

Genome-wide analysis of phenobarbital-inducible genes in *Drosophila melanogaster*

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Abstract

An oligoarray analysis was conducted to determine the differential expression of genes due to phenobarbital exposure in *Drosophila melanogaster* (w^{1118} strain) third instar larvae. Seventeen genes were observed to be induced with increased expression by a statistical analysis of microarrays approach with a $q \leq 0.05$. At $q \leq 0.12$, four more genes (*Cyp12d1*, *DmGstd4*, and two genes with unknown function) were found to be up-regulated, and 11 genes with unknown function were found to be down-regulated. Fifteen of these genes, *Cyp4d14*, *Cyp6a2*, *Cyp6a8*, *Cyp12d1*, *Cyp6d5*, *Cyp6w1*, *CG2065*, *DmGstd6*, *DmGstd7*, *Amy-p/Amy-d*, *Ugt86Dd*, *GC5724*, *Jheh1*, *Jheh2* and *CG11893*, were verified using quantitative real time polymerase chain reaction. Some of these genes have been shown to be over-transcribed in metabolically DDT-resistant *Drosophila* strains.

Keywords: P450, GST, carbohydrate metabolism, oxido-reductase.

Introduction

Phenobarbital (PB) treatment has a variety of effects on mammals: (1) reduces frequency of epileptic seizures (Baumann, 2001; Hernandez *et al.*, 2002; Kwan & Brodie,

2004; Zhong *et al.*, 2004); (2) induces resistance/tolerance to insecticides in rodents, cows and humans (Fries *et al.*, 1971; Zavon, 1971; Vitarius *et al.*, 1995); and (3) causes liver cancer (Henry, 1997; Ferko *et al.*, 2003; Calvisi *et al.*, 2004; Hokaiwado *et al.*, 2004). PB induction of P450s has also been demonstrated across insect species, including *Drosophila hydei* (Danielson *et al.*, 1997), *Helicoverpa zea* (Li *et al.*, 2002), *Blatella germanica* (Brown *et al.*, 2003), *D. melanogaster* (Amichot *et al.*, 1994), *Aedes aegypti* (Sousa-Polezzi & Bicudo, 2004) and across multiple P450 families (Danielson *et al.*, 1998). In insects, the best-studied P450 response element for plant allelochemicals has been the xenobiotic response element to xanthotoxin (XRE-xan) (Petersen *et al.*, 2003; McDonnell *et al.*, 2004) and this element has been identified as an important component in the induction of the *Cyp6b1v3* gene in the black swallowtail butterfly (*Papilio polyxenes*).

In *Drosophila*, treatment with PB induces multiple cytochrome P450 genes, including *Cyp6a2* and *Cyp6a8* (Amichot *et al.*, 1994; Dunkov *et al.*, 1997; Maitra *et al.*, 2000). PB causes a 15-fold increase in expression of *Cyp6a2* in the DDT-susceptible strain Canton-S (Brun *et al.*, 1996). Numerous DDT laboratory-selected (e.g. 91R) and field-collected DDT-resistant strains (e.g. Hikone-R and Wisconsin) have been shown to constitutively over-transcribe several P450s, including *Cyp6a2* and *Cyp6a8* (Brun *et al.*, 1996; Pedra *et al.*, 2004). PB treatment of normally pesticide-susceptible (Canton-S) *Drosophila* also (1) induces increases in cytochrome P450 enzymatic activity, and (2) causes these flies to become more resistant to pesticides such as DDT and certain pyrethroids (Amichot *et al.*, 1994, 1998). Thus, evidence exists that PB treatment of *Drosophila* results in increased expression of multiple P450 genes, some of which may be associated with metabolic pesticide resistance.

In house-fly (*Musca domestica*), PB has been shown to induce expression of several P450s. While using a cDNA expression library from PB-induced house-fly, Feyereisen *et al.* (1989) isolated a P450 gene with similarity to mammalian P450III family of proteins. PB increased the expression of *Cyp6d3* in both insecticide resistant and susceptible strains by 16-fold and 1.6-fold, respectively (Kasai & Scott, 2001). PB treatment increased total P450 proteins in the susceptible house-fly adult and larvae (Scott *et al.*, 1996). There was a 4.2-fold increase in *Cyp6d1* mRNA expression

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in susceptible strain treated with PB (Scott *et al.*, 1996). A *trans*-acting factor of PB inducible element has been hypothesized to exist for *Cyp6d1* gene (Liu & Scott, 1997b). These results are in keeping with the hypothesis that PB induction and P450 mediated metabolic resistance to insecticides share some common regulatory pathways.

Metabolic resistance is a common mechanism by which insects, including lepidopteran, coleopteran and dipteran species, develop resistance to insecticides (Hemingway *et al.*, 1991; Ottea *et al.*, 1995; Rose *et al.*, 1997; Chandre *et al.*, 1998; Kasai *et al.*, 1998; Stuart *et al.*, 1998; Feyereisen, 1999; Scott, 1999; Kasai *et al.*, 2000; Li *et al.*, 2000; Scharf *et al.*, 2000). Additionally, metabolic pesticide resistance in insects is (1) typically polygenic, and (2) associated with over-transcription of detoxification enzymes, including cytochrome P450s and GSTs (Haupt *et al.*, 1988; Daly & Fisk, 1992; Heckel *et al.*, 1998; Scott, 1999; Maitra *et al.*, 2000; Tang *et al.*, 2000; Kranthi *et al.*, 2001; Rajurkar *et al.*, 2003).

The cloning of numerous P450 and GST genes that are over-transcribed in resistant insects, but which fail to map back to a major resistance locus, has led workers in the field to hypothesize that there is a PB-sensitive *trans*-regulatory protein that, in the absence of PB, represses expression of these detoxification enzymes (Plapp, 1984; Terriere, 1984; Grant & Hammock, 1992; Liu & Scott, 1997b; Dombrowski *et al.*, 1998; Scott & Kasai, 2004). It has also been hypothesized that mutations, in the genes that code for these regulatory proteins, reduce the regulatory proteins' ability to suppress transcription of the P450 genes, resulting in insects that constitutively over-transcribe these cytochrome P450s (Plapp, 1984; Terriere, 1984; Grant & Hammock, 1992; Carino *et al.*, 1994; Maitra *et al.*, 1996; Liu & Scott, 1997a; Dombrowski *et al.*, 1998; Kasai *et al.*, 1998; Scott & Kasai, 2004). To date, no such *trans*-acting PB-sensitive repressor has been identified in insects. Additionally, in bacterial systems this repressor-mechanism hypothesis of PB induction has been challenged (Shaw *et al.*, 1998). Regardless of the exact mechanism of regulation, strong evidence suggests a relationship between constitutive over-transcription of normally PB inducible gene(s) and metabolic resistance to pesticide. Although we know the complement of array of genes over-transcribe in some DDT-resistant *Drosophila* strains (Pedra *et al.*, 2004), we do not know the complement of genes in *Drosophila* that are induced in response to PB.

In the current study we used the Affymetrix *Drosophila* Genome 2.0 Arrays to investigate differential expression of genes in PB-untreated and PB-treated *w*¹¹¹⁸ third instar *Drosophila*. This allowed us to define if there is an overlap between the array of the genes constitutively over-transcribed in metabolic DDT-resistant strains and PB inducible genes in a DDT-susceptible strain. We also used quantitative real-time PCR to verify the differential transcription of a set

of these candidate genes. Our transcriptome approach identified a manageable number of genes to investigate further the molecular response of *Drosophila* to PB treatment. Here we show that PB induces genes associated with detoxification, development, and carbohydrate metabolism.

