

Genome-wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*

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Genome-wide microarray analysis (Affymetrix array) was used (i) to determine whether only one gene, the cytochrome P450 enzyme *Cyp6g1*, is differentially transcribed in dichlorodiphenyltrichloroethane (DDT)-resistant vs. -susceptible *Drosophila*; and (ii) to profile common genes differentially transcribed across a DDT-resistant field isolate [*Rst(2)DDT^{Wisconsin}*] and a laboratory DDT-selected population [*Rst(2)DDT^{91-R}*]. Statistical analysis (ANOVA model) identified 158 probe sets that were differentially transcribed among *Rst(2)DDT^{91-R}*, *Rst(2)DDT^{Wisconsin}*, and the DDT-susceptible genotype Canton-S ($P < 0.01$). The cytochrome P450 *Cyp6a2* and the diazepam-binding inhibitor gene (*Dbi*) were over transcribed in the two DDT-resistant genotypes when compared to the wild-type *Drosophila*, and this difference was significant at the most stringent statistical level, a Bonferroni correction. The list of potential candidates differentially transcribed also includes 63 probe sets for which molecular function ontology annotation of the probe sets did not exist. A total of four genes (*Cyp6a2*, *Dbi*, *Uhg1*, and *CG11176*) were significantly different ($P < 5.6 \times 10^{-6}$) between *Rst(2)DDT^{91-R}* and Canton-S. Additionally, two probe sets encoding *Cyp12d1* and *Dbi* were significantly different between *Rst(2)DDT^{Wisconsin}* and Canton-S after a Bonferroni correction. Fifty-two probe sets, including those associated with pesticide detoxification, ion transport, signal transduction, RNA transcription, and lipid metabolism, were commonly expressed in both resistant lines but were differentially transcribed in Canton-S. Our results suggest that more than *Cyp6g1* is overtranscribed in field and laboratory DDT-resistant genotypes, and the number of commonalities suggests that similar resistance mechanisms may exist between laboratory- and field-selected DDT-resistant fly lines.

The evolution of insecticide resistance is often, but not always, based on major effect alleles (1–4). It has been hypothesized that high selection pressure in the field will favor monogenic forms of pesticide resistance, and that selection for resistance in the laboratory will favor polygenic resistance (5–7). In early genetic studies in *Drosophila*, dichlorodiphenyltrichloroethane (DDT) resistance was mapped to multiple locations on chromosomes II and III (8–14). Subsequently, low-level DDT resistance was mapped to 64.5 ± 2 centiMorgans on the second chromosome (15), a locus (loci) known as *Rst(2)DDT*.

Recently, Daborn *et al.* (16) suggested that resistance to DDT in the field is monogenic and is due to the overexpression of a single P450 gene, *Cyp6g1*. Le Goff *et al.* (17) suggested that resistance in field isolates of both *Drosophila melanogaster* and *Drosophila simulans* is associated with overtranscription of *Cyp6g1*, whereas prolonged laboratory selection with DDT apparently coselects additional genes such as *Cyp12d1* (18) and *Cyp6a8* (19). In contrast to the hypothesis presented by Le Goff *et al.* (17), Brandt *et al.* (18) observed that both *Cyp6g1* and *Cyp12d1* are overexpressed in the field-selected isochromosomal line *Rst(2)DDT^{Wisconsin}*.

Daborn *et al.* (16) provided a transcriptional profile of a DDT-resistant *Drosophila* field isolate and suggested that DDT resistance found in many field strains of *Drosophila* was due to

a specific P450 enzyme (*Cyp6g1*). Daborn *et al.* (16) used custom-made microarrays comprised of all known members of *Drosophila* cytochrome P450 genes and metabolic enzymes such as esterases and GSTs in addition to housekeeping genes. Overexpression of *Cyp6g1* in transgenic *Drosophila* showed only slight increases in resistance, suggesting that there is much more to resistance than a single gene.

To date, no genome-wide expression profile has been evaluated to investigate the extent to which gene transcription varies between genotypes that are resistant and susceptible to DDT. This comparison would allow for the testing of the hypothesis that only a single detoxification enzyme, *Cyp6g1*, is being overexpressed in DDT-resistant strains (16, 17). Additionally, expression profiles of (i) a field-collected isolate and (ii) a laboratory-selected isolate would allow for testing of the hypothesis that different sets of genes are overexpressed in field-collected and laboratory-selected strains. In the following study, we used a laboratory DDT-selected population, *Rst(2)DDT^{91-R}*, and a field-collected DDT-resistant isolate, *Rst(2)DDT^{Wisconsin}*, to test both of these aforementioned hypotheses. Our transcriptome approach identified a manageable number of genes for further investigation of the molecular basis of pesticide resistance. This analysis also strongly suggests that DDT resistance in *Drosophila* is more complex than previously described.

