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Carboxylesterase genes in pyrethroid resistant house flies, *Musca domestica*

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ABSTRACT

Carboxylesterases are one of the major enzyme families involved in the detoxification of pyrethroids. Up-regulation of carboxylesterase genes is thought to be a major component of insecticide resistant mechanisms in insects. Based on the house fly transcriptome and genome database, a total of 39 carboxylesterase genes of different functional clades have been identified in house flies. In this study, eleven of these genes were found to be significantly overexpressed in the resistant ALHF house fly strain compared with susceptible aabys and wild-type CS strains. Eight up-regulated carboxylesterase genes with their expression levels were further induced to a higher level in response to permethrin treatments, indicating that constitutive and inductive overexpression of carboxylesterases are co-responsible for the enhanced detoxification of insecticides. Spatial expression studies revealed these up-regulated genes to be abundantly distributed in fat bodies and genetically mapped on autosome 2 or 3 of house flies, and their expression could be regulated by factors on autosome 1, 2 and 5. Taken together, these results demonstrate that multiple carboxylesterase genes are co-upregulated in resistant house flies, providing further evidence for their involvement in the detoxification of insecticides and development of insecticide resistance.

1. Introduction

Carboxylesterases (COEs) represents a multigene superfamily that is widely distributed in insects (Montella et al., 2012), mammals (Satoh and Hosokawa, 1998), plants (Marshall et al., 2003) and microbes (Bornscheuer, 2002), playing a major role in hydrolyzing a broad range of ester-containing xenobiotics, including drugs, environmental toxicants, insecticides and carcinogens. In insects, COEs are of particular interest due to their roles in metabolizing insecticides (Wheelock et al., 2005; Zhang et al., 2010; Ranson et al., 2002; Sogorb and Vilanova, 2002; Hemingway and Karunaratne, 1998; Devonshire and Moores, 1982; Farnsworth et al., 2010). Qualitative changes (mutations occurring in the active sites of COEs) and quantitative changes (overexpressed COE genes and enhanced COE activities) resulting from constitutive gene amplification or transcriptional up-regulation of COEs are the predominant mechanisms implicated in the development of insecticide resistance in insects (Hemingway et al., 2004; Devonshire et al., 1986; Li et al., 2007; Zhang et al., 2010). The overexpression of carboxylesterases has been detected in many resistant insect species, including *Aphis gossypii*, *Culex quinquefasciatus*, *Bemisia tabaci*, *Myzus persicae*, *Musca domestica*, *Boophilus microplus*, *Aedes aegypti* and *Helicoverpa armigera* (Cao et al., 2008; Vaughan and Hemingway, 1995; Alon et al., 2008; Foster et al., 2003; Zhang et al., 2010; Hernandez et al., 2002; Poupardin et al., 2014; Wu et al., 2011), indicating that the COE-mediated metabolism due to constitutive gene overexpression plays a key role in governing increased levels of detoxification in insecticides, thus conferring insecticide resistance. In addition, the induction of carboxylesterases by insecticides is of considerable importance in the increased metabolic detoxification of insecticides in insect species such as *Anopheles gambiae* (Vontas et al., 2005), *Aedes aegypti* (Poupardin et al., 2008), *Tetranychus cinnabarinus* (Wei et al., 2016) and *Leptinotarsa decemlineate* (Lü et al., 2015). Both constitutive and inductive overexpressions of COEs are thought to be responsible for the increased levels of detoxification of insecticides.

The house fly, *M. domestica*, is a major cosmopolitan pest that is

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capable of transmitting more than 100 human and animal intestinal diseases, including major illnesses such as cholera, typhoid fever, salmonellosis and polio (Hewitt, 2011; Abbas et al., 2014; Scott et al., 2014). Although insecticides from the pyrethroid family, especially permethrin, are currently widely applied to control house flies, their extensive application is known to lead to resistance issues in insects (Liu and Yue, 2001; Kaufman et al., 2010; Scott et al., 2013; Scott, 2017). In a previous study, our group reported that house flies can and do develop resistance and cross-resistance to pyrethroids, organophosphates as well as some relatively new insecticides such as fipronil, imidacloprid and spinosad (Liu and Yue, 2000). Now that a transcriptome and genome database for the house fly *M. domestica* has become available (Li et al., 2013; Scott et al., 2014), a total of 39 COE genes have been identified and their constitutive and inductive expression profiles were compared in different resistant and susceptible populations to demonstrate their involvement in detoxifying insecticides and conferring insecticide resistance in house flies. In the current study, which was designed to decipher and understand the importance of COE genes in insecticide resistance, we classified and annotated COE genes from the house fly genome by constructing a phylogenic tree with those genes from other insect species, characterizing the expression profiles of COEs in resistant and susceptible house flies, investigating the spatial expression patterns of the COE genes, and examining COE gene expression in response to a permethrin challenge, as well as genetically mapping the COE genes in house flies.

2. Material and methods

2.1. House flies

Three house fly strains were used in this study. ALHF, a highly insecticide resistant strain, was originally collected from a poultry farm in Alabama. This strain exhibited a high level of resistance after subsequent selection with permethrin for six generations and has been annually selected with permethrin to maintain its highly resistant status (Liu and Yue, 2000; Tian et al., 2011). The first of the insecticide susceptible strains used in this study, aabys, bears five recessive morphological markers: ali-curve (*ac*), aristapedia (*ar*), brown body (*bwb*), yellow eyes (*ye*) and snipped wings (*snp*) located on autosomes 1, 2, 3, 4 and 5, respectively. The second, CS, is a wild type susceptible strain bearing the same phenotype as ALHF. The aabys and CS strains were originally obtained from Dr. J. G. Scott (Cornell University). All house flies were reared at 25 ± 2 °C under a photoperiod of 12:12 h (L: D), and fed with sugar and water.

A genetic cross of ALHF females with aabys males was performed. The F1 males (~400 flies) were then backcrossed with aabys females. Five back-cross (BC_1) lines with the following genotypes were isolated: *ac*/*ac*, +/*ar*, +/*bwb*, +/*ye*, +/*snp*; +/*ac*, *ar*/*ar*, +/*bwb*, +/*ye*, +/*snp*; +/*ac*, +/*ar*, *bwb*/*bwb*, +/*ye*, +/*snp*; +/*ac*, +/*ar*, +/*bwb*, *ye*/*ye*, +/*snp*; and +/*ac*, +/*ar*, +/*bwb*, +/*ye*, *snp*/*snp* (Li et al., 2013). Homozygous house fly lines *ac*/*ac*, +/+, +/+, +/+, +/+ (A2345); +/+, *ar*/*ar*, +/+, +/+,+/+ (A1345); (+/+, +/+, *bwb*/*bwb*, +/+, +/+ (A1245); +/+, +/+, +/+, *ye*/*ye*, +/+ (A1235) and +/+, +/+, +/+, +/+, *snp*/*snp* (A1234) were generated by sorting for appropriate phenotypic markers and selecting with permethrin at corresponding doses causing ~70% mortality for each of the lines for three generations. One hundred single-pair crossings of each of the lines for the desired phenotype and genotype were then set up for each line (Liu and Yue, 2000; Tian et al., 2011). The name of each line indicates which of its autosomes bear wild-type markers from ALHF. For instance, the A2345 strain has wild-type markers on autosomes 2, 3, 4 and 5 from ALHF, with a mutant marker on autosome 1 from aabys.

2.2. Phylogenetic analysis of M. domestica carboxylesterase genes

To comprehensively classify and annotate the carboxylesterases in *M. domestica*, a phylogeny tree was created based on the COEs of *M. domestica* extracted from the first adult whole transcriptome database and genome database (https://www.ncbi.nlm.nih.gov/assembly/GCF_000371365.1/) (Li et al., 2013; Scott et al., 2014), and the COEs of *An. gambiae* and *D. melanogaster*, downloaded from their respective genome databases (https://www.ncbi.nlm.nih.gov/genome/?term=Drosophila+melanogaster) and (https://www.ncbi.nlm.nih.gov/genome/?term=Anopheles+Gambiae). The repertoires of COEs of all the species used in the present project were aligned using Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/) before constructing the phylogeny tree using FastTree utilizing the default settings (Price et al., 2009). An approximate-maximum-likelihood phylogeny tree was constructed based on the JTT model of amino acid evolution and adopting a Bayesian approach (Drummond and Rambaut, 2007) to compute the local support values. The phylogeny trees were run on MEGA6.0 for visualization (Tamura et al., 2013).

2.3. Bioassay

An insecticide bioassay was conducted on each of the strains by dropping 0.5 μl of permethrin (dissolved in acetone) at a range of concentrations on the thoracic notum of 2-day old female house flies (Liu and Yue, 2000; Tian et al., 2011). Twenty flies were tested per dose with a total of 5–6 doses designed to produce >0% and <100% mortality. Control groups received acetone alone. Treated flies were reared in a paper box and fed with 15% sugar water. Mortality was assessed after 24 h and any flies that did not move were scored as dead. All tests were performed at room temperature (25 ± 2 °C). Three replications were prepared with house flies emerging on different days for each of three consecutive generations. Bioassay data were pooled and analyzed by PROBIT analysis in SPSS. Statistical analyses of LD_{50} values were based on non-overlapping 95% confidence intervals.

2.4. Permethrin treatment

In this study, two different permethrin treatment experiments were conducted: 1) Hundreds of female house flies of each strain were treated with permethrin at their corresponding LD_{10}, LD_{50} and LD_{90} doses and the surviving flies (20 flies/treatment) were collected for RNA extraction after 24 h treatment with permethrin; and 2) Hundreds of female house flies of each strain were treated with permethrin at their corresponding LD_{50} dose and the surviving flies (20 flies/treatment) were collected 12 h, 24 h, 48 h and 72 h after permethrin treatment for RNA extraction. Control groups treated with acetone only (with no exposure to permethrin) were collected at the same time points as their permethrin treated counterparts. Three replications with different preparations were conducted.

2.5. RNA extraction and cDNA preparation

Twenty 2-day old virgin female house flies of each strain (ALHF, aabys and CS) and 20 surviving flies with permethrin treatment at different permethrin doses (LD_{10}, LD_{50} and LD_{90}) and time points (12 h, 24 h, 48 h and 72 h) and their respective counterparts (acetone treated only) were collected and total RNA was extracted using the acidic guanidine thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). The DNA was removed from total RNA using DNase (TURBO DNA-free, Ambion), after which the DNA-free RNA (500 ng per sample) was reverse-transcribed to cDNA