGSTt1*	*LsGSTs1*	*LsGSTs2*	*LsGSTs3*	*LsGSTo1*	*LsGSTz1*
LsGSTd1	–	37.95	26.16	11.67	11.81	12.66	13.1	15.77
LsGSTe1		–	23.43	12.18	12.77	14.47	13.55	14.64
LsGSTt1			–	14.11	11.2	12.45	12.25	19.42
LsGSTs1				–	*38.32*	*36.92*	15.06	14.85
LsGSTs2					–	*64.53*	13.25	11.95
LsGSTs3						–	11.49	11.89
LsGSTo1							–	25
LsGSTz1								–

collected from the 1.5 mL tubes. All the tubes mentioned above were treated with DEPC to protect the RNA from RNase.

3 RESULTS AND DISCUSSION

3.1 Identification and classification of *L. striatellus* GSTs

Nine different GST transcripts were identified from three transcriptome datasets of *L. striatellus* and comparison with the non-redundant database at NCBI. Based on the phylogenetic analysis and similarity of these amino acid residues to other known insect GSTs, eight sequences were placed into six cytosolic classes and named according to the classification and order of discovery. Specifically, one gene was grouped into the microsomal subgroup and named *LsGSTm*, as it shows little similarity to cytosolic GSTs but a significantly high level of similarity to microsomal GSTs from other insects (Fig. 4). The percentages of deduced amino acid identities are 11.2–64.53% among all *L. striatellus* GSTs and 36.92–64.53% among three sigma-class GSTs (Table 2). Similarly to other insect GSTs, the length of the deduced amino acid sequences of the cloned *L. striatellus* GSTs was between 202 and 236 for cytosolic GSTs and 152 for microsomal GSTs. All of the GST genes identified were deposited in GenBank (Table 1).

L. striatellus GST genes, similarly to those in dipteran (*D. melanogaster, A. gambiae* and *A. aegypti*), coleopteran (*T. castaneum*) and lepidopteran (*B. mori*) insects, belong to each of the six classes (Table 3) and the microsomal group. This is in contrast to another hemipteran, *A. pisum*, the genome of which was previously sequenced. No gene in *A. pisum* was identified in the classes epsilon, omega or zeta. The abbreviation of gene class within the family may spring from the long-term ecological adaptation of Hemiptera to different feeding habits and 'food recipes'.[29] The aphid *A. pisum* is a typical polyphagous species, living on many annual plants (e.g. pea and broad bean) and perennial plants (e.g. red clover and alfalfa). In contrast, the oligophagous insect *L. striatellus* has a much narrower host spectrum, living mainly on Poaceae hosts.

3.2 Comparison of GSTs from *L. striatellus* and other insects

Insect GSTs have a much longer history of evolution than those of mammals.[30] The division of GSTs is associated with the ecological niches and feeding types that insects have evolved. According to the phylogenic tree (Fig. 1), there is greater similarity between GST genes from the sigma class (except for *LsGSTs2*) and the omega class of the hemipteran species *L. striatellus, A. pisum* and *T. citricida* compared with those of other families (Fig. 1). In contrast, GST genes from the classes theta, delta and epsilon are more diverse between these species, indicating that their functional roles vary. For example, the *LsGSTt1* (from the theta class) and the *LsGSTz1* (from the zeta class) have greater similarity to some GSTs from hymenopteran species and the *LsGSTd1* has greater similarity to

Table 3. Numbers of validated glutathione *S*-transferases in the insect genomes or expressed sequence tag dataset

	Insect order											
	Diptera				Hymenoptera		Coleoptera	Lepidoptera	Orthoptera	Hemiptera		
GST class	*D.m*	*A.g*	*A.a*	*C.t*[a]	*A.m*	*N.v*	*T.c*	*B.m*	*L.m*[a]	*A.p*[b]	*M.p*[a]	*L.s*[a]
Delta	11	12	8	2	1	5	3	4	1	10 (6)	8	1
Epsilon	14	8	8	0	0	0	19	8	0	0	0	1
Omega	5	1	1	1	1	2	4	4	0	0	0	1
Sigma	1	1	1	4	4	8	7	2	7	6 (5)	8	3
Theta	4	2	4	0	1	3	1	1	1	2 (2)	2	1
Zeta	2	1	1	0	1	1	1	2	0	0	0	1
Microsomal	1	3	1	0	2	0	1	1	0	2 (2)	2	1
Unclassified	0	3	3	4	0	0	0	2	1	0	0	0
Total	**38**	**31**	**27**	**11**	**10**	**19**	**36**	**24**	**10**	**20 (15)**	**20**	**9**

D. m = D. melanogaster;[9] A. g = A. gambiae;[26] A. a = A. aegypti;[11] C. t = C. tentans;[27] A. m = A. mellifera;[28] N. v = N. vitripennis;[16] T. c = T. castaneum;[17] B. m = B. mori;[14] L. m = L. migratoria manilensis;[12] A. p = A. pisum; M. p = M. persicae;[17] L. s = L. striatellus.
[a] Collected from EST dataset instead of genomes.
[b] Numbers in brackets indicate the number of genes identified from the EST dataset.

DmGSTd (Fig. 1) compared with related GSTs of other hemipterans. The percentage identities of amino acid residues in the delta class are usually above 40% in insects. *LsGSTd1* and *DmGSTd1* have the largest amino acid identity level (40.2%) compared with related GSTs of other insects. These differences reflect function as the amino acids form the catalytic pocket and determine protein folding (Fig. 2).

