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Regulation of cytochrome P450 expression in *Drosophila*: Genomic insights

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Abstract

Genomic tools such as the availability of the *Drosophila* genome sequence, the relative ease of stable transformation, and DNA microarrays have made the fruit fly a powerful model in insecticide toxicology research. We have used transgenic promoter-GFP constructs to document the detailed pattern of induced *Cyp6a2* gene expression in larval and adult *Drosophila* tissues. We also compared various insecticides and xenobiotics for their ability to induce this cytochrome P450 gene, and show that the pattern of *Cyp6a2* inducibility is comparable to that of vertebrate *CYP2B* genes, and different from that of vertebrate *CYP1A* genes, suggesting a degree of evolutionary conservation for the “phenobarbital-type” induction mechanism. Our results are compared to the increasingly diverse reports on P450 induction that can be gleaned from whole genome or from “detox” microarray experiments in *Drosophila*. These suggest that only a third of the genomic repertoire of *CYP* genes is inducible by xenobiotics, and that there are distinct subsets of inducers / induced genes, suggesting multiple xenobiotic transduction mechanisms. A relationship between induction and resistance is not supported by expression data from the literature. The relative abundance of expression data now available is in contrast to the paucity of studies on functional expression of P450 enzymes, and this remains a challenge for our understanding of the toxicokinetic aspects of insecticide action.

1. Introduction

Insecticidal action requires the presence of an active form of the insecticide at the target site at an effective concentration and for a sufficient time. The determinants of this bioavailability, transport, metabolism and sequestration, are therefore the toxicokinetic parameters [1] in insecticidal action. They have an equal importance to the molecular details of toxicodynamic, or mode of action, parameters. Efficacy, selective toxicity, and resistance can all be determined by either toxicokinetic or toxicodynamic differences between a sensitive species and a less sensitive species, a target or a non-target organism, and a susceptible or a resistant strain.

The increased use of *Drosophila* as a model insect in toxicology studies over the last decade is a logical consequence of the advantages it offers [2,3]. In the specific area of insecticide metabolism and resistance, the *Drosophila* genome sequence provided a first complete picture of cytochrome P450 diversity and abundance in insects with about 85 active CYP genes [4] while other insect genomes harbor more or fewer CYP genes [5]. The genomic tools available

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in *Drosophila* now allow both the detailed study of single genes and global approaches on the whole family of P450s in an insect.

Here, we provide examples of both approaches. First we focus on the *Cyp6a2* gene, a gene abundantly expressed in insecticide-resistant strains [6–9]. The CYP6A2 enzyme metabolizes organochlorine and organophosphorus insecticides [10] as well as dimethylbenzanthracene and aflatoxin B1 [11]. A mutant form of CYP6A2 has been reported to metabolize DDT as well [12]. The detoxification function of CYP6A2 is thus well established. *Cyp6a2* is also known to be inducible by barbiturates [7,9,10,13]. We report here the fine-scale mapping of the tissues in which the *Cyp6a2* gene is induced, and we report the pattern of *Cyp6a2* induction by various classes of chemicals using a screening approach with a transgenic GFP marker.

Secondly, we analyze the literature for induction of other CYP genes in *Drosophila* in order to place *Cyp6a2* in the context of the whole CYP family. The use of DNA microarrays allowed a genome-wide or “CYPome”-wide assessment of transcript abundance, and many important studies have been published since this field was reviewed [14]. Our analysis shows that only a third of the genomic repertoire of CYP genes is inducible by xenobiotics, and that there are distinct subsets of inducers / induced genes, suggesting multiple xenobiotic transduction mechanisms.

2. Materials and methods

2.1 Transgenic *Drosophila*

The 1.3 kb promoter fragment of the *Cyp6a2* gene [10] was cloned upstream of the Green Fluorescent Protein coding sequence in the pCasper P-element vector and this was used to transform the w^{1119} line of *Drosophila melanogaster*. The P[w+1.3-*Cyp6a2*-GFP]-transformed lines were screened for high inducibility of GFP fluorescence following phenobarbital treatment. A double X-chromosome transformant line 1E; 11B; Sco/SM6b was used for further study.