Methods

Drosophila Strains. Three *D. melanogaster* lines were used in the following experiments: (i) the DDT-susceptible Canton-S line; (ii) the DDT-resistant lines *Rst(2)DDT^{91-R}*; and (iii) *Rst(2)DDT^{Wisconsin}*. The Canton-S strain was obtained from the *Drosophila* Stock Center (Bloomington, IN) in January 2000 and was used as a standard susceptible strain. Field collected flies from Door County in Wisconsin were exposed to 200 μg of DDT per vial for 24 h, and a single male survivor was mated with the *Drosophila* balancer line $w^{\text{sc}};T(2,3)ap^{\text{Xa}}/TM3, Sb^1$ (18). A single DDT-resistant progeny was again crossed with the balancer line, and the resultant progeny were used to establish an isochromosomal line, *Rst(2)DDT^{Wisconsin}*. The *Drosophila* populations were reared in an environmentally controlled chamber at 28°C, 80% humidity, and 14 h of light per day. Experimental adult flies were collected as virgins (3 h after eclosion) by using light CO₂ anesthesia. For expression analysis, flies were sorted in a 1:1 male/female ratio, flash frozen in liquid nitrogen, and transferred to -80°C .

Experimental Design and Statistical Analysis. Twelve independent vials from each genotype were established; four vials were pooled for each replicate for a total of three replicates for each

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Abbreviation: DDT, dichlorodiphenyltrichloroethane.

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genotype and nine samples overall. This strategy was intended to reduce between-vial variations. Total RNA was isolated by using the TRI Reagent extraction protocol (Molecular Research Center, Cincinnati). Messenger RNA, cRNA synthesis, and labeling reactions were performed independently for each replicate following the recommendations of the GeneChip Expression Analysis technical manual (Affymetrix, Santa Clara, CA). The nine Affymetrix *Drosophila* genome chips were hybridized to the fragmented cRNA, stained, and washed according to the recommendations of the GeneChip Expression Analysis technical manual (Affymetrix) at the Purdue Genomics and Microarray Core Facility. Image data were quantified by using genechip analysis suite/microarray suite 5.0 (MAS 5.0). The identification of informative probe sets was performed by using default settings (α_1 , 0.04; α_2 , 0.06; δ , 0.015; scale factor, 1.0; norm factor, 1.0). If all nine replicates for a particular probe set were deemed “absent,” the probe set was removed from further consideration. The remaining probe sets (8,974) were analyzed, and transcript levels were normalized to the chip median and log transformed. For each probe set, which represents the combined expression data from all relevant probe pairs on the chip, the generalized linear model $Y_i = \mu + B_1L_i + \epsilon_{ij}$ was fit (20–22). In each ANOVA, Y_i is a log-normalized transcript for the i th line and j th replicate, μ is the overall mean expression for the probe sets, and the L_i is the i line represented (Canton-S, *Rst(2)DDT^{91-R}*, and *Rst(2)DDT^{Wisconsin}*). An F test of the effect of genotype for each gene was conducted as the ratio of the mean squares for line over the mean squares for error. The P value for the test of the null hypothesis $\lambda_2 = \lambda_1 = \lambda_0$ (i.e., mean expression not different among the three genotypes) was also calculated. We examined the model for conformation to the assumption of normality of the residuals testing the null hypothesis that the residuals for each gene were normally distributed by using the Shapiro–Wilkes Test. All analyses were performed in SAS (SAS Institute, Cary, NC). A Bonferroni significance level was used as an initial criterion for rejecting the null hypothesis of a significant treatment effect (0.05/8974), corresponding to a false discovery rate (FDR) of 0.045. We used a second arbitrary nominal threshold of $\alpha < 0.01$, because type I and II errors are inversely related, with decreases in false positives (type I) being associated with increases in false negatives (type II), and because the Bonferroni correction is overly conservative as tests are correlated (20–22). This threshold corresponded to a FDR of 0.56. We also considered the test for normality of the residuals. If the test of the null hypothesis of difference across genotypes was rejected at the Bonferroni level, and we had no evidence for departure from normality of the residuals, we declared the gene differentially expressed across genotypes and examined additional contrasts comparing the effect of the genotypes [Canton vs. *Rst(2)DDT^{91-R}* and Canton vs. *Rst(2)DDT^{Wisconsin}*]. If the P value for the test of differences over genotypes was ≤ 0.01 (56% FDR) but larger than the Bonferroni level, and we had no evidence for departure from normality of the residuals, we considered a gene as being differentially transcribed.