Results

The statistical analysis of microarrays (SAM) of the oligoarrays data revealed 17 putative differentially expressed genes between the PB and control treatments at the 95% level ($q \leq 0.05$) (Table 1). All 17 genes were over-transcribed in the PB treatment, of which 15 genes have known functions. When the false discovery rate (FDR) was relaxed to 88% ($q \leq 0.12$) five additional putative genes were identified as being over-transcribed and 11 genes as under-transcribed (Table 1). The over-transcribed genes included those coding for cytochrome P450s, oxido-reductase, glutathione S-transferases, steroid metabolism, calcium ion binding, α -amylase, juvenile hormone epoxide hydrolases, and genes that code for proteins with unknown function. Interestingly, all the genes observed to be under-transcribed (at the $q \leq 0.12$ level) in the PB-treated flies have currently unknown functions (Table 1).

We performed quantitative real-time PCR (qRT-PCR) on 17 of the genes described in Table 1. Fifteen of the 17 genes were confirmed to be differentially transcribed in the PB treatment (Table 2, Fig. 1a,b). From each of the following categories, we verified one or more genes to be over-transcribed in the presence of PB: cytochrome P450s, oxido-reductase, glutathione S-transferases, steroid metabolism, calcium ion binding, α -amylase, juvenile hormone epoxide hydrolases, and a gene that codes for a protein with unknown function.

Even though *Cyp6g1* was not identified in the initial oligoarrays experiments, at the FDR level of $q \leq 0.12$, we elected to determine if PB treatment of *Drosophila* resulted in differential transcription of *Cyp6g1*, as this gene has been implicated as one of the two P450s associated with a major metabolic pesticide resistance locus (loci) in *Drosophila*, known as *Rst(2)DDT* (Tang *et al.*, 2000; Brandt *et al.*, 2002; Daborn *et al.*, 2002; Pedra *et al.*, 2004, 2005; Festucci-Buselli *et al.*, 2005). Using qRT-PCR we observed a 5.82-fold increase in transcription of *Cyp6g1* in third instars treated with PB as compared with the control (Table 2). These data suggest that PB does induce *Cyp6g1*, but not at levels equivalent to other genes investigated in this study.

We also investigated the impact of PB on expression of several P450 genes, *Cyp12d1*, *Cyp6a2*, and *Cyp6g1*, at the adult stage of development with qRT-PCR (Fig. 2). Both *Cyp12d1* and *Cyp6a2* were highly over-transcribed in response to PB treatment at the adult stage, whereas *Cyp6g1* was only 3.6-fold over-transcribed (Fig. 2).

Table 1. Genes that were differentially expressed in the oligoarray experiments at the $q = 95\%$ or $q = 88\%$ levels in *Drosophila* after a 24 h exposure to 0.1% phenobarbital in the diet of third-instar larvae as compared with larvae reared on control diet

Gene category/name	Probe Id	Ratio†	q-value	Cytogenetic map
Monoxygenase activity (GO:0004497)				
<i>Cyp4d14</i>	1627180_at	11.31	0.05	2E1
<i>Cyp6w1</i>	1634143_at	89.07	0.05	42A13
<i>Cyp6a2</i>	1626401_at	39.60	0.05	47D4
<i>Cyp6a8</i>	1640755_at	79.28	0.05	51D1
<i>Cyp6d5</i>	1638562_a_at	20.44	0.05	88A4
<i>Cyp12d1</i>	1633401_s_at	32.67	0.12	47D4
Oxidoreductase activity (GO:0016491)				
CG2065	1630212_at	27.11	0.05	43E9
Cytochrome b5-related	1632623_at	9.20	0.05	36B1
Glutathione S Transferases (GO:0004364)				
<i>DmGstd6</i>	1626136_at	57.06	0.05	87B8
<i>DmGstd7</i>	1624793_at	27.90	0.05	87B8
<i>DmGstd9</i>	1636174_at	9.10	0.05	87B8
<i>DmGstd4</i>	1626253_at	15.71	0.12	87B8
Steroid metabolism (GO:0008202)				
<i>Ugt86Dd</i>	1641481_at	14.43	0.05	86D5
CG5724	1638903_at	17.17	0.05	87C8
Alpha-amylase activity (GO:0004556)				
Amy-p/Amy-d	1639374_s_at	35.76	0.05	53F12
Alpha-glucosidase activity				
CG8693	1631806_at	12.79	0.12	44D1
Epoxide hydrolase activity (GO:0004301)				
Juvenile hormone epoxide hydrolase 1 (Jheh1)	1626414_at	15.00	0.05	55F7
Juvenile hormone epoxide hydrolase 2 (Jheh2)	1627773_a_at	10.60	0.05	55F8
Nucleotide phosphatase activity (GO:0019204)				
CG5150	1637660_at	6.68	0.12	64D5
Genes that code for proteins with unknown function				
CG11893	1635512_at	6.62	0.05	
X01472	1626392_s_at	6.62	0.05	
CG6908	1622946_at	15.50	0.12	
CG30043	1624823_at	0.09	0.12	
CG10513	1623265_at	0.08	0.12	
CG10514	1638424_at	0.10	0.12	
CG11892	1624892_s_at	0.14	0.12	
CG31745	1638743_at	0.14	0.12	
CG15919	1637672_at	0.18	0.12	
CG14129	1623779_at	0.15	0.12	
1624823_at	1624823_at	0.09	0.12	
1638347_at	1638347_at	0.14	0.12	
1635966_at	1635966_at	0.10	0.12	
1630583_at	1630583_at	0.13	0.12	

†Ratio of >1 indicates upregulation of mRNA in the presence of PB; and a ratio of <1 indicates downregulation of mRNA in the presence of PB.

Discussion

Interestingly, some of the genes observed to be over-transcribed (in the PB treatment) in the top 88% ($q \leq 0.12$), and hence inclusive of the 95% ($q \leq 0.05$) probability level using SAM, have previously been implicated in metabolic pesticide resistance both in *Drosophila* and other insects (Haupt *et al.*, 1988; Daly & Fisk, 1992; Liu & Scott, 1996; Heckel *et al.*, 1998; Tang *et al.*, 2000; Kranthi *et al.*, 2001; Rajurkar *et al.*, 2003). These included cytochrome P450s, cytochrome b5-related, and glutathione S-transferases. Additionally, we observed differential expression of genes coding for proteins involved in epoxide hydrolase activity, carbohydrate/lipid steroid metabolism, along with a series of genes with a currently unknown functions. All of the

under-transcribed genes were in the unknown category, making it difficult at this time to hypothesize how (or if) their differential expression may play a part in altering the biology of the treated flies.