Delta is the largest GST class of the cytosolic subgroup in *D. melanogaster, A. gambiae, A. aegypti, N. vitripennis* and *A. pisum*, while most *T. castaneum* and *B. mori* genes are within the epsilon class (Table 3). The majority of GSTs from these two classes have been implicated in insect metabolism of xenobiotics.[32,33] In this study, *L. striatellus* was found to have only one delta gene and one epsilon gene. The relatively few delta and epsilon genes but greater numbers of sigma GSTs identified in *L. striatellus* are possibly due to the samples collected for transcriptome sequencing being limited. Within-species variation cannot be ruled out. The amino acid sequences of *LsGSTd1* were aligned with those from four other insect species in order to predict functionally conserved loci. Two amino acid residues (S12 and N50) of *L. striatellus* represent a possible catalytic pocket, which is important for the catalytic activity of GST enzymes. The amino acid residues P56-L146-G154-D161 may determine protein folding and structure.[31]

In addition to the delta class, the sigma class also has a wide taxonomic distribution (found in all 12 insect species) (Table 3) and may be essential for some housekeeping-related roles.[35] In *D. melanogaster*, a sigma-class GST-2 enzyme exhibits considerable conjugation activity for a lipid peroxidation product, indicating a possible role as an antioxidant.[36] Moreover, even though some insecticide metabolism activity has been detected in insects, such as *L. migratoria manilensis*[12] and *Callosobrochus maculatus*,[37] it is not clear whether this response is directly induced or is an indirect reaction to insecticide stress. Sigma-class genes appear to be the most common or second most common genes among hemipteran, orthopteran, coleopteran and hymenopteran insects (Table 3). In contrast, only one sigma-class gene was identified in the genome of *D. melanogaster, A. gambiae* and *A. aegypti* (Table 3). Three of the *L. striatellus* sigma GSTs shared characteristics with sigma GSTs of other insects. The percentage identities of amino acids among sigma GSTs range from 36.9 to 64.5%. Aligning the *L. striatellus* sigma GSTs with *D. melanogaster DmGSTs1* revealed several key residues involved in catalytic function (Fig. 3): the putative GSH binding site, the electrophilic binding site, the putative H-site (interacting with GSH) and the bulge-inducing site.[34] These residues were not strictly conserved in *L. striatellus*, which correlates with the metabolic diversity of these GSTs.

Microsomal glutathione transferase (MGST) has mostly been studied in mammals. It is involved in protecting the cell from oxidative damage and/or xenobiotics by catalysing reactions involving a multitude of substrates ranging from products of lipid peroxidation to cytostatic drugs.[38] *Drosophila melanogaster* MGST null mutants show no obvious defects in morphology, but their lifespan is significantly reduced compared with controls. This indicates that MGST is not essential for development but is involved in the processes related to ageing.[39] Nearly all the amino acid identities among the MGST gene from 12 insects, including *L. striatellus*, are above 40%, suggesting a conservation of function in this gene subgroup. In addition, the MGST gene *LsGSTm* from *L. striatellus* has greater similarity to *A. pisum* than other insects, reflecting the fact that these species are from the same family.

The omega-class gene can bind organophosphate insecticides instead of metabolising them. For example, *AcGSTO*1-1 in *Anopheles cracens* shows high affinity for temephos,[40] and *BmGSTO* in *B. mori* has high affinity for fenitrothion.[41] The theta-class genes had a peroxidase function and acted as binding protein for organophosphates in *Anopheles cracens*.[40] As to the zeta class, this enzyme is abundantly present in the permethrin-resistant strain of *B. mori*, suggesting that it is involved in the detoxification of xenobiotics containing chloride.[42] In this study, *L. striatellus* has one gene in each of the GST classes omega, theta and zeta.

3.3 Transcription profiling of GSTs in nymph tissues, life stages and sex

Third-instar nymphs were used for the analysis of tissue-specific expression patterns of the nine *L. striatellus* GST genes. Five different tissues, namely the head, fat body, hemolymph, midgut and malpighian tubules, were analysed by qRT-PCR. As expected, six genes (*LsGSTd1, LsGSTm, LsGSTs1, LsGSTs2, LsGSTs3, LsGSTt1*) were noticeably more highly expressed in the midgut and/or the malpighian tubules, tissues well known for their crucial function in metabolism of xenobiotics. The widespread expression of GST genes in these organs has also been reported in other insects such as *C. tentans*,[27] *Manduca sexta*[43] and *D. melanoganster*.[44] In contrast, *LsGSTe1* was highly expressed in the head and fat body. The role of the fat body in storage and metabolism of insecticides, thereby retarding the spread of the insecticide and decreasing its toxic effects, is well known. *LsGSTe1* was constantly expressed across all life stages (Fig. 5) but expected to be closely related to insecticide metabolism. Indeed, the expression of this gene was found to increase after insecticide exposure (Fig. 6). As for *LsGSTo1* and *LsGSTz1*, their expression levels did not change significantly within the tissues analysed.

The stage-specific expression patterns of *L. striatellus* were determined in a total of ten different samples, namely egg, five instar nymph stages, macropterous adult male, macropterous adult female, brachypterous adult males and brachypterous adult females. Five genes (*LsGSTe1, LsGSTs1, LsGSTs2, LsGSTs3, LsGSTz1*) were relatively more highly expressed in the first- or second-instar nymph stages. Compared with the adults, early nymphs of planthoppers are covered with soft cuticles and tend to be influenced by environmental chemicals, and a high level of metabolic enzymes will facilitate their adaptation to both host plants and toxic compounds. Among the three sigma-class genes, *LsGSTs2* and *LsGSTs3* show a similar expression pattern to *LsGSTs1*. As *LsGSTs2* and *LsGSTs3* are highly conserved in the amino acid sequence level (Fig. 3), they may all play identical functional roles in *L. striatellus*.

