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Differential transcription of cytochrome P450s and glutathione S transferases in DDT-susceptible and -resistant *Drosophila melanogaster* strains in response to DDT and oxidative stress

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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/).