Several of the genes induced by PB, in this study, have previously been associated with metabolic DDT-resistance phenotype in *Drosophila*: *Cyp6a2* (Waters *et al.*, 1992; Dombrowski *et al.*, 1998; Maitra *et al.*, 2000), *Cyp6a8* (Maitra *et al.*, 1996, 2000), *Cyp6a9* (Maitra *et al.*, 1996), *Cyp6g1* (Daborn *et al.*, 2001, 2002; Brandt *et al.*, 2002) and *Cyp12d1* (Brandt *et al.*, 2002; Le Goff *et al.*, 2003; Pedra *et al.*, 2004; Festucci-Buselli *et al.*, 2005). Interestingly, *Cyp6g1*, which has been implicated as one of many genes putatively involved in the polygenic DDT resistance phenotype, was not observed in the 5% or 12% FDR

Table 2. Genes that were differentially expressed, based on qRT-PCR, in *Drosophila* after a 24 h exposure to 0.1% phenobarbital in the diet of third instar larvae as compared with larvae reared on control diet

Gene category/name	Δ CT* (SE)	P-value
Monoxygenase activity (GO:0004497)		
<i>Cyp4d14</i>	3.75 (0.33)	< 0.0001
<i>Cyp6w1</i>	5.92 (0.23)	< 0.0001
<i>Cyp6a2</i>	7.37 (0.26)	< 0.0001
<i>Cyp6a8</i>	9.04 (0.23)	< 0.0001
<i>Cyp6d5</i>	6.40 (0.18)	< 0.0001
<i>Cyp12d1</i>	5.03 (0.33)	< 0.0001
† <i>Cyp6g1</i>	2.54 (0.23)	< 0.0001
Oxidoreductase activity (GO:0016491)		
CG2065	5.49 (0.35)	< 0.0001
Cytochrome b5-related	3.93 (0.29)	< 0.0001
Glutathione S transferases (GO:0004364)		
<i>DmGstd6</i>	6.85 (0.39)	< 0.0001
<i>DmGstd7</i>	7.56 (0.52)	< 0.0001
<i>DmGstd9</i>	-0.02 (0.58)	0.977
Steroid metabolism (GO:0008202)		
<i>Ugt86Dd</i>	4.10 (0.30)	< 0.0001
GC5724	3.54 (0.58)	< 0.0001
Alpha-amylase activity (GO: 0004556)		
Amy-p/Amy-d	7.08 (0.40)	< 0.0001
Epoxide hydrolase activity (GO:0004301)		
Juvenile hormone epoxide hydrolase 1 (Jheh1)	3.89 (0.28)	< 0.0001
Juvenile hormone epoxide hydrolase 2 (Jheh2)	4.52 (0.22)	< 0.0001
Genes that code for proteins with unknown function		
CG11893	3.20 (0.39)	< 0.0001
1626392_s_at	-0.02 (0.24)	0.923

* Δ CT is extra number of PCR cycles needed for control sample to reach same level of amplifications as PB-treated samples.

†Not identified in the oligoarrays experiment (Table 1), but previously shown to be associated with metabolic resistance to pesticides in *Drosophila* (Brandt *et al.*, 2002; Daborn *et al.*, 2002; Pedra *et al.*, 2004).

groups. Subsequent qRT-PCR analysis revealed 5.8-fold and 3.6-fold levels of induction, respectively, in the larval and adult stages of development.

PB treatment of *Drosophila* has previously been shown to cause a rapid increase in both *Cyp6a2* mRNA and protein level (Dunkov *et al.*, 1997). *Cyp6a2* has been shown to catalyse the metabolism of several insecticides including aldrin and heptachlor (Dunkov *et al.*, 1997). As well, *Cyp6a8* oxidizes the endogenous substrate lauric acid (C12:0), a short-chain unsaturated fatty acid (Helvig *et al.*, 2004). PB inducible cytochrome P450s involved in lauric acid metabolism has been identified across a diverse set of species ranging from rats to wheat (Tanaka *et al.*, 1990; Forthoffer *et al.*, 2001).

Increases in *Cyp6a2* mRNA (and the resultant *Cyp6a2* protein) have been associated with metabolic pesticide resistance both in terms of over-transcription of this gene and in terms of changes in catalytic properties. *Cyp6a2* expression levels in pesticide resistant and susceptible strains of *Drosophila* have been measured using western blots (Bride *et al.*, 1997) and a correlation was observed between *Cyp6a2* titre and resistance (Bride *et al.*, 1997). However, *Cyp6a2* expression level was not a predictor of resistance level. *Cyp6a2* has also been sequenced in RDDT^R

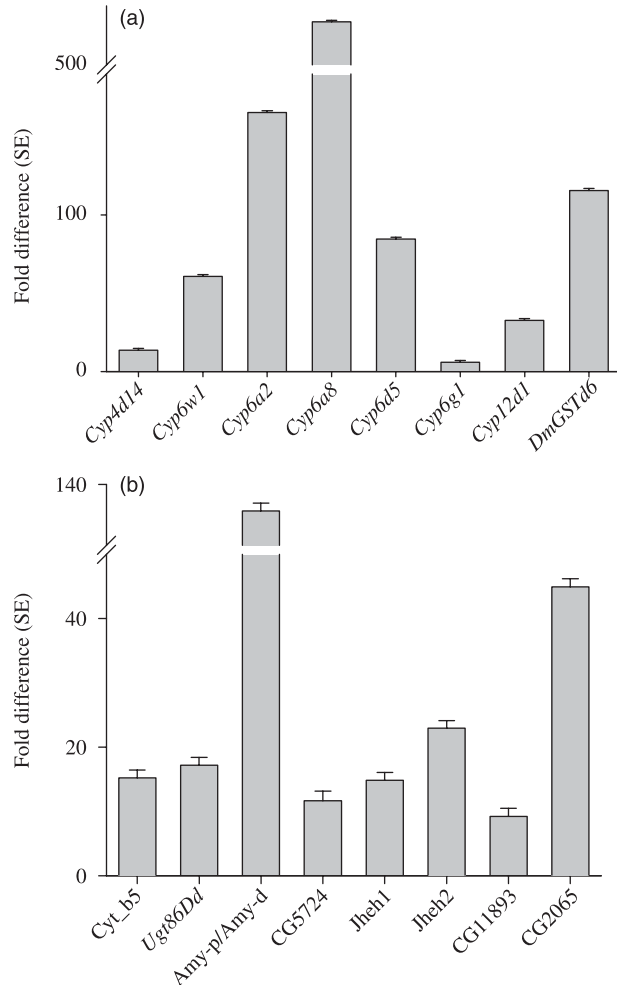


Figure 1. Fold induction of genes in third instars treated with phenobarbital for a 24 h interval as compared with larvae not treated. The expression level of the genes in the control group was 1 for all the genes and hence is not shown. Quantitative real-time PCR was used to distinguish between non-treated (control) and treated (0.1% phenobarbital in the diet) larvae. In (a) differential expression of cytochrome P450s and glutathione S-transferases are given. In (b) are given differential expression of genes associated with oxidoreductase, epoxide hydrolases (Jheh1 and Jheh2), Alpha-amylase activity/steroid metabolism (Amy-p/Amy-d, *Ugt86Dd*, and GC5724), and genes that code for proteins with unknown function. All genes displayed significant increases in expression due to phenobarbital treatment (Table 2).

strain. Using heterologous expression Amichot *et al.* (2004) demonstrated that several point mutations in this gene account for an increase in DDT metabolism by the *Cyp6a2* protein. Thus, resistance is not only associated with increased P450 expression levels, but also with qualitative changes in the enzyme that results in increased metabolism.

Oligoarray analysis revealed that third instar *Drosophila* treated with PB over-transcribed multiple glutathione S-transferases as compared with the untreated larvae. We also verified, through qRT-PCR, that *DmGSTd6* and *DmGSTd7* were over-transcribed in the PB treated third instars. These GSTs belong to the insect specific delta

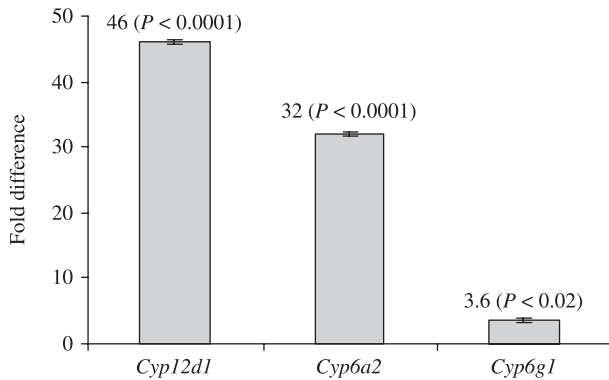


Figure 2. Fold induction of genes in adult *Drosophila* treated with phenobarbital for a 24-h interval as compared with adults not treated. The expression level of the genes in the control group was 1 for all the genes and hence is not shown. Quantitative real time PCR (qRT-PCR) was used to distinguish between non-treated (control – apple juice adjusted to pH 7 with extra 5% sucrose added) and treated (5 mg/ml of phenobarbital in apple juice adjusted to pH 7 with extra 5% sucrose added) adults. All three genes were cytochrome P450s previously associated with metabolic pesticide resistance in adult *Drosophila*. Fold-levels of induction and significance levels for each gene are given.