The gene *LsGSTd1*, closely related to xenobiotic metabolism, is highly expressed in all nymph stages and macropterous wing adult females (Fig. 5). The expression of *LsGSTm* shows a similar expression pattern in all stages and wing forms. The expression pattern is also similar between different wing forms of adult males but not for adult females. Four genes (*LsGSTd1, LsGSTe1, LsGSTo1, LsGSTz1*) show increased expression in macropterous adult females compared with brachypterous ones. The migration of *L. striatellus* in different geographical areas in the east of Asia has been recorded by many researchers. Carried by the wind, macropterous adults can travel thousands of miles before being brought to earth by rain.[45] Macropterous adults, especially the female ones, are crucial for the establishment of new populations.[46] It is speculated that the increase in the four GSTs in macropterous adult female improves their adaptability to different ecological niches.

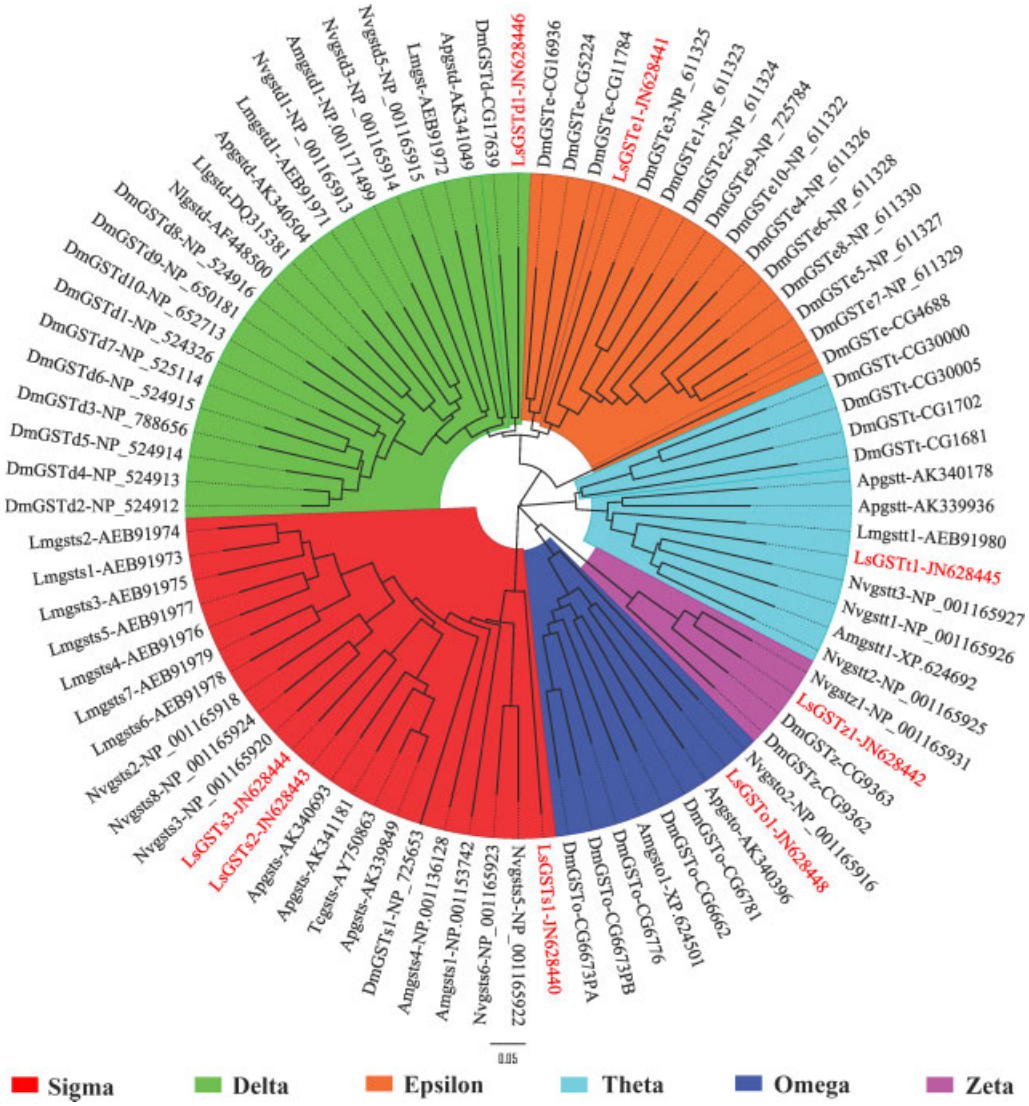

Figure 1. Phylogenetic relationships of 84 GST proteins from the nine insect species *D. melanogaster* (Dm, 37), *N. vitripennis* (Nv, 13), *A. mellifera* (Am, 5), *L. migratoria manilensis* (Lm, 10), *A. pisum* (Ap, 8), *Toxoptera citricida* (Tc, 1), *Lygus lineolaris* (Ll, 1), *Nilaparvata lugens* (Nl, 1) and *L. striatellus* (Ls, 8). Branches of genes from the same class are coloured by FigTree software. The eight *L. striatellus* GSTs are shown in red.

3.4 Transcription profiling in nymphs exposed to xenobiotics

In metabolic resistance assessments of rice planthoppers, specific enzymes within mixtures of proteins extracted from insect bodies cannot be separated owing to their similar molecular weights, leading to deficiencies in the understanding of enzyme functions. In order to gain an understanding of enzyme functions, the induction capacities of the nine GSTs studied were investigated after exposing nymphs to sublethal concentrations (LC_{50} for 24 h) of six different xenobiotics: the organochloride pesticide DDT, the organophosphate insecticide chlorpyrifos, the phenylpyrazole insecticide fipronil, the neonicotinoid insecticide imidacloprid, the heterocyclic synthesis of insect chitin buprofezin and the synthetic pyrethroid insecticide beta-cypermethrin (Fig. 6). For each GST gene identified, the transcription level change in third-instar nymphs exposed to each xenobiotic was measured for up to 48 h after the exposure and normalised according to controls (unexposed nymphs).

