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ABSTRACT

Metabolic DDT resistance in *Drosophila melanogaster* has previously been associated with constitutive over-transcription of cytochrome P450s. Increased P450 activity has also been associated with increased oxidative stress. In contrast, over-transcription of glutathione S transferases (GSTs) has been associated with resistance to oxidative stress. However, little is known in regards to the impact of xenobiotics on induction of P450s and GSTs and if there exist differences in inducibility between the pesticide susceptible and resistant strains. Thus, we investigated the transcriptional expression of GSTs and P450s in DDT resistant (*Wisconsin*) and susceptible (*Canton-S*) *Drosophila* strains in response to exposure to DDT and the oxidative stressor H_2O_2. *Wisconsin* constitutively over-transcribed P450s, constitutively under-transcribed 27% of its total GSTs, and was more susceptible to H_2O_2 than *Canton-S*. DDT exposure induced GST expression only in the *Wisconsin* strain and not in the *Canton-S* strain. These results suggest that there are potentially more differences between pesticide susceptible and resistant strains than just constitutive expression of P450s; there may also exist, at least in some strains, differences in their patterns of inducibility of P450s and GSTs. Within the context of the *Wisconsin* strain, these differences may be contributing to the fly lines increased susceptibility to oxidative stress.

1. Introduction

Researchers have suggested that alleles causing resistance to some pesticides may be costly for an insect population and that, if the pesticide were no longer applied, these costly alleles and resistance would revert to low frequency [1–5]. Few studies have detailed the exact nature of "costs for resistance" and their underlying molecular mechanisms [6,7].

Only a few studies have focused on compounds or environmental factors that confer a cost to resistance [6–17]. Such compounds are termed negative cross-resistance toxins and the environmental factors are termed ecological negative cross-resistance factors [17]. In negative cross-resistance, increased resistance to one compound or environmental factor causes increased susceptibility to another compound or environmental factor. In some or many cases, development of negative cross-resistance toxins may not be economically viable for use in managing resistance that may occur to pesticides that are currently on the market [17,18]. However, understanding environmental parameters (e.g., plant varieties, abiotic stresses, or biological control agents) [6,7] that increase fitness costs (*i.e.*, ecological negative cross-resistance) may provide the basis for economically viable integrated pest management strategies to minimize pesticide resistance in insect populations.

Successful strategies have been developed for minimizing certain forms of recessive resistance (e.g., refuges are used to minimize resistance in insect populations to transgenic plants expressing *Bt*), but such resistance management strategies do not work for dominant resistance traits [18–20]. Metabolic pesticide resistance is often a dominant trait [21,22]. This form of resistance has typically been associated with over-transcription or over-translation or both of detoxification enzymes, including glutathione S transferases (GSTs), cytochrome P450 enzymes, and

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esterases. Some strains of dipteran species, including houseflies and mosquitoes (*Aedes aegypti*, *Anopeheles gambiae*, and *Anopheles albimanus*), appear to be resistant to DDT (dichloro-diphenyl-trichloroethane), and other pesticides, through GST-catalyzed reactions [23–35].

In addition to detoxifying pesticides, GSTs also allow organisms to reduce oxidative stress, an important environmental challenge faced by many organisms [36,37]. In fact, some plants use lipoxygenases to defend against herbivorous insects, presumably by increasing the herbivore's oxidative stress [38,39]. In *Anopeheles gambiae*, GSTs associated with pesticide resistance also respond to H_2O_2 [40,41], an oxidative stressor, suggesting the potential for positive cross-resistance between metabolic pesticide resistance and oxidative stress. Like GSTs, the metabolite trehalose is an important protectant against oxidative and other environmental stresses in a diversity of organisms, including insects [42–50].

In contrast to GSTs and trehalose, some cytochrome P450 enzymes have been associated with increased cellular oxidative stress [51] and are often down-regulated in response to oxidative stressors [52,53]. In *Drosophila melanogaster*, metabolic resistance to DDT has been associated with increased cytochrome P450 expression across a series of fly lines, including the strains known as *Wisconsin* [54,55] and Oregon R [56]. In the *Wisconsin* strain, three P450s (*CYP6G1*, *CYP12D1*, and *CYP6A2*) were induced by DDT or in some cases constitutively over-transcribed (CYP12D1and CYP6G1 proteins have also been shown to be over-translated), and *CYP6G1*and *CYP12D1* (as well as other genes) are thought to be associated with the DDT-resistant phenotype [55,57–59].

Transgenic flies over-expressing *CYP6G1* are more tolerant to DDT than non-transgenic flies [57,60]. Additionally, tissue-directed (midgut, Malpighian tubules, and fat body) over-expression of eight P450s genes in separate fly lines produced DDT-resistant survivors only in the *CYP6G1* and *CYP12D1* strains [59]. Over-expression of *CYP6A2* did not produce additional DDT-treatment survivors [59]. *CYP6A2* expressed in *Escherichia coli* did not metabolize DDT [61].

Over-transcribed *CYP6G1* has been observed in many DDT-resistant *Drosophila* strains from many parts of the world, with apparently little or no cost to insect fitness [62,63]. Over-transcription of *CYP6G1* on its own, however, is associated with low-level DDT resistance. Higher-level DDT resistance, as observed in the *Wisconsin* strain, is associated with over-transcription of multiple P450s [55,58]. It is not known whether resistance, beyond the low-level *CYP6G1*-based resistance, has any costs. However, before we begin to understand the ecological "costs" associated with resistance, we first must understand the differences in how resistant and susceptible strains respond to potential environmental challenges, such as oxidative stress.

As over-expression of P450s has been associated with increased susceptibility to oxidative stress, we analyzed the Half Lethal Concentration (LC_{50}) and molecular responses of *Wisconsin* and the DDT-susceptible strain *Canton-S* to dietary H_2O_2. Constitutive and induced (in the presence of DDT and an oxidative stressor) GST and P450 expression patterns in both *Wisconsin* and *Canton-S* were analyzed. Additionally, we quantified trehalose levels of *Wisconsin* and *Canton-S* males in the presence and absence of H_2O_2.

2. Material and methods

2.1. Strains

Four *D. melanogaster* lines were used: the DDT-susceptible strains *91-C* and *Canton-S*, and the DDT-resistant strains *Wisconsin* and *Hikone-R*. The origins of these strains have previously been described [54,55,58]. The *91-R* strain was not tested because most of its resistance is due to factors other than P450s [16,64]. The *Drosophila* populations were cultured in a controlled chamber at approximately 25 °C, 80% humidity, and 14 h of light per day.

2.2. Bioassays for DDT and H_2O_2 and correlations between LC_{50} values

The four strains of *Drosophila* were bioassayed with the following concentrations of H_2O_2: 0 (water control), 5, 7.5, 10, 12.5, 15, 20, 25, and 30%. A 5% sucrose solution was included in all these treatments. Twenty adult *Drosophila* (3 days old, 1:1 male:female ratio) were anesthetized using CO_2 and transferred into a 15 ml scintillation vial. The vial opening was covered with a cotton ball (lid), and then 5 ml of a H_2O_2 solution or the water control was pipetted onto the cotton lid. Each vial also received a 5% sucrose solution, which was a food source for the flies and which was applied in 5 ml to each cotton lid. Three replicate vials were used for each concentration of H_2O_2. For the H_2O_2 treatments, the 5% sucrose was combined with the H_2O_2 into one solution. After 30 h, the number of dead flies was recorded, and the LC_{50} was calculated using SAS (SAS Institute Inc., Cary, NC). The LC_{25}s and LC_{50}s of DDT for the four fly lines were generated as previously described in Festucci-Buselli et al. [58]. A regression analysis was performed using the LC_{50}s from the four fly strains to determine whether DDT and oxidative stress resistance were correlated.