2.2 CYP6A2-GFP induction

Induction of *Cyp6a2*-GFP by phenobarbital and by the polychlorobiphenyl mixture Aroclor 1254 (PCB) in different tissues/organs in larva, pupa and adult of the transgenic stock 1E; 11B; Sco/SM6b was determined using confocal microscopy. Late first instar or early second instar larvae, and 1-day-old adults were fed for 3–5 days on Ward's *Drosophila* instant diet mixed with phenobarbital (1g dry instant diet + 2.5ml 0.4% phenobarbital sodium in distilled water). One-day-old flies were also treated with PCB, by contact for 4–5 days; for this, 15–20 flies were kept in 20ml glass scintillation vials coated with 0.1mg ($3\mu\text{g}/\text{cm}^2$) PCB dissolved in acetone. PCB-treated flies were also fed wet instant diet placed at the bottom of the vial. For controls, larvae and adults were similarly but without phenobarbital sodium or PCB. Whole larvae after molting in to 2nd or 3rd instar, and adults, and freshly dissected organs from larvae and adults were mounted on microscope slide in 80% glycerin in PBS and were examined for GFP fluorescence using a laser scanning confocal microscope (BioRad 1024 confocal scanning head attached to a Nikon Optiphot 2 microscope with PlanApo objectives). Confocal images were processed, using Lasershar software (BioRad, Hercules, CA) and Adobe Photoshop. Treatment of flies with other chemicals was either by contact (DDD:0.1 mg/vial; DDE:0.01 mg/vial; dicofol: 0.1mg/vial; aldrin: 1 $\mu\text{g}/\text{vial}$; clofibrate: 10 $\mu\text{g}/\text{vial}$) or by incorporation in the media i.e. ingestion (*trans*-stilbene oxide: 0.3%; limonene: 0.1%)

3. Results

3.1 Tissue specificity of *Cyp6a2*-GFP induction

Cyp6a2-GFP fluorescence was induced in many but not all the tissues/organs in treated larvae and adults (Table 1). In addition to this non-ubiquitous expression, there were also apparent quantitative differences among different tissues/organs. Yellow/orange auto-fluorescence of chitinous cuticle and cuticular lining of internal structures (trachea, crop, foregut, proventriculus/cardia, hindgut, rectum, salivary gland duct), food content (digestive system), or other inclusions (distal part of the Malpighian tubules, nephrocytes and pericardial cells) sometimes masked or modified the green fluorescence (GF). All muscles/ muscle fibers in both larvae and adults showed GF, but in the flight muscles GF was less intense than in other muscular components. Heart also showed only weak GF. In adults, regions of the body covered with only the membranous cuticle showed uniform green fluorescence because of the GFP in hemolymph (hemocytes) and epidermis. The fat body and the entire intestinal wall were intensely green fluorescent, but not the gastric caecum. GFP appeared to be induced throughout the central and peripheral nervous system, and sensory cells and their axons; but GF was weak in the larval brain and ganglion. In adult brain and ganglion, GF was more intense in the neuropile and lamina of the optic lobes than in the neurons. The ring gland and ovary did not show detectable GF, however, accessory glands showed faint GF. In the newly formed pupa, produced from phenobarbital-fed larva, fat body and digestive system were green (Fig. 1 L); other internal structures were not distinguishable in whole-mounted pupae examined. None of the tissues/organs in control larvae, pupae and adult flies showed green fluorescence (Fig. 1. J, K & M, Fig. 2. L–N).