class (Ding *et al.*, 2003) and all are in the same general cytological region (87B8). Over-transcription of insect specific delta and epsilon classes of glutathione S-transferases has been shown to be associated with metabolic pesticide resistance in a variety of insect species (Clark & Shamaan, 1984; Ku *et al.*, 1995; Ranson *et al.*, 2001; Vontas *et al.*, 2001; Pedra *et al.*, 2004; Ranson & Hemingway, 2005). In *Anopheles gambiae*, GSTs play an important role in conferring insecticide resistance (Ding *et al.*, 2003). Additionally, glutathione S-transferases have also been shown to be PB inducible in both insects and mammals (Sasaki *et al.*, 1989; Pinkus *et al.*, 1993; Tang & Tu, 1995).

DmGSTd7 was originally thought to be a pseudogene, but recent work by Sawicki *et al.* (2003) has revealed that *DmGSTd7* does code for a functional protein. Sawicki *et al.* (2003) also observed that *DmGSTd7* is effective at metabolizing 4-hydroxynonenal (4-HNE). 4-HNE is a highly reactive aldehyde derived from other reactive oxygen species (ROS) and is associated with oxidative stress (Sawicki *et al.*, 2003). 4-HNE-protein adducts have been observed both in *Drosophila* and house-flies (Yan & Sohal, 1998; Singh *et al.*, 2001). The major route for 4-HNE metabolism in mammals is through glutathione conjugation (Alin *et al.*, 1985; Videla *et al.*, 2000). Additionally, PB has been shown to have an impact on oxidative stress response in mammals. Findings by Sawicki *et al.* (2003) suggest a potential role of *DmGSTd7* in oxidative stress in *Drosophila*.

Third instar *Drosophila* treated with PB over-transcribed both juvenile hormone epoxide hydrolase 1 and 2 (Jheh1 and Jheh2) as compared with untreated larvae. Epoxide hydrolases have been implicated in juvenile hormone degradation in insects (Anspaugh & Roe, 2004). PB has been

shown to increase expression of juvenile hormone epoxide hydrolases both in house-flies, *Musca domestica* (Yu & Terriere, 1978) and in the cabbage looper, *Trichoplusia ni* (Anspaugh & Roe, 2004). Epoxide hydrolases have been shown to be inducible by a diversity of xenobiotics, including compounds with and without epoxides, leading Anspaugh & Roe (2004) to hypothesize that these enzymes may play a role in xenobiotic metabolism.

We also observed over-transcription of four genes associated with lipid/steroid and carbohydrate metabolism in the PB-treated flies (Table 1). Using qRT-PCR we verified differential expression of three of these genes: *Amy-p/Amy-d*, *Ugt86Dd*, and *GC5724* (Table 2). PB has been shown to influence carbohydrate metabolism levels in rat liver (Schaub & Freksa, 1976). Total glycogen synthetase was also increased by PB treatment in rats during prenatal development, but this activity decreased during postnatal development (Schaub & Freksa, 1976). Both the active and the inactive forms of phosphorylase were increased significantly by PB during the prenatal period (Schaub & Freksa, 1976). In the postnatal period the active form of phosphorylase was influenced to a greater extent, as compared with the inactive form, by PB (Schaub & Freksa, 1976). PB treatment also increased α -glucosidase activity during the prenatal period (Schaub & Freksa, 1976). The activity of Fructose-6-P-kinase (F-6-PK) and 6-phosphogluconate dehydrogenase (6-PGDH) were also decreased by PB during the prenatal periods, as was Glucose-6-phosphate dehydrogenase (G-6-PDH) activity during both pre- and postnatal periods (Schaub & Freksa, 1976).

Metabolic pesticide resistance is also associated with differential expression of genes and proteins associated with carbohydrate and lipid metabolism. Pedra *et al.* (2004) observed over-transcription of genes associated with carbohydrate and lipid/steroid metabolism in metabolically DDT-resistant strains of *Drosophila*, including *Ugt86Dd*, which we also observed in the current study to be induced by PB (Tables 1 and 2, Fig. 1b). Additionally, two-dimensional gel comparisons between DDT resistant and susceptible *Drosophila* protein patterns revealed differential expression of proteins associated with carbohydrate and lipid metabolism (Pedra *et al.*, 2005). In insects DDT metabolism has been shown to be associated with glucose utilization (Plapp, 1970; Maltseva & Golovleva, 1982) and in mammals DDT has also been shown to affect β -oxidation of fatty acids (Hinton *et al.*, 1978; Oda *et al.*, 1994). Maltseva & Golovleva (1982) observed that the activity of isocitrate and glucose-6-phosphate dehydrogenases was increased in *Pseudomonas aeruginosa* strains capable of metabolizing DDT.

Rencurel *et al.* (2005) have recently shown that in hepatocytes, and in a newly derived human hepatoma cell line, PB influences AMP-activated protein kinase (AMPK), which activates CAR through phosphorylation. CAR then binds with the NR1 site of PBREM (PB Responsive Enhancer

Module), which in turn is responsible for activating increases in P450 expression (Rencurel *et al.*, 2005). The AMPK is important for regulating energy levels in the cell and AMPK is activated when cells have experienced energy depletion. Constitutive over-expression of AMPK in mammalian cells results in constitutive over-expression of P450s and loss of function of AMPK inhibits induction of P450s by PB. AMPK is important for regulation of glucose uptake in skeletal muscle cells during exercise and has been proposed to be a target site for antidiabetic drugs (Zangar & Novak, 1997). In *Drosophila*, DDT resistance is associated with constitutive over-expression of genes and proteins involved in regulating energy levels in the cell (Pedra *et al.*, 2004, 2005) and metabolically resistant insects have a greater metabolic rate than susceptible insects (Plapp, 1970).

Although considerable work has been done in vertebrates regarding PB inducible response elements and genes, we know very little about such regulatory mechanisms in insects. PB alters gene expression of mammalian P450s by activating the pregnane X receptor (PXR) and constitutive androstane receptor (CAR). In non-mammalian animals only one xenosensor gene has been found (e.g. *Drosophila*, *Caenorhabditis elegans* and chicken) and sequence-wise this xenosensor is about equally related to the mammalian PXR and CARs (Handschin *et al.*, 2004). Interestingly, chicken xenobiotic receptor and mammalian PXR and CAR can be freely interchanged in *trans*-activation and electrophoretic mobility shift assays suggesting evolutionary conservation of the fundamental drug-induction mechanisms from birds to mammals (Handschin *et al.*, 2001). Of the 18 nuclear receptors in the *Drosophila* genome only DHR96 bears some similarity to the non-mammalian xenosensors, but it remains to be determined whether DHR96 plays a role in PB induction (Handschin *et al.*, 2004).

In conclusion, PB induces differential expression of genes associated with detoxification, metabolism, oxidative stress, juvenile hormone esterases, as well as genes that code for proteins with unknown function. Interestingly, metabolic pesticide resistance, which can be induced by PB, is also associated with increases in genes associated with both detoxification and metabolism. These results support the hypothesis that constitutive over-expression of certain detoxification enzymes and metabolic proteins in pesticide-resistant *Drosophila* are associated with perturbations of regulatory mechanisms associated with PB induction.