According to the resistance monitoring of rice brown planthopper *N. lugens* in laboratory conditions (continually exposed to specific insecticides), metabolic resistance plays a key role in insecticide resistance, especially under the low dose selection.[47] The expression level of mRNA after insecticide treatments for *L. striatellus* GSTs was increased 2/3–3-fold (Fig. 6) which is similar to other insects such as *C. tentans*[27] and *L. migratoria manilensis*.[12] Four of the nine genes (*LsGSTe1*, *LsGSTo1*, *LsGSTs2* and *LsGSTs3*) were more highly expressed when treated with organophosphate insecticide chlorpyrifos. This finding is consistent with former studies in *Anopheles cracens*,[48] *L. migratoria manilensis*[12] and *Callosobrochus maculatus*.[37]

The expression of epsilon-class gene *LsGSTe1* was activated soon (6 h) after treatment with all insecticides, indicating that this gene quickly responded to stimulation by the xenobiotics. The response to imidacloprid and buprofezin lasted for 24 h. A similarly rapidly induced reaction also occurred in sigma-class genes *LsGSTs2* and *LsGSTs3*. *LsGSTs2* was highly expressed at 6 h when exposed to the three insecticides chlorpyrifos, fipronil and beta-cypermethrin, and *LsGSTs3* showed increased expression 6 h after chlorpyrifos exposure. In contrast, for the *LsGSTm* gene,

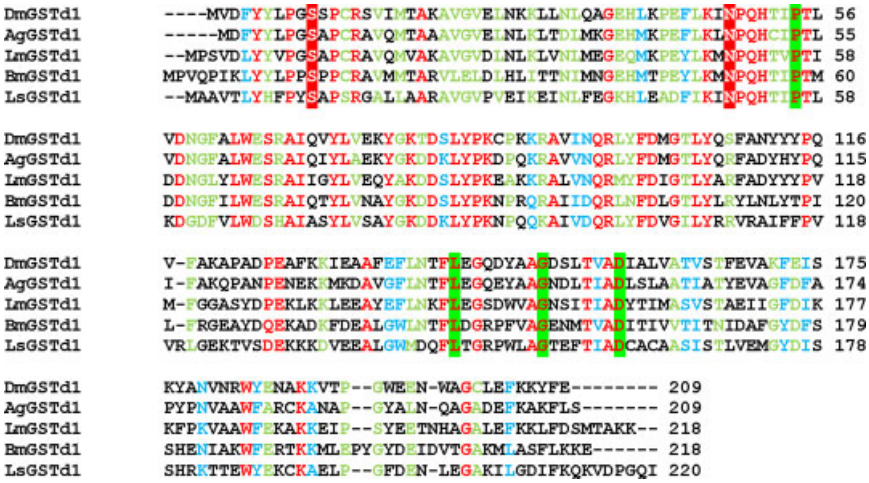

Figure 2. Similarity comparisons of the amino acid sequences of *L. striatellus* delta GST (*LsGSTd1*) with *D. melanogaster* (*DmGSTd1*, AAM52032), *A. gambiae* (*AgGSTd1*, 40889324), *L. migratoria manilensis* (*LmGSTd1*, HM131834) and *B. mori* (*BmGSTd1*, AJ006502). S12 and N50 of *L. striatellus*, which represent the catalytic pocket, are shaded in red. Amino acid residues, shaded in green, determine folding.[31] The colour of the letter represents the similarity of the amino acid in this locus between the sequences aligned (red = high similarity; blue = low similarity; green = middle). Dashes are used to denote gaps introduced for a maximum alignment.

