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Cis-Regulatory Elements in the *Accord* Retrotransposon Result in Tissue-Specific Expression of the *Drosophila melanogaster* Insecticide Resistance Gene *Cyp6g1*

Henry Chung,*,† Michael R. Bogwitz,*,† Caroline McCart,‡ Alex Andrianopoulos,† Richard H. ffrench-Constant,§ Philip Batterham*,†,1 and Phillip J. Daborn*,†

**Centre for Environmental and Stress Adaptation Research, Bio21 Molecular Science and Biotechnology Institute, †Department of Genetics, University of Melbourne, Melbourne, Victoria, 3010, Australia, ‡Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, United Kingdom and §Center for Ecology and Conservation, University of Exeter, Penryn, Cornwall, TR10 9EZ, United Kingdom*

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ABSTRACT

Transposable elements are a major mutation source and powerful agents of adaptive change. Some transposable element insertions in genomes increase to a high frequency because of the selective advantage the mutant phenotype provides. *Cyp6g1*-mediated insecticide resistance in *Drosophila melanogaster* is due to the upregulation of the cytochrome P450 gene *Cyp6g1*, leading to the resistance to a variety of insecticide classes. The upregulation of *Cyp6g1* is correlated with the presence of the long terminal repeat (LTR) of an *Accord* retrotransposon inserted 291bp upstream of the *Cyp6g1* transcription start site. This resistant allele (*DDT-R*) is currently at a high frequency in *D. melanogaster* populations around the world. Here, we characterize the spatial expression of *Cyp6g1* in insecticide-resistant and -susceptible strains. We show that the *Accord* LTR insertion is indeed the resistance-associated mutation and demonstrate that the *Accord* LTR carries regulatory sequences that increase the expression of *Cyp6g1* in tissues important for detoxification, the midgut, Malpighian tubules, and the fat body. This study provides a significant example of how changes in tissue-specific gene expression caused by transposable-element insertions can contribute to adaptation.

TRANSPOSABLE elements (TEs), present in most sequenced genomes to date, were once referred to as "selfish DNA," alluding to the presumptively parasitic nature of these highly repetitive elements (Doolittle and Sapienza 1980; Orgel and Crick 1980). There is now increasing evidence that TEs play an important role in driving and shaping genome evolution (Kazazian 2004). For example, in humans it has been shown that TEs contribute a substantial number of regulatory sequences (Britten 1996; Jordan *et al.* 2003) and form part of the coding sequences of some genes (Britten 2004). There are numerous types of mutations associated with TE insertions. TEs can cause mutations by inserting into the coding regions of genes and disrupting or altering gene function. There are also examples of TEs inserting into the untranslated regions (UTRs) of genes, changing transcript length and stability (Landry *et al.* 2002; Dunn *et al.* 2003; Marsano *et al.* 2005). Insertions of TEs into the regulatory regions of genes can also disrupt or alter gene expression, by introducing regulatory elements such as enhancers (Argeson *et al.* 1996; Conte *et al.* 2002) or insulators (Gdula *et al.* 1996; Conte *et al.* 2002; Parnell *et al.* 2003).

Insecticide resistance is a model for studying evolutionary change, as the selective agent is known (the insecticide), and the response to selection (the evolution of resistance) is usually rapid (McKenzie and Batterham 1994). There has been a long predicted role of TEs in the evolution of insecticide resistance (Wilson 1993). However, it is only recently that resistance mechanisms potentially involving TEs have been identified in natural insect populations (ffrench-Constant *et al.* 2006), where resistance is associated with either the deletion or the inactivation of an insecticide target protein (Gahan *et al.* 2001), the truncation of a gene resulting in the generation of a protein with novel function (Aminetzach *et al.* 2005), or the increased expression of a gene associated with metabolic insecticide resistance (Daborn *et al.* 2002; Schlenke and Begun 2004; Marsano *et al.* 2005). Both for insecticide resistance examples and for other examples of TE-associated insertions, rarely has the effect of the mutation caused by the TE been fully characterized at the molecular level.

In *Drosophila melanogaster*, insecticide resistance mapping to the *DDT-R* locus is due to the overexpression of

[1]*Corresponding author:* Centre for Environmental and Stress Adaptation Research, Bio21 Molecular Science and Biotechnology Institute, Department of Genetics, University of Melbourne, Melbourne, Victoria, 3010, Australia. E-mail: p.batterham@unimelb.edu.au

the cytochrome P450 gene *Cyp6g1* (Daborn *et al.* 2001). In populations collected from different locations around the world, increased expression of *Cyp6g1* is correlated with the presence of the 491-bp long terminal repeat (LTR) of an *Accord* retrotransposon 291 bp upstream from the transcription start site of this gene (Daborn *et al.* 2002; Catania *et al.* 2004). In this study we investigate if the insertion of the *Accord* LTR is responsible for the increased expression of *Cyp6g1*. We characterize the expression pattern of *Cyp6g1* in third instar larvae of strains with and without the *Accord* LTR insertion upstream of *Cyp6g1* and demonstrate that the molecular mechanism of *Cyp6g1* overexpression is due to the *Accord* LTR insertion. We also demonstrate that the *Accord* LTR carries regulatory sequences that change the spatial expression of *Cyp6g1*, resulting in an insecticide resistance phenotype.