Experimental procedures

Experimental design

The experiment was designed as two treatments (control vs. PB) with three biological replicates for each treatment. The bioassays were conducted as pairs (control vs. PB) on separate days. The six separate RNA samples were processed on the same day and were later used in the oligoarray and qRT-PCR analyses.

Bioassays, RNA extraction and Affymetrix experiments

The *w¹¹¹⁸* strain used in this experiment was obtained from Dr Misha Ludwig (University of Chicago). The larvae were reared on Formula 24® *Drosophila* diet (Carolina Biological Supply, Burlington, NC, USA) at room temperature ($\approx 23^\circ\text{C}$ and 60–70% R.H) until they became third instar larvae. The third-instar *w¹¹¹⁸* were transferred to Formula 24® *Drosophila* diet containing apple juice (Great Value®, WalMart, Bentonville, AR, USA), in order to assure larval feeding. The larvae were equally split between one of two treatments: (1) no additional chemicals (control), and (2) 0.1% PB (Sigma-Aldrich, St Louis, MO, USA). The concentration of PB in diet (0.1%) was the maximum dose that did not retard the growth of the larvae, which we determined in dose–response experiment (data not shown). All larvae were 120–122 h of age (recorded from the time the eggs were laid) at the time of transfer. After transfer the larvae were allowed to feed on the media for 24 h. At the end of the 24 h exposure the larvae were removed from the media, immediately placed in RNA later, then flash-frozen in liquid nitrogen and stored at -80°C .

For each treatment of each biological replicate 30 larvae were combined for a single RNA extraction. For the adult experiments, adult flies were first starved (with no water either) overnight (12 h), then fed with pH adjusted (pH 7.0) apple juice (with extra 5% sucrose added) containing 0 or 5 mg/ml of PB for 24 h. The dose (5 mg/ml) for PB was the maximum non-lethal dose that we determined in a dose–response experiment for *w¹¹¹⁸* adults (data not shown). Thirty adults were used for each control and treatment replicate. For both larval and adult experiments, a total of three biological replicates were performed for both the control and the PB treatments. At the end of 24-h period, the flies were flash frozen in liquid nitrogen and stored at -80°C until total RNA extraction.

Messenger RNA was extracted and Affymetrix oligoarray experiments were performed as described in Pedra *et al.* (2004), with the exception that we used the Affymetrix *Drosophila* Genome 2.0 Arrays (Affymetrix, Santa Clara, CA, USA). Image data were quantified by using genechip-operating software (GCOS 1.2). The identification of the informative probe sets were performed by using default settings (α_1 , 0.05; α , 0.065; δ , 0.015; scale factor, 1.0; normalization factor, 1.0).

Statistical analysis of oligoarrays

The data were analysed using the SAM permutation based statistical analysis software (Tusher *et al.*, 2001). A list of genes with associated *q*-values (Storey & Tibshirani, 2003) was generated. The *q*-value gives the minimum value for the FDR at which that gene will be declared significant.

Quantitative real-time polymerase chain reaction

For each treatment and replicate RNA was extracted from 30 flies using the Qiagen RNeasy kit (Qiagen, Valencia, CA, USA) with an on column DNase digestion procedure. The first stranded cDNA was synthesized by using 1 μg of total RNA with iScript cDNA kit from Bio-Rad (Hercules, CA, USA) in a 20 μl reaction volume. A 25-fold dilution was made to each first stranded cDNA for quantitative PCR. qRT-PCR was performed with iQ SYBR Green Supermix from Bio-Rad with SYBR Green dye on an iCycler Thermal Cycler. For each cDNA, three qRT-PCR reactions were conducted. To minimize the positional effect on PCR reactions due to variations of block temperature, PCR reactions for target gene and reference gene were placed on the cycler with a randomized block design.

Table 3. Primer sequences for qRT-PCR

Gene name	Accession no.	Primer sequence (5'-3')
rp49	U92431	CGGTTACGGATCGAACAAAGCG TTGGCGCGCTCGACAATCT
<i>Cyp12d1</i>	NM_206090	CAACGAGGGTATCTGGCCACGT GGCATCCACACCAGCGAATAGG
<i>Cyp6g1</i>	NM_136899	AACTCCTTTGGGATGCACCTATCG GGTTGTGGAACCGATTGAAGTCCT
1626392	X01472	CCTACCAGATTGGCAATGTGGAG AGGCTCCAATGCAGCGCA
Amy-p/Amy-d	NM_080421	CAGGTCTCCCCTGTGAACGAGAA GGCGGCCATGTGGTTGAA
CG11893	NM_143118	GCGACTCACATTTGTTCTCCACC TGCGGCACCAAATCCTCG
CG2065	NM_136465	CCTGCTCACCCACTTGCTGC GGCCAATCCCAGGTGAA
CG5724	NM_141971	GAATGTCCTTCCGACAGCGCT TGCGTGGGAGCGAAGAA
<i>Cyp4d14</i>	NM_143763	AGATGTTGGGCGAAAGTGGCT TGATCTTCTGCGGCGGA
<i>Cyp6a2</i>	NM_078904	TTCACCACCGATGTGATTGGC TCGGGCATCATGCGCATT
<i>Cyp6a8</i>	NM_079025	ACGAACTGGCAAAGAATCCGGAC TGCGGCCATTGATCCA
<i>Cyp6d5</i>	NM_169567	GTGAAAATCGGCTGGAAACAGG TGCTTTCCGCCGACAAA
<i>Cyp6w1</i>	NM_136362	AGCCCATAGGGTTCTAGCCGG CCATCGCCTCGGCATCAA
Cyt-b5	NM_057806	GGACGATGGAGCCGAGGA TTTCGGGAGCCGTGGTCA
<i>DmGstd6</i>	NM_080176	ACCCTGGCCGTTATGATGA TGGCCCGAGTTCCCAA
<i>DmGstd7</i>	NM_080375	AGGCTTTGGGTCTGGAGTGTA TGGGGAATCGGGTTTGCC
<i>DmGstd9</i>	NM_141924	TATATGCTCTACTCGGCACCTTG TACTCGCAGCCCTCCAGTT
Jheh1	NM_137541	GTGCTGGAGTACTGGCGCGA GGCCATCCGTGGAGCAGC
Jheh2	NM_137542	CAAATGGAGCGAACGGGA ATTTCGGAACGGTTCCCG
<i>Ugt86Dd</i>	NM_144367	GCCTGGAAAGCCAGCTAATGTG GGCGCGCTGCACATTCAT

The threshold cycle (CT) was calculated by the iCycler IQ software. The relative expression levels were calculated as given in Pfaffl *et al.* (2002), and the statistical analysis of the relative gene expression level were performed using SAS (SAS Institute Inc., Cary, NC, USA). *Rp49* was used as the reference gene. The primers used for qRT-PCR and gene bank accession numbers were listed in the Table 3. Statistical analysis followed a General Linear Model (with log transformation for linearization and normalization) and planned contrasts between the control and PB treatments.