2.3. H_2O_2 and DDT treatments as well as sample preparation for qRT-PCRs

Canton-S and *Wisconsin* showed the greatest inverse relationship in resistance to DDT and H_2O_2, and we therefore used these two strains to investigate GST constitutive expression as well as GST expression after exposure to DDT and H_2O_2. Because the P450 enzymes *CYP6G1, CYP12D1,* and *CYP6A2* have all previously been documented to be over-transcribed (and in the case of CYP6G1 and CYP12D1 proteins over-translated) [55,58] in the *Wisconsin* strain, we also investigated the expression of these transcripts after exposure to DDT and H_2O_2.

Male and female flies that were 3 days old were prepared separately for each fly strain. The fly strains were treated with the LC_{25}s of H_2O_2 (15.1% for *Canton-S* and 7.5% for *Wisconsin*) and a 5% sucrose solution in 15 ml scintillation vials as described for the H_2O_2 bioassay. For each fly strain, the control group was treated with only a 5% sucrose solution and the experimental group was treated with H_2O_2 + 5% sucrose for 30 h. The males and female flies were then flash-frozen separately at −80 °C. These samples represented a single biological replicate for RNA extraction, which was performed with the RNeasy mini-kit (Qiagen Inc., Valencia, CA). Three separate biological replicates were used per treatment.

We also determined the effect of DDT on induction of all the GSTs in the *Drosophila* genome and the three P450s (*CYP12D1*, *CYP6G1*, and *CYP6A2*) in 3-day-old male and female flies. We used the LC_{25} of DDT (0.15 μg for *Canton-S* and 34.68 μg for *Wisconsin*). The DDT was coated on the inside surface of the 15 ml scintillation vials, the *Wisconsin* and *Canton-S* flies were placed in separate vials [54], and a 5% sucrose solution was added to the cotton lid. The adults were exposed to their respective treatments for 24 h as described by Brandt et al. [54] before being flash-frozen and stored at −80 °C. The samples were prepared as described for H_2O_2 exposure in the previous paragraph.

2.4. Primers

We designed 37 pairs of primers for all 37 *GST* genes found in the *Drosophila* genome (Supplemental Table 1). Primers were designed using the PCR Now™ program (http://pathogene.swmed.edu/rt_primer/).

2.5. Quantitative real time PCR (qRT-PCR)

For each biological replicate of each treatment, we performed three technical replicates for the qRT-PCRs. For each biological replicate, RNA was extracted from 16 3-day-old flies using the Qiagen RNeasy kit (Qiagen, Valencia, CA) with the column DNase digestion procedure. A minimum of three biological replicates was performed for each experiment. cDNA was synthesized using 0.5 μg of total RNA with the iScript cDNA kit from Bio-Rad (Hercules, CA) in a 10 μl reaction volume. We used a 25-fold dilution for each cDNA for the qRT-PCRs. qRT-PCRs were performed with the iQ SYBR Green Supermix from Bio-Rad (Hercules, CA) on an iCycler Thermal Cycler. The threshold cycle (CT) was calculated using iCycler IQ software. The relative expression levels were calculated as given in Pfaffl et al. [65], and the statistical analyses of the relative gene expression level were performed using SAS (SAS Institute Inc., Cary, NC). *Rp49* was used as the reference gene, and the transcription of 37 GST and three P450 genes was analyzed.

2.6. Metabolomics and GC/MS procedure

Three-day-old male flies were prepared separately for *Canton-S* and *Wisconsin* flies. For each fly strain, the control group was treated with only a 5% sucrose solution and the experimental group was treated with the LC_{25}-level of H_2O_2 for the given strain + 5% sucrose for 30 h (in 15 ml scintillation vials). The flies were then flash-frozen in liquid nitrogen and transferred separately to −80 °C. Four biological replicates (15 flies per replicate) were tested for trehalose levels.

For trehalose extraction, each sample was removed from the freezer, and 200 μl of 100% ethanol was added to each 1.5 ml centrifuge tube. The sample was ground for 3 min with a sterile plastic pellet pestle. The samples were then placed into a heating block at 80 °C. After 10 min, 400 μl of a methanol/water (50:50 v/v) mixture was added and placed on a vortex for 30 min at room temperature. Once the extraction was complete, the tubes were centrifuged at 13,000*g* for 10 min. The supernatant was transferred to a new tube and dried using a rotary evaporator at 43 °C for 3 h. The samples were derivatized with 20 μl of a *O*-Methylhydroxylamine-HCl solution (20 mg/ml anhydrous pyridine) by heating to 60 °C for 30 min. Subsequently, 30 μl of MSTFA labeling reagent was added to each tube and incubated at 60 °C for 1 h. Each sample was allowed to cool to room temperature and was then transferred to a glass autosampler vial.

The instruments used for GC–MS were the Pegasus 4D GCxGC-TOFMS from Leco Corp. (St. Joseph, Michigan), an Agilent 6890 N GC, and an Agilent 7683B Series autosampler. The first dimension column was an HP-5MS phase, 30 m long, 0.250 mm I.D., 0.25 μm film. The second dimension column was a DB-17 phase, 1 m long, 0.100 mm I.D., 0.10 μm film. Both columns were from Agilent Technologies. A 3 μl injection was made for each sample using helium as a carrier gas at a flow rate of 1 ml/min. The front inlet split was set to 20 and the inlet temperature was 280 °C. The temperature gradient was as follows: 50 °C for 0.20 min; ramp 10 °C/min to 250 °C and held for 10 min; and ramp 25 °C/min to 300 °C and held for 5 min. The second dimension temperature profile was exactly the same only +20 °C. The transfer line between GC and MS was set to 250 °C. The MS had a solvent delay of 150 s. Data were collected from 30–1000 m/z with an acquisition rate of 100 spectra/s. The detector voltage was 1700, and electron energy was -70 V. The ion source was set to 200 °C. All data were processed using Leco ChromaTOF software (Version 3.32). Area and height calculations were based on the 73 ion. Standard curves for the trehalose metabolite were generated using an eqimolar mixture of standards at five concentrations (0.5, 0.25, 0.05, 0.025, 0.005 μmol).

The method of analysis was by absolute quantification whereby a standards curve was completed for trehalose where the area under the curve was regressed to a known concentration of the metabolite. Density was regressed on concentration to obtain the linear coefficient. This was then used to convert observed densities in the experimental data to quantities (μ mol) of metabolites. The quantified data were then analyzed by SAS using Proc Mixed.

3. Results

3.1. Significant difference in Canton-S and Wisconsin in respectively LC_{50}s and LT_{50}s for DDT and H_2O_2

We observed an inverse linear correlation ($r^2 = 0.96$) between DDT resistance and H_2O_2 resistance (Fig. 1) in the fly strains *Canton-S*, *91-C*, *Hikone-R*, and *Wisconsin*. The *91-R* strain was not included, as the major form of resistance in this strain is not metabolic [66]. The strain most susceptible to DDT, *Canton-S*, was the most resistant to H_2O_2, while the strain most resistant to DDT, *Wisconsin*, was the most susceptible to H_2O_2. Additionally, the LC_{50}s and 95% confidence intervals for *Wisconsin* and *Canton-S* exposed to H_2O_2 did not overlap, and there was no overlap between the mortalities of *Wisconsin* and *Canton-S* in response to H_2O_2 (Fig. 2), showing a significant difference between these two strains ($P < 0.05$). Thus, as these two strains responded differently to DDT and H_2O_2, they were used for further comparisons in terms of transcription of GSTs and P450s.

3.2. Constitutive GST and P450 transcription in the Canton-S and Wisconsin strains

Compared to the *Canton-S* adults, *Wisconsin* adults (males and females collectively) constitutively under-transcribed ten GSTs ($P < 0.01$; Table 1). Of these GSTs, female and male *Wisconsin* flies shared only five genes that were constitutively under-transcribed relative to same sexed *Canton-S* flies (Table 1). After a Bonferonni correction, the number of under-transcribed GST genes dropped to six in *Wisconsin* males and five in *Wisconsin* females. Thus, the *Wisconsin* strain had generally lower expression of GSTs as compared to their *Canton-S* counterparts. Compared to *Canton-S* adults, *Wisconsin* adults constitutively over-transcribed only one P450, *CYP6A2* (Table 1).