Gene Ontology Analysis. We used BLAST to link Affymetrix probes to a FlyBase annotation 2, to determine the molecular function, biological process, and cellular component of each gene (www.geneontology.org). We described the transcripts using the molecular function ontology, because it serves well as a foundation for unifying the growing amount of expression information related to genome-wide pesticide studies in different organisms, and it is designed to facilitate the transfer of gene/protein function information among other organisms. However, the three separate ontologies are available which is published as supporting information on the PNAS web site.

Results and Discussion

Multiple Genes Are Differentially Transcribed Between DDT-Resistant and -Susceptible Lines. High-density Affymetrix oligoarrays were used to identify transcripts differentially expressed among (i) the laboratory DDT-selected *Rst(2)DDT^{91-R}*, (ii) the isochromosomal DDT-resistant field isolate *Rst(2)DDT^{Wisconsin}*, and (iii) the DDT-susceptible Canton-S line. The GeneChip contained 13,966 probe sets, of which 4,992 were uninformative (absent for gene expression in all nine replicates). We identified 158 probe sets that were significantly different among the genotypes ($P < 0.01$; false discovery rate of 0.56) and the probe sets 143127.at [*Cyp6a2* (cytochrome P450)] and 143608.at [*Dbi* (Diazepam-binding inhibitor gene)] were differentially transcribed at the Bonferroni corrected level.

We then investigated the individual contrasts for *Rst(2)DDT^{91-R}* and Canton-S and *Rst(2)DDT^{Wisconsin}* and Canton-S. A total of four probe sets (*Cyp6a2*, *Dbi*, *Uhg1*, and CG11176) were significantly different between *Rst(2)DDT^{91-R}* and Canton-S (Tables 1 and 2) at the 5.6×10^{-6} . The P values of two probe sets encoding for *Cyp12d1* and *Dbi* were significantly different between *Rst(2)DDT^{Wisconsin}* and Canton-S after a Bonferroni correction (Table 1). Two cytochrome P450 genes (*Cyp6g1* and *Cyp12d1*) were previously identified in our laboratory (18) and elsewhere (16, 17) as putative transcripts associated with DDT resistance in both *Rst(2)DDT^{91-R}* and *Rst(2)DDT^{Wisconsin}*.

Of the 158 probe sets differentially transcribed, 63 were categorized in this group that were not annotated for molecular function using the Gene Ontology (GO) database and that are not discussed hereafter (Table 2). Ninety-five probe sets were identified as belonging to a particular GO molecular function. A large group of detoxification enzymes were differentially expressed (Table 1). Also, transcripts involved in neuronal function were identified. Four transcription factors were differentially expressed (Cf2, NFAT, Odd, and Nut2). The transcript NFAT (Misexpression Suppressor of Ras 1) was overtranscribed in both DDT-resistant lines and may represent extracellular signals that can modulate DDT resistance.

Differential transcription was found in six probe sets coding for peptidase activity. This finding is in keeping with Saleem *et al.* (23) and Ahmed *et al.* (24), who observed that proteases have higher enzymatic activities in DDT-resistant houseflies as compared with susceptible ones. The increased proteolytic activity may serve a role to meet energy demands during stress, thus balancing protein degradation and synthesis. Intracellular proteases may play a role in protein biosynthesis or in modification of the conformation of enzymes as part of this induction process (23, 24). Further investigation of protease expression should help to determine the possible role of proteases in pesticide-resistant insects. The following groups of genes were also overexpressed in the resistant lines: (i) transcripts of nearly all forms of molecule binding were overtranscribed in the resistant lines, including DNA, protein, carbohydrate, vitamin, lipid, drug, and ATP binding; (ii) genes involved in perception of abiotic stimuli and oxidoreductase activity; (iii) genes associated with immune defense and signal transduction; as well as (iv) phosphatases, kinases, and structural molecules.