MATERIALS AND METHODS

Drosophila strains and insecticide bioassays: All Drosophila strains were maintained on standard medium at 25°. Unless otherwise indicated, all strains were obtained from the Bloomington Drosophila Stock Center (Indiana University). DNA constructs were transformed into the w^{1118} strain using standard techniques (Rubin and Spradling 1982; Spradling and Rubin 1982). The w^{1118} strain does not contain the *Accord* LTR insertion upstream of *Cyp6g1* (data not shown). Expression of *Cyp6g1* in transgenic flies was achieved using the GAL4-UAS system (Brand and Perrimon 1993). UAS-*Cyp6g1* strains (Daborn *et al.* 2002) were generated by cloning the open reading frame of *Cyp6g1* from the Canton-S strain into the p*UAST* vector. Independent transformants in a w^{1118} background were then isolated using standard techniques. The *6g1*HR-*GAL4*-6c strain (generated and characterized in this study) carries 1608 bp to the translational start of *Cyp6g1* from the Hikone-R strain cloned upstream of GAL4 and inserted onto chromosome III. This strain expresses GAL4 in the gastric cecum, midgut, Malpighian tubules, and fat body of larvae and was used to drive *Cyp6g1* in these tissues in crosses to UAS-*Cyp6g1* strains. Insecticide resistance bioassays using dichloro–diphenyl–trichloroethane (DDT), dicyclanil, and nitenpyram were performed using standard techniques (Daborn *et al.* 2001; Pyke *et al.* 2004; Magoc *et al.* 2005). Survival to adulthood from five replicates of 50 larvae (first instar) per vial was used to assay for resistance to nitenpyram and dicyclanil. For DDT, five replicates of 20 adult females (4 days posteclosion) were used in a 24-hr contact assay (Daborn *et al.* 2001).

***In situ* hybridization:** The open reading frame (ORF) of *Cyp6g1* was amplified from *y; cn bw sp* cDNA by PCR using *Taq* polymerase (Promega, Madison, WI) and the primers ORF6g1F (CGA CAG CGG CCG CAT GGT GTT GAC CAG GT) and ORF6g1R (GCG ATT CTA GAT CAT TGG AGC GAT GGA CC). The resulting PCR product was cloned into *pGEM-T Easy* (Promega) in both sense and antisense orientations with respect to the T7 RNA polymerase transcription initiation site. Plasmid constructs were then linearized with *Sal*I and digoxigenin labeled using Megascript T7 polymerase (Ambion) and DIG-labeled dNTP mix (Roche, Indianapolis) following the manufacturer's instructions. The final concentration and purity of probes was determined by UV spectrophotometry and agarose gel electrophoresis. Third instar larvae were dissected in PBS and fixed in 8% paraformaldehyde. *In situ* hybridization was performed as previously described (Tautz and Pfeifle 1989).

Real-time PCR: Malpighian tubules, midgut, and fat bodies were hand dissected from 200 feeding and wandering third instar larvae. RNA isolation and real-time PCR were performed as previously described (Bogwitz *et al.* 2005). PCR primers used were RpL11F (CGA TCC CTC CAT CGG TAT CT) and RpL11R (AAC CAC TTC ATG GCA TCC TC) for *RpL11* and Cyp6g1aF (GCC CGC TGC GAT CCC CAT) and Cyp6g1aR (CCT TTC CAA TCT CCT GCA TA) for *Cyp6g1*.

Reporter constructs: Transgenic strains carrying *Cyp6g1* promoter regions in front of GAL4 were generated from different strains to determine if differences in *Cyp6g1* expression between strains were the result of differences in *Cyp6g1* promoter DNA sequence. DNA from insecticide-resistant *Accord* LTR carrying strain Hikone-R and two different insecticide-susceptible strains not carrying the *Accord* LTR insertion (Canton-S and *y; cn bw sp*) were used in this study. The upstream region of *Cyp6g1* consisting of 1608 bp to the translational start site was PCR amplified from Hikone-R using the Expand High-Fidelity PCR system (Roche) and the primers MBF1 (ATT TGA TCC CGT CAT TTC GCC) and 5p2R (TTT GGG GAT GTC GAT GTA ATG). The equivalent 1200- and 1197-bp fragments were amplified from Canton-S and *y; cn bw sp*, respectively, using the same primers. The Hikone-R and Canton-S fragments were cloned into the plasmid *pGEM-T Easy* (Promega) and subcloned into *pBC SK–* (Stratagene, La Jolla, CA), using the *Eco*RI restriction sites. The *GAL4* gene from *pGATB* (gift from Lucy Cherbus, Indiana University) was ligated into *pBC SK–* containing the promoter fragment via the *Bam*HI and *Not*I restriction sites. The promoter fragment and the *GAL4* gene were then ligated into the Drosophila transformation vector *pW8*, using the *Kpn*I and *Not*I restriction sites. The 1197-bp upstream region of *Cyp6g1* from *y; cn bw sp* was also cloned directly in front of *GFP.nls* in the *pStinger* vector, using the *Eco*RI restriction site (Barolo *et al.* 2000).

To determine if the *Accord* LTR by itself could drive *Cyp6g1* expression, the 491-bp *Accord* LTR was PCR amplified from the Hikone-R strain, using primers accordF (CGT GAG TTA CGG GTG CCT CCG) and accordR (AGT TAC CAT GCC CAG CAT TAA C). This fragment was cloned into the *pGEM-T Easy* vector (Promega), sequenced, and subcloned in both directions into *pH-Stinger*, which contains a minimal *Hsp70* promoter, and *pStinger*, which does not contain a minimal *Hsp70* promoter (Barolo *et al.* 2000). Final constructs were microinjected (0.5 μg/μl) into <1-hr-old embryos of the w^{1118} strain, along with a Δ2–3 transposase source (0.1 μg/μl) as per standard procedures (Rubin and Spradling 1982; Spradling and Rubin 1982). The w^{1118} strain does not contain the *Accord* LTR insertion upstream of *Cyp6g1* (data not shown). Independent transformed lines were made homozygous and the inserted construct was mapped to a chromosome using the *w; If/CyO; MKRS/TM6b,Tb* strain (gift from G. Hime, University of Melbourne). Expression in at least three independent transformants was compared. All GFP images were captured using the SZX12 stereomicroscope system (Olympus, Lake Success, NY).