3.3. Differential expression of GST and P450 transcripts by DDT in Wisconsin and Canton-S adults

Multiple GSTs were responsive to LC_{25} DDT exposure in *Wisconsin* adults (DDT resistant) but not in the *Canton-S* adults (DDT susceptible). Eight of the 37 GST genes were significantly over-transcribed in DDT-treated vs. non-treated male *Wisconsin* flies ($P < 0.01$; Table 2A). Nine of the 37 GST genes were significantly over-transcribed in DDT-treated vs. non-treated female *Wisconsin* flies (Table 2B). Compared to non-treated flies, DDT-treated *Wisconsin* male and female flies shared four over-transcribed GSTs (Table 2A and B). In *Canton-S* males and females, none of 37 GSTs genes were differentially transcribed due to LC_{25} treatment with DDT (Table 2A and B). Only *CYP12D1* was over-transcribed in DDT-treated vs. non-treated *Wisconsin* males; three P450 genes (*CYP6G1*, *CYP12D1*, and *CYP6A2*) were over-transcribed in DDT-treated vs. non-treated *Wisconsin* females (Table 2). In *Canton-S* adults, the transcriptional levels of the three P450 genes did not significantly change after DDT treatment (Table 2).

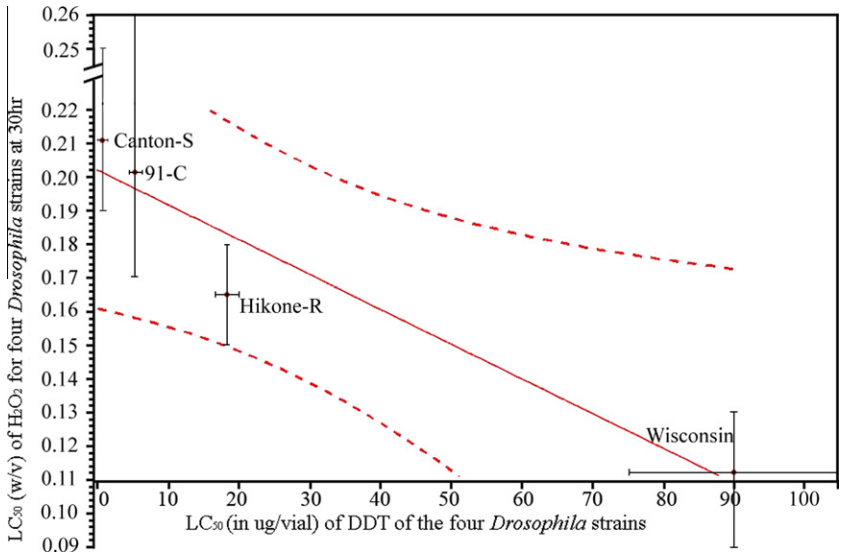

Fig. 1. Inverse relationship in four *Drosophila melanogaster* strains (*Canton-S*, *91-C*, *Hikone-R*, and *Wisconsin*) between resistance to DDT (LC_{50}) and resistance to dietary H_2O_2 (LC_{50}) ($r^2 = 0.96$). DDT bioassays were performed for 24 h and H_2O_2 bioassays were performed for 30 h [58]. The 95% CI error bars are given for both the DDT and H_2O_2 assays.

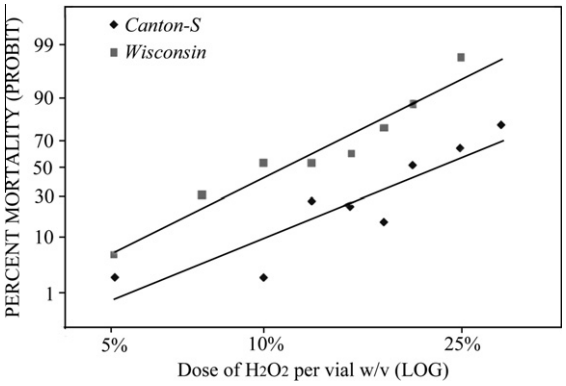

Fig. 2. Dose–response curves for the *Drosophila melanogaster* strains *Canton-S* and *Wisconsin* using H_2O_2. The *x*-axis shows the dose of H_2O_2 in logarithm; the *y*-axis shows mortality in probit.

3.4. Induction and repression of GST and P450 transcripts in the presence of dietary H_2O_2 in Wisconsin and Canton-S adults

When exposed to an LT_{25} of H_2O_2, both the *Wisconsin* and *Canton-S* flies responded by differentially expressing their GSTs. However, in the *Wisconsin* adults, all the responsive GSTs were over-transcribed in the presence of an LC_{25} treatment by H_2O_2 ($P < 0.01$; Table 3A and B). In *Canton-S* males, GST genes were both over- and under-transcribed ($P < 0.01$; Table 3A and B). Additionally, both *Canton-S* and *Wisconsin* under-transcribed *CYP6A2* as a result of LC_{25} H_2O_2 exposure (Table 3). However, *CYP6A2* still had higher transcription in *Wisconsin* as compared to *Canton-S* flies in response to H_2O_2 treatment (Table 4).

3.5. Impact of dietary H_2O_2 on trehalose levels

Because there were few differences in how males and females within a strain responded to H_2O_2 (based on our observations with GST and P450 expression patterns), we arbitrarily choose males to determine the impact of H_2O_2 on trehalose levels. Specifically, we compared *Canton-S* and *Wisconsin* males for trehalose levels in the absence and presence of LC_{25} H_2O_2 levels. Trehalose levels were significantly decreased ($P < 0.001$) in H_2O_2-treated vs. nontreated *Wisconsin* males; the integrated area of chromatographic peak corresponding to trehalose from the GC–MS data (see Material and methods) was $5.93 \times 10^7 \pm 4.36 \times 10^6$ counts (mean ± SE) for the treated males and $1.3 \times 10^8 \pm 1.3 \times 10^7$ counts for the non-treated males, with four replications and 15 insects per replication. The same significant decrease ($P < 0.001$) in trehalose was also observed for H_2O_2-treated vs. nontreated *Canton-S* males; the integrated area of trehalose peak (see Material and methods) was $6.71 \times 10^7 \pm 9.15 \times 10^6$ (mean ± SE) for the treated males and 1.67×10^8 counts $\pm 5.60 \times 10^7$ (mean ± SE) counts for the non-treated males, with four replications and 15 insects per replication.

4. Discussion

The *Drosophila* strain that was most resistant to DDT (*Wisconsin*) was also the most susceptible to the dietary oxidative stressor, H_2O_2. The opposite was true for *Canton-S*, which was the most susceptible to DDT and the most resistant to dietary H_2O_2. These differences were consistent with observed changes in the constitutive and induced expression of enzymes (GSTs and cytochrome P450s) associated with metabolic resistance to DDT and with resistance/susceptibility to H_2O_2. Compared to *Canton-S* adults, *Wisconsin* adults constitutively over-transcribed several cytochrome P450s and constitutively under-transcribed >27% of the GST found in the *Drosophila* genome genes. The *Wisconsin* strain was also far more responsive than the *Canton-S* strain to DDT in terms of GST transcript induction. In response to DDT treatment, GSTs were over-transcribed in the *Wisconsin* strain but were unchanged in the *Canton-S* strain.

Of the genes encoding the P450s and GSTs that responded to DDT treatment in this study, *GSTD1*, *CYP6G1*, and *CYP12D1* have been previously implicated in coding for enzymes that directly metabolize DDT or have at least been previously associated with the DDT-resistant phenotype [54–60,67–69]. The P450 *CYP6A2* is over-transcribed in some DDT-resistant strains, although it is not currently thought to have a direct role in resistance [55,70]. Tang and Tu [67] also observed low-level DDT-ase activity in *GSTD2* (they termed the gene *GSTD21*). Our results are consistent with the concept that, in addition to having constitutive over-expression of resistance traits, some resistant insects may up-regulate genes associated with detoxification when exposed to a toxin

Table 1
Relative constitutive transcription levels for GST and P450 *Drosophila melanogaster* genes by male and female *Wisconsin* flies.