To examine whether the positions of genes overexpressed in DDT-resistant *Drosophila* were random or clustered together in a region, we checked the cytological position of each differentially transcribed probe set. With the exception of a moderate representation of transcripts in the right arm of the second chromosome, transcripts appear to be widely distributed across all chromosomes [Tables 1 (“Cytological position”) and 2]. These results are consistent with the findings of Dapkus and Merrel (12), who reported that *Rst(2)DDT^{91-R}* has

Table 1. Genes annotated for molecular function with differential transcription among three *Drosophila* genotypes

Molecular function	GO code	Symbol	Probe set	P value	Cytological position	91-R	Wisconsin		
Cytochrome P450 activity	GO:0015034	Cyp6a2*	143127	8.6 e-7	42C8	2.8 e-7*	5.9 e-6		
		Cyp12d1	146978	7.2 e-6	47D4	1.0 e-3	2.5 e-6*		
		Cyp6a17	152313	4.2 e-5	51D1	0.43	2.5 e-5		
		Cyp6a8	142189	6.0 e-5	51D1	7.1 e-5	0.17		
		Cyp12d1	154692	1.5 e-4	47D4	6.9 e-3	5.0 e-5		
		Cyp6w1	152494	1.8 e-4	42A13	6.2 e-5	0.01		
		Cyp6g1	152900	6.9 e-4	48E8	2.9 e-4	1.1 e-3		
		Cyp6a14	146815	7.5 e-4	44D3	9.4 e-3	7.3 e-3		
		Cyp9c1	141780	7.9 e-4	60D10	6.5 e-4	0.74		
		Cyp4p1	143782	1.0 e-3	45B7	4.1 e-4	0.11		
		Cyp6a23	147225	3.3 e-3	51D1	0.03	0.03		
		Glutathione transferase activity	GO:0004364	CG17530	142537	2.9 e-5	55C7	1.9 e-5	2.4 e-5
				CG17522	147434	1.8 e-3	55C6	1.6 e-3	9.9 e-4
CG1681	152675			8.3 e-4	11F4	0.75	4.7 e-3		
Gst3-1	141930			8.4 e-4	55C8	0.17	3.1 e-3		
CG6673	142740			9.3 e-4	66D5	3.8 e-3	0.01		
Glucuronosyltransferase activity	GO:0015020	Ugt86Dh	149669	6.6 e-4	86D6	2.2 e-3	0.05		
		Ugt86Dd	149663	3.5 e-4	86D4	2.2 e-3	0.91		
		Ugt35b	142271	3.6 e-4	86D5	1.9 e-4	3.8 e-4		
Tyrosine-ester sulfotransferase activity	GO:0017067	CG5431	141306	8.7 e-4	59F6	3.5 e-4	0.12		
Oxidoreductase activity	GO:0016491	Pdh	153433	2.6 e-3	72E3	8.7 e-4	0.01		
		CG30019	152569	1.1 e-3	47C3	0.10	4.1 e-4		
Oxidoreductase activity	GO:0016491	CG3301	151819	3.5 e-3	93D2	0.01	0.06		
		CG12224	149723	4.1 e-3	87A4	1.3 e-3	0.03		
		CG8888	152990	4.3 e-3	48D8	0.02	0.05		
		CG9360	144893	4.4 e-3	10E2	4.1 e-3	2.3 e-3		
		CG3603	144428	4.5 e-3	3C3	1.5 e-3	0.09		
		CG3842	144594	7.0 e-3	5F2	0.01	2.6 e-3		
		CG15531	150879	5.9 e-3	99E3	3.0 e-3	5.9 e-3		
		CG9747	152137	6.4 e-3	99E1	0.05	2.2 e-3		
		CG15093	152083	7.6 e-3	55F2	2.7 e-3	0.02		
		InaF	143971	4.3 e-5	10E2	2.1 e-5	5.5 e-5		
		CG17142	147913	1.3 e-5	61B2	0.01	4.4 e-4		