5′ RACE: To determine the start site of *Cyp6g1* transcription in strains carrying the *Accord* LTR and wild-type strains, 5′ RACE was conducted using a SMART RACE cDNA amplification kit (Clontech, Palo Alto, CA) and a primer within exon 1 of *Cyp6g1* (CAG TGC GGC GAC CAC CAA AAG AG).

RESULTS

Increased expression of *Cyp6g1* is tissue specific: To determine the spatial distribution of increased expression of *Cyp6g1* in insecticide-resistant flies, *in situ* hybridization of a DIG-labeled antisense *Cyp6g1* probe was

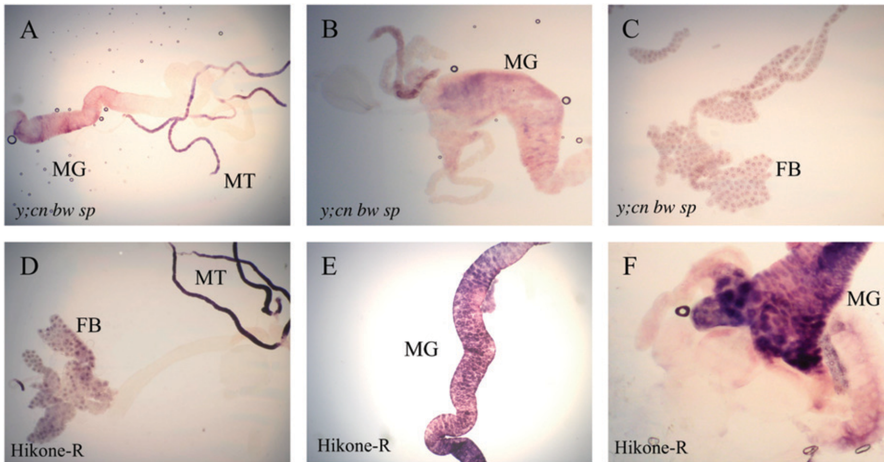

Figure 1.—*In situ* hybridization of *Cyp6g1* antisense digoxgenin-labeled RNA probe on tissues dissected from *y; cn bw sp* and Hikone-R third instar wandering larvae. (A–C) Third instar larvae of *y; cn bw sp*. *Cyp6g1* mRNA is detected in midgut (MG), Malpighian tubules (MT), and fat body (FB). (D–F) Third instar larvae of Hikone-R. *Cyp6g1* mRNA is detected in midgut, Malpighian tubules, and fat body. Staining is more intense in Hikone-R than in *y; cn bw sp*, suggesting that *Cyp6g1* mRNA is present in these tissues at a higher level.

conducted in third instar larvae. In the insecticide-susceptible strain *y; cn bw sp*, *Cyp6g1* mRNA was detected in parts of the midgut and in the Malpighian tubules in early third instar larvae. In wandering third instar larvae, *Cyp6g1* mRNA was detected in parts of the midgut, the Malpighian tubules, and the fat body (Figure 1, A–C). In the insecticide-resistant strain Hikone-R that carries the *Accord* LTR inserted upstream of *Cyp6g1* (Daborn *et al.* 2002), *Cyp6g1* mRNA was detected in the gastric cecum, the entire midgut, the Malpighian tubules, and the fat body in both feeding and wandering third instar larvae (Figure 1, D–F). Additionally, the intensity of staining in these three tissues is higher in Hikone-R when compared to *y; cn bw sp*. *Cyp6g1* mRNA was not detected in the hindgut, salivary glands, central nervous system, or any of the imaginal discs in either of these strains. To quantify the abundance of *Cyp6g1* mRNA in larvae with and without the *Accord* LTR insertion, real-time PCR was performed on RNA isolated from midguts, Malpighian tubules, and fat body dissected from both feeding and wandering third instar larvae. Increases in the abundance of *Cyp6g1* mRNA in the midgut, Malpighian tubules, and the fat body were detected in Hikone-R, when compared to *y; cn bw sp* at a magnitude of ∼10–40 folds in each tissue measured (Figure 2).

Characterization of *Cyp6g1* expression using transgenes: To determine if the 5′ upstream region of *Cyp6g1* contains all the essential elements for the expression of *Cyp6g1* in third instar larvae, transgenic reporter constructs containing the upstream regions of *Cyp6g1* were constructed. In strains driving GFP under the control of the *Cyp6g1* promoter of Canton-S, GFP was detected in sections of the midgut and the Malpighian tubules in both feeding and wandering third instar larvae (Figure 3, A and B). In strains driving GFP under the control of the *Cyp6g1* promoter of *y; cn bw sp*, GFP was detected in parts of the midgut, in the Malpighian tubules in feeding third instar larvae, and additionally in the fat body in late third instar larvae (Figure 3, C and D). Finally, strains driving GFP under the control of the *Cyp6g1* promoter of Hikone-R express GFP in the gastric cecum, the entire midgut, Malpighian tubules, and the fat body in both feeding and wandering third instar larvae (Figure 3, E and F). The intensity of GFP expression was much higher in the Hikone-R-driven strains than in the other two strains. The upstream region of *Cyp6g1* is sufficient to replicate the native expression of *Cyp6g1* in third instar larvae as detected using *in situ* hybridization in both *Accord* (Hikone-R) and non-*Accord* (*y; cn bw sp*) strains and quantified using real-time PCR. This indicates that all of the elements required for *Cyp6g1* expression in third instar larvae are located within 1.2 kb upstream of the translational start.