3.2. *Cyp6a2* induction by xenobiotics

The clear difference in green fluorescence between induced and non-induced insects allowed us to screen for the ability of various chemicals to induce *Cyp6a2*. Table 2 shows the response of the *Cyp6a2*-GFP transgenic line to treatment by various chemicals, compared to the induction pattern of vertebrate *CYP1A* and *CYP2B* genes [15–17]. Phenobarbital, pentobarbital, organochlorines (DDT and aldrin), *trans*-stilbene oxide and limonene were all inducers of *Cyp6a2* and these are known inducers of the *CYP2B* genes, but not of the *CYP1A* genes. In contrast, *Cyp6a2* was not inducible by β -naphthoflavone or 3-methylcholanthrene, known inducers of the *CYP1A* genes. The case of polychlorobiphenyls is particularly interesting, as a mixture of coplanar and non-coplanar PCBs (Aroclor 1254) induces all three types of genes, but pure isomers show a distinguishable pattern: 2,4,5,2',4',5'-HCB (non-coplanar) induces *Cyp6a2* and the *CYP2B* genes, but 3,4,5,3',4',5'-HCB (coplanar) only induces the *CYP1A* genes [18,19]. Ethanol and clofibrate, typical inducers of the vertebrate *CYP2E* and *CYP4A* genes respectively did not induce *Cyp6a2*.

3.3. From *Cyp6a2* induction to induction of the CYP-ome

Cyp6a2 was initially reported to be phenobarbital (or barbital) inducible, and it is also regulated by ecdysone [20]. It has recently been shown to be also induced by caffeine [21]. Moreover, a number of DNA microarray studies have now identified *Cyp6a2* as being responsive to chemical treatments. We have therefore compared and evaluated the results of ten published studies from 17 datasets on xenobiotic treatment of *Drosophila melanogaster* that used either custom DNA arrays (“detox chips” [22,23]) or whole [24–28] (and partial [29–31]) genome arrays (Table 3). These studies covered ten chemicals, with different treatment regimes (concentrations and exposure times), as well as different developmental stages (third instar larvae and adults). Despite the differences in techniques and statistical treatments, we feel that the consensus outcome of this comparison provides an interesting first glimpse of the patterns of xenobiotic response of the fruit fly. Negative results, i.e. chemical treatments that did not induce P450 genes were not considered, although these are also informative [22]. We included

the studies on exposure to *Piper nigrum* extracts [30] because the main component piperamides are well known. We did not include the study on dietary shift in larvae (standard cornmeal to bananas) [32] that showed a slight induction of *Cyp9b2* and a moderate decrease in *Cyp6g1* and *Cyp4d2* expression. It is known that P450 genes are involved in the adaptation to diet in other *Drosophila* species, for instance *Cyp28a1* and *Cyp4d10* in *D. mettleri* [33], and that the transcriptome is affected by starvation. Every diet should be considered as a collection of inducers and inhibitors of various potencies and concentrations, but here we focus on identified xenobiotics.

The results of this survey show that between one and twelve CYP genes are affected by each chemical treatment, and that no more than a third (27 P450s) of the CYP-ome is responsive to xenobiotics. Different classes of compounds induce different CYP genes, and there is considerable overlap between some chemicals while others stand out (Figure 3). For instance, paraquat (11 genes) and tunicamycin (7 genes) seem to induce a set of CYP genes with little overlap with the set induced by the other chemicals. Tunicamycin causes a stress at the level of the endoplasmic reticulum and paraquat causes stress through the generation of reactive oxygen species [26]. The CYP genes seen induced in those treatments are perhaps best described as stress-responsive. *Cyp6d4*, *Cyp28a5* and *Cyp4p1* are the common genes in that category. A broader overlap is seen in the response to the other chemicals, with 11 genes retained in the consensus for phenobarbital induction (from six experimental conditions), 12 genes for piperonyl butoxide and 11 genes for caffeine. *Piper nigrum* extracts, atrazine and pyrethrum induced fewer genes, whereas DDT and ethanol had only one major target in the CYPome (*Cyp12d1* and *Cyp6a8* respectively). Those common genes responding to a chemical challenge are *Cyp6a2*, *Cyp12d1*, *Cyp6a8*, *Cyp6d5* and *Cyp6w1*. The wide chemical responsiveness of *Cyp6a2* that we observed using the transgenic fly model is thus confirmed by the various microarray studies.