Calcium channel regulator activity	GO:0005246	InaF	143971	4.3 e-5	10E2	2.1 e-5	5.5 e-5		
Calcium channel activity	GO:0005262	CG17142	147913	1.3 e-5	61B2	0.01	4.4 e-4		
Calcium ion binding	GO:0005509	Cpn	152156	2.2 e-4	87B1	7.3 e-4	0.01		
		CG2185	153946	4.2 e-3	83B8	1.9 e-3	0.36		
Voltage-sensitive calcium binding	GO:0005245	Ca- α 1D	151837	3.6 e-3	35E5	0.09	0.01		
Calcium-dependent cell adhesion molecule activity	GO:0008014	Mys	143507	7.0 e-3	7D5	2.5 e-3	0.10		
Organic ion transporter activity	GO:0015101	CG16727	150207	8.3 e-3	92A10	3.2 e-3	0.22		
Ryanodine-sensitive calcium-release channel activity	GO:0005219	Rya-r44F	143650	3.4 e-3	44F1	0.63	3.1 e-3		
Sodium-dependent multivitamin transporter activity	GO:0008523	CG8932	150628	8.7 e-4	96F8	8.1 e-3	4.6 e-3		
Monosaccharide transporter activity	GO:0015145	CG15407	145701	2.4 e-4	23E5	0.02	0.02		
Maleylacetoacetate isomerase activity	GO:0016034	CG9362	141293	2.7 e-3	85D7	1.0 e-3	5.3 e-3		
Long-chain fatty acid transporter activity	GO:0005324	CG5568	141286	6.6 e-3	64F3	4.3 e-3	4.6 e-3		
Cholesterol O-acyltransferase	GO:0017066	CG5397	151961	1.3 e-3	21F3	1.9 e-3	5.5 e-4		
Triacylglycerol lipase activity	GO:0004806	CG17192	150700	2.0 e-3	97D14	0.5	1.0 e-3		
Argininosuccinate lyase activity	GO:0004056	CG9510	141703	5.2 e-3	29F2	2.2 e-3	7.7 e-3		
Acetyl-CoA C-acyltransferase	GO:0003988	Yip2	153437	1.1 e-3	30D1	3.6 e-4	0.03		
Diacylglycerol binding	GO:0019992	CG10737	153286	9.9 e-3	56C1	0.89	6.2 e-3		
Acetylglucosaminyltransferase activity	GO:0008375	Ext2	151844	9.9 e-3	52F3				
Lipid binding	GO:0008289	Dbi*	143608	1.2 e-6	65E8	7.9 e-7*	1.2 e-6		
Drug binding	GO:0008144	CG14715	142488	4.2 e-3	86E19	4.9 e-3	1.9 e-3		
ATP-binding cassette transporter activity	GO:0004009	CG9892	141801	8.8 e-3	23A2	0.01	3.7 e-3		
Protein binding	GO:0005515	Arr1	152756	5.1 e-4	36D3	2.3 e-4	6.6 e-4		
		Arr2	143078	1.7 e-3	66D9	9.1 e-4	1.7 e-3		
		Dia	143636	1.0 e-3	38E5	1.1 e-3	5.5 e-4		
		Ank2	151478	2.1 e-3	66A10	7.3 e-4	7.5 e-3		
		Map205	153771	3.7 e-5	100F5	1.6 e-5	1.5 e-5		
DNA binding	GO:0003677	Sox100B	151112	2.3 e-3	100B1	1.2 e-3	2.2 e-3		
RNA polymerase II transcription factor activity	GO:0003702	Cf2	143101	5.1 e-3	25B1	1.8 e-3	0.09		
		NFAT	153719	2.8 e-3	12A9	3.7 e-3	1.2 e-3		
		Odd	143286	2.2 e-3	24A1	0.71	1.3 e-3		
		Nut2	148854	7.8 e-3	72D10	3.5 e-3	0.38 *		
Vitamin binding	GO:0019842	CG3091	142199	5.1 e-3	2F2	0.42	2.4 e-3		
Galactose-binding lectin	GO:0005531	Lectin-galC1	143876	7.2 e-4	37D1	0.05	2.0 e-3		