The *Accord* element carries tissue-specific enhancers: Although there are some single-base differences in the upstream region of *Cyp6g1* between *y; cn bw sp*, Canton-S, and Hikone-R (Daborn *et al.* 2002), by far the biggest difference is the *Accord* LTR insertion in Hikone-R. This motivated our characterization of the effect of the Accord LTR insertion on *Cyp6g1* expression. It has been reported previously that TEs can carry their own promoters that are capable of changing the transcription

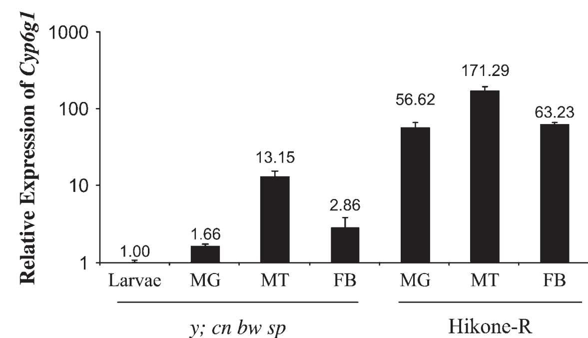

Figure 2.—Real-time PCR on dissected tissues from third instar larvae. All values are relative to *RPL11* and normalized to *y; cn bw sp* whole third instar larvae. An increase in *Cyp6g1* mRNA is detected in the midgut (MG), Malpighian tubules (MT), and the fat body (FB) of Hikone-R. Values are the mean of two replicates ± SEM.

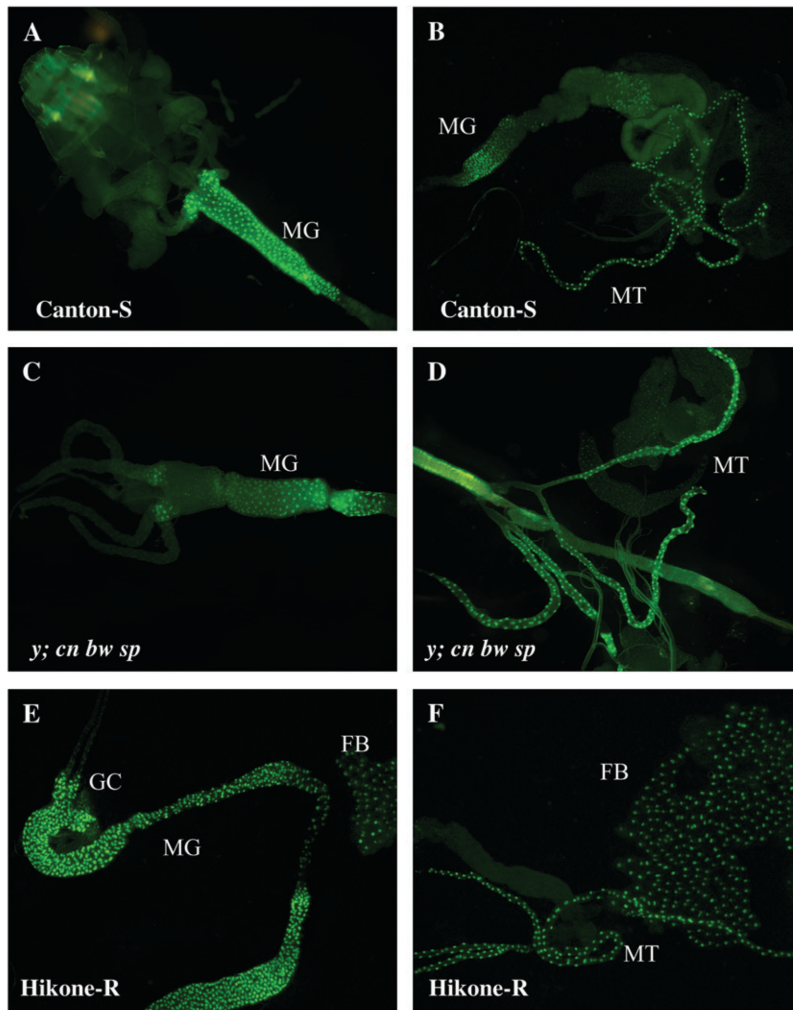

FIGURE 3.—Promoter constructs of *Cyp6g1* from Canton-S, *y; cn bw sp*, and Hikone-R (containing the *Accord* LTR) expressing nuclear-localized GFP replicate the expression pattern seen by *in situ* hybridization. (A and B) Expression of GFP in the midgut (MG) and Malpighian tubules (MT) is detected using the *Cyp6g1* promoter of Canton-S in third instar larvae. (C and D) Expression of GFP in the midgut (MG) and Malpighian tubules (MT) is detected using the *Cyp6g1* promoter of *y;cn bw sp*. Faint expression of GFP in the fat body was detected only in wandering third instar larvae but not feeding third instar larvae. (E and F) Expression of GFP in the MG, MT, gastric caecae (GC), and the fat body (FB) is detected throughout the third instar larval stage when GFP is driven by the promoter of Hikone-R (containing the *Accord* LTR).