3.4 Induction and insecticide resistance

Several reports in the literature have noted that CYP genes that are constitutively overexpressed in insecticide-resistant strains are also inducible by xenobiotics in susceptible strains, in *Drosophila* and in other insect species. We thus compared the data on CYP gene induction by xenobiotics that were collected in DNA microarray experiments with the list of genes that are currently known to be constitutively overexpressed in resistant strains or otherwise related to resistance (Fig. 4). Of the 27 genes known to be inducible by one or the other xenobiotics, and of the 12 genes known to be associated with resistance, eight are overlapping. Of these eight only three (*Cyp6a2*, *Cyp6g1* and *Cyp12d1*) have been shown to be causally related to resistance. Two (*Cyp12a4* and *Cyp6g2*) have been experimentally linked to resistance but have not been shown to be inducible by any of the nine compounds tested to date.

4. Discussion and conclusions

4.1. Tissue-specific expression

The midgut, Malpighian tubules and fat body are the tissues recognized as the major sites of P450-mediated detoxification in insects [34–36]. DNA microarray analyses in *Drosophila* have confirmed that P450 transcripts are enriched in these tissues. In Malpighian tubules of adult flies, the *Cyp6a18* transcript was enriched >25 fold and *Cyp6a2* enriched 8 fold [37]. The data of that study reveal that while 1,457 genes (10% of all genes) are expressed more than 2-fold in the Malpighian tubules, 29 P450 genes (or 28% of all P450 genes) are expressed over 2.8 fold. Similarly, 36 P450 genes (40%), including *Cyp6a2*, are represented in the larval midgut transcriptome [38]. A distillation of FlyAtlas data on tissue expression of P450 genes [39] shows that *Cyp6a2* is not expressed at high levels in normal flies, with Malpighian tubules, hindgut and head being the major sites of expression. Our results also show that *Cyp6a2* basal

expression is very low, although our technique does not measure the *Cyp6a2* transcript levels but the accumulation of GFP protein driven by the *Cyp6a2* promoter, and is therefore qualitatively different and perhaps less sensitive. Following induction, however, the gene is highly expressed both in larvae and in adults, in the midgut, Malpighian tubules and fat body. These results confirm and expand in detail the early results obtained by in situ hybridization in adult flies [7]. They also clearly show that *Cyp6a2*, after induction, is expressed in many other tissues as well as in the recognized sites of detoxification. The expression in the nervous system, in both larvae and adults is of significance, as the majority of insecticides have nervous system targets, such as the organophosphorus insecticides that are metabolized by CYP6A2 [10]. Expression of *CYP6D1* in the thoracic ganglia of adult house flies has also been reported to contribute to cypermethrin resistance in the Lpr strain [40]. The metabolism of xenobiotics by P450 enzymes is highly << uncoupled >>, costing much NADPH and generating considerable amounts of reactive oxygen species, as shown recently in a detailed study of house fly CYP6A1 [53]. The uninduced, low basal production of P450 enzymes is therefore probably an optimal state in the absence of chemical challenge. The observation that induced *Cyp6a2* should be found in a wide variety of tissues, beyond those in the front line of defense against xenobiotics such as the midgut and Malpighian tubules, may be an evolved response of insects who lack a closed circulatory system and therefore have all their tissues exposed to xenobiotics, once these have crossed the midgut or integument barriers. *Cyp6a2* has a pattern of expression that is similar to that of *Cyp6g1*. Chung et al [50] have used in situ hybridization as well as GFP reporter constructs to study the spatial expression of the *Cyp6g1* gene. They showed that the wild-type *Cyp6g1* is expressed in parts of the midgut and in the Malpighian tubules. In wandering larvae, expression is also detected in the fat body. In resistant strains carrying a fragment of the Accord transposable element, the expression is higher in those tissues, the whole midgut, and also includes the gastric caeca. Manipulation (RNAi or overexpression) of *Cyp6g1* transcript levels in transgenic flies under the control of a Malpighian tubule promoter is sufficient to affect DDT resistance, whereas RNAi suppression of *Cyp6g1* expression in fat body or brain is without effect [39]. Thus, the tissue expression pattern is critical in determining the toxicodynamics of insecticides (or more generally xenobiotics) that are metabolized by P450 enzymes.