Table 1. (continued)

Molecular function	GO code	Symbol	Probe set	P value	Cytological position	91-R	Wisconsin
Mannose-binding lectin	GO:0005532	CG11211	146673	3.0 e-3	42A14	0.08	1.1 e-3
UV-sensitive opsin	GO:0015064	Rh4	143321	1.3 e-3	73D2	5.4 e-4	2.2 e-3
		Rh3	151860	2.8 e-3	92C5	1.5 e-3	2.7 e-3
Ligand binding or carrier	GO:0005488	Glob1	152233	6.9 e-4	89A8	3.8 e-4	6.5 e-4
Blue-sensitive opsin	GO:0015059	Nina E	143283	3.6 e-3	92B4	1.8 e-3	3.7 e-3
Protein serine/threonine kinase	GO:0004674	Nina C	152330	8.1 e-3	27F3	3.7 e-3	9.4 e-3
CDP-alcohol phosphotransferase	GO:0008414	CG10355	141512	3.7 e-3	37D3	3.7 e-3	0.52
Chaperone activity	GO:0003754	CG7409	148360	4.3 e-3	66A12	0.43	5.0 e-3
Aldose 1-epimerase activity	GO:0004034	CG10467	148265	4.4 e-3	65A6	3.7 e-3	2.5 e-3
Structural constituent of cuticle (sensu Insecta) activity	GO:0005214	CG8505	141213	5.4 e-3	49A2	2.1 e-3	0.19
Peptidase activity	GO:0008233	CG1304	145450	5.1 e-4	19E6	3.2 e-4	0.78
		CG10477	148251	9.7 e-4	65A3	0.07	3.6 e-4
		CG11034	145847	1.0 e-3	25A5	0.3	1.2 e-3
		CG9897	147730	1.4 e-3	59C1	6.5 e-3	4.7 e-4
		Ser12	143698	3.0 e-3	22D1	1.4 e-3	3.3 e-3
		BG:BACR44L 22	144331	4.7 e-3	35D3	1.6 e-3	0.02
Nitrophenylphosphatase activity	GO:0003869	EG:100G10.4	144046	8.2 e-3	3B3	4.3 e-3	7.7 e-3
Protein serine/threonine kinase activity	GO:0004674	InaC	152951	5.7 e-4	53E2	2.8 e-4	6.3 e-4
Signal transducer activity	GO:0004871	Ggamma30A	146078	1.9 e-4	30A2	6.9 e-5	5.9 e-4
		Gbeta76C	153427	5.6 e-3	76C1	0.21	5.8 e-3
		Sr-CI	143747	8.6 e-3	24D6	2.9 e-3	0.08
		Or92a	151722	9.6 e-3	92E14	0.78	5.6 e-3
Defense/immunity protein activity	GO:0003793	LysD	143466	1.9 e-4	61F3	0.06	3.8 e-4
		LysB	143464	6.4 e-4	61F3	0.13	1.1 e-3
		LysC	143465	2.4 e-4	61F3	0.05	5.3 e-4
		LysE	143467	1.7 e-3	61F3	0.08	4.6 e-3
		LysP	143468	1.8 e-3	61F4	8.3 e-3	0.03
Hydrogen-translocating F-type ATPase activity	GO:0016467	I(2)06225	146216	1.5 e-3	32B3	0.82	1.1 e-3
Structural molecule activity	GO:0005198	InaD	143203	2.7 e-3	59B3	1.1 e-3	5.5 e-3
Farnesyltransferase activity	GO:0004311	Qm	143901	8.8 e-3	65F4	0.7	7.6 e-3
Phosphatidylinositol 3-kinase activity	GO:0016303	Pi3K59F	151517	4.6 e-4	59E4	1.6 e-3	0.02

Transcripts differentially expressed among three *Drosophila* genotypes ($P < 0.01$). "Molecular function" and "GO code" represent the molecular function category and the gene ontology number according to the Gene Ontology annotation. "Symbol" and "Cytological position" columns are given based on Flybase information, release 2 (<http://flybase.bio.indiana.edu>). "Cytological position" is the gene location in the *Drosophila* cytological map. A single asterisk indicates a highly significant association between the probe set and the DDT-resistant phenotype ($P < 5.6 \text{ e-}6$). "Probe set" is the Affymetrix name for the probe set on the chip. "P value" shows the statistical P value for the test of null hypothesis $\lambda_2 = \lambda_1 = \lambda_0$ (i.e., mean expression not different among the three genotypes), as described in *Methods*. "91-R" and "Wisconsin" indicate the P value for t tests when the mean differences of each DDT-resistant *Drosophila* genotype is compared to the wild-type Canton-S.

multifactorial resistance to DDT and is associated with all three of the major chromosomes in *Drosophila*.

Transcripts Coding for Detoxification Enzymes. We identified 19 differentially transcribed probe sets representing cytochrome P450s, GSTs, or glucuronosyltransferases (Table 1). Just over half (11) of these 19 probe sets were annotated as cytochrome P450 genes. Some of these P450 genes, such as *Cyp6g1* and *Cyp12d1* (16–18) and *Cyp6a2* and *Cyp6a8* (19, 25), were expected to show differential transcription. Other P450 genes are potentially interesting for further study, because they have not yet been shown to be overtranscribed in resistant genotypes. The gene *Cyp12d2* has been recently described in the literature as differing by only three nucleotide substitutions from *Cyp12d1* (<http://P450.antibes.inra.fr>); although this gene sequence was deposited neither in National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) nor in FlyBase (<http://flybase.bio.indiana.edu>). Le Goff *et al.* (17) were not able to separate overtranscription of either *Cyp12d1* or *Cyp12d2* using a cDNA spotted array.