Gene name	*Wisconsin* vs. *Canton-S* (Male)			*Wisconsin* vs. *Canton-S* (Female)		
	Ratio	ΔCT[a] (SE)	*P*-value	Ratio	ΔCT[a] (SE)	*P*-value
GSTD5[d]	0.12	3.04(0.56) ↓	<0.0001[b]	0.19	2.37(0.50) ↓	0.0002[b]
CG6776	0.48	1.07(0.25) ↓	0.0006[b]	0.32	1.66(0.32) ↓	<0.0001[b]
CG5224	0.33	1.60(0.22) ↓	<0.0001[b]			NS
GSTE3	0.42	1.24(0.34) ↓	0.0020	0.50	1.01(0.26) ↓	0.0015
GSTD8[d]			NS	0.35	1.52(0.46) ↓	0.0047
GSTE1[c]	0.30	1.72(0.47) ↓	0.0020			NS
CG9362	0.40	1.32(0.34) ↓	0.0014[b]	0.40	1.31(0.29) ↓	0.0003[b]
GSTD4[d]	0.29	1.80(0.45) ↓	0.0010[b]	0.13	2.90(0.57) ↓	0.0001[b]
GSTD6[d]	0.34	1.55(0.52) ↓	0.0085			NS
GSTE7	0.48	1.07(0.25) ↓	0.0007[b]			NS
CG30000	0.67	0.58(0.19) ↓	0.0092			NS
GSTD10[c]			NS	0.45	1.16(0.32) ↓	0.0024
GSTE10			NS	0.52	0.93(0.30) ↓	0.0066
CG1702			NS	0.50	0.99(0.22) ↓	0.0004[b]
CYP6A2	4.82	−2.27(0.71) ↑	0.0037[b]	4.53	−2.18(0.48) ↑	0.0001[b]

All other GSTs were not tested by Sawicki et al. [37] for 4-HNE substrate activities.
NS, Not significant.
[a] ΔCT is the extra number of PCR cycles needed for *Wisconsin* samples to reach the same level of amplification as *Canton-S* [88]. The positive ΔCT value means that *Wisconsin* had a lower transcription level of the gene as compared to *Canton-S* as given in the ratio column. The negative sign associated with the ΔCT value indicated *Wisconsin* over-transcribed P450 gene as compared to *Canton-S*. ↓ means under-transcribed and ↑ means over-transcribed.
[b] Expression of these genes was significantly different even after a Bonferonni correction. For the 37 GSTs, the Bonferonni correction was $P = 0.00143$. For the three P450s, the Bonferonni correction was $P = 0.0167$.
[c] GSTs known to accept 4-hydroxynonenal (4-HNE) as a substrate [37].
[d] GSTs reported not to accept 4-HNE as a substrate [37].

Table 2
Relative constitutive transcription levels for *Drosophila melanogaster* GST and P450 genes, in response to DDT treatment (LC_{25} DDT treatment for 24 h), in *Wisconsin* and *Canton-S* males and females. QRT-PCR was used to determine expression levels of each transcript.

Gene name	*Wisconsin* + DDT vs. *Wisconsin*			*Canton-S* + DDT vs. *Canton-S*		
	Ratio	ΔCT[a] (SE)	*P*-value	Ratio	ΔCT[a] (SE)	*P*-value
(A) Male						
GSTE8	1.74	0.80(0.23) ↑	−0.0035			NS
GSTE3	3.23	−1.69(0.34) ↑	0.0001[b]			NS
GSTD2[c]	4.06	−2.02(0.48) ↑	0.0007[b]			NS
GSTD1[c]	2.51	−1.33(0.33) ↑	0.0011[b]			NS
CG6781	1.79	−0.84(0.27) ↑	0.0061			NS
GSTE9	1.84	−0.88(0.24) ↑	0.0021			NS
GSTE6	1.74	−0.80(0.25) ↑	0.0062			NS
GSTE5	2.17	−1.12(0.36) ↑	0.0066			NS
CYP12D1	8.75	−3.13(0.62) ↑	0.0002[b]			NS
(B) Female						
GSTE1	2.08	−1.06(0.33) ↑	0.0050			NS
CG1702	1.67	−0.74(0.22) ↑	0.0043			NS
CG16936	2.00	−1.00(0.28) ↑	0.0026			NS
GSTE3	4.11	−2.04(0.26) ↑	<0.0001[b]			NS
GSTD2[c]	2.48	−1.31(0.40) ↑	0.0045			NS
CG1681	1.83	−0.87(0.28) ↑	0.0073			NS
GSTE9	1.85	−0.89(0.27) ↑	0.0044			NS
GSTE5	2.57	−1.36(0.33) ↑	0.0008[b]			NS
GSTD4[d]	4.56	−2.19(0.57) ↑	0.0014[b]			NS
CYP6G1	2.87	−1.52(0.52) ↑	0.0065[b]			NS
CYP12D1	9.19	−3.20(0.64) ↑	0.0001[b]			NS
CYP6A2	6.28	−2.65(0.48) ↑	<0.0001[b]			NS

All other GSTs were not tested by Sawicki et al. [37] for 4-HNE substrate activities.
NS, Not significant.
[a] ΔCT was the extra number of PCR cycles between control group and DDT treated group [88]. *p*-value shows the level of significance. The negative sign associated with the ΔCT value indicated induction of the GSTs or P450s in the presence of DDT. The positive ΔCT value means that *Canton-S* treated by DDT had a lower transcription level of the gene as compared to *Canton-S* as given in the ratio column. ↓ means under-transcribed and ↑ means over-transcribed.
[b] Expression of these genes was significantly different even after a Bonferonni correction. For the 37 GSTs, the Bonferonni correction was $P = 0.00143$. For the three P450s, the Bonferonni correction was $P = 0.0167$.
[c] GSTs known to accept 4-hydroxynonenal (4-HNE) as a substrate [37].
[d] GSTs reported not to accept 4-HNE as a substrate [37].

[54,55,58–60,71]. Whether this up-regulation contributes to resistance, however, remains unclear.

Evidence exists that some of the GSTs that we observed to be differentially expressed are involved in reducing oxidative stress. Sawicki et al. [37] cloned Delta-class GSTs and one Epsilon-class GSTs of *Drosophila* and transformed them into *E. coli*. They then tested for the role that these proteins might have in reducing oxidative stress by determining whether the GSTs accept

Table 3
Differential expression of *Drosophila melanogaster* GST and P450 genes in response to H_2O_2 treatment (LC_{25} H_2O_2 treatment for 30 h) in *Wisconsin* and *Canton-S* males and females. QRT-PCR was used to determine expression levels of each transcript.

Gene name	*Wisconsin* + H_2O_2 vs. *Wisconsin*			*Canton-S* + H_2O_2 vs. *Canton-S*		
	Ratio	ΔCT[a] (SE)	*P*-value	Ratio	ΔCT[a] (SE)	*P*-value
A (Male)						
GSTD5[d]	9.99	−3.32(0.48) ↑	<0.0001[b]	4.56	−2.19(0.48) ↑	0.0003[b]
GSTD6[d]	5.24	−2.39(0.38) ↑	<0.0001[b]	2.25	−1.17(0.38) ↑	0.0078
GSTD2[c]	10.27	−3.36(0.73) ↑	0.0003[b]	5.74	−2.52(0.73) ↑	0.0032
CG6776	2.58	−1.37(0.43) ↑	0.0053			NS
GSTD4[d]			NS	4.23	−2.08(0.53) ↑	0.0012[b]
GSTE1[c]			NS	0.05	4.38(0.76) ↓	<0.0001[b]
GSTE4			NS	0.52	0.93(0.31) ↓	0.0077
CG9363			NS	0.43	1.21(0.33) ↓	0.0020
GSTE10			NS	0.28	1.82(0.52) ↓	0.0029
Gfzf			NS	0.47	1.08(0.36) ↓	0.0083
CYP6A2	0.50	0.99(0.35) ↓	0.0121[b]			NS
B (Female)						
GSTD5[d]	10.34	−3.37(0.72) ↑	0.0003[b]	15.56	−3.96(0.72) ↑	<0.0001[b]
GSTD4[d]	3.92	−1.97(0.43) ↑	0.0003[b]	4.06	−2.02(0.43) ↑	0.0002[b]
GSTD3[c]	2.87	−1.52(0.35) ↑	0.0006[b]			NS
GSTD2[c]	9.51	−3.25(0.47) ↑	<0.0001[b]	10.34	−3.37(0.47) ↑	<0.0001[b]
GSTD6[d]			NS	3.12	−1.64(0.50) ↑	0.0046
GSTD8[d]	2.25	−1.17(0.36) ↑	0.0054			NS
GSTD9[c]	2.04	−1.03(0.34) ↑	0.0073			NS
GSTE9	1.89	−0.92(0.27) ↑	0.0039			NS
CYP6A2	0.49	1.04(0.40) ↓	0.0185	0.83	0.27(0.40) ↓	<0.0001[b]