4.2 Specificity of induction

The *Cyp6a2* gene appears to be responding to multiple chemical challenges, but not to all chemical treatments. Other genes in the CYPome also appear to have a specific range of response. It is obvious that the detailed study of one gene or the cursory analysis of the whole CYPome as seen through various microarray experiments does not allow definitive conclusions to be drawn, and that more detailed analyses should focus on the time course of induction and its dose response (see [22] for time courses). Nonetheless, it seems that about a third of the CYPome is inducible, while the majority of P450 genes is not regulated by xenobiotic exposure. The non-inducible genes probably include the Halloween genes encoding enzymes of ecdysteroid biosynthesis [41] but also include many genes of unknown function, that are expressed at low levels in the whole insect and may in fact have a very restricted physiological function and pattern of expression. Among the inducible genes, there are obvious subsets, evidenced in our survey as stress responsive genes and genes responding to chemical challenge. This suggests that the chemical diversity of xenobiotics is perceived by different, probably overlapping transduction mechanisms, and this leads to an adapted response. In vertebrates, this is achieved by two types of receptors, the members of the nuclear receptor family including the xenosensors PXR and CAR, and the bHLH-PAS proteins such as the Ah receptor. Cross-talk between these receptors and with receptors for endogenous hormones probably allows a fine-tuning of the response [42].

The inducibility of certain P450 genes in bacteria, plants, and insects by phenobarbital remains somewhat mysterious. However, the remarkable parallel between *Cyp6a2* induction and the known induction pattern of mammalian *CYP2B* genes strongly suggests that the “phenobarbital-type” induction in insects and mammals may share some characteristic that has been conserved during evolution. The nuclear receptor CAR is an essential component of the mouse response to the drug [43;44]. Analysis of the evolutionary relationships between *Drosophila*, *Caenorhabditis elegans* and vertebrate nuclear receptors revealed that the orphan receptor DHR96 was most closely related to the nematode NHR-8 and DAF-12 receptors and to vertebrate CAR, vitamin D receptor (VDR) and Pregnane X receptor (PXR) [45,46]. The nematode NHR-8 receptor is essential for wild-type resistance to colchicine and chloroquine [45]. Recently, King-Jones et al. [24] presented evidence for the role of DHR96 in the response of *Drosophila* to phenobarbital. They obtained DHR96 null mutants and showed that the expression of some but not all phenobarbital-inducible genes was affected in the mutants.

We attempted to silence the DHR96 gene by RNAi, using the UAS-GAL4 system wherein a transgenic line with a promoter driven-GAL4 expression was crossed to a transgenic line with UAS-driven expression of DHR96 dsRNA (line 11783R-2 kindly provided by Dr. Ueno, NIG-Fly, Japan). The heat shock promoter (hsp70-GAL4) or the promoter with moderate expression from the *Cyp6g1* gene (*Cyp6g1*-GAL4; line kindly provided by Dr. French-Constant, Univ. Exeter) were insufficient as drivers to obtain significant downregulation of DHR96, whereas a stronger, ubiquitous promoter (tubulin-GAL4) led to high mortality in both male and female crosses (results not shown). These negative results nonetheless indicate a complex role for DHR96 that may be involved in essential physiological or developmental process in addition to its reported implication in the response to xenobiotics.