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References

- Alin, P., Danielson, U.H. and Mannervik, B. (1985) 4-Hydroxyalk-2-enals are substrates for glutathione transferase. *FEBS Lett* **179**: 267–270.
- Amichot, M., Brun, A., Cuany, A., Helvig, C., Salaun, J.P., Durst, F. and Berge, J.B. (1994) Expression study of CYP genes in *Drosophila* strains resistant or sensitive to insecticides. In: *Cytochrome P-450. 8th International Conference* (Lechner, M.C., ed.), pp. 689–692. Paris: Eurotext-John Libbey.
- Amichot, M., Brun, A., Cuany, A., De Souza, G., Le Mouel, T., Bride, J.M., *et al.* (1998) Induction of cytochrome P450 activities in *Drosophila melanogaster* strains susceptible of resistant to insecticides. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* **121**: 311–319.
- Amichot, M., Tares, S., Brun, A., Arthaud, L., Bride, J.M. and Berge, J.B. (2004) Point mutations associated with insecticide resistance in the *Drosophila* cytochrome P450 *Cyp6a2* enable DDT metabolism. *Eur J Biochem* **271**: 1250–1257.
- Anspaugh, D.D. and Roe, R.M. (2004) Regulation of JH epoxide hydrolase versus JH esterase activity in the cabbage looper, *Trichoplusia ni*, by juvenile hormone and xenobiotics. *J Insect Physiol* **51**: 523–535.
- Baumann, R.J. (2001) Prevention and management of febrile seizures. *Paediatr Drugs* **3**: 585–592.
- Brandt, A., Scharf, M.E., Pedra, J.H.F., Holmes, G., Dean, A., Kreitman, M. and Pittendrigh, B.R. (2002) Differential expression and induction of two *Drosophila* cytochrome P450 genes near the *Rst (2) DDT* locus. *Insect Mol Biol* **11**: 337–342.
- Bride, J.M., Cuany, A., Amichot, M., Brun, A., Babault, M., Mouel, T.L., *et al.* (1997) Cytochrome P-450 field insecticide tolerance and development of laboratory resistance in grape vine populations of *Drosophila melanogaster* (Diptera: Drosophilidae). *J Econ Entomol* **90**: 1514–1520.
- Brown, D., Zhang, L., Wen, Z. and Scott, J.G. (2003) Induction of P450 monooxygenases in the German cockroach, *Blattella germanica* L. *Arch Insect Biochem Physiol* **53**: 119–124.
- Brun, A., Cuany, A., Le Mouel, T., Berge, J. and Amichot, M. (1996) Inducibility of the *Drosophila melanogaster* cytochrome P450 gene, CYP6A2, by phenobarbital in insecticide susceptible or resistant strains. *Insect Biochem Mol Biol* **26**: 697–703.
- Calvisi, D.F., Ladu, S., Factor, V.M. and Thorgeirsson, S.S. (2004) Activation of beta-catenin provides proliferative and invasive advantages in c-myc/TGF-alpha hepatocarcinogenesis promoted by phenobarbital. *Carcinogenesis* **25**: 901–908.
- Carino, F.A., Koener, J.F., Plapp, F.W. Jr and Feyereisen, R. (1994) Constitutive overexpression of the cytochrome P450 gene CYP6A1 in a house fly strain with metabolic resistance to insecticides. *Insect Biochem Mol Biol* **24**: 411–418.
- Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J.M., Pasteur, N. and Guillet, P. (1998) Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med Vet Entomol* **12**: 359–366.
- Clark, A.G. and Shamaan, N.A. (1984) Evidence that DDT-dehydrochlorinase from the house fly is a glutathione S-transferase. *Pest Biochem Physiol* **22**: 249–261.

- Daborn, P., Boundy, S., Yen, J., Pittendrigh, B.R. and Ffrench-Constant, R. (2001) DDT resistance in *Drosophila* correlates with *Cyp6g* over-expression and confers cross-resistance to the neonicotinoid imidacloprid. *Mol Genet Genomics* **266**: 556–563.
- Daborn, P.J., Yen, J.L., Bogwitz, M.R., Le Goff, G., Feil, E., Jeffers, S., et al. (2002) A single P450 allele associated with insecticide resistance in *Drosophila*. *Science* **297**: 2253–2256.
- Daly, J.C. and Fisk, J.H. (1992) Inheritance of metabolic resistance to the synthetic pyrethroids in Australian *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Bull Entomol Res* **82**: 5–12.
- Danielson, P.B., MacIntyre, R.J. and Fogleman, J.C. (1997) Molecular cloning of a family of xenobiotic-inducible drosophilid cytochrome P450s: evidence for involvement in host-plant allelochemical resistance. *Proc Natl Acad Sci USA* **94**: 10797–10802.
- Danielson, P.B., Foster, J.L., McMahill, M.M., Smith, M.K. and Fogleman, J.C. (1998) Induction by alkaloids and phenobarbital of Family 4 Cytochrome P450s in *Drosophila*: evidence for involvement in host plant utilization. *Mol Gen Genet* **259**: 54–59.
- Ding, Y., Ortell, F., Rossiter, L.C., Hemingway, J. and Ranson, H. (2003) The *Anopheles gambiae* glutathione transferase supergene family: annotation, phylogeny and expression profiles. *BMC Genomics* **4**: 1–16.
- Dombrowski, S.M., Krishnan, R., Witte, M., Maitra, S., Diesing, C., Waters, L.C. and Ganguly, R. (1998) Constitutive and barbital-induced expression of the *Cyp6a2* allele of a high producer strain of CYP6A2 in the genetic background of a low producer strain. *Gene* **221**: 69–77.
- Dunkov, B., Guzov, V.M., Mocelin, G., Shotkoski, F.A., Brun, A., Amichot, M., Ffrench-Constant, R. and Feyereisen, R. (1997) The *Drosophila* cytochrome P450 gene *Cyp6a2*: structure, localization, heterologous expression, and induction by phenobarbital. *DNA Cell Biol* **16**: 1345–1356.
- Ferko, A., Bedrna, J. and Nozicka, J. (2003) Pigmented hepatocellular adenoma of the liver caused by long-term use of phenobarbital. *Rozhl Chir* **82**: 192–195.
- Festucci-Buselli, R.A., Carvalho-Dias, A.S., de Oliveira-Andrade, M., Caixeta-Nunes, C., Li, H.M., Stuart, J.J., et al. (2005) Expression of *Cyp6g* and *Cyp12d*. DDT resistant and susceptible strains of *Drosophila melanogaster*. *Insect Mol Biol* **14**: 69–77.
- Feyereisen, R. (1999) Insect P450 enzymes. *Annu Rev Entomol* **44**: 507–533.
- Feyereisen, R., Koener, J.F., Farnsworth, D.E. and Nebert, D.W. (1989) Isolation and sequence of cDNA encoding a cytochrome P-450 from an insecticide-resistant strain of the house fly, *Musca domestica*. *Proc Natl Acad Sci USA* **86**: 1465–1469.
- Forthoffer, N., Helvig, C., Dillon, N., Benveniste, I., Zimmerlin, A., Tardif, F. and Salaun, J.P. (2001) Induction and inactivation of a cytochrome P450 conferring herbicide resistance in wheat seedlings. *Eur J Drug Metab Pharmacokinet* **26**: 9–16.
- Fries, G.F., Marrow, G.S., Jr, Lester, J.W. and Gordon, C.H. (1971) Effect of microsomal enzyme inducing drugs on DDT and dieldrin elimination from cows. *J Dairy Sci* **54**: 364–368.
- Grant, D.F. and Hammock, B.D. (1992) Genetic and molecular evidence for a trans-acting regulatory locus controlling glutathione S-transferase-2 expression in *Aedes aegypti*. *Mol Gen Genet* **234**: 169–176.
- Handschin, C., Podvinec, M., Stöckli, J., Hoffmann, K. and Meyer, U.A. (2001) Conservation of signaling pathways of xenobiotic-sensing orphan nuclear receptors, chicken xenobiotic receptor, constitutive androstane receptor, and pregnane X receptor, from birds to humans. *Mol Endocrinol* **15**: 1571–1585.
- Handschin, C., Blaettler, S., Roth, A., Looser, R., Oscarson, M., Kaufmann, M.R., et al. (2004) The evolution of drug-activated nuclear receptors: one ancestral gene diverged into two xenosensor genes in mammals. *Nucl Receptor* **2**: 7.
- Heckel, D.G., Gahan, L.J., Daly, J.C. and Trowell, S. (1998) A genomic approach to understanding *Heliothis* and *Helicoverpa* resistance to chemical and biological insecticides. *Philos Trans R Soc* **353**: 1713–1722.
- Helvig, C., Tijet, N., Feyereisen, R., Walker, F.A. and Restifo, L.L. (2004) *Drosophila melanogaster* CYP6A8, an insect P450 that catalyzes lauric acid (omega-1)-hydroxylation. *Biochem Biophys Res Commun* **325**: 1495–1502.
- Hemingway, J., Miyamoto, J. and Herath, P.R.J. (1991) A possible novel link between organophosphorus and DDT insecticide resistance genes and in *Anopheles* – supporting evidence from fenitrothion metabolism studies. *Pestic Biochem Physiol* **39**: 49–56.
- Henry, J.A. (1997) *Management of Poisoning: a handbook for health care workers*. World Health Organization, Geneva.
- Hernandez, E.J., Williams, P.A. and Dudek, F.E. (2002) Effects of fluoxetine and TFMPP on spontaneous seizures in rats with pilocarpine-induced epilepsy. *Epilepsia* **43**: 1337–1345.
- Hinton, D.E., Galumann, H., Trump, B.F. and Virchows, A. (1978) Studies on the cellular toxicity of polychlorinated biphenyls (PCBs). I. Effect of PCBs on microsomal enzymes and on synthesis and turnover of microsomal and cytoplasmic lipids of rat liver – a morphological and biochemical study. *Virchows Arch B Cell Pathol* **27**: 279–306.
- Hokaiwado, N., Asamoto, M., Tsujimura, K., Hirota, T., Ichihara, T., Satoh, T. and Shirai, T. (2004) Rapid analysis of gene expression changes caused by liver carcinogens and chemopreventive agents using a newly developed three-dimensional microarray system. *Cancer Sci* **95**: 123–130.
- Haupt, D.R., Pursey, J.C. and Morton, R.A. (1988) Genes controlling malathion resistance in a laboratory-selected population of *Drosophila melanogaster*. *Genome* **30**: 844–853.
- Kasai, S. and Scott, J.G. (2001) Expression and regulation of *Cyp6d3* in the house fly, *Musca domestica* (L.). *Insect Biochem Mol Biol* **32**: 1–8.
- Kasai, S., Weerasinghe, I.S. and Shono, T. (1998) P450 Monooxygenases are an important mechanism of permethrin resistance in *Culex quinquefasciatus* Say larvae. *Arch Insect Biochem Physiol* **37**: 47–56.
- Kasai, S., Weerasinghe, I.S., Shono, T. and Yamakawa, M. (2000) Molecular cloning, nucleotide sequence and gene expression of a cytochrome P450 (CYP6F1) from the pyrethroid-resistant mosquito, *Culex quinquefasciatus* Say larvae. *Insect Biochem Mol Biol* **30**: 163–171.
- Kranthi, K.R., Jadhav, D., Wanjari, R., Kranthi, S. and Russell, D. (2001) Pyrethroid resistance and mechanisms of resistance in field strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J Econ Entomol* **94**: 253–263.
- Ku, S.S., Chiang, F.M., Hsin, C.T., Yao, Y.E. and Sun, C.N. (1995) Glutathione transferase isozymes involved in insecticide resistance in diamondback moth larvae. *Pestic Biochem Physiol* **50**: 191–197.
- Kwan, P. and Brodie, M.J. (2004) Phenobarbital for the treatment of epilepsy in the 21st century: a critical review. *Epilepsia* **45**: 1141–1149.
- Le Goff, G., Boundy, S., Daborn, P.J., Yean, J.L., Sofer, L., Lind, R., et al. (2003) Microarray analysis of cytochrome P450 mediated