All other GSTs were not tested by Sawicki et al. [37] for 4-HNE substrate activities.
NS, Not significant.
[a] ΔCT was the extra number of PCR cycles between control group and H_2O_2 treated group [88]. *P*-value shows the level of significance. The negative sign associated with the ΔCT value indicated induction of the GSTs in the presence of H_2O_2. The positive ΔCT value means under-transcription of the GSTs or P450s in the presence of H_2O_2. ↓ means under-transcribed and ↑ means over-transcribed.
[b] The transcripts for these genes were observed to still be significantly differentially expressed even after a Bonferonni correction. For the 37 GSTs, the Bonferonni correction was $P = 0.00143$. For the three P450s, the Bonferonni correction was $P = 0.0167$.
[c] GSTs known to accept 4-hydroxynonenal (4-HNE) as a substrate [37].
[d] GSTs reported not to accept 4-HNE as a substrate [37].

Table 4
Transcription of *Drosophila melanogaster* GSTs and P450s, based on qRT-PCR analysis, in *Wisconsin* flies treated with H_2O_2 as compared to *Canton-S* flies treated with H_2O_2.

Gene name	*Wisconsin* + H_2O_2 vs. *Canton-S* + H_2O_2 (Male)			*Wisconsin* + H_2O_2 vs. *Canton-S* + H_2O_2 (Female)		
	Ratio	ΔCT[a] (SE)	*P*-value	Ratio	ΔCT[a] (SE)	*P*-value
GSTD5[c]	0.26	1.94(0.48) ↓	0.0009[b]	0.11	3.20(0.72) ↓	0.0004[b]
GSTD4[c]	0.13	3.00(0.53) ↓	<0.0001[b]	0.19	2.39(0.43) ↓	<0.0001[b]
GSTE5	2.80	−1.49(0.49) ↑	0.0078	2.75	−1.46(0.36) ↑	0.0009[b]
CG9363	1.97	−0.98(0.33) ↑	0.0089			NS
GSTE6			*NS*	1.95	−0.97(0.32) ↑	0.0088
CYP6A2	5.74	−2.52(0.35) ↑	<0.0001[b]	4.23	−2.08(0.40) ↑	<0.0001[b]

All other GSTs were not tested by Sawicki et al. [37] for 4-HNE substrate activities.
NS, Not significant.
[a] ΔCT is the extra number of PCR cycles needed for *Wisconsin* treated with H_2O_2 samples to reach the same level of amplification of *Canton-S* treated with H_2O_2 [88]. The positive ΔCT value means that *Wisconsin* treated with H_2O_2 had a lower transcription level of the gene as compared to *Canton-S* treated with H_2O_2 as given in the ratio column. The negative sign associated with the ΔCT value indicated *Wisconsin* treated with H_2O_2 over-transcribed GST gene as compared to *Canton-S* treated with H_2O_2. The *P*-value of < 0.01 was significant. ↓ means under-transcribed and ↑ means over-transcribed.
[b] The transcripts for these genes were observed to still be significantly differentially expressed even after a Bonferonni correction. For the 37 GSTs, the Bonferonni correction was $P = 0.00143$. For the three P450s, the Bonferonni correction was $P = 0.0167$.
[c] GSTs reported not to accept 4-HNE as a substrate [37].

4-hydroxynonenal (4-HNE) as a substrate. Sawicki et al. [37] observed that *GSTD1*, *GSTD2*, *GSTD3*, *GSTD7*, *GSTD9*, *GSTD10*, and *GSTE1* had 4-HNE conjugating activity, demonstrating their potential to reduce oxidative stress in *Drosophila*.

Sawicki et al. [37] also identified GSTs that lacked 4-HNE conjugating activity: GSTD4, GSTD5, GSTD6, and GSTD8. Additionally, Sawicki et al. [37] assayed for glutathione peroxidase activity for these GSTs. They observed that only *GSTD1* (they termed it *DmGSTD1-1*) showed glutathione peroxidase activity to the substrate cumene hydroperoxide.

Interestingly, we observed differential expression in the presence of H_2O_2 for GSTs known to have 4-HNE conjugating activity (e.g., *GSTD1*, *GSTD2*, *GSTD3*, *GSTD9*, and *GSTE1*; see Table 3) and for GSTs lacking this activity (e.g., *GSTD4*, *GSTD5*, *GSTD6*, and *GSTD8*; see Table 3). It is not known whether *GSTD4*, *GSTD5*, *GSTD6*, and *GSTD8* may have some other function in oxidative stress or whether they are simply induced as part of a general response to the oxidative stressor, H_2O_2. It also remains to be determined whether those GSTs not tested by Sawicki et al. [37] have the ability to play any direct role in the response of *Drosophila* to oxidative stressors.

Wisconsin's lower constitutive transcription of multiple GSTs and over-expression of P450s, as compared to *Canton-S*, are consistent with its relative susceptibility to H_2O_2. *Wisconsin*

constitutively over-transcribed *CYP6A2*, and previous work with northern and western blots has demonstrated that CYP6G1 and CYP12D1 proteins are over-expressed in *Wisconsin* [58]. The constitutive over-expression of any one or a combination of P450s may contribute to the *Wisconsin* strain being more susceptible than the *Canton-S* strain to H_2O_2 because at least some P450s generate reactive oxygen species (ROS) as a by-product of catalysis [72–75], thereby increasing cellular oxidative stress. Oxidative stress is known to have negative impact on biological systems [76,77]. The greater susceptibility of the *Wisconsin* vs. *Canton-S* to H_2O_2 could also be due, in part, to *Wisconsin*'s overall lower constitutive and H_2O_2-induced expression of GSTs. Indeed, GSTs play a key role in the defense against the deleterious effects of oxidative stress [78–81]. It is likely that a combination of over-expression of P450s and under-expression GSTs strongly contributes to *Wisconsin's* greater susceptibility to H_2O_2.

Like GSTs, cellular trehalose levels have also been associated with an organism's ability to protect itself from oxidative stress [47]. Trehalose can act as an antioxidant, and thus, is itself destroyed by oxidative stress. This observation offers one potential explanation for the lower trehalose levels observed in both *Canton-S* and *Wisconsin* H_2O_2-treated males; both strains showed a similar response in their reduction of trehalose levels. In addition, trehalose is the major blood sugar in *Drosophila* and decreased trehalose levels may therefore reflect increased carbohydrate metabolism, possibly because of increased flux through the pentose phosphate pathway (PPP). The PPP plays a key role in eukaryotes of combating oxidative stress because it generates NADPH [82,83], which is used to maintain levels of reduced glutathione, a major cellular antioxidant. In fact, it has been shown that oxidative stress increases flux through the pentose phosphate pathway [84,85]. We cannot, however, rule out the possibility that other metabolic sinks of carbohydrates account for the decrease in trehalose, including, but not limited to, increases in the following: glycolysis, oxidative respiration, glycogen synthesis, protein glycosylation/glycation, and polyol synthesis. It also is possible that a combination of the processes mentioned above leads to decreased trehalose in the presence of H_2O_2. Alternatively, it also is possible that combination of the processes mentioned above leads to decreased trehalose in the presence of H_2O_2.