Six detoxification enzyme genes were constitutively overtranscribed in both *Rst(2)DDT^{Wisconsin}* and *Rst(2)DDT^{91-R}* (*Cyp6g1*, *Cyp12d1*, *Cyp6a2*, *Cyp6w1*, *Ugt35b*, and *CG17530*)

(Fig. 1). *Cyp6a17* and *CG17522* had higher transcript abundance in wild-type flies compared to resistant genotypes. This result suggests potential endogenous functions for these respective gene products. The relative transcript expression of *Cyp6a2* in *Rst(2)DDT^{91-R}* was 255- and 9.1-fold greater than Canton-S and *Rst(2)DDT^{Wisconsin}*. It is important to note that the *Rst(2)DDT^{91-R}* line is more resistant to DDT than *Rst(2)DDT^{Wisconsin}* and Canton-S (data not shown). The respective LC₅₀s for these three genotypes are 1,304, 89, and 0.71 μg of DDT per vial (with a 24-h bioassay; data not shown). Dunkov *et al.* (26) reported that *Cyp6a2* is able to metabolize xenobiotics, which suggests that *Cyp6a2* may play a role in the higher level of DDT resistance observed in *Rst(2)DDT^{91-R}* as compared to *Rst(2)DDT^{Wisconsin}*. However, we do not rule out the possible occurrence of target site insensitivity in combination with metabolic resistance by *Rst(2)DDT^{91-R}*. Three cytochrome P450s (*Cyp6g1*, *Cyp12d1*, and *Cyp6a2*) were previously described as being associated with pesticide resistance in laboratory-selected *Drosophila* (16–19, 25), whereas it has been hypothesized that only *Cyp6g1* is overexpressed in DDT-resistant flies collected from the field (16, 17). To the contrary, our report shows that multiple cytochrome P450s are overexpressed and potentially contribute to the DDT resistance phenotype.

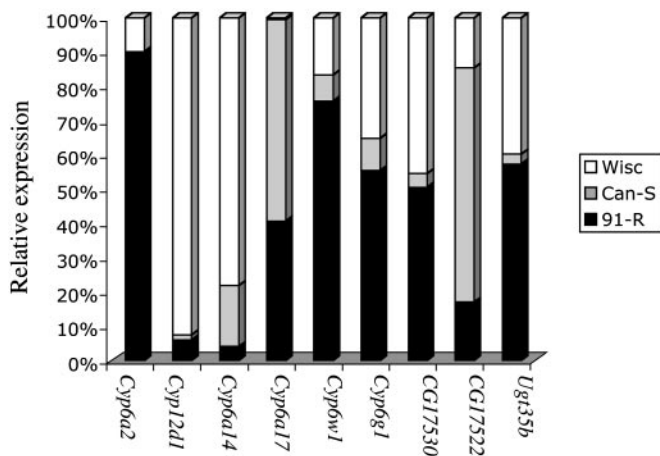


Fig. 1. Relative expression for probe sets derived from detoxification enzyme coding genes among three *Drosophila* fly lines. Bars show the percentage of each genotype contributing to a total transcriptional level across the probe sets. The wild-type Canton-S is represented by gray (Can-S), and the two DDT resistant genotypes, *Rst(2)DDT^{91-R}* and *Rst(2)DDT^{Wisconsin}*, are represented by black (91-R) and white (Wisc) columns, respectively. Detoxification enzymes were annotated as cytochrome P450 enzymes (GO: 0015034) (*Cyp6a2*, *Cyp12d1*, *Cyp6a14*, *Cyp6a17*, *Cyp6w1*, and *Cyp6g1*), GSTs (GO, 0004363) (*CG17530* and *CG17522*) and UDP-glucuronosyltransferases (GO, 0015020) (*Ugt35b*).

GSTs are able to conjugate glutathione to xenobiotics, thus converting the xenobiotics to nonreactive water-soluble conjugates that are easily excreted (27). GSTs have been associated with organochlorine and organophosphorous insecticide resistance both in the malaria mosquito (*Anopheles gambiae*) and in *Drosophila* (28–31). We found five GST transcripts overexpressed in the DDT-resistant lines (Fig. 1 and Table 1). Another group of conjugative detoxification enzymes, UDP-glucuronosyltransferases (UGTs), catalyzes the conjugation of glucuronic acid to a wide variety of endobiotics and xenobiotics (32, 33). Okazaki and Katayama (34) have shown that dietary DDT in rats increases enzymatic activity of hepatic 4-nitrophenol-UDP glucuronosyltransferases. We also have identified three UGT transcripts as being differentially transcribed in the resistant genotypes (Fig. 1 and Table 1).