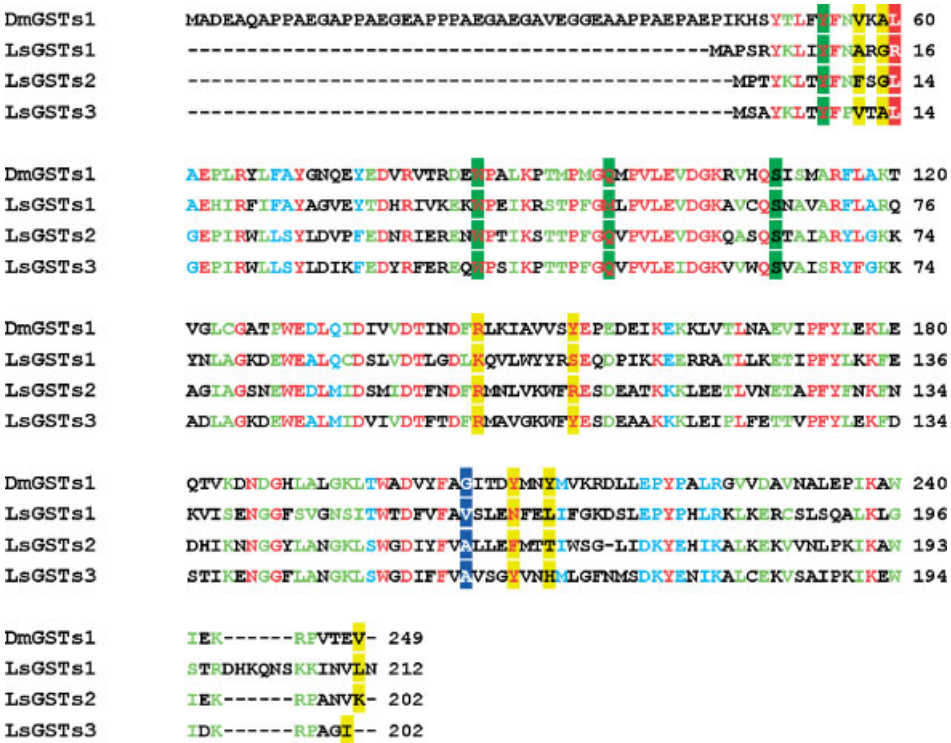

Figure 3. Similarity comparisons of the amino acid sequences of three *L. striatellus* sigma GSTs (*LsGSTs1*, *LsGSTs2*, *LsGSTs3*) with a *Drosophila melanogaster* sigma GST (*DmGSTs1*, AAM48357). The amino acid residues of the sigma GSTs, shaded in green and yellow, represent residues that constitute the putative glutathione (GSH) and electrophilic substrate binding sites respectively. The putative H-site residue of *L. striatellus* (L14 in *LsGSTs2* and *LsGSTs3*), which contacts GSH, is shaded in red. The putative bulge-inducing residues (V160 in *LsGSTs1*, A158 in *LsGSTs2* and A158 in *LsGSTs3*) are shaded in blue.[34] The colour of the letter represents the similarity of the amino acid in this locus between the sequences aligned (red = high similarity; blue = low similarity; green = middle). Dashes are used to denote gaps introduced for a maximum alignment.

belonging to the microsomal subgroup, expression increased after 24 or 48 h. Additionally, *LsGSTo1* responded quickly to chlorpyrifos (6 h) but slowly to imidacloprid (24 h). The mRNA level of *LsGSTd1*, *LsGSTs1*, *LsGSTt1* and *LsGSTz1* remained unchanged between different treatments. The same phenomenon was found in *Culex quinquefasciatus*.[49] Nevertheless, the lack of response does not necessarily mean that these enzymes are not involved in resistance to the insecticides tested. It is possible that high expression levels are maintained generally to respond to xenobiotics. In addition, it cannot be ruled out that these enzymes are involved in resistance to other insecticides not tested.

GSTs mediate resistance to many insecticides, including organophosphates, carbamates and pyrethroids. A direct rela-

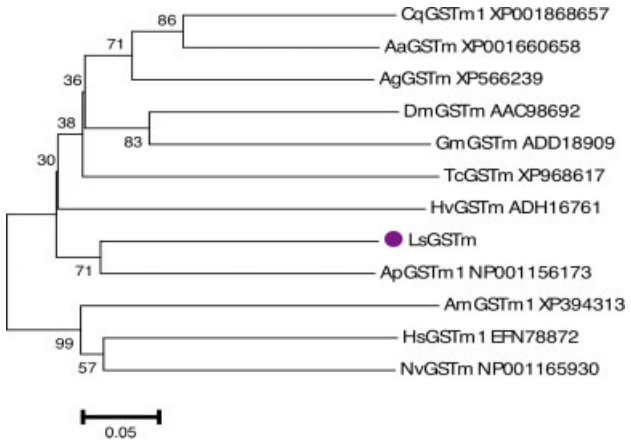

Figure 4. Phylogenetic relationships of 12 microsomal GST proteins from the 12 insect species *L. striatellus* (Ls), *A. pisum* (Ap), *Culex quinquefasciatus* (Cq), *A. gambiae* (Ag), *D. melanogaster* (Dm), *A. aegypti* (Aa), *T. castaneum* (Tc), *Heliothis virescens* (Hv), *Glossina morsitans* (Gm), *A. mellifera* (Am), *Harpegnathos saltator* (Hs) and *N. vitripennis* (Nv). Amino acid sequences were aligned using ClustalW (www.ebi.ac.uk/clustalW), and a distance neighbour-joining tree was generated using MEGA. Nodes with distance bootstrap values (1000 replicates) are shown. The *L. striatellus* microsomal GST is marked with filled circles.

tionship between GST overexpression and resistance has been reported during the past decades. As shown in Fig. 6, the numbers of *L. striatellus* GST genes with increased expression for the six insecticides were as follows: chlorpyrifos (four genes) > fipronil (three genes) = imidacloprid (three genes) = beta-cypermethrin (three genes) > buprofezin (two genes) > DDT (one gene). This diversity may be caused by the difference in insecticide accumulation and targeting in insect bodies, or by the adaptation of *L. striatellus* itself to the field application of these toxics. Chlorpyrifos is a typical organophosphate insecticide that may induce *L. striatellus* to express significantly more (threefold) of the epsilon gene *LsGSTe1*. In the diamondback moth, *Plutella xylostella*, the epsilon gene *PxGST3* is strongly correlated with resistance of organophosphorus insecticides. *PxGST3* is the first cloned GST gene with a well-defined role, and it encodes for an enzyme capable of degrading organophosphorus insecticides.[50] *LsGSTe1* may have a similar function to *PxGST3*. The organochloride pesticide DDT has been banned in most countries, including China, for decades but was once used to control rice pests. In DDT-resistant populations of *A. aegypti, GSTe2, GSTe5* and *GSTe7* are expressed at elevated levels. Additionally, partial silencing of either *GSTe7* or *GSTe2* by RNA interference in *A. aegypti* was found to result in increased susceptibility to the pyrethroid deltamethrin.[51] Therefore, an elevated level of *LsGSTe1* activity may also be required for resistance to the organochloride DDT and the pyrethroid beta-cypermethrin in *L. striatellus*.

Until now, little direct evidence has been available for a connection between a specific GST gene and resistance to insecticides such as fipronil, imidacloprid and buprofezin. *L. striatellus* shows elevated expression of *LsGSTe1* and *LsGSTm* when exposed to these insecticides. Therefore, *LsGSTe1* and *LsGSTm* may be related to the metabolism of these insecticides. Furthermore, the increased expression of *LsGSTo1* in the presence of imidacloprid (24 h) and *LsGSTs2* in the presence of fipronil (6 h) suggests that the genes may be involved in resistance to the respective insecticides.