Although we have only investigated one pair of DDT-resistant and -susceptible strains, our results demonstrate that these two strains respond differently to DDT and oxidative stress. Although our results support the idea that P450 over-expression is associated with increased susceptibility to oxidative stress, further work needs to be done to determine if there is a causal link. Additionally, it remains to be determined whether this translates into a "cost" for resistance in the field for other insects that over-express P450s, and if so, whether such "costs" can be exploited to reduce P450-based metabolic resistance. For example, insects growing on plant varieties expressing higher levels of lipoxygenases may experience reduced fitness if they are metabolically resistant to pesticides via P450 over-translation. Increased oxidative stress, however, may select for GST over-expression, which may in turn confer GST metabolic resistance.

It is not known if an environment with high levels of oxidative stress would select, in insect populations, for metabolic resistance to pesticides via GSTs. For example, one important form of oxidative stress is UV-B light, which occurs in areas with intense sunlight. Larvae of the mosquito *Anopeheles gambiae* are likely to experience intense UV-B exposure in their natural environment and tend to be resistant to pesticides via GSTs, which are in some cases responsive to oxidative stress [40,41]. High levels of oxidative stress could make the use of certain P450s in xenobiotic metabolism a "costly" approach and would favor the use of constitutive over-transcription of GSTs as opposed to constitutive over-transcription of P450s.

Whether or not such differential expression of P450s and GSTs causes any cost to the insects in the field, our results have revealed that the genomes of both a DDT-resistant and a DDT-susceptible *Drosophila* strain responded differently to LC_{25} DDT and H_2O_2 exposure. These results suggest that the genomes of pesticide-resistant and pesticide-susceptible insects may respond differently to environmental stresses. Exploiting these differences may ultimately be useful for minimizing pesticide resistance. For example, by better understanding the mechanisms of resistance, we may be able to use environmental negative cross-resistance, in which the environment can be altered to increase the costs of resistance (e.g., biocontrol agents that selectively kill resistant insects), to minimize resistance in pest populations [86,87].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pestbp.2011.01.009.

References

[1] C. Chevillon, D. Bourguet, F. Rousset, N. Pasteur, M. Raymond, Pleiotropy of adaptive changes in populations: comparisons among insecticide resistance genes in *Culex pipiens*, Genet. Res. 70 (1997) 195–203.

[2] R. Eritja, C. Chevillon, Interruption of chemical mosquito control and evolution of insecticide resistance genes in *Culex pipiens* (Diptera: Culicidae), J. Med. Entomol. 36 (1999) 41–49.

[3] T. Miyo, S. Akai, Y. Oguma, Seasonal fluctuation in susceptibility to insecticides within natural populations of *Drosophila melanogaster*: empirical observations of fitness costs of insecticide resistance, Genes Genet. Syst. 75 (2000) 97–104.

[4] B. Raymond, A.H. Sayyed, D.J. Wright, Genes and environment interact to determine the fitness costs of resistance to *Bacillus thuringiensis*, Proc. Biol. Sci. 272 (2005) 1519–1524.

[5] Z. Liu, Z. Han, Fitness costs of laboratory-selected imidacloprid resistance in the brown planthopper, Nilaparvata lugens Stal, Pest Manag. Sci. 62 (2006) 279–282.

[6] Y. Carriere, C. Ellers-Kirk, R. Biggs, D.M. Higginson, T.J. Dennehy, B.E. Tabashnik, Effects of gossypol on fitness costs associated with resistance to Bt cotton in pink bollworm, J. Econ. Entomol. 97 (2004) 1710–1718.

[7] A.J. Gassmann, S.P. Stock, Y. Carriere, B.E. Tabashnik, Effect of entomopathogenic nematodes on the fitness cost of resistance to Bt toxin crylac in pink bollworm (Lepidoptera: Gelechiidae), J. Econ. Entomol. 99 (2006) 920–926.

[8] Z. Ogita, An attempt to reduce and increase insecticide-resistance in *D. Melanogaster* by selection pressure. Genetical and biochemical studies on negatively correlated cross-resistance in *Drosophila melanogaster* I, Botyu-Kagaku 26 (1961) 7–17.

[9] Z. Ogita, Relationship between the structure of compounds and negatively correlated activity. Genetical and biochemical studies on negatively correlated cross-resistance in *Drosophila melanogaster* II, Botyu-Kagaku 26 (1961) 18–19.

[10] Z. Ogita, Genetical studies on actions of mixed insecticides with negatively correlated substances. Genetical and biochemical studies on negatively correlated cross-resistance in *Drosophila melanogaster* III, Botyu-Kagaku 26 (1961) 88–93.

[11] R.B.a.P. Chapman, D.R., Negatively correlated cross-resistance to a synthetic pyrethroid in organophosphorous-resistant *Tetranychus urticae*, Nature (London) 218 (1979) 298–299.

[12] J.E. Cilek, D.L. Dahlman, F. Knapp, Possible mechanism of diazinon negative cross-resistance in pyrethroid-resistant horn flies (Diptera: Muscidae), J. Econ. Entomol. 88 (1995) 520–524.

[13] B.R. Pittendrigh, P. Gaffney, L.L. Murdock, Deterministic modeling of negative cross-resistance strategies for use in transgenic host-plant resistance, J. Theor. Biol. 204 (2000) 135–150.

[14] B.P. Khambay, I. Denholm, G.R. Carlson, R.M. Jacobson, T.S. Dhadialla, Negative cross-resistance between dihydropyrazole insecticides and pyrethroids in houseflies *Musca domestica*, Pest. Manag. Sci. 57 (2001) 761–763.

[15] B.R. Pittendrigh, P.J. Gaffney, Pesticide resistance. can we make it a renewable resource?, J Theor. Biol. 211 (2001) 365–375.

[16] J.H.F. Pedra, A. Hostetler, P.J. Gaffney, R.A. Reenan, B.R. Pittendrigh, Hypersusceptibility to deltamethrin in para ts-1 DDT resistant *Drosophila melanogaster*, Pestic. Biochem. Physiol. 78 (2004) 58–66.

[17] B.R. Pittendrigh, V. Margam, L. Sun, J. Huesing, Resistance in the post-genomics era. in: D. Onstad, (Ed.), Insect Resistance Management, 2008, pp. 39–68.

[18] B.R. Pittendrigh, P.J. Gaffney, J.E. Huesing, D.W. Onstad, R.T. Roush, L.L. Murdock, Active Refuges can inhibit the evolution of resistance in insects towards transgenic insect-resistant plants, J. Theor. Biol. 231 (2004) 461–474.