- insecticide resistance in *Drosophila*. *Insect Biochem Mol Biol* **33**: 701–708.
- Li, X., Zangerl, A.R., Schuler, M.A. and Berenbaum, M.R. (2000) Cross-resistance to alpha-cypermethrin after xanthotoxin ingestion in *Helicoverpa zea* (Lepidoptera: Noctuidae). *J Econ Entomol* **93**: 18–25.
- Li, X., Berenbaum, M.R. and Schuler, M.A. (2002) Plant allelochemicals differentially regulate *Helicoverpa zea* cytochrome P450 genes. *Insect Mol Biol* **11**: 343–351.
- Liu, N. and Scott, J.G. (1996) Genetic analysis of factors controlling high-level expression of cytochrome P450, CYP6D1, cytochrome b5, P450 reductase, and monooxygenase activities in LPR house flies, *Musca domestica*. *Biochem Genet* **34**: 133–148.
- Liu, N. and Scott, J.G. (1997a) Inheritance of CYP6D1-mediated pyrethroid resistance in house fly (Diptera: Muscidae). *J Econ Entomol* **90**: 1478–1481.
- Liu, N. and Scott, J.G. (1997b) Phenobarbital induction of Cyp6d1 is due to a trans acting factor on autosome 2 in house fly, *Musca domestica*. *Insect Mol Biol* **6**: 77–81.
- Maitra, S., Dombrowski, S.M., Waters, L.C. and Ganguly, R. (1996) Three second chromosome-linked clustered *Cyp6* genes show differential constitutive and barbital-induced expression in DDT-resistant and susceptible strains of *Drosophila melanogaster*. *Gene* **180**: 165–171.
- Maitra, S., Dombrowski, S.M., Basu, M., Raustol, O., Waters, L.C. and Ganguly, R. (2000) Factors on the third chromosome affect the level of *Cyp6a2* and *Cyp6a8* expression in *Drosophila melanogaster*. *Gene* **248**: 147–156.
- Maltseva, O.V. and Golovleva, L.A. (1982) Central metabolic characteristics of a *Pseudomonas aeruginosa* culture degrading DDT. *Mikrobiologiya* **51**: 5–11.
- McDonnell, C. and Brown, Berenbaum, R., M. and Schuler, M. (2004) Conserved regulatory elements in the promoters of two allelochemical-inducible cytochrome P450 genes differentially regulate transcription. *Insect Biochem Mol Biol* **34**: 1129–1139.
- Oda, H., Matsushita, N., Hirabayashi, A. and Yoshida, A. (1994) Cholesterol-rich very low density lipoproteins and fatty liver in rats fed polychlorinated biphenyls. *Biosci Biotechnol Biochem* **58**: 2152–2158.
- Ottea, J.A., Ibrahim, S., Youins, A.M., Young, R.J., Leonard, B.R. and McCaffery, A.R. (1995) Biochemical and physiological mechanisms of pyrethroid resistance in *Heliothis virescens* (F.). *Pestic Biochem Physiol* **51**: 117–128.
- Pedra, J.H., McIntyre, L.M., Scharf, M.E. and Pittendrigh, B.R. (2004) Genome-wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. *Proc Natl Acad Sci USA* **101**: 7034–7039.
- Pedra, J.H., Festucci-Buselli, R.A., Sun, W., Muir, W.M., Scharf, M.E. and Pittendrigh, B.R. (2005) Profiling of abundant proteins associated with dichlorodiphenyltrichloroethane (DDT)-resistance in *Drosophila melanogaster*. *Proteomics* **5**: 258–269.
- Petersen, R., Niamsup, H., Berenbaum, M.R. and Schuler, M.A. (2003) Transcriptional response elements in the promoter of *Cyp6b1*, an insect P450 gene regulated by plant chemicals. *Biochim Biophys Acta* **1619**: 269–282.
- Pfaffl, M.W., Horgan, G.W. and Dempfle, L. (2002) Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* **30**: Art. no. e36.
- Pinkus, R., Bergelson, S. and Daniel, V. (1993) Phenobarbital induction of AP-1 binding activity mediates activation of glutathione S-transferase and quinone reductase gene expression. *Biochem J* **290**: 637–640.
- Plapp, F.W. (1970) Changes in glucose metabolism associated with resistance to DDT and dieldrin in the house fly. *J Econ Entomol* **63**: 1768–1772.
- Plapp, F.W. Jr (1984) The genetic basis of insecticide resistance in the house fly: evidence that a single locus plays a major role in metabolic resistance to insecticides. *Pesticide Biochem Physiol* **22**: 194–201.
- Rajurkar, R.B., Khan, Z.H. and Gujar, G.T. (2003) Studies on levels of glutathione S-transferase, its isolation and purification from *Helicoverpa armigera*. *Curr Sci India* **85**: 1355–1360.
- Ranson, H. and Hemingway, J. (2005) Glutathione transferases. In: *Comprehensive Molecular Insect Science* (Gilbert, L.I. Iatrou, K. and Gill, S.S., eds), Vol. 5, pp. 383–402. Elsevier Pergamon, Oxford.
- Ranson, H., Rossiter, L., Ortelli, F., Jensen, B., Wang, X., Roth, C.W., et al. (2001) Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. *Biochem J* **359**: 295–304.
- Rencurel, F., Stenhouse, A., Hawley, S., Friedberg, T., Hardie, D., Sutherland, C. and Wolf, C.R. (2005) AMP-activated protein kinase mediates phenobarbital induction of CYP2B gene expression in hepatocytes and a newly derived human hepatoma cell line. *J Biol Chem* **280**: 4367–4373.
- Rose, R.L., Goh, D., Thompson, D.M., Verma, K.D., Heckel, D.G., Gahan, L.J., et al. (1997) Cytochrome P450 *Cyp9a1*. *Heliothis virescens*: the first member of a new CYP family. *Insect Biochem Mol Biol* **27**: 605–615.
- Sasaki, H., Miyaura, S., Horie, K. and Isono, H. (1989) Induction of glutathione S-transferase by phenobarbital in rat hepatocyte culture. *J Pharmacobiodyn* **12**: 775–780.
- Sawicki, R., Singh, S.P., Mondal, A.K., Benes, H. and Zimniak, P. (2003) Cloning, expression and biochemical characterization of one Epsilon-class (GST-3) and ten Delta-class (GST-1) glutathione S-transferases from *Drosophila melanogaster*, and identification of additional nine members of the Epsilon class. *Biochem J* **370**: 661–669.
- Scharf, M.E., Siegfried, B.D., Meinke, L.J. and Chandler, L.D. (2000) Fipronil metabolism, oxidative sulfone formation and toxicity among organophosphate- and carbamate-resistant and susceptible western corn rootworm populations. *Pest Manag Sci* **56**: 757–766.
- Schaub, J. and Freksa, A.F. (1976) The influence of phenobarbital on carbohydrate metabolism in developing rat liver. *Eur J Pediatr* **122**: 249–256.
- Scott, J.G. (1999) Cytochromes P450 and insecticide resistance. *Insect Biochem Mol Biol* **29**: 757–777.
- Scott, J. and Kasai, S. (2004) Evolutionary plasticity of monooxygenase-mediated resistance. *Pestic Biochem Physiol* **78**: 171–178.
- Scott, J.G., Sridhar, P., Liu, N. (1996) Adult specific expression and induction of cytochrome P450Ipr in house flies. *Arch Insect Biochem Physiol* **31**: 313–323.
- Shaw, G.C., Sung, C.C., Liu, C.H. and Lin, C.H. (1998) Evidence against the Bm1P1 protein as a positive transcription factor for barbiturate-mediated induction of cytochrome P450BM-1 in *Bacillus megaterium*. *J Biol Chem* **273**: 7996–8002.
- Singh, S.P., Coronella, J.A., Benes, H., Cochrane, B.J. and Zimniak, P. (2001) Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1–1 (GST-2) in conjugation of lipid peroxidation end products. *Eur J Biochem* **268**: 2912–2923.

