Insect science

Insect Science (2018) 25, 928–945, DOI 10.1111/1744-7917.12498

ORIGINAL ARTICLE

High throughput sequencing reveals *Drosophila suzukii* responses to insecticides

Ruchir Mishra[1], Joanna C. Chiu[2], Gang Hua[1], Nilesh R. Tawari[4], Michael J. Adang[1,3] and Ashfaq A. Sial[1]

[1]*Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, Athens, Georgia, USA;* [2]*Department of Entomology and Nematology, College of Agricultural and Environmental Sciences, University of California, Davis, California, USA;* [3]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia, USA and* [4]*Computational and Systems Biology, Genome Institute of Singapore, Singapore*

Abstract Global climate change and acquired resistance to insecticides are threats to world food security. *Drosophila suzukii*, a devastating invasive pest in many parts of the world, causes substantial economic losses to fruit production industries, forcing farmers to apply broad-spectrum insecticides frequently. This could lead to the development of insecticide resistance. We determined the Lethal Concentration 50 (median lethal concentration, LC_{50}) values of zeta-cypermethrin, spinosad, and malathion insecticides against *D. suzukii* colonies established from Clarke and Pierce county Georgia, United States. The LC_{50} values were 3 fold higher in the Pierce county population for all insecticide treatments. We then used RNA sequencing to analyze the responses of Pierce and Clarke population flies surviving a LC_{50} treatment of the 3 insecticides. We identified a high number of differentially expressed genes that are likely involved in detoxification and reduced cuticular penetration, especially in the Pierce population, with extensive overlap in differentially expressed genes between the 3 insecticide treatments. Finally, we predicted fewer nonsynonymous single nucleotide variants having deleterious effects on protein function among detoxification, insecticide target, and cuticular protein encoding genes in Pierce flies. Thus a combination of increased gene expression and fewer deleterious single nucleotide variants highlights molecular mechanisms underlying the higher LC_{50} values for Pierce population flies.

Key words differentially expressed genes; *Drosophila suzukii*; high throughput sequencing; insecticide response; insertions and deletions; single nucleotide variants

Introduction

Global fruit production is being threatened by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), a recently invasive insect pest commonly known as spotted wing Drosophila (SWD) (Lee *et al.*, 2011). *D. suzukii* is indigenous to Eastern and South Eastern Asia and over the past 5 years it has dramatically expanded its range globally to include Europe, North America, and South America (Walsh *et al.*, 2011; Cini *et al.*, 2012; Deprá *et al.*, 2014). *D. suzukii* was first detected in California in 2008 (Hauser, 2011; Walsh *et al.*, 2011). Since then it has spread throughout the United States (Burrack *et al.*, 2012) causing significant losses in crop yield and quality. *D. suzukii* is a polyphagous insect pest which causes significant damage to soft and thin-skinned fruits including blueberries, blackberries, raspberries, cherries, strawberries, peaches, and grapes worldwide.

Correspondence: Michael J. Adang, Department of Entomology, College of Agricultural and Environmental Sciences, University of Georgia, Athens, GA 30602–2603, USA. Tel: +1 706 202 9341; fax: +1706 542 2279; email: adang@uga.edu

Preventive applications of insecticides have been used as a primary strategy to manage *D. suzukii* (Beers *et al.*, 2011; Bruck *et al.*, 2011; Walsh *et al.*, 2011; Haviland & Beers, 2012). The most effective insecticides used against *D. suzukii* are relatively broad-spectrum chemicals such as pyrethroids, spinosyns and organophosphates. These chemicals are neurotoxins and their primary targets are sodium channels, nicotinic acetylcholine receptors (nAChR), and acetylcholinesterase, respectively (ffrench-Constant *et al.*, 1998; Baxter *et al.*, 2010). A zero damage tolerance policy among growers for *D. suzukii* has led to the frequent application of broad-spectrum insecticides, which could lead to resistance development in *D. suzukii*. This is of particular concern in *D. suzukii* because it has a short generation time (Kopp & True, 2002) comparable to the closely related *D. melanogaster*. A species which, has been shown to develop insecticide resistance at a much faster rate than anticipated by prior population models (Karasov *et al.*, 2010). Alarmingly, a significant level of resistance to permethrin in a field population of *D. suzukii* has been reported (Bolda, 2011).

Insecticide resistance mechanisms documented in insects include metabolic resistance, target-site/receptor mutations, reduced cuticular penetration, and avoidance behavior (Li *et al.*, 2007; Rivero *et al.*, 2010; Silva *et al.*, 2012). Metabolic and target site receptor-based resistance have been the most extensively studied at genetic and molecular levels (Hemingway & Ranson, 2000). Metabolic resistance involves overproduction of detoxification enzymes that degrade or sequester insecticides (Hemingway *et al.*, 1998; Vontas *et al.*, 2001; ffrench-Constant *et al.*, 2004; Li *et al.*, 2007), which may be achieved by gene amplification (Karunaratne *et al.*, 1998; Puinean *et al.*, 2010), mutations in coding sequences (Claudianos *et al.*, 1999; Zhu *et al.*, 1999; Aminetzach *et al.*, 2005; Magwire *et al.*, 2012), or mutations and insertions/deletions (Indels) in *cis*-acting promoter sequences (Daborn *et al.*, 2002; Seifert & Scott, 2002; Guio *et al.*, 2014) and/or *trans*-acting regulatory loci (Kasai & Scott, 2001; Maitra *et al.*, 2002; Feyereisen *et al.*, 2005; Mateo *et al.*, 2014).

The effective management of insecticide resistance depends ultimately on a thorough understanding of resistance mechanisms at the genetic and molecular levels. Since the invasion of *D. suzukii* in the United States and the heavy use of insecticides to control it in fruit crops is relatively recent phenomenon, it is therefore timely to take a proactive approach to access the risk of resistance development in *D. suzukii*. Recently, transcriptomic analyses using high throughput sequencing and microarray have revealed that insecticide resistance is more complex than previously anticipated, often being associated to multiple genes rather than a single locus (Pedra *et al.*, 2004; Puinean *et al.*, 2010; Kalajdzic *et al.*, 2012; Silva *et al.*, 2012; David *et al.*, 2014).

In the current study, we used RNA sequencing-based transcriptomic analyses to determine the responses of 2 populations of *D. suzukii* that survived LC_{50} (median lethal concentration) level treatments of 3 broad-spectrum insecticides commonly used against *D. suzukii*. This approach allowed a comparison at the transcriptome level between populations of *D. suzukii* with differing insecticide susceptibility, and then a view of the transcriptomic responses in surviving flies at the end of the selective period. We further predicted single nucleotide variants (SNVs) and insertions and deletions (indels) in these *D. suzukii* populations with a focus on variants in genes involved in pesticide detoxification. By correlating analyses of genomic sequence differences between the Pierce and Clarke *D. suzukii* populations, and their observed differential response to multiple classes of insecticides, we establish baseline chemical response profile at an early stage of an invasive species. Information from this study will facilitate future comparisons to *D. suzukii* populations that emerge as threats to effective control with chemical pesticides.

Materials and methods

D. suzukii rearing and field collection

The Clarke population was established in July 2013 by collecting *D. suzukii* from unsprayed blueberries from Clarke County (Georgia, USA, approximately 250 flies were collected to start the colony) and at the time of bioassays flies had been in the colony for approximately 12 generations. The flies were reared in plastic fly bottles using a cornmeal and molasses-based artificial diet (Haviland & Beers, 2012). The colony was maintained in upright growth chambers (model I-41 LLVLC8, Percival Scientific, Perry, IA, USA) operating at 50% RH, 25 ± 2 °C with a 14 : 10 (L : D) photoperiod. A stock of minimum 2000 flies was maintained for each generation of the Clarke population. The *D. suzukii* Pierce population used in this study was collected in June 2014 from a commercial blueberry orchard in Pierce County (Georgia, USA). Approximately 200 flies were collected and maintained in the laboratory for one generation using the same methods as for the Clarke colony. In order to collect the Pierce population, blueberries infested with *D. suzukii* larvae were collected into 52 oz ventilated plastic containers and placed into upright growth chambers. Adult flies were aspirated and transferred into plastic fly

bottles with artificial diet and reared for one generation to obtain pupae. Pupae were then separated into individual 1.5 oz Solo® clear polystyrene cups (Solo® Cup Co., Urbana, IL, USA) until adults emerged. Newly emerged adults were separated by sex and 5- to 7-d-old females were used for the bioassays.

Insecticide bioassays

Standardized glass vial bioassay protocols were utilized to assess the susceptibility of adult *D. suzukii* females to zeta-cypermethrin, spinosad, and malathion. As these 3 insecticides have contact activity, a residual bioassay protocol was developed. Insecticide was diluted in acetone for zeta-cypermethrin and malathion, or deionized water for spinosad to prepare 20–100 mL of stock solution. A series of dilutions at desired concentrations were prepared for each of the selected insecticides. The bioassay chambers, 225 mL glass jars (cat. no. 02-911-460, Fisher Scientific, Pittsburgh, PA, USA) and their lids were labeled with appropriate dilution using labeling tape. The glass jars used for spinosad bioassays contained approximately 1 cm deep layer of fly diet at the bottom. One milliliter of appropriate insecticide dilution was added to each of the prelabeled glass jars. The lids were put back on the corresponding glass jars. The glass jars were then swirled and inverted to insure that all surfaces inside the bottle were coated with insecticide residue. The glass jars were then opened and placed inside a fume hood to air dry for 30 min to 1 h depending on the selected insecticide. While glass jars were drying in the fume hood, 5- to 7-d-old *D. suzukii* females were aspirated from the vials used to maintain *D. suzukii* colonies in the laboratory into 22 mL glass vials. Once glass jars and lids were completely dry, a set of 10 *D. suzukii* females was transferred to each of the pretreated glass jars. Glass jars treated with acetone or deionized water served as the controls. Fly mortality was evaluated after 2–6 h of exposure depending on nature of the insecticides tested. The exposure time was 2 h for zeta-cypermethrin and malathion, and 6 h for spinosad. All bioassays were replicated 6–10 times. The LC_{50} values were generated using a range of concentrations of 0.01–3, 0.3–100, and 0.3–100 ppm for zeta-cypermethrin, spinosad, and malathion, respectively. Median lethal concentration (LC_{50}) values were estimated using probit option of the POLO software (Software, 1987) and lethal concentration ratios (LCR) at LC_{50} values and their corresponding 95% confidence limits (CL) were calculated using a lethal concentration ratio significance test (Robertson *et al.*, 2007). The Clarke colony served as the reference susceptible population for comparison purposes and was assigned a ratio of 1.0. The LC_{50} values of the Pierce population were considered significantly different from those of the Clarke population if the 95% CL of their corresponding LCR did not include the value of 1.0 ($\alpha = 0.05$).

Sample collection, RNA extraction, and high throughput sequencing

Once the LC_{50} values were established for zeta-cypermethrin, spinosad, and malathion, the 5- to 7-d-old *D. suzukii* Pierce and Clarke populations female flies were treated with their respective LC_{50} concentration values with the same exposure time as for bioassays. For controls, flies were placed in jars coated with either acetone for zeta-cypermethrin and malathion or water for spinosad. A total of 10 survivors per replicate were collected from both insecticide treated (i.e., survivors of LC_{50} dose) and control groups of Clarke and Pierce populations, and immediately stored at −80 °C. All bioassays for extraction of RNA from flies were replicated 3 times.

Total RNA was extracted from survivors of treated and control groups using TRIzol (Ambion) as follows. Ten survivors from each group of Pierce and Clarke populations were pooled separately and homogenized in 200 μL of TRIzol reagent using a cordless motor-driven pellet pestle (Grainger, Lake Forest, IL) and processed for total RNA according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). The purity and concentrations of RNA samples were determined using NanoDrop spectrophotometer (N-1000) and samples were submitted to the Georgia Genomic Facility (GGF), University of Georgia. GGF performed RNA integrity determination, Poly (A) enrichment of mRNA, cDNA synthesis, library preparations, and sequencing using 75 bp paired-end Illumina NextSeq 500 platform.

Transcriptome assembly, differential expression, and statistical analysis

Due to high sequencing base quality at both 3′ and 5′ ends according to the FastQC output (Mortazavi *et al.*, 2008) and the availability of a reference genome, preprocessing steps were not performed on raw reads. RNA-seq raw datasets were analyzed using the protocol developed by Trapnell *et al.* (2012). The 24 samples (paired-end reads, including 3 biological replicates) were independently mapped onto the *D. suzukii* genome (SpottedWingFlybase v.1) (Chiu *et al.*, 2013) downloaded from http://spottedwingflybase.oregonstate.edu/ by using

Tophat v2.0.13 (Trapnell *et al.*, 2009; Kim *et al.*, 2013), which uses Bowtie2 (Langmead *et al.*, 2009) as an aligner. After the alignment, Cufflinks v2.2.1 (Trapnell *et al.*, 2010) was used to estimate the expression values of the transcripts in FPKM (fragments per kilobase per million mapped reads) with the Cuffdiff 2 default geometric normalization. Differentially expressed genes (FDR < 0.05 after Benjamini–Hochberg correction for multiple-testing) were identified for insecticide-treated versus untreated control for either (1) Clarke population or (2) Pierce population. CummeRbund tools (Trapnell *et al.*, 2012), a package in R v2.15.3, were used to generate scatter and squared coefficient of variation plots, and to calculate Pearson's correlation coefficient for biological replicates. All sequences are deposited in the NCBI database (GEO series accession number: GSE73595) and SRA accession number (SRP064328).

Comparative analyses across insecticide treatments as well as between Clarke and Pierce populations

In-house written Perl scripts, Awk and Linux commands were used to pull out significantly differentially expressed genes, across 3 insecticide treatments in Pierce and Clarke population flies, from Cuffdiff outputs. Awk command was used to extract significantly differentially expressed genes from the Cuffdiff outputs. Only genes with FDR < 0.05, FPKM values ≥ 0.1 were used for differential expression analyses. An in-house written Perl script was used to generate a list of significantly differentially expressed detoxification, receptor, and defense response genes. Another Perl script was written to identify significantly up- and downregulated *D. suzukii* genes with *D. melanogaster* orthologs. Venn Diagram Plotter (Littlefield & Monroe, 2008) was used to generate diagrams illustrating overlapping differential expression of genes between treatments.

GO enrichment analyses

GO Biological processes (BP) and Molecular Function (MF) terms enrichment analyses were performed using DAVID v6.8 algorithm (Huang *et al.*, 2009a, 2009b) with the Benjamini–Hochberg correction for multiple testing and fold change. The Flybase gene ID for the *D. melanogaster* orthologs to significantly up- and downregulated *D. suzukii* genes were used as input for GO enrichment analysis. A maximum of top 15 GO terms were selected on the basis of number of sequences with corrected *P* value (Benjamini) <0.05 and fold enrichment ≥1.5 to be presented in our results.

SNV and Indel analyses

Bam files (output of Tophat v2.0.13) from individual replicates were first sorted and then combined using SAMtools (Li *et al.*, 2009) mpileup for Pierce and Clarke untreated populations separately. VarScan v2.3.8 (Koboldt *et al.*, 2012), which uses SAMtools mpileup data as input, was used to call for SNVs and indels. Selected VarScan v2.3.8 (Koboldt *et al.*, 2012) options included: minimum read depth at a position to make a call (10), minimum base quality at a position to count a read (25), *P*-value threshold (0.01), minimum supporting reads at a position to call variants (2), and for minimum variant frequency the threshold default option of 0.2. We further used SnpEff v4.1i (build 2015-08-14) (Cingolani *et al.*, 2012), in which our *D. suzukii* database was built, to annotate and predict the effects of SNVs and indels.

To predict the effect of nonsynonymous SNVs on protein function, SIFT4G (Vaser *et al.*, 2016) was used. Before using SIFT4G, *D. suzukii* database was built to be used in SIFT4G. The nonsynonymous SNV is predicted to be deleterious on protein function when the SIFT score is ≤0.05. SIFT median measures the diversity of the sequences used for prediction. A warning with low confidence occurs when the sift median is greater than 3.5 because this indicates that the prediction was based on closely related sequences. The low confidence in SIFT score means that the protein alignment does not have enough sequence diversity because the position artificially appears to be conserved, an amino acid substitution may incorrectly be predicted to be damaging.

An in house python script was written to pull out the SNVs and indels on genes of interest from SnpEff and SIFT4G vcf outputs.

Results

Insecticide bioassays indicate that Pierce population is less susceptible to 3 classes of insecticides than Clarke population

The LC_{50} values of zeta-cypermethrin, spinosad, and malathion for *D. suzukii* females from the Clarke population were 0.49, 2.78, and 10.25 ppm and those for the Pierce population were 1.50, 7.60, and 27.34 ppm, respectively (Table 1). The Pierce population was significantly less susceptible to zeta-cypermethrin, spinosad, and malathion than the Clarke population with LCRs of 3.07, 2.73, and 2.66, respectively.

Table 1 The median lethal concentration (LC_{50}) values of zeta-cypermethrin, spinosad, and malathion against Pierce and Clarke county *D. suzukii* population.

Populations	$N^{\dagger}$	Slope (± SE)	χ^2	LC_{50} (ppm) (95% FL)[‡]	LCR[§] at LC_{50} (95% CL)[¶]
Zeta-cypermethrin					
Pierce	100	2.27 (± 0.52)	0.97	1.50 (0.87–2.66)	3.07 (1.58–5.96)*
Clarke	100	3.18 (± 0.85)	0.45	0.49 (0.30–0.80)	
Spinosad					
Pierce	100	1.66 (± 0.33)	1.10	7.60 (4.072–14.32)	2.73 (1.29–5.81)*
Clarke	100	2.69 (± 0.67)	0.44	2.78 (1.64–4.67)	
Malathion					
Pierce	70	2.37 (± 0.56)	0.45	27.34 (15.84–47.41)	2.66 (1.41–5.05)*
Clarke	60	3.40 (± 0.83)	3.34	10.25 (6.49–15.94)	

$^{\dagger}N$ = number of adults assayed.

[‡]95% fiducial limits estimated using POLO (LeOra Software, 1987).

[§]LCR, lethal concentration ratio = LC_{50} (Pierce population)/LC_{50} (Clarke population).

[¶]95% confidence limits estimated using lethal concentration ratio significance test.

*LC_{50} of Pierce collected population significantly different from that of the Clarke population at $\alpha = 0.05$.

χ^2 = Chi-squared values indicate that the bioassay data fit the probit model and the distribution of responses is binomial.

Examination of transcriptomic changes upon insecticide treatments by RNA sequencing

To reveal possible trends that could account for differences in the LC_{50} values of *D. suzukii* Clarke and Pierce populations treated with zeta-cypermethrin, spinosad, or malathion, we used high throughput RNA sequencing to examine transcriptomic changes by performing pairwise comparisons between Clarke and Pierce population flies surviving the different insecticide treatments versus their respective untreated controls. The total paired-end reads obtained per sample ranged from 28 million to 51 million, of which 17 million to 27 million reads had aligned pairs (Table S1). Estimates of gene expression abundance expressed as FPKM were highly correlated with *r* values ranging from 0.89 to 0.99 (Fig. 1).

The expression scatter plots show strong positive correlation with Pearson's correlation coefficient (*r*) values ranging from 0.88 to 0.99, between control and treated samples for Pierce and Clarke population flies across 3 treatments (Fig. 1). The *r* values between treated and control samples are lower for the Pierce population as compared to the Clarke population suggesting a higher variance in the Pierce population. Similar results were obtained when squared coefficient of variance (SCV) were calculated (Fig. S1), suggesting more genetic variability among the Pierce population flies.

Comparisons of differentially expressed genes in Clarke and Pierce population flies surviving an insecticide treatment

Surviving Pierce population flies had 2411, 2330, and 1310 significantly upregulated genes after exposure to zeta-cypermethrin, spinosad, or malathion, respectively (Fig. 2A). Of the upregulated genes, 1223 were common between Pierce flies regardless of the insecticide treatment; while 737, 56, and 21 genes overlapped between the pairs of zeta-cypermethrin and spinosad, zeta-cypermethrin and malathion, and spinosad and malathion treatments, respectively (Fig. 2A). In contrast, only 168, 186, and 86 genes were significantly upregulated in Clarke flies surviving a zeta-cypermethrin, spinosad, or malathion treatment, respectively (Fig. 2C), with only 29 genes differentially upregulated in common between the 3 treatments. Twenty, 28, and 10 genes were commonly upregulated between zeta-cypermethrin and spinosad, zeta-cypermethrin and malathion, and spinosad and malathion treatments, respectively (Fig. 2C). Similar trends were observed in significantly downregulated genes of Pierce and Clarke population flies (Figs. 2B and D). A greater number of genes were downregulated in Pierce than Clarke population flies and the number of common genes downregulated across the 3 insecticide treatments was also higher in Pierce population flies.

Comparisons of the total number of upregulated genes in the Pierce and Clarke populations after

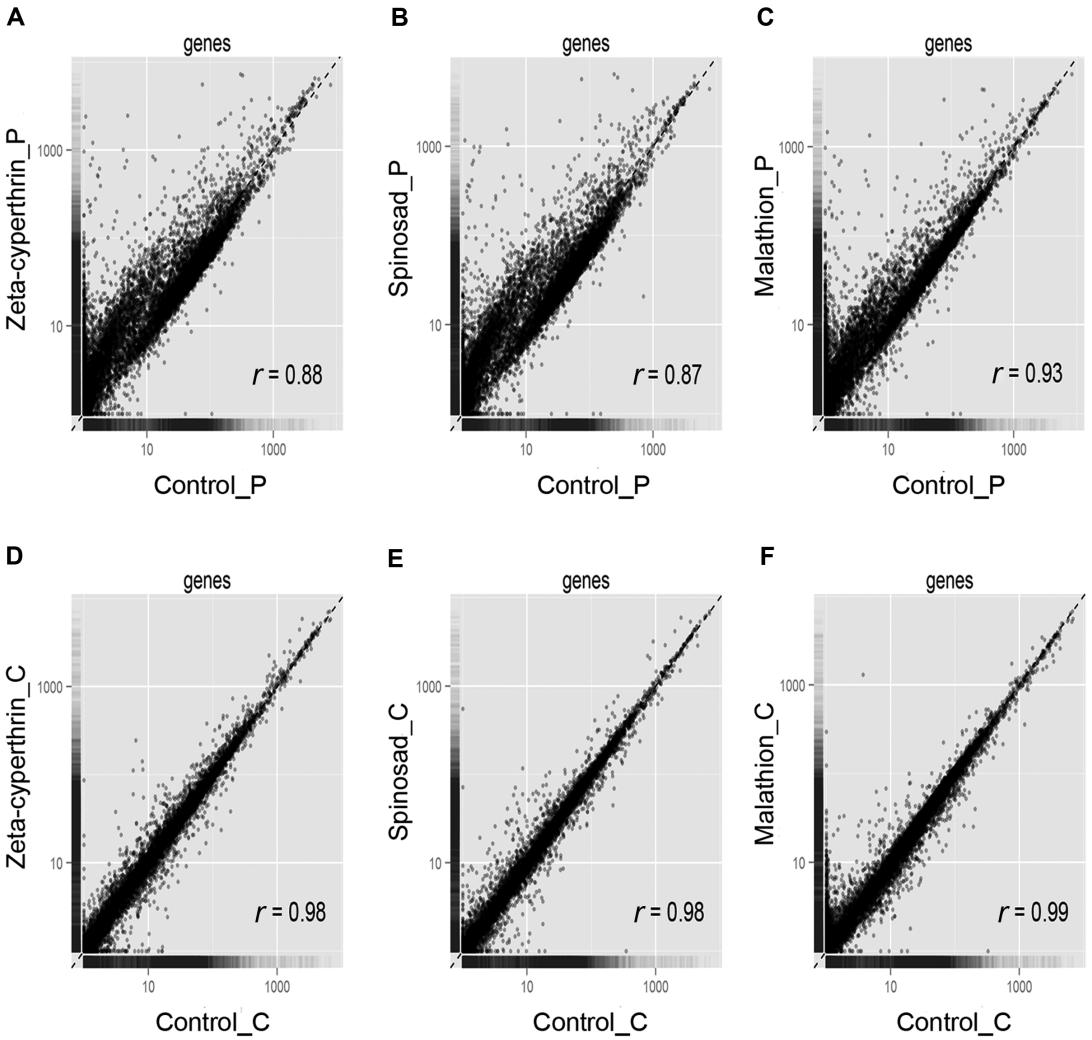

Fig. 1 Comparisons of gene expression levels between control and insecticide-treated *D. suzukii* Pierce and Clarke populations. Scatter plots of $\log_{10}$ (FPKM) showing pairwise comparisons between control and insecticide-treated *D. suzukii* Pierce and Clarke population. Results for Pierce population treatments are shown in (A)–(C) and comparisons for Clarke population are shown in (D)–(F). The FPKM values for all transcripts were plotted for control and treated samples by averaging across the biological replicates and normalization. Pairwise comparisons generated include (A) and (D) control versus zeta-cypermethrin, (B) and (E) control versus spinosad, and (C) and (F) control versus malathion. Each dot on the scatterplot represents a gene. CummeRbund was used for statistical analyses and generation of plots.

zeta-cypermethrin, spinosad and malathion treatments illustrate a more substantial transcriptomic response in Pierce population flies to the insecticides (Figs. 3A–C). While the Clarke population response was lower, the majority of upregulated genes in the Clarke population were also upregulated in the Pierce population (Figs. 3A, C, and E, yellow region). As with upregulated genes, the total number of downregulated genes was greater in Pierce as compared to Clarke population flies (Figs. 3B, D, and E). In contrast to upregulated genes, there were relatively few downregulated genes common between Pierce and Clarke populations after insecticide treatments (Figs. 3B, D, and F, yellow region).

GO analysis identified enriched functional gene classes in differentially expressed genes upon insecticide treatments

We performed GO enrichment on significantly upregulated and downregulated genes in zeta-cypermethrin, spinosad, and malathion-treated Pierce and Clarke

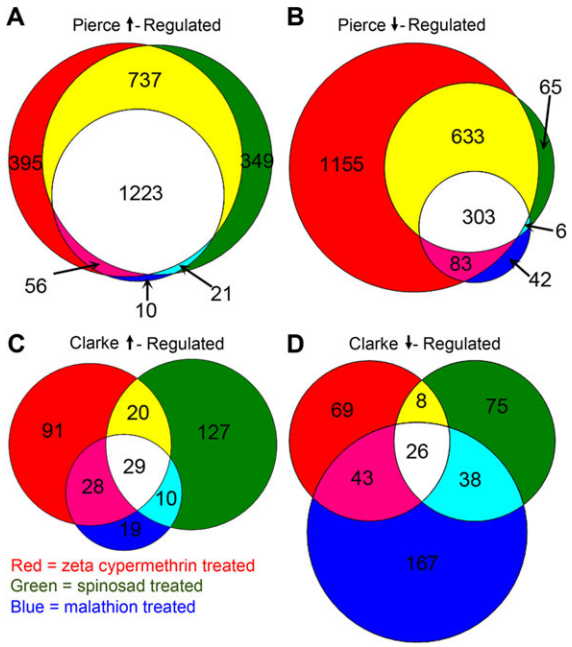

Fig. 2 Venn diagrams depicting the overlap among differentially expressed genes in *D. suzukii* surviving a treatment with 1 of 3 distinct insecticides. Panels A (Pierce) and C (Clarke) show overlap between upregulated genes in flies surviving a zeta-cypermethrin, spinosad, or malathion treatment. Panels B (Pierce) and D (Clarke) show overlap between downregulated genes in zeta-cypermethrin, spinosad, or malathion treated *D. suzukii*. The color representations of overlap between differentially expressed genes are as follows: red, green, and blue sections show genes that are exclusively differentially regulated in zeta-cypermethrin, spinosad, and malathion-treated flies, respectively; white sections show overlap between genes that are common to 3 treatments; yellow shows overlapping differential gene expression exclusive to zeta-cypermethrin and spinosad; cyan shows overlap exclusive to spinosad and malathion; while magenta shows overlap between differentially expressed genes exclusive to zeta-cypermethrin and malathion. Venn diagram plotter was used to generate proportional Venn diagrams.

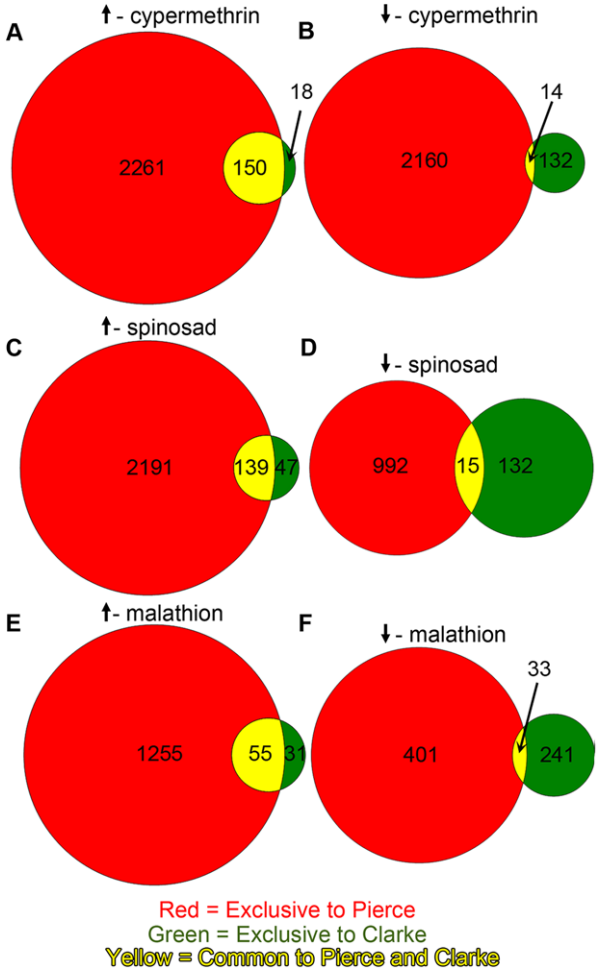

Fig. 3 Venn diagrams depicting the overlap among differentially expressed genes between *D. suzukii* Pierce and Clarke populations surviving insecticide treatments. (A), (C), and (E): Comparisons of upregulated genes between *D. suzukii* Pierce and Clarke population in zeta-cypermethrin (A), spinosad (C), and malathion (E) treatments. (B), (D), and (F): Comparisons of downregulated genes between *D. suzukii* Pierce and Clarke populations in zeta-cypermethrin (B), spinosad (D), and malathion (F) treatments. Color representation for up- and downregulated genes comparisons between Pierce and Clarke population are as follows: red: exclusive to Pierce population; green: exclusive to Clarke population; yellow: common to Pierce and Clarke populations.

populations to identify significantly enriched biological processes (BP) and molecular functions (MF) terms. The top 15 significantly enriched BP and MF terms on the basis of number of genes included in each term are presented in Tables S2 and S3.

GO enrichment analysis of upregulated genes The top enriched BP and MF terms for significantly upregulated genes across the 3 treatments in Pierce flies, were cell-cell signaling or oxidation-reduction process (BP), and transporter or receptor activity (MF) (Tables S2 and S3). Those terms, oxidation-reduction process (BP) and receptor activity (MF), were not enriched in Clarke flies surviving insecticide treatments (Tables S2 and S3).

The enriched GO terms oxidoreductase activity, G-protein coupled receptor signaling pathway and receptor activity in Pierce population flies were of particular interest because they are relevant to the actions of insecticides

(Tables S2 and S3). Oxidoreductase activity (MF term) includes cytochrome P450 monooxygenases (CYP), which are involved in phase I detoxification process of insecticides (Mitchell *et al.*, 2014) and have been implicated in insecticide resistance across a wide variety of insects (Rivero *et al.*, 2010). The oxidoreductase activity, G-protein coupled receptor signaling pathway and receptor activity MF terms were not enriched in Clarke flies (Table S3). The MF terms enriched in Clarke flies were structural constituent of cuticle, structural constituent of chorion and structural constituent of vitelline membrane (Table S3).

GO enrichment analysis of downregulated genes
Similar BP and MF terms were enriched in significantly downregulated genes across Pierce flies treated with the different insecticides (Tables S4 and S5). The significantly enriched GO terms for insecticide-surviving Pierce flies include genes involved in gene expression, mitotic cell cycle, protein modification process, chromosome organization, nervous system development and regulation of signal transduction (Tables S4 and S5). Relatively few BP and MF terms were enriched for downregulated genes in Clarke flies surviving zeta-cypermethrin, spinosad, and malathion treatments.

Genes encoding proteins involved in metabolic detoxification are differentially expressed upon insecticide treatments

A greater number of detoxification genes (*Est*, *Gst*, *Ugt*, and *Mdr*) were significantly upregulated with a fold change ≥2 in Pierce flies surviving zeta-cypermethrin, spinosad or malathion treatments relative to untreated Pierce flies (Table 2, Tables S6–S20). In contrast only 2 detoxification genes (*Cyp4e3* and *Cyp6w1*) were significantly upregulated with a fold change ≥2 in Clarke flies surviving insecticide treatments relative to the untreated flies (Table 2, Tables S21–S35). Furthermore, there were many shared differentially expressed detoxification genes in Pierce flies across treatments (Table 2).

The *Cyp* genes, which encode for enzymes involved in Phase I detoxification, were notably more responsive to the different classes of insecticide treatments in Pierce flies relative to other detoxification gene classes (*Est*, *Gst*, and *Ugt*) (Table 2). The significantly upregulated (with fold changes ≥2) *Cyp* genes in zeta-cypermethrin, spinosad, and malathion treated Pierce population belong to *Cyp* families 4, 6, 9, 12, 28, 49, 301, 304, 308, and 310 (Table 2, Tables S9–S11). The upregulation of members of *Cyp* families 4, 6, 9, and 12 are implicated in insecticide resistance (Li *et al.*, 2007).

Mdr genes encoding P-glycoproteins are upregulated upon insecticide treatments

Mdr genes encode P-glycoproteins (P-gp) (also called ATP-binding cassette [ABC] transporters), which have recently gained attention for their contributions to insecticide and Bt Cry resistance in insects (Heckel, 2012; Merzendorfer, 2014). P-gps protect *Culex pipiens* larvae from cypermethrin, endosulfan, and ivermectin toxicity (Buss *et al.*, 2002). Pierce flies surviving zeta-cypermethrin and spinosad treatments were upregulated for *Mdr65* and *Mdr50* gene expression (Table 2, Tables S18–S20). No *Mdr* genes were significantly upregulated with a fold change ≥2 in treated Clarke population flies (Table 2, Tables S33–S35). However, Clarke flies surviving a malathion treatment were downregulated for *Mdr65* and *Mdr49* gene expression (Tables S35).

Altered expression of cuticular protein genes is a potential mechanism to reduce penetration of pesticides

We analyzed the *Cpr* gene transcript levels in Pierce and Clarke population flies surviving insecticide treatments as higher expression levels of cuticular protein genes have been observed in pyrethroid resistant bed bugs (*Cimex lectularius* L.) (Koganemaru *et al.*, 2013). A total of 13, 12, and 13 *Cpr* genes were significantly upregulated in zeta-cypermetrin, spinosad, and malathion treatments, respectively, with a fold change ≥2 in Pierce population (Table 3). The *Cpr92F*, *Cpr100A*, *Cpr47Ec*, *Cpr62Bc*, *Cpr49Ah*, *Cpr76Bd*, and *Cpr49Ae* genes were significantly upregulated with a fold change ≥5 across 3 treatments in Pierce population (Tables S36–S38). In contrast no *Cpr* genes were significantly upregulated with fold change ≥ 2 in zeta-cypermethrin, spinosad, and malathion treated Clarke population (Table 3, Tables S39–S41). The *Cpr78Cc* and *Cpr49Ae* genes were significantly downregulated in zeta-cypermethrin-treated Pierce and spinosad-treated Clarke populations, respectively (Tables S36, Tables S40).

Comparing the basal levels of gene expression in Pierce and Clarke D. suzukii without insecticide treatments

In total, 2860 and 3302 genes were significantly up- and downregulated, respectively, in untreated Pierce

Table 2 Significantly upregulated detoxification genes with corrected *P* value (Benjamini) <0.05 and fold change ≥2.

Detox genes	Zeta-cypermethrin		Spinosad		Malathion	
	Pierce	Clarke	Pierce	Clarke	Pierce	Clarke
Cyp	*Cyp4e3* *Cyp6a14* *Cyp308a1* *Cyp310a1* *Cyp304a1* *Cyp4p2* *Cyp6a20* *Cyp6a21* *Cyp28c1* *Cyp6w1* *Cyp6d5* *Cyp12d1* *Cyp4d14* *Cyp4g15* *Cyp49a1* *Cyp4s3* *Cyp9b2* *Cyp6d5* *Cyp301a1*	*Cyp6w1*	*Cyp4e3* *Cyp49a1* *Cyp4d14* *Cyp4g15* *Cyp9b2* *Cyp28c1* *Cyp318a1* *Cyp301a1* *Cyp6a14* *Cyp4d8* *Cyp308a1* *Cyp6d5* *Cyp313b1* *Cyp304a1* *Cyp6d5*	*Cyp4e3*	*Cyp4e3* *Cyp304a1* *Cyp28c1* *Cyp6a14*	None
Est	*DS10_00002107*	None	*DS10_00002107*	None	None	None
Gst	*GstD10* *GstZ2* *GstE14*	None	*GstD10* *GstZ2* *GstE14* *GstE10*	None	*GstD10* *GstZ2*	None
Ugt	*Ugt86Dd* *Ugt58Fa* *Ugt36Bb*	None	*Ugt36Ba* *Ugt58Fa* *Ugt37b1* *Ugt35b* *Ugt58Fa* *Ugt36Bb*	None	None	None
Mdr	*Mdr50* *Mdr65*	None	*Mdr50* *Mdr65*	None	None	None

population samples relative to Clarke population samples (Tables S42 and S43). The expression levels of genes encoding proteins involved in detoxification process and target sites of insecticides were significantly downregulated in *D. suzukii* Pierce population when compared to Clarke population in control samples with the exceptions of *Cyp6a22*, *Cyp6v1*, *GstO2*, *GstD5*, and *rpk*, which were significantly upregulated in Pierce population control samples (Table S44). The genes encoding cuticular proteins were significantly downregulated in Pierce population (Table S45).

Higher number of predicted nonsynonymous SNVs and indels observed in genes encoding detoxification enzymes, insecticide targets, and cuticular proteins in Clarke population

Variants in expressed genes were analyzed by single nucleotide variant (SNV) and indel analyses as a means to predict the functionality of expressed DEGs. We predicted a total of 19432 and 18315 nonsynonymous SNVs in all expressed genes in Pierce and Clarke populations, respectively (Fig. 4). We then focused our analysis on genes

Table 3 Significantly upregulated cuticular protein encoding genes with corrected *P* value (Benjamini) <0.05 and fold change ≥ 2.

Defense genes	Zeta-cypermethrin		Spinosad		Malathion	
	Pierce	Clarke	Pierce	Clarke	Pierce	Clarke
Cpr	*Cpr92F*	None	*Cpr92F*	None	*Cpr92F*	None
	Cpr100A		*Cpr100A*		*Cpr100A*	
	Cpr47Ec		*Cpr47Ec*		*Cpr47Ec*	
	Cpr62Bc		*Cpr62Bc*		*Cpr62Bc*	
	Cpr49Ah		*Cpr49Ah*		*Cpr49Ah*	
	Cpr76Bd		*Cpr76Bd*		*Cpr76Bd*	
	Cpr49Ae		*Cpr49Ae*		*Cpr49Ae*	
	Cpr47Ee		*Cpr47Ee*		*Cpr47Ee*	
	Cpr50Cb		*Cpr50Cb*		*Cpr50Cb*	
	Cpr62Bb		*Cpr62Bb*		*Cpr62Bb*	
	Cpr49Ac		*Cpr49Ac*		*Cpr49Ac*	
	Cpr67B		*Cpr73D*		*Cpr73D*	
	Cpr73D					

encoding detoxification enzymes, insecticide targets and cuticular proteins; specifically identifying SNVs and indels in genes that were significantly differentially expressed in Pierce and Clarke flies surviving treatment with at least 1 insecticide. In comparison to Clarke population, fewer nonsynonymous SNVs were predicted in *CYPs*, *ESTs*, *GSTs*, *UGTs*, *MDRs* and cuticular protein encoding genes in Pierce population (Fig. 5). Furthermore we predicted the effects of nonsynonymous SNVs on the