[19] J.D. Tang, H.L. Collins, T.D. Metz, E.D. Earle, J.Z. Zhao, R.T. Roush, A.M. Shelton, Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies, J. Econ. Entomol. 94 (2001) 240–247.
[20] C. Vacher, D. Bourguet, M. Desquilbet, S. Lemarie, S. Ambec, M.E. Hochberg, Fees or refuges: which is better for the sustainable management of insect resistance to transgenic Bt corn?, Biol. Lett. 2 (2006) 198–202.
[21] D. Bourguet, M. Prout, M. Raymond, Dominance of insecticide resistance presents a plastic response, Genetics 143 (1996) 407–416.
[22] H.C. Bagheri, G.P. Wagner, Evolution of dominance in metabolic pathways, Genetics 168 (2004) 1713–1735.
[23] A.G.a.S. Clark, N.A., Evidence that DDT dehydrochlorinase from the house fly is a glutathione S transferase. Pest. Biochem. Physiol. 22 (1984) 249–261.
[24] A.G. Clark, N.A. Shamaan, M.D. Sinclair, W.C. Dauterman, Insecticide metabolism by multiple glutathione S transferases in two strains of the house fly, *Musca domestica* (L.), Pest Biochem. Physiol. 25 (1986) 169–175.
[25] J.Y. Wang, S. McCommas, M. Syvanen, Molecular cloning of a glutathione S-transferase overproduced in an insecticide-resistant strain of the housefly (*Musca domestica*), Mol. Gen. Genet. 227 (1991) 260–266.
[26] D. Fournier, J.M. Bride, M. Poirie, J.B. Berge, F.W. Plapp Jr., Insect glutathione S-transferases Biochemical characteristics of the major forms from houseflies susceptible and resistant to insecticides, J. Biol. Chem. 267 (1992) 1840–1845.
[27] L. Prapanthadara, J. Hemingway, A.J. Ketterman, Partial purification and characterization of glutathione Stransferase involved in DDT resistance from the mosquito *Anopheles gambiae*, Pest. Biochem. Physiol. 47 (1993) 119–133.
[28] L.A. Prapanthadara, S. Koottathep, N. Promtet, J. Hemingway, A.J. Ketterman, Purification and characterization of a major glutathione S-transferase from the mosquito Anopheles dirus (species B), Insect. Biochem. Mol. Biol. 26 (1996) 277–285.
[29] R.P. Penilla, A.D. Rodriguez, J. Hemingway, J.L. Torres, J.I. Arredondo-Jimenez, M.H. Rodriguez, Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against Anopheles albimanus in Mexico, Med. Vet. Entomol. 12 (1998) 217–233.
[30] H. Ranson, L. Rossiter, F. Ortelli, B. Jensen, X. Wang, C.W. Roth, F.H. Collins, J. Hemingway, Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*, Biochem. J. 359 (2001) 295–304.
[31] S.H. Wei, A.G. Clark, M. Syvanen, Identification and cloning of a key insecticide-metabolizing glutathione S-transferase (MdGST-6A) from a hyper insecticide-resistant strain of the housefly Musca domestica, Insect. Biochem. Mol. Biol. 31 (2001) 1145–1153.
[32] H. Ranson, C. Claudianos, F. Ortelli, C. Abgrall, J. Hemingway, M.V. Sharakhova, M.F. Unger, F.H. Collins, R. Feyereisen, Evolution of supergene families associated with insecticide resistance, Science 298 (2002) 179–181.
[33] H. Ranson, J. Hemingway, Mosquito glutathione transferases, Methods Enzymol. 401 (2005) 226–241.
[34] A.A. Enayati, H. Ranson, J. Hemingway, Insect glutathione transferases and insecticide resistance, Insect Mol. Biol. 14 (2005) 3–8.
[35] N. Lumjuan, L. McCarroll, L.A. Prapanthadara, J. Hemingway, H. Ranson, Elevated activity of an Epsilon class glutathione transferase confers DDT resistance in the dengue vector *Aedes aegypti*, Insect. Biochem. Mol. Biol. 35 (2005) 861–871.
[36] Y.C. Awasthi, P. Zimniak, S. Awasthi, S.S. Singhal, S.K. Srivastava, J.T. Piper, M. Chaubey, D.R. Petersen, N.G. He, R. Sharma, S.V. Sigh, M.F. Khan, G.A.S. Ansari, P.J. boor, A new group of glutathione S-transferases with protective role against lipid peroxidation. In Glutathione S-transferases: Structure, Function and Clinical Implications Taylor & Francis, London, 1996.
[37] R. Sawicki, S.P. Singh, A.K. Mondal, H. Benes, P. Zimniak, 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 (2003) 661–669.
[38] R.H.a.M. Shukle, L.L., Determination of plant proteinase inhibitors by agar gel radial diffusion assay. Environ. Entomol. 12 (1983) 255–259.
[39] G.W. Felton, J.L. Bi, C.B. Summers, A.J. Mueller, S.S. Duffey, Potential role of lipoxygenases in defense against insect herbivory, J. Chem. Ecol. 20 (1994) 651–666.
[40] J.D. Hayes, D.J. Pulford, The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance, Crit. Rev. Biochem. Mol. Biol. 30 (1995) 445–600.
[41] Y. Ding, N. Hawkes, J. Meredith, P. Eggleston, J. Hemingway, H. Ranson, Characterization of the promoters of Epsilon glutathione transferases in the mosquito *Anopheles gambiae* and their response to oxidative stress, Biochem. J. 387 (2005) 879–888.
[42] Q. Chen, G.G. Haddad, Role of trehalose phosphate synthase and trehalose during hypoxia: from flies to mammals, J. Exp. Biol. 207 (2004) 3125–3129.
[43] J.C. Arguelles, Physiological roles of trehalose in bacteria and yeasts: a comparative analysis, Arch. Microbiol. 174 (2000) 217–224.
[44] N. Benaroudj, D.H. Lee, A.L. Goldberg, Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals, J. Biol. Chem. 276 (2001) 24261–24267.
[45] T. Matsuo, Trehalose protects corneal epithelial cells from death by drying, Br. J. Ophthalmol. 85 (2001) 610–612.
[46] A.K. Garg, J.K. Kim, T.G. Owens, A.P. Ranwala, Y.D. Choi, L.V. Kochian, R.J. Wu, Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses, Proc. Natl. Acad. Sci. USA 99 (2002) 15898–15903.
[47] F.J. Alvarez-Peral, O. Zaragoza, Y. Pedreno, J.C. Arguelles, Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in Candida albicans, Microbiology 148 (2002) 2599–2606.
[48] Q. Chen, K.L. Behar, T. Xu, C. Fan, G.G. Haddad, Expression of Drosophila trehalose-phosphate synthase in HEK-293 cells increases hypoxia tolerance, J. Biol. Chem. 278 (2003) 49113–49118.
[49] Q. Chen, E. Ma, K.L. Behar, T. Xu, G.G. Haddad, Role of trehalose phosphate synthase in anoxia tolerance and development in *Drosophila melanogaster*, J. Biol. Chem. 277 (2002) 3274–3279.
[50] A.D. Elbein, Y.T. Pan, I. Pastuszak, D. Carroll, New insights on trehalose: a multifunctional molecule, Glycobiology 13 (2003) 17R–27R.
[51] M. Strolin-Benedetti, G. Brogin, M. Bani, F. Oesch, J.G. Hengstler, Association of cytochrome P450 induction with oxidative stress in vivo as evidenced by 3-hydroxylation of salicylate, Xenobiotica 29 (1999) 1171–1180.
[52] Y. Morel, R. Barouki, Down-regulation of cytochrome P450 1A1 gene promoter by oxidative stress. Critical contribution of nuclear factor 1, J. Biol. Chem. 273 (1998) 26969–26976.
[53] C.W. Barker, J.B. Fagan, D.S. Pasco, Down-regulation of P4501A1 and P4501A2 mRNA expression in isolated hepatocytes by oxidative stress, J. Biol. Chem. 269 (1994) 3985–3990.
[54] A. Brandt, M. Scharf, J.H. Pedra, G. Holmes, A. Dean, M. Kreitman, B.R. Pittendrigh, Differential expression and induction of two Drosophila cytochrome P450 genes near the Rst(2)DDT locus, Insect Mol. Biol. 11 (2002) 337–341.
[55] J.H. Pedra, L.M. McIntyre, M.E. Scharf, B.R. Pittendrigh, Genome-wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant Drosophila, Proc. Natl. Acad. Sci. USA 101 (2004) 7034–7039.
[56] G. Le Goff, F. Hilliou, B.D. Siegfried, S. Boundy, E. Wajnberg, L. Sofer, P. Audant, R.H. Ffrench-Constant, R. Feyereisen, Xenobiotic response in *Drosophila melanogaster*: sex dependence of P450 and GST gene induction, Insect Biochem. Mol. Biol. 36 (2006) 674–682.
[57] P.J. Daborn, J.L. Yen, M.R. Bogwitz, G. Le Goff, E. Feil, S. Jeffers, N. Tijet, T. Perry, D. Heckel, P. Batterham, R. Feyereisen, T.G. Wilson, R.H. Ffrench-Constant, A single p450 allele associated with insecticide resistance in *Drosophila*, Science 297 (2002) 2253–2256.
[58] R.A. Festucci-Buselli, A.S. Carvalho-Dias, M. De Oliveira-Andrade, C. Caixeta-Nunes, H.M. Li, J.J. Stuart, W. Muir, M.E. Scharf, B.R. Pittendrigh, Expression of Cyp6g1 and Cyp12d1 in DDT resistant and susceptible strains of Drosophila melanogaster, Insect Mol. Biol. 14 (2005) 69–77.
[59] P.J. Daborn, C. Lumb, A. Boey, W. Wong, R.H. Ffrench-Constant, P. Batterham, Evaluating the insecticide resistance potential of eight *Drosophila melanogaster* cytochrome P450 genes by transgenic over-expression, Insect Biochem. Mol. Biol. 37 (2007) 512–519.
[60] G. Le Goff, S. Boundy, P.J. Daborn, J.L. Yen, L. Sofer, R. Lind, C. Sabourault, L. Madi-Ravazzi, R.H. Ffrench-Constant, Microarray analysis of cytochrome P450 mediated insecticide resistance in *Drosophila*, Insect Biochem. Mol. Biol. 33 (2003) 701–708.
[61] M. Amichot, S. Tares, A. Brun-Barale, L. Arthaud, J.M. Bride, J.B. Berge, Point mutations associated with insecticide resistance in the Drosophila cytochrome P450 Cyp6a2 enable DDT metabolism, Eur. J. Biochem. 271 (2004) 1250–1257.
[62] C. McCart, R.H. Ffrench-Constant, Dissecting the insecticide-resistance-associated cytochrome P450 gene Cyp6g1, Pest. Manag. Sci. 64 (2008) 639–645.
[63] C. McCart, A. Buckling, R.H. Ffrench-Constant, DDT resistance in flies carries no cost, Curr. Biol. 15 (2005) R587–R589.
[64] B. Pittendrigh, R. Reenan, R.H. Ffrench-Constant, B. Ganetzky, Point mutations in the Drosophila sodium channel gene para associated with resistance to DDT and pyrethroid insecticides, Mol. Gen. Genet. 256 (1997) 602–610.
[65] M.W. Pfaffl, T.M. Georgieva, I.P. Georgiev, E. Ontsouka, M. Hageleit, J.W. Blum, Real-time RT-PCR quantification of insulin-like growth factor (IGF)-1, IGF-1 receptor, IGF-2, IGF-2 receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species, Domest. Anim. Endocrinol. 22 (2002) 91–102.
[66] B. Pittendrigh, K. Aronstein, E. Zinkovsky, O. Andreev, B. Campbell, J. Daly, S. Trowell, R.H. Ffrench-Constant, Cytochrome P450 genes from Helicoverpa armigera: expression in a pyrethroid-susceptible and -resistant strain, Insect. Biochem. Mol. Biol. 27 (1997) 507–512.
[67] A.H. Tang, C.P. Tu, Biochemical characterization of Drosophila glutathione S-transferases D1 and D21, J. Biol. Chem. 269 (1994) 27876–27884.
[68] I. Denholm, G.J. Devine, M.S. Williamson, Evolutionary genetics insecticide resistance on the move, Science 297 (2002) 2222–2223.
[69] N. Joussen, D.G. Heckel, M. Haas, I. Schuphan, B. Schmidt, Metabolism of imidacloprid and DDT by P450 CYP6G1 expressed in cell cultures of Nicotiana tabacum suggests detoxification of these insecticides in Cyp6g1-overexpressing strains of *Drosophila melanogaster*, leading to resistance, Pest Manag. Sci. 64 (2008) 65–73.
[70] L.C. Waters, A.C. Zelhof, B.J. Shaw, L.Y. Ch'ang, Possible involvement of the long terminal repeat of transposable element 17.6 in regulating expression of an insecticide resistance-associated P450 gene in *Drosophila*, Proc. Natl. Acad. Sci. USA 89 (1992) 4855–4859.
[71] P. Daborn, S. Boundy, J. Yen, B. Pittendrigh, And R. ffrench-Constant, DDT resistance in Drosophila correlates with Cyp6g1 over-expression and confers cross-resistance to the neonicotinoid imidacloprid, Mol. Genet. Genomics 266 (2001) 556–563.