4 CONCLUSION

In the present study, nine GST genes were identified from *L. striatellus*, an important rice insect pest in Asian countries. By phylogenetic analysis and amino acid identity comparison, these genes were placed into two subgroups and six classes. The GST genes of *L. striatellus* were different to those found in another hemipteran, *A. pisum*, indicating divergent evolutionary paths related to the differing ecological adaptations of the insects. The expression profile of these GSTs in different tissues and developmental stages can contribute to the functional prediction of them in *L. striatellus*. After exposure to six insecticides commonly applied in rice fields (with the exception of DDT), mRNA increased for half of the nine GSTs. The expression of the epsilon-class GST (*LsGSTe1*) was elevated by all of the insecticides tested. Therefore, *LsGSTe1* is a possible factor in the cross-resistance of *L. striatellus* to many different insecticides. Insecticide resistance has become an urgent problem for the control of *L. striatellus* and other

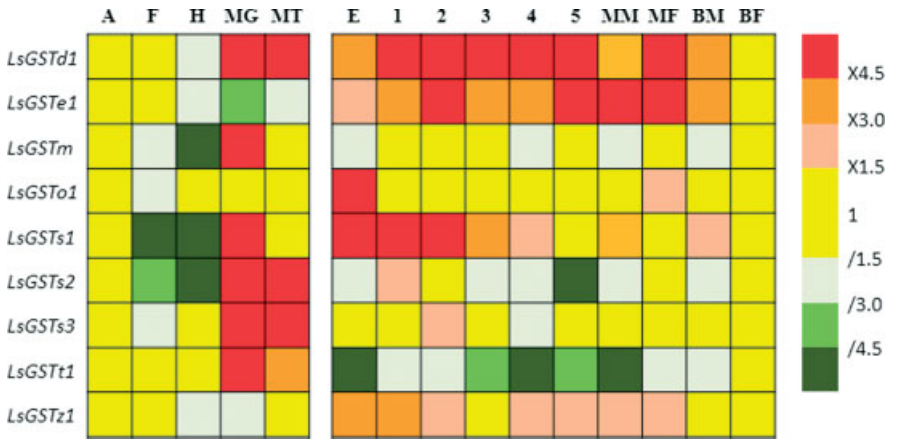

Figure 5. Constitutive transcription profiles of nine *L. striatellus* GSTs across different nymph tissues (left), life stages, sexes and wing-forms (right). Tissues analysed were: head (A), fatbody (F), hemolymph (H), midgut (MG) and malpighian tubules (MT). Life stages analysed were: egg (E), first-instar nymph (1), second-instar nymph (2), third-instar nymph (3), fourth-instar nymph (4), fifth-instar nymph (5), macropterous adult male (MM), macropterous adult female (MF), brachypterous adult male (BM) and brachypterous adult female (BF). Transcription levels are expressed as mean fold transcription relative to head (tissues) or brachypterous adult females (life stages). Red and green indicate significant over- and undertranscription respectively (ratio of >1.5-fold in either direction, and Mann–Whitney test *P*-value of <0.05; data were converted by SPSS v.19.0). Yellow indicates no significant transcription variations.

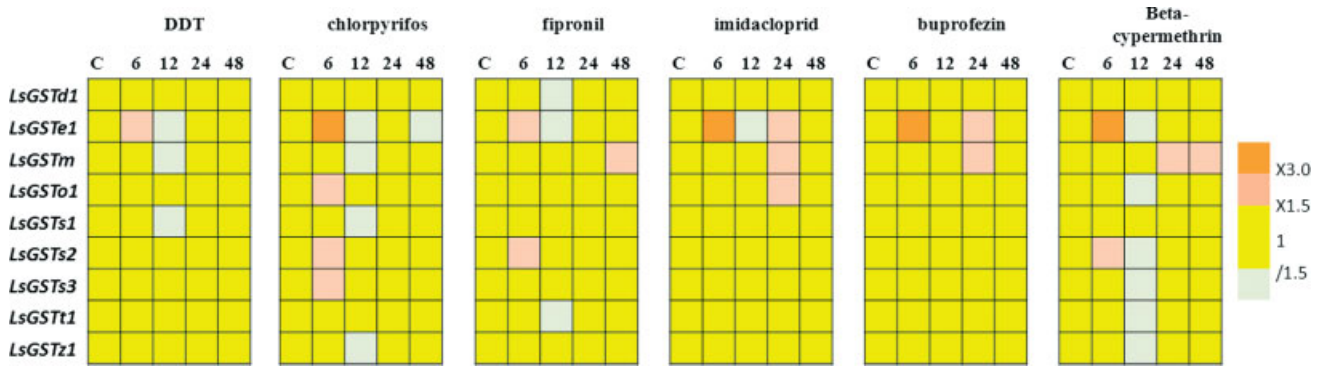

Figure 6. Transcription profiles of nine GSTs (*LsGSTd1, LsGSTe1, LsGSTm, LsGSTo1, LsGSTs1, LsGSTs2, LsGSTs3, LsGSTt1, LsGSTz1*) in *L. striatellus* third-instar nymphs exposed for 6–48 h to sublethal concentrations of six different insecticides (DDT, chlorpyrifos, fipronil, imidacloprid, buprofezin and beta-cypermethrin). For each time point, transcription levels are expressed as mean fold transcription relative to controls (unexposed nymphs). Orange and white indicate significant over- and undertranscription respectively (ratio of >1.5-fold in either direction, and Mann–Whitney test *P*-value of <0.05; data were converted by SPSS v.19.0). Yellow indicates no significant transcription variations.

Delphacidae such as the rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera*. The present characterisation of GSTs in *L. striatellus* may shed light on delphacid resistance to insecticides and even rice cultivars resistant to the rice planthoppers.

ACKNOWLEDGEMENTS

This work was partially supported by the 973 programme (2010CB126200), the National Natural Science Foundation of China (30971908) and the 863 programme (2007AA10Z220). Thanks to Professor Kun-Yan Zhu from Kansas State University for useful suggestions concerning this research. The authors thank DMY Read from Charles Sturt University (Australia), who helped greatly in the construction and writing of this article. Many thanks also to the reviewers of this article, who made very valuable suggestions for improving this paper.