- Sousa-Polezzi, R.C. and Bicudo, H.E.M.C. (2004) Effect of phenobarbital on inducing insecticide tolerance and esterase changes in *Aedes aegypti* (Diptera: Culicidae). *Genet Mol Biol* **27**: 275–283.
- Storey, J.D. and Tibshirani, R. (2003) Statistical significance for genomewide studies. *Proc Natl Acad Sci USA* **100**: 9440–9445.
- Stuart, J.J., Ray, S., Harrington, B.J., Neal, J.J. and Beeman, R.W. (1998) Genetic mapping of a major locus controlling pyrethroid resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J Econ Entomol* **91**: 1232–1238.
- Tanaka, S., Imaoka, S., Kusunose, E., Kusunose, M., Maekawa, M. and Funae, Y. (1990) Omega- and (omega-1)-hydroxylation of arachidonic acid, lauric acid and prostaglandin A1 by multiple forms of cytochrome P-450 purified from rat hepatic microsomes. *Biochim Biophys Acta* **1043**: 177–181.
- Tang, A.H. and Tu, C.-P.D. (1995) Pentobarbital-induced changes in *Drosophila* glutathione S-transferase D21 mRNA stability. *J Biol Chem* **270**: 13819–13825.
- Tang, F., Yue, Y.D. and Hua, R.M. (2000) The relationships among MFO, glutathione S-transferases, and phoxim resistance in *Helicoverpa armigera*. *Pestic Biochem Physiol* **68**: 96–101.
- Terriere, L.C. (1984) Induction of detoxication enzymes in insects. *Annu Rev* **29**: 71–88.
- Tusher, V., Tibshirani, R. and Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* **98**: 5116–5121.
- Videla, L.A., Arisi, A.C., Fuzaro, A.P., Koch, O.R. and Junqueira, V.B. (2000) Prolonged phenobarbital pretreatment abolishes the early oxidative stress component induced in the liver by acute lindane intoxication. *Toxicol Lett* **115**: 45–51.
- Vitarius, J.A., O'Shaughnessy, J.A. and Sultatos, L.G. (1995) The effects of phenobarbital pretreatment on the metabolism and toxicity of paraoxon in the mouse. *Pharmacol Toxicol* **77**: 16–22.
- Vontas, J.G., Small, G.J. and Hemingway, J. (2001) Glutathione S-transferases as antioxidant defense agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochem J* **357**: 65–72.
- Waters, L.C., Zelhof, A.C., Shaw, B.J. and Chang, L.Y. (1992) 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**: 4855–4859.
- Yan, L.J. and Sohal, R.S. (1998) Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. *Proc Natl Acad Sci USA* **95**: 12896–12901.
- Yu, S.J. and Terriere, L.C. (1978) Juvenile hormone epoxide hydrolase in house flies, flesh flies and blow flies. *Insect Biochem* **8**: 349–352.
- Zangar, R.C. and Novak, R.F. (1997) Effects of fatty acids and ketone bodies on cytochromes P450, 2B, 4A, and 2E1 expression in primary cultured rat hepatocytes. *Arch Biochem Biophys* **337**: 217–224.
- Zavon, M.R. (1971) Treatment of organophosphorus and chlorinated hydrocarbon insecticide intoxications. *Modern Treatment* **8**: 503–510.
- Zhong, W.Z., Zhao, Y.J., Gao, G.M., Yang, Z.H. and Yue, W. (2004) Effect of combination of dizocilpine with general antiepileptic drugs on amygdala kindling models in rats. *Yao Xue Xue Bao* **39**: 89–92.