[72] E. Cadenas, H. Sies, Low level chemiluminescence of liver microsomal fractions initiated by tert-butyl hydroperoxide Relation to microsomal hemoproteins, oxygen dependence, and lipid peroxidation, Eur. J. Biochem. 124 (1982) 349–356.
[73] E. Cadenas, H. Sies, H. Graf, V. Ullrich, Oxene donors yield low-level chemiluminescence with microsomes and isolated cytochrome P-450, Eur. J. Biochem. 130 (1983) 117–121.
[74] G.M. Cleator, A.G. Lewis, P.E. Klapper, H.L. Sharma, A.M. Smith, HM-PAO-imaging and herpes encephalitis, Arch. Virol. 109 (1989) 263–268.
[75] D.F. Lewis, C. Ioannides, D.V. Parke, Molecular orbital studies of oxygen activation and mechanisms of cytochromes P-450-mediated oxidative metabolism of xenobiotics, Chem. Biol. Interact. 70 (1989) 263–280.
[76] E. Mariani, M.C. Polidori, A. Cherubini, P. Mecocci, Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 827 (2005) 65–75.
[77] J. Ling, D. Soll, Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. Proc. Natl. Acad. Sci. USA 107 4028–33.
[78] H. Franciosa, J.B. Berge, Glutathione S-transferases in housefly (Musca domestica): location of GST-1 and GST-2 families, Insect Biochem. Mol. Biol. 25 (1995) 311–317.
[79] S.P. Singh, J.A. Coronella, H. Benes, B.J. Cochrane, P. Zimniak, Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1–1 (GST-2) in conjugation of lipid peroxidation end products, Eur. J. Biochem. 268 (2001) 2912–2923.
[80] J.G. Vontas, G.J. Small, J. Hemingway, Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in Nilaparvata lugens, Biochem. J. 357 (2001) 65–72.
[81] P. Hyrsl, E. Buyukguzel, K. Buyukguzel, The effects of boric acid-induced oxidative stress on antioxidant enzymes and survivorship in *Galleria mellonella*, Arch. Insect Biochem. Physiol. 66 (2007) 23–31.
[82] K.H. Slekar, D.J. Kosman, V.C. Culotta, The yeast copper/zinc superoxide dismutase and the pentose phosphate pathway play overlapping roles in oxidative stress protection, J. Biol. Chem. 271 (1996) 28831–28836.
[83] K. Brehms, B. Juhnke, P. Kötter, K.D. Entian, Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress, Mol. Gen. Genet. 252 (1996) 456–464.
[84] S.W. Tuttle, A. Maity, P.R. Oprysko, A.V. Kachur, I.S. Ayene, J.E. Biaglow, C.J. Koch, Detection of reactive oxygen species via endogenous oxidative pentose phosphate cycle activity in response to oxygen concentration: implications for the mechanism of HIF-1alpha stabilization under moderate hypoxia, J. Biol. Chem. 282 (2007) 36790–36796.
[85] A.C. Williams, W.C. Ford, Functional significance of the pentose phosphate pathway and glutathione reductase in the antioxidant defenses of human sperm, Biol. Reprod. 71 (2004) 1309–1316.
[86] A.J. Gassmann, D.W. Onstad, B.R. Pittendrigh, Evolutionary analysis of herbivorous insects in natural and agricultural environments, Pest Manag. Sci. 65 (2009) 1174–1181.
[87] B.R. Pittendrigh, L. Sun, P. Gaffney, J. Huesing, Negative Cross Resistance. in: D. Onstad, (Ed.), Insect Resistance Management, 2008, pp. 108–124.
[88] W. Sun, V.M. Margam, L. Sun, G. Buczkowski, G.W. Bennett, B. Schemerhorn, W.M. Muir, B.R. Pittendrigh, Genome-wide analysis of phenobarbital-inducible genes in *Drosophila melanogaster*, Insect Mol. Biol. 15 (2006) 455–464.