REFERENCES

1 Blanchette B, Feng X and Singh BR, Marine glutathione *S*-transferasess. *Mar Biotechnol* **9**:513–542 (2007).
2 Mannervik B and Danielson UH, Glutathione transferases – structure and catalytic activity. *CRC Crit Rev Biochem* **23**:283–337 (1988).
3 Loyall L, Uchida K, Braun S, Furuya M and Frohnmeyer H, Glutathione and a UV light-induced glutathione *S*-transferase are involved in signaling to chalcone synthase in cell cultures. *Plant Cell* **12**:1939–1950 (2000).
4 Che-Mendoza A, Penilla RP and Rodriguez DA, Insecticide resistance and glutathione *S*-transferases in mosquitoes: a review. *Afr J Biotechnol* **8**:1386–1397 (2009).
5 Lumjuan N, Stevenson BJ, Prapanthadara LA, Somboon P, Brophy PM, Loftus BJ, *et al*, The *Aedes aegypti* glutathione transferase family. *Insect Biochem Mol Biol* **37**:1026–1035 (2007).
6 Oztetik E, A tale of plant glutathione *S*-transferases: since 1970. *Bot Rev* **74**:419–437 (2008).
7 Enayati AA, Ranson H and Hemingway J, Insect glutathione transferases and insecticide resistance. *Insect Mol Biol* **14**:3–8 (2005).
8 Ranson H, Collins F and Hemingway J, The role of alternative mRNA splicing in generating heterogeneity within the *Anopheles gambiae* class I glutathione *S*-transferase family. *PNAS* **95**:14 284–14 289 (1998).
9 Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, *et al*, The genome sequence of *Drosophila melanogaster*. *Science* **287**:2185–2195 (2000).
10 Ding YC, Ortelli F, Rossiter LC, Hemingway J and Ranson H, The *Anopheles gambiae* glutathione transferase supergene family: annotation, phylogeny and expression profiles. *BMC Genomics* **4**:35 (2003).
11 Ranson H, Strode C, Wondji CS, David JP, Hawkes NJ, Lumjuan N, *et al*, Genomic analysis of detoxification genes in the mosquito *Aedes aegypti*. *Insect Biochem Mol Biol* **38**:113–123 (2008).
12 Ma EB, Qin GH, Jia M, Liu T, Xuan T, Zhu KY, *et al*, Identification and characterisation of ten glutathione *S*-transferase genes from oriental migratory locust, *Locusta migratoria manilensis* (Meyen). *Pest Manag Sci* **67**:697–704 (2011).
13 Richards S, Gibbs RA, Weinstock GM, Brown SJ, Denell R, Beeman RW, *et al*, The genome of the model beetle and pest *Tribolium castaneum*. *Nature* **452**:949–955 (2008).
14 Yu QY, Lu C, Li B, Fang SM, Zuo WD, Dai FY, *et al*, Identification, genomic organization and expression pattern of glutathione *S*-transferase in the silkworm, *Bombyx mori*. *Insect Biochem Mol Biol* **38**:1158–1164 (2008).
15 Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, Berenbaum MR, *et al*, A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. *Insect Mol Biol* **15**:615–636 (2006).
16 Oakeshott JG, Johnson RM, Berenbaum MR, Ranson H, Cristino AS and Claudianos C, Metabolic enzymes associated with xenobiotic and chemosensory responses in *Nasonia vitripennis*. *Insect Mol Biol* **19**:147–163 (2010).
17 Ramsey JS, Rider DS, Walsh TK, De Vos M, Gordon KHJ, Ponnala L, *et al*, Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae*. *Insect Mol Biol* **19**:155–164 (2010).
18 Kisimoto R, Flexible diapause response to photoperiod of a laboratory selected line in the small brown planthopper, *Laodelphax striatellus* Fallen. *Appl Entomol Zool* **24**:157–159 (1989).
19 Kisimoto R, Genetic variation in ability of a planthopper vector – *Laodelphax striatellus* (Fallen) to acquire rice stripe virus. *Virology* **32**:144–152 (1967).
20 Sanada-Morimura S, Sakumoto S, Ohtsu R, Otuka A, Huang SH, Thanh DV, *et al*, Current status of insecticide resistance in the small brown planthopper, *Laodelphax striatellus*, in Japan, Taiwan, and Vietnam. *Appl Entomol Zool* **46**:65–73 (2011).
21 Qian W, Zhang FJ, Guo HY, Zheng HJ, Zhou T, Zhou YJ, *et al*, Massively parallel pyrosequencing-based transcriptome analyses of small brown planthopper (*Laodelphax striatellus*), a vector insect transmitting rice stripe virus (RSV). *BMC Genomics* **11**:303 (2010).
22 Thompson JD, Higgins DG and Gibson TJ, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific ga penalties and weight matrix choice. *Nucleic Acids Res* **22**:4673–4680 (1995).
23 Tamura K, Dudley J, Nei M and Kumar S, MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**:1596–1599 (2007).
24 Zhang J, Liu J, Qin XW, Yuan FH and Zhang RJ, Analysis of time–and concentration–mortality relationship of nitenpyram against different larval stages of *Nilaparvata lugens* (Hemiptera: Delphacidae). *J Econ Entomol* **103**:1665–1669 (2010).
25 Livak KJ and Schmittgen TD, Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* **25**:402–408 (2001).

26 Holt RA, Subramanian GM, Halpern A, Sutton GG, Charlab R, Nusskern DR, *et al*, The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* **298**:129–149 (2002).

27 Li XW, Zhang X, Zhang JZ, Zhang X, Starkey SR and Zhu KY, Identification and characterization of eleven glutathione *S*-transferase genes from the aquatic midge *Chironomus tentans* (Diptera: Chironomidae). *Insect Biochem Mol Biol* **39**:745–754 (2009).