Transcripts Coding for Lipid Metabolism Genes. DDT and its metabolites have been shown to act as estrogen receptor agonists, thereby producing estrogen-like effects (35). We detected differential transcript levels of (i) long-chain fatty acid transporter, (ii) triacylglycerol lipase, (iii) cholesterol *O*-acyl transferase, and (iv) “diacylglycerol binding” in both resistant genotypes. These findings suggest a possible relationship between DDT resistance and lipid metabolism (Fig. 2). Diazepam-binding inhibitor (DBI) is a polypeptide found in several organisms that has been shown to be involved in benzodiazepine receptor modulation, acyl CoA metabolism, steroidogenesis, insulin secretion, and drug dependence (36, 37). The relative transcript expression of DBI in *Rst(2)DDT^{91-R}* and *Rst(2)DDT^{Wisconsin}* was 108- and 89-fold greater than Canton-S, suggesting a possible association between DDT resistance and cholesterol metabolism (Fig. 2).

Resistance appears more complex than the overtranscription of *Cyp6g1*, as suggested by Daborn and colleagues (16, 17). Also, RNA expression profiles in *Rst(2)DDT^{91-R}* and *Rst(2)DDT^{Wisconsin}* *Drosophila* genotypes identified several common metabolic pathways components associated with DDT resistance. The comparison of microarray data among different research groups is difficult. Differences in experi-

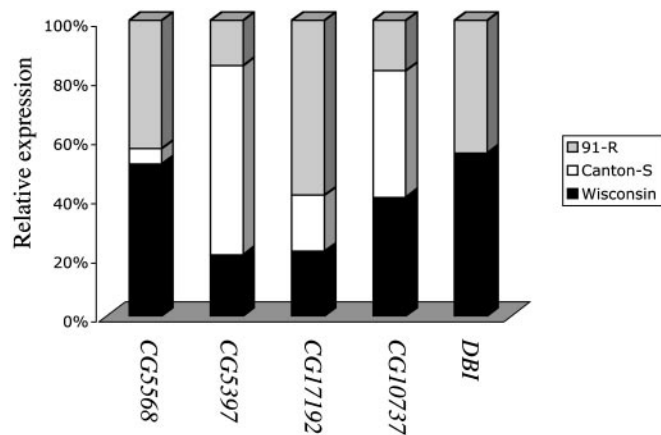


Fig. 2. Relative expression for probe sets derived from lipid metabolism coding genes among three *Drosophila* fly lines. Bars show the percentage of each genotype contributing to a total transcriptional level across the probe sets. The wild-type Canton-S is represented by white (Canton-S), and the two DDT-resistant genotypes, *Rst(2)DDT^{91-R}* and *Rst(2)DDT^{Wisconsin}*, are represented by gray (91-R) and black (Wisconsin) columns, respectively. Transcripts associated with lipid metabolism were annotated as long chain fatty acid transporter, CG5568 (GO, 0005324); cholesterol *O*-acyltransferase, CG5397 (GO, 0017066); triacylglycerol lipase, CG17192 (GO, 0004806); diacylglycerol binding, CG10737 (GO, 0019992); and lipid binding, DBI (GO, 0008289).

mental design of microarrays, techniques used (cDNA spotted array vs. high-density oligoarrays), and statistical analysis make such comparisons challenging. Sexual dimorphism plays a pivotal role in *Drosophila* gene expression. Studies of the transcriptome of sexually mature males and females have shown clear sex-dependent gene regulation (38–41). Finally, aging is associated with changes in the expression of many genes. Whole genome transcript profiles showed that nearly 23% of the expressed genome changed with age (42). Therefore, discrepancies between our findings and those of Daborn and colleagues (16, 17) may be due to differences in statistical methodologies, array technology used, age, or gender.

Nonetheless, the use of genome-wide microarray technology allows an efficient and quantitative evaluation of transcripts in insecticide-resistant genotypes and has the potential to suggest interesting genes for further study. To confirm the role of any genes described above from DDT-resistant *Drosophila*, validation studies such as quantitative PCR, Northern blots, RNA interference, P element transformation, and messenger RNA and protein integration are essential. Our group (18) and others (19, 43) have validated, by using Northern blot, that *Cyp6g1*, *Cyp12d1* (*Cyp12d2*), *Cyp6a2*, and *Cyp6a8* are differentially transcribed in DDT-resistant *Drosophila* genotypes. Pesticide metabolism studies in *Escherichia coli* and yeast using recombinant detoxification enzymes should shed more light on DDT metabolism by resistant *Drosophila*. This study has also allowed for the identification of several other gene transcripts with undefined relationships to detoxification processes. Functional characterizations of these mechanisms will almost certainly yield highly informative findings not previously considered in pesticide resistance research.

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