start site of the genes adjacent to the TE insertion point (LANDRY *et al.* 2002; DUNN *et al.* 2003). The presence or absence of the *Accord* LTR upstream of *Cyp6g1* did not alter the transcription start site of *Cyp6g1* as determined by 5′ RACE (Figure 4). Some studies have shown that TEs can alter the spatial expression of genes they insert near either by disrupting regulatory elements (LERMAN and FEDER 2004) or by carrying their own enhancers (CONTE *et al.* 2002). To determine if the *Accord* LTR itself carries an enhancer, we cloned the 491-bp *Accord* LTR into *pH-Stinger*, which contains a *Hsp70* minimal promoter driving nuclear-localized GFP (BAROLO *et al.* 2000), and transformed w^{1118} flies with this construct. The expression pattern was the same as that in strains carrying the *Accord* insertion upstream of *Cyp6g1*, *i.e.*, expression in the gastric cecum, the entire midgut, Malpighian tubules, and the fat body throughout third instar (Figure 5). This suggests that the *Accord* LTR carries its own tissue-specific enhancer(s) capable of expressing *Cyp6g1* in these tissues. No GFP expression was detected when the *Accord* LTR was cloned into *p-Stinger*, which does not contain the *Hsp70* minimal promoter (data not shown).

Expression of *Cyp6g1* in the 5′ *6g1*-Hikone-R pattern results in insecticide resistance: It has previously been shown that the increased expression of *Cyp6g1* in *D. melanogaster* results in resistance to multiple insecticides (DABORN *et al.* 2001) and that driving the expression of *Cyp6g1* ubiquitously using the *GAL4*/UAS system results in insecticide resistance (DABORN *et al.* 2002; LE GOFF *et al.* 2003). These studies have made use of *GAL4* drivers with a ubiquitous expression pattern, not the exact expression pattern found in resistant strains. To formally link the *Accord* LTR insertion allele with insecticide resistance, we have used the *6g1*HR-GAL4-6c strain to express *Cyp6g1* in the same tissues as those in strains

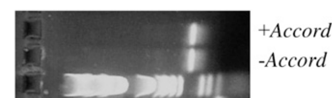

FIGURE 4.—5′ RACE performed on *Cyp6g1* in flies with and without the *Accord* LTR shows identical-sized products. This indicates that the *Accord* LTR does not change the transcription start site of *Cyp6g1*.

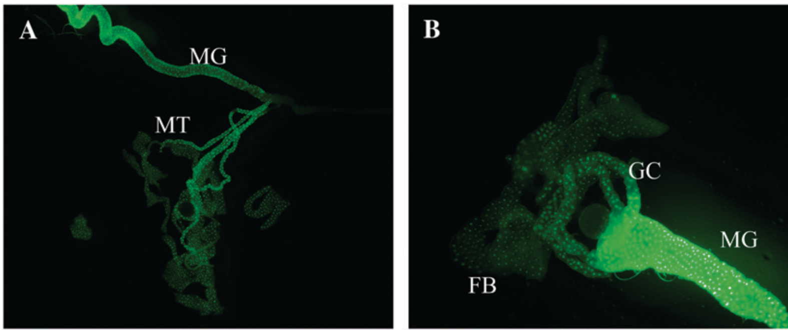

Figure 5.—Expression pattern of GFP driven by the 491-bp *Accord* LTR placed in front of the *Hsp70* minimal promoter. (A) Expression of GFP is seen in the midgut (MG), Malpighian tubules (MT), and (B) fat bodies (FB) and gastric caecae (GC), indicating that the *Accord* LTR carries *cis*-regulatory sequences for expression in these tissues. No GFP expression was detected when the *Hsp70* minimal promoter is not present (data not shown).

carrying the *Accord* LTR insertion allele. Expressing *Cyp6g1* in these tissues does result in resistance for the three insecticides tested (Figure 6), confirming that the *Accord* LTR insertion upstream of *Cyp6g1* is sufficient to confer insecticide resistance. Resistance is seen at both larval and adult life stages. We have presented a detailed characterization of *Cyp6g1* expression in larvae and the effect of the *Accord* LTR. *Cyp6g1* expression in adults is increased in *Accord*-containing strains (Daborn *et al.* 2002). Both the *6g1*HR-GAL4-6c and the *pH-Stinger-Accord* LTR constructs drive gene expression in similar tissues in adults as in larvae (data not shown), which probably accounts for resistance to DDT at adult life stages.

DISCUSSION

In this study, we have documented the native expression pattern of the insecticide resistance gene *Cyp6g1* in third instar larvae by *in situ* hybridization and showed using transgenic constructs that all *cis*-regulatory elements driving the expression of this gene are located within 1.2 kb of its promoter. We also demonstrated that increased expression of *Cyp6g1*, which leads to insecticide resistance, is due to the presence of the *Accord* LTR insertion upstream of *Cyp6g1*. The *Accord* LTR does not change the transcription start site of *Cyp6g1* but instead carries tissue-specific enhancers that result in *Cyp6g1* expression in the Drosophila gastric cecum, midgut, Malpighian tubules, and fat body.

There are an increasing number of examples of TEs contributing to shape the genomes of many organisms (Brookfield 2003; Kazazian 2004), possibly creating new functions for genes (Aminetzach *et al.* 2005), or contributing novel regulatory sequences (Jordan *et al.* 2003). The *Accord* LTR insertion allele of *Cyp6g1* has swept to a high frequency in *D. melanogaster* populations around the world (Catania *et al.* 2004), indicating that it confers an adaptive phenotype to its host. Our finding that the *Accord* LTR contains tissue-specific enhancers that function within the host genome is of significance, as there are very few examples where the molecular consequences of TE insertions have been studied in such detail.

In insects, evidence of retrotransposon-derived enhancer sequences that independently regulate the expression of endogenous genes in a tissue-specific manner is limited. One example is the *ZAM* retrotransposon from *D. melanogaster*. *Cis*-acting regulatory sequences in the 5′-untranslated region of *ZAM* were shown to increase transcription of the *white* gene (Conte *et al.* 2002). It is not known if these regulatory sequences are tissue specific, however, as the adult eye was the only tissue where the expression of *white* was characterized. In another study, an enhancer found in a *17.6* element in *D. melanogaster* has been characterized that directs the expression in the eye lamina (Mozer and Benzer 1994). This element, however, is not known to regulate the expression of any gene. We provide an example where not only does the TE provide tissue-specific regulatory elements, but these elements actually contribute to a selected phenotype in natural populations, due to its effect on the insecticide resistance-conferring gene, *Cyp6g1*. The regulatory elements found within the *Accord* LTR actually independently drive expression in similar tissues to the endogenous *Cyp6g1* gene.

TEs have been shown to adapt to the host genome by evolving sequences within them that provide novel regulatory sequences. This is not the case for the *Accord* LTR in this study. The *Accord* LTR sequence upstream of *Cyp6g1* is identical to the LTR of the canonical *Accord* elements present in the *y; cn bw sp* strain (Kaminker *et al.* 2002). It is therefore likely that the *Accord* LTR intrinsically carries the regulatory sequences to confer the tissue-specific expression. Other TEs have also been shown to carry tissue-specific enhancers in their LTR or 5′-UTR (Pi *et al.* 2004; Ruda *et al.* 2004). Enhancer sequences are presumably involved in the expression of TEs in temporal and tissue-specific patterns as shown by the BDGP embryonic *in situ* hybridization project (Tomancak *et al.* 2002). The fact that the *Accord* LTR carries tissue-specific enhancers for the midgut, Malpighian tubules, and the fat body and has inserted upstream of a gene capable of detoxifying insecticides makes this story remarkable. These tissues play major roles in toxin metabolism and excretion (Hoshizaki 2005; Dow and Davies 2006), although little work has

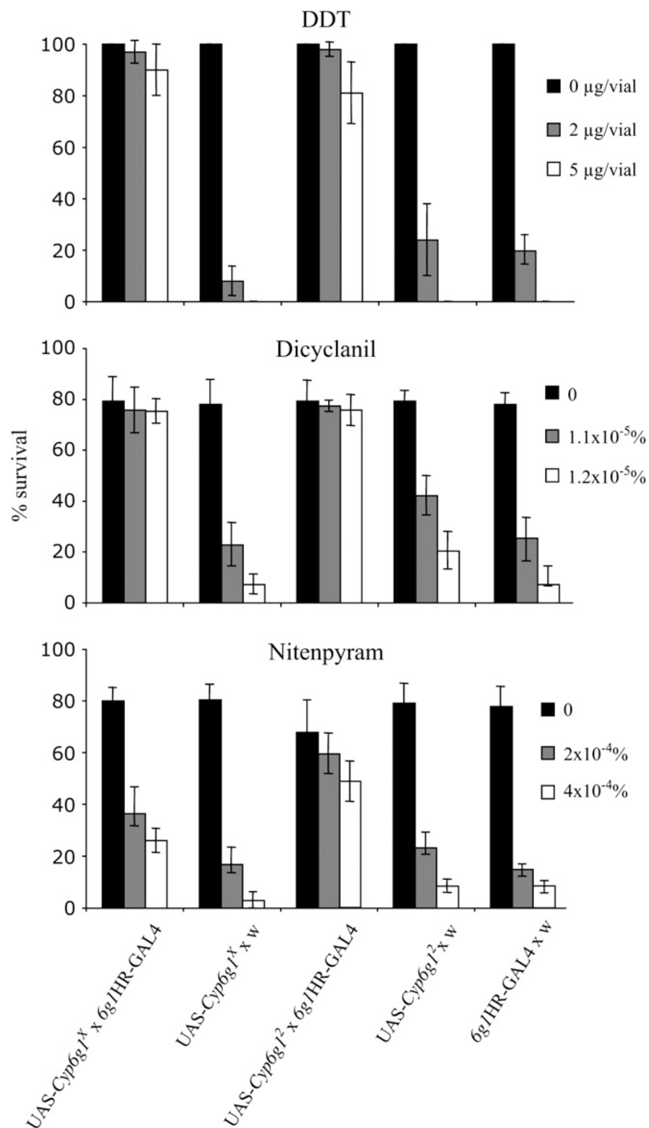

Figure 6.—Percentage of survival on discriminating concentrations of the insecticides DDT, dicyclanil, and nitenpyram in two independent *UAS-Cyp6g1* lines (*UAS-Cyp6g1^X* where the insert maps to the X chromosome and *UAS-Cyp6g1^2* where the insert maps to the second chromosome). *Cyp6g1* is driven in the midgut, Malpighian tubules, and the fat body using the *6g1HR-GAL4-6c* strain generated in this study. Increased survival is not seen in controls that do not drive the tissue-specific expression of *Cyp6g1* (UAS-Cyp6g1 × w and w × *6g1HR-GAL4-6c*). Values are the mean of five replicates ± SEM.

so far been published showing these tissues to directly metabolize insecticides (Suchail *et al.* 2004).

D. melanogaster is not the only species where a TE has inserted upstream of *Cyp6g1*. In a striking example of parallel evolution, a *Doc* element has inserted into the upstream region of the *D. simulans Cyp6g1* ortholog and is associated with the overexpression of this gene (Schlenke and Begun 2004). The exact mechanism of *D. simulans Cyp6g1* overexpression has thus far not been characterized, although it is likely that *Doc*-associated sequences affect the expression of the gene. This highlights the importance of increased or tissue-specific expression of *Cyp6g1* in providing a selective advantage in natural Drosophila populations and the potential importance of TEs in rapidly providing the suitable molecular changes upon which selection can act.

A recent study has shown that some retrotransposon insertions associated with specific genes are fixed in diverse populations of *D. melanogaster* (Franchini *et al.* 2004). There is evidence that the *Accord* allele has rapidly swept to high frequencies in *D. melanogaster* populations around the world (Daborn *et al.* 2002; Catania *et al.* 2004). It is not clear exactly what has driven this sweep, although selection by insecticides is possible. Our data indicate that the *Accord* insertion confers resistance to a broad spectrum of widely used chemical insecticides. Drnevich *et al.* (2004) present evidence indicating that there may be a fitness cost in terms of male reproductive success under competitive conditions in the laboratory. Chemical selection could provide a cogent explanation for the observed sweep of the *Accord* allele even in the face of selective disadvantage in the field. McCart *et al.* (2005) have examined a range of life history traits and reported a female reproductive advantage associated with the *Accord* allele. This advantage may therefore have some capacity to explain the selective sweep as an alternative hypothesis to chemical selection. Clearly further analysis of the fitness of *Cyp6g1* genotypes in the field is required, as is a thorough investigation of other possible functions of *Cyp6g1*. This example indicates that the detailed analysis of other retrotransposon insertions at high frequencies in natural populations may be fruitful in investigating the molecular basis of adaptation.

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LITERATURE CITED

Aminetzach, Y. T., J. M. Macpherson and D. A. Petrov, 2005 Pesticide resistance via transposition-mediated adaptive gene truncation in *Drosophila*. Science **309:** 764–767.

Argeson, A. C., K. K. Nelson and L. D. Siracusa, 1996 Molecular basis of the pleiotropic phenotype of mice carrying the hypervariable yellow (*Ahvy*) mutation at the *agouti* locus. Genetics **142:** 557–567.

Barolo, S., L. A. Carver and J. W. Posakony, 2000 GFP and beta-galactosidase transformation vectors for promoter/enhancer analysis in *Drosophila*. Biotechniques **29:** 726, 728, 730, 732.

Bogwitz, M. R., H. Chung, L. Magoc, S. Rigby, W. Wong *et al.*, 2005 *Cyp12a4* confers lufenuron resistance in a natural population of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **102:** 12807–12812.

Brand, A. H., and N. Perrimon, 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development **118:** 401–415.

Britten, R. J., 1996 Cases of ancient mobile element DNA insertions that now affect gene regulation. Mol. Phylogenet. Evol. **5:** 13–17.

Britten, R. J., 2004 Coding sequences of functioning human genes derived entirely from mobile element sequences. Proc. Natl. Acad. Sci. USA **101:** 16825–16830.

Brookfield, J. F., 2003 Mobile DNAs: the poacher turned gamekeeper. Curr. Biol. **13:** R846–R847.

Catania, F., M. O. Kauer, P. J. Daborn, J. L. Yen, R. H. ffrench-Constant *et al.*, 2004 World-wide survey of an *Accord* insertion and its association with DDT resistance in *Drosophila melanogaster.* Mol. Ecol. **13:** 2491–2504.

Conte, C., B. Dastugue and C. Vaury, 2002 Coupling of enhancer and insulator properties identified in two retrotransposons modulates their mutagenic impact on nearby genes. Mol. Cell. Biol. **22:** 1767–1777.

Daborn, P., S. Boundy, J. Yen, B. Pittendrigh and R. ffrench-Constant, 2001 DDT resistance in *Drosophila* correlates with *Cyp6g1* over-expression and confers cross-resistance to the neonicotinoid imidacloprid. Mol. Genet. Genomics **266:** 556–563.

Daborn, P. J., J. L. Yen, M. R. Bogwitz, G. Le Goff, E. Feil *et al.*, 2002 A single p450 allele associated with insecticide resistance in *Drosophila.* Science **297:** 2253–2256.

Doolittle, W. F., and C. Sapienza, 1980 Selfish genes, the phenotype paradigm and genome evolution. Nature **284:** 601–603.

Dow, J. A., and S. A. Davies, 2006 The Malpighian tubule: rapid insights from post-genomic biology. J. Insect. Physiol. **52:** 365–378.

Drnevich, J. M., M. M. Reedy, E. A. Ruedi, S. Rodriguez-Zas and K. A. Hughes, 2004 Quantitative evolutionary genomics: differential gene expression and male reproductive success in *Drosophila melanogaster.* Proc. Biol. Sci. **271:** 2267–2273.

Dunn, C. A., P. Medstrand and D. L. Mager, 2003 An endogenous retroviral long terminal repeat is the dominant promoter for human *beta1,3-galactosyltransferase 5* in the colon. Proc. Natl. Acad. Sci. USA **100:** 12841–12846.

ffrench-Constant, R., P. Daborn and R. Feyereisen, 2006 Resistance and the jumping gene. BioEssays **28:** 6–8.

Franchini, L. F., E. W. Ganko and J. F. McDonald, 2004 Retrotransposon-gene associations are widespread among *D. melanogaster* populations. Mol. Biol. Evol. **21:** 1323–1331.

Gahan, L. J., F. Gould and D. G. Heckel, 2001 Identification of a gene associated with Bt resistance in *Heliothis virescens.* Science **293:** 857–860.

Gdula, D. A., T. I. Gerasimova and V. G. Corces, 1996 Genetic and molecular analysis of the *gypsy* chromatin insulator of *Drosophila.* Proc. Natl. Acad. Sci. USA **93:** 9378–9383.

Hoshizaki, D. K., 2005 Fat-cell development, pp. 315–345 in *Comprehensive Molecular Insect Science*, edited by L. I. Gilbert, K. Iatrou and S. S. Gill. Elsevier, Amsterdam/New York.

Jordan, I. K., I. B. Rogozin, G. V. Glazko and E. V. Koonin, 2003 Origin of a substantial fraction of human regulatory sequences from transposable elements. Trends Genet. **19:** 68–72.

Kaminker, J. S., C. M. Bergman, B. Kronmiller, J. Carlson, R. Svirskas *et al.*, 2002 The transposable elements of the *Drosophila melanogaster* euchromatin: a genomics perspective. Genome Biol. **3:** RESEARCH0084.

Kazazian, Jr., H. H., 2004 Mobile elements: drivers of genome evolution. Science **303:** 1626–1632.

Landry, J. R., A. Rouhi, P. Medstrand and D. L. Mager, 2002 The Opitz syndrome gene *Mid1* is transcribed from a human endogenous retroviral promoter. Mol. Biol. Evol. **19:** 1934–1942.

Le Goff, G., S. Boundy, P. J. Daborn, J. L. Yen, L. Sofer *et al.*, 2003 Microarray analysis of cytochrome P450 mediated insecticide resistance in *Drosophila.* Insect Biochem. Mol. Biol. **33:** 701–708.

Lerman, D. N., and M. E. Feder, 2004 Naturally occurring transposable elements disrupt *hsp70* promoter function in *Drosophila melanogaster.* Mol. Biol. Evol. **22**(3): 776–783. (erratum: Mol. Biol. Evol. **22**(4): 1160).

Magoc, L., J. L. Yen, A. Hill-Williams, J. A. McKenzie, P. Batterham *et al.*, 2005 Cross-resistance to dicyclanil in cyromazine-resistant mutants of *Drosophila melanogaster* and *Lucilia cuprina.* Pestic. Biochem. Physiol. **81:** 129–135.

Marsano, R. M., R. Caizzi, R. Moschetti and N. Junakovic, 2005 Evidence for a functional interaction between the *Bari1* transposable element and the cytochrome P450 *Cyp12a4* gene in *Drosophila melanogaster.* Gene **357:** 122–128.

McCart, C., A. Buckling and R. H. ffrench-Constant, 2005 DDT resistance in flies carries no cost. Curr. Biol. **15:** R587–589.

McKenzie, J. A., and P. Batterham, 1994 The genetic, molecular and phenotypic consequences of selection for insecticide resistance. Trends Ecol. Evol. **9:** 166–169.

Mozer, B. A., and S. Benzer, 1994 Ingrowth by photoreceptor axons induces transcription of a retrotransposon in the developing *Drosophila* brain. Development **120:** 1049–1058.

Orgel, L. E., and F. H. Crick, 1980 Selfish DNA: the ultimate parasite. Nature **284:** 604–607.

Parnell, T. J., M. M. Viering, A. Skjesol, C. Helou, E. J. Kuhn *et al.*, 2003 An endogenous suppressor of hairy-wing insulator separates regulatory domains in *Drosophila.* Proc. Natl. Acad. Sci. USA **100:** 13436–13441.

Pi, W., Z. Yang, J. Wang, L. Ruan, X. Yu *et al.*, 2004 The LTR enhancer of *ERV-9* human endogenous retrovirus is active in oocytes and progenitor cells in transgenic zebrafish and humans. Proc. Natl. Acad. Sci. USA **101:** 805–810.

Pyke, F. M., M. R. Bogwitz, T. Perry, A. Monk, P. Batterham *et al.*, 2004 The genetic basis of resistance to diazinon in natural populations of *Drosophila melanogaster.* Genetica **121:** 13–24.

Rubin, G. M., and A. C. Spradling, 1982 Genetic transformation of *Drosophila* with transposable element vectors. Science **218:** 348–353.

Ruda, V. M., S. B. Akopov, D. O. Trubetskoy, N. L. Manuylov, A. S. Vetchinova *et al.*, 2004 Tissue specificity of enhancer and promoter activities of a HERV-K(HML-2) LTR. Virus Res. **104:** 11–16.

Schlenke, T. A., and D. J. Begun, 2004 Strong selective sweep associated with a transposon insertion in *Drosophila simulans.* Proc. Natl. Acad. Sci. USA **101:** 1626–1631.

Spradling, A. C., and G. M. Rubin, 1982 Transposition of cloned *P* elements into *Drosophila* germ line chromosomes. Science **218:** 341–347.

Suchail, S., G. De Sousa, R. Rahmani and L. P. Belzunces, 2004 In vivo distribution and metabolisation of 14C-imidacloprid in different compartments of *Apis mellifera* L. Pest Manag. Sci. **60:** 1056–1062.

Tautz, D., and C. Pfeifle, 1989 A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback.* Chromosoma **98:** 81–85.

Tomancak, P., A. Beaton, R. Weiszmann, E. Kwan, S. Shu *et al.*, 2002 Systematic determination of patterns of gene expression during *Drosophila* embryogenesis. Genome Biol. **3:** 1–14.

Wilson, T. G., 1993 Transposable elements as initiators of insecticide resistance. J. Econ. Entomol. **86:** 645–651.

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