28 Weinstock GM, Robinson GE, Gibbs RA, Worley KC, Evans JD, Maleszka R, *et al*, Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* **443**:931–949 (2006).

29 Brambila J and Hodges GS, Bugs (Hemiptera), in *Encyclopedia of Entomology*, ed. by Capinera JL. Springer, Dordrecht, The Netherlands, p. 593 (2008).

30 Ketterman AJ, Saisawang C and Wongsantichon J, Insect glutathione transferases. *Drug Metab Rev* **43**:253–265 (2011).

31 Mittapalli O, Neal JJ and Shukle RH, Tissue and life stage specificity of glutathione *S*-transferase expression in the Hessian fly, *Mayetiola destructor*: implications for resistance to host allelochemicals. *J Insect Sci* **7**:1–13 (2007).

32 Wang YJ, Qiu L, Ranson H, Lumjuan N, Hemingway J, Setzer WN, *et al*, Structure of an insect epsilon class glutathione *S*-transferase from the malaria vector *Anopheles gambiae* provides an explanation for the high DDT-detoxifying activity. *J Struct Biol* **164**:228–235 (2008).

33 Lerksuthirat T and Ketterman AJ, Characterization of putative hydrophobic substrate binding site residues of a Delta class glutathione transferase from *Anopheles dirus*. *Arch Biochem Biophys* **479**:97–103 (2008).

34 Agianian B, Tucker PA, Schouten A, Leonard K, Bullard B and Gros P, Structure of a *Drosophila* sigma class glutathione *S*-transferase reveals a novel active site topography suited for lipid peroxidation products. *J Mol Biol* **326**:151–165 (2003).

35 Wildenburg G, Liebau E and Henkle-Duhrsen K, *Onchocerca volvulus*: ultrastructural localization of two glutathione *S*-transferases. *Exp Parasitol* **88**:34–42 (1998).

36 Singh SP, Coronella JA, Benes H, Cochrane BJ and Zimniak P, 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 (2001).

37 Kolawole AO, Ajele JO and Sirdeshmuhk R, Studies on glutathione transferase of cowpea storage bruchid, *Callosobrochus maculatus* F. *Pestic Biochem Phys* **100**:212–220 (2011).

38 Morgenstern R, Microsomal glutathione transferase 1. *Gluthione Transferases and Gamma-Glutamyl Transpeptidases* **401**:136–146 (2005).

39 Aigaki T and Toba G, Disruption of the microsomal glutathione *S*-transferase-like gene reduces life span of *Drosophila melanogaster*. *Gene* **253**:179–187 (2000).

40 Wongtrakul J, Pongjaroenkit S, Leelapat P, Nachaiwieng W, Prapanthadara LA and Ketterman AJ, Expression and characterization of three new glutathione transferases, an epsilon (AcGSTE2-2), omega (AcGSTO1-1), and theta (AeGSTT1-1) from *Anopheles cracens* (Diptera: Culicidae), a major Thai malaria vector. *J Med Entomol* **47**:162–171 (2010).

41 Yamamoto K, Nagaoka S, Banno Y and Aso Y, Biochemical properties of an omega-class glutathione *S*-transferase of the silkmoth, *Bombyx mori*. *Comp Biochem Phys C* **149**:461–467 (2009).

42 Yamamoto K, Shigeoka Y, Aso Y, Banno Y, Kimura M and Nakashima T, Molecular and biochemical characterization of a zeta-class glutathione *S*-transferase of the silkmoth. *Pestic Biochem Phys* **94**:30–35 (2009).

43 Snyder MJ, Walding JK and Feyereisen R, Glutathione *S*-transferases from larval *Manduca sexta* midgut – sequence of 2 cDNAs and enzyme-induction. *Insect Biochem Mol Biol* **25**:455–465 (1995).

44 Nakamura A, Yoshizaki I and Kobayashi S, Spatial expression of Drosophila glutathione *S*-transferase-D1 in the alimentary canal is regulated by the overlying visceral mesoderm. *Dev Growth Differ* **41**:699–702 (1999).

45 Matsumura M, Otuka A, Sanada-Morimura S, Takeuchi H, Watanabe T, Ohtsu R, *et al*, The 2008 overseas mass migration of the small brown planthopper, *Laodelphax striatellus*, and subsequent outbreak of rice stripe disease in western Japan. *Appl Entomol Zool* **45**:259–266 (2010).

46 Novotny V, Adaptive significance of wing dimorphism in males of *Nilaparvata lugens*. *Entomol Exp Appl* **76**:233–239 (1995).

47 Liu ZW and Han ZJ, Fitness costs of laboratory-selected imidacloprid resistance in the brown planthopper, *Nilaparvata lugens* Stål. *Pest Manag Sci* **62**:279–282 (2006).

48 Wongtrakul J, Wongsantichon J, Vararattanavech A, Leelapat P, Prapanthadara LA and Ketterman AJ, Molecular cloning and expression of several new *Anopheles cracens* epsilon class glutathione transferases. *Protein Peptide Lett* **16**:75–81 (2009).

49 Kasai S, Komagata O, Okamura Y and Tomita T, Alternative splicing and developmental regulation of glutathione transferases in *Culex quinquefasciatus* Say. *Pestic Biochem Phys* **94**:21–29 (2009).

50 Huang HS, Hu NT, Yao YE, Wu CY, Chiang SW and Sun CN, 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 (1998).

51 Lumjuan N, Rajatileka S, Changsom D, Wicheer J, Leelapat P, Prapanthadara LA, *et al*, The role of the *Aedes aegypti* epsilon glutathione transferases in conferring resistance to DDT and pyrethroid insecticides. *Insect Biochem Mol Biol* **41**:203–209 (2011).