4.3 Resistance and induction

Cyp6a2 and *Cyp12d1* are both inducible by at least 4 compounds and their constitutive overexpression has been causally linked to resistance. CYP6A2 metabolizes organophosphorus insecticides [10] and CYP12D1, when overexpressed in transgenic flies, confers resistance to dicyclanil and to DDT [47]. We note here that *Cyp12d1* is a recently duplicated gene (i.e. *Cyp12d1* and *Cyp12d2* that differ by only three non-neutral nucleotide changes (<http://p450.sophia.inra.fr>), *Cyp12d2* having been masked by a gap in the initial release of the *Drosophila* genome sequence). A third gene, *Cyp6g1*, is inducible by phenobarbital and by caffeine, and its overexpression in natural populations of *Drosophila* [48,49] as well as in transgenic flies [48,50,47,39] causes resistance to DDT and to neonicotinoids. Five other genes, *Cyp4e2*, *Cyp4p1*, *Cyp6a17*, *Cyp6a8* and *Cyp6w1* are both inducible and overexpressed in some resistant strains, but there is no evidence for their causal relationship to resistance. The case of *Cyp6a8* is noteworthy, as the recombinant enzyme metabolizes aldrin to a modest degree but not heptachlor or DDT [51], and its overexpression in transgenic flies does not confer resistance to DDT, nitenpyram, dicyclanil or diazinon [47]. Two genes are known to be linked to resistance but have not been shown to be inducible in any of the studies that we analyzed. *Cyp12a4* confers a selective resistance to lufenuron in field strains and in laboratory, transgenic strains [52]. *Cyp6g2* is a gene expressed at very low levels in the head [39] but its transgenic overexpression in gut and Malpighian tubules confers diazinon and dicyclanil resistance [47]. These discrepancies highlight the danger of associating resistance with constitutive overexpression, and resistance with induction. We have argued [23] that inducibility of a P450 gene implies the presence of *cis*-regulatory sequences in addition to the sequences controlling the correct tissue and developmental expression. The broader target (*cis*-sequences of the induced gene and genes for the *trans*-factors that are necessary for its induction) for mutational events is therefore a risk factor for resistance when the gene encodes a xenobiotic-metabolizing enzyme. It is well known that inducibility is under genetic control with different insect strains showing different induction patterns (see e.g. [54–56]). Hällström [57] has compared a resistant

strain in which the resistance mutation has caused increased constitutive expression and has abolished further inducibility to a “constitutive mutant”. Any other link between induction and resistance is probably coincidental rather than causal, because the time needed for induction and its dose-response would not sufficiently improve the toxicokinetics of most fast-acting neurotoxins used as insecticides to explain resistance.

While this paper was under review, Chung et al. [58] reported on the constitutive expression of CYP genes in *Drosophila* larvae. They obtained expression patterns for about two thirds of the CYPome, of which half were expressed in the fat body, midgut and Malpighian tubules, including all the genes that we highlighted in Figure 3. Many other genes had a more discrete expression pattern, sometimes specific to small organs or cell types. Their results and our survey lead to a similar conclusion, that only a restricted subset of P450 enzymes are likely to be involved in detoxification. Many endogenous functions remain to be discovered for the other P450 enzymes.

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Fig. 1. Phenobarbital-induced GFP in third instar larva and pupa. **A:** Early third instar larva. **B:** Brain-ganglion complex and anterior part of the digestive system. and **C:** Abdominal region showing body wall and internal organs. **D** and **E:** anterior and posterior ends of the larva. **F:** Anterior part of the salivary gland. **G:** Heart and pericardial cells. **H:** Anterior spiracle and spiracular glands. **I:** Body wall showing muscles. **J** and **K:** Anterior and posterior ends of control larva (without Phenobarbital). **L:** Newly formed pupa, treated with Phenobarbital at larval stage. **M:** Control pupa. Br, brain; Ed, epidermis; Es, esophagus; Fb, fat body; Gn, ganglion; He, heart; Hg, hindgut; Mg, midgut; Ms, muscles; Pv, proventriculus; Sbr, spiracular branches; So, sense organ; Sp, spiracle; Spg, spiracular glands; Tr, trachea.

Fig. 2.

Phenobarbital (Pheno)- or PCB-treated adults. **A:** Female fly (Pheno). **B** and **C:** Apex of proboscis (frontal view), surface and interior (PCB). **D:** Brain, thoracic ganglion and anterior region of the digestive system (Pheno). **E:** Part of midgut and hindgut and proximal region of Malpighian tubules (PCB). **F:** Wing (PCB). **G:** Abdominal wall showing tergosternal muscles and epidermis (PCB). **H:** Hindgut and rectum (Pheno). **I:** Abdominal fat body, heart and pericardial cells (Pheno). **J:** Part of the salivary gland (Pheno). **K:** Part of male reproductive system (PCB). **L–N** Control female, and parts of the digestive system. AcGl, Accessory gland; Br, brain; Cr, crop; EjD, ejaculatory duct; Fb, fatbody; He, heart; Hg, hindgut; Mg, midgut; Mt, malpighian tubule; Mu, muscles; Pc, pericardial cells; Pr, proventriculus; Pt, pseudotrachea; Re, rectum; Rv, rectal valve; SeCe, sensory cells; SeNe, sensory nerve; Sg, salivary gland Te,

testis; Vde, vas deferens. Scale bar: D, 40 μm , E, F, H, K, M and N, 100 μm ; B, C, G and I, 50 μm ; J, 10 μm .

Fig. 3.

Venn diagram summarizing the inducibility of *Drosophila* CYP genes by various chemicals. 1: ethanol; 2: pyrethrum; 3: phenobarbital; 4: caffeine; 5: piperonyl butoxide ; 6: *Piper nigrum* extract ; 7: atrazine; 8: tunicamycin ; 9: paraquat. The limited overlap between the CYP genes induced by paraquat and tunicamycin and those induced by the other chemicals led us to draw an arbitrary line to separate the genes that we designate as representing the response to stress from those representing the response to chemical challenge.

Fig. 4.

Venn diagram showing the overlap between *CYP* genes inducible by chemicals and *CYP* genes associated to resistance in field or laboratory studies. The overlapping genes are identified, and of those, the three genes that have been causally linked to resistance in field-derived strains or experimental studies are in bold.

Table 1

PCB / Phenobarbital- induced GFP in various tissues/organs in larvae and adults

Organs/tissues	Larva ¹	Adult
Cuticle	–	–
Tormogen cells of bristles	–	+
Epidermis	+	+
Imaginal disc	–	na
Skeletal muscles	+	+
Flight muscles	na	+
Fat body	+	+
Heart	– ²	– ²
Pericardial cells and nephrocytes	–	+ ³
Hemolymph (hemocytes)	–	+
Pharynx, esophagus, proventriculus/cardia	+	+
Midgut, hindgut, rectum	+	+
Caecum	–	–
Crop muscles	na	+
Salivary gland	+ ⁴	+
Malpighian tubules	+ ⁵	+ ⁵
Tracheal cells/lining	+	+
Spiracular glands	+	na
Brain, thoracic ganglion, peripheral nerves	+	+
Ring gland	–	–
Sense cells/organs	+	+
Compound eye and ocellus	na	+
Ovary/eggs	na	–
Uterus	na	+
Accessory gland	na	– ²
Testis, vas deferens, ejaculatory duct, sperm pump	na	+

The results are expressed as presence or absence of green fluorescence and are not meant to be interpreted quantitatively.

¹ Third instar larva

² only very weak green fluorescence (GF)

³ strong yellow/orange auto-fluorescence masked GF

⁴ GF only in the anterior region of the salivary gland in the larva

⁵ weak GF in the distal region; na, not applicable.

Table 2

Inducers of mammalian *CYP1A* and *CYP2B* genes compared with inducers of fruit fly *Cyp6a2*.

inducer	mammalian P450		<i>Drosophila</i>
	<i>CYP1A</i>	<i>CYP2B</i>	<i>Cyp6a2</i>
phenobarbital	–	+	+
pentobarbital	–	+	+
OCs DDT /DDD/DDE/dicofol	–	+	+
aldrin	–	+	+
<i>trans</i> -stilbene oxide	–	+	+
PCBs Aroclor 1254	+	+	+
2,4,5,2',4',5' HCB	–	+	+
3,4,5,3',4',5' HCB	+	–	–
limonene	–	+	+
β-naphthoflavone	+	–	–
3-methylcholanthrene	+	–	–
ethanol	–	–	–
clofibrate	–	–	–

Induction of *Cyp6a2* was estimated *in vivo* by the green fluorescence of treated transgenic *6a2*-GFP flies, see materials and methods for details. Induction patterns of *CYP1A* and *CYP2B* genes were compiled from the literature.

Table 3

Induction of the CYPome

Phenobarbital	Atrazine	Caffeine	<i>Piper nigrum</i> extract	Piperonyl butoxide	Pyrethrum	Ethanol or DDT	Paraquat	Tunicamycin
6 datasets [22,23,24,27] Ad-, L-, F-, M-	2 datasets [23] Ad-, L-, F-, M-	1 dataset [22] Ad- M-	1 dataset [30] Ad- F-	2 datasets [25] Ad- M-	1 dataset [31] Ad- F-	1 dataset [28] Ad- M-	2 datasets [26,29] Ad- M-	1 dataset [26] Ad- M-
<i>Cyp4d4</i>	<i>Cyp6a2</i>	<i>Cyp4ae1</i>	<i>Cyp6a8</i>	<i>Cyp4ae1</i>	<i>Cyp9f2</i>	<i>Cyp6a8 (ethanol)</i> <i>Cyp12d1 (DDT)</i>	<i>Cyp4ac3</i>	<i>Cyp4e1</i>
<i>Cyp4e2</i>	<i>Cyp6d5</i>	<i>Cyp4d14</i>	<i>Cyp6d4</i>	<i>Cyp4e2</i>	<i>Cyp12d1</i>		<i>Cyp4p1</i>	<i>Cyp4p1</i>
<i>Cyp6a2</i>	<i>Cyp6g1</i>	<i>Cyp4e2</i>	<i>Cyp6d5</i>	<i>Cyp4p1</i>			<i>Cyp6a13</i>	<i>Cyp6a23</i>
<i>Cyp6a8</i>	<i>Cyp6w1</i>	<i>Cyp6a2</i>	<i>Cyp6w1</i>	<i>Cyp6a2</i>			<i>Cyp6a22</i>	<i>Cyp6d4</i>
<i>Cyp6a17</i>	<i>Cyp12d1</i>	<i>Cyp6a8</i>	<i>Cyp9b2</i>	<i>Cyp6a8</i>			<i>Cyp6a23</i>	<i>Cyp9b2</i>
<i>Cyp6a23</i>	<i>Cyp304a1</i>	<i>Cyp6a21</i>	<i>Cyp12d1</i>	<i>Cyp6a21</i>			<i>Cyp6d4</i>	<i>Cyp28a5</i>
<i>Cyp6d5</i>		<i>Cyp6d4</i>		<i>Cyp6a23</i>			<i>Cyp6d5</i>	<i>Cyp313a1</i>
<i>Cyp6g1</i>		<i>Cyp6d5</i>		<i>Cyp6d5</i>			<i>Cyp28a5</i>	
<i>Cyp6w1</i>		<i>Cyp6g1</i>					<i>Cyp309a1</i>	
<i>Cyp9b2</i>		<i>Cyp6w1</i>		<i>Cyp12b2</i>				
<i>Cyp12d1</i>		<i>Cyp12d1</i>		<i>Cyp12c1</i>				
				<i>Cyp12d1</i>				

L-, Ad- : third instar larvae or Adults

M-, F- : Male or Female

Genes listed in bold are the genes most representative of inducibility following chemical challenge or stress response (see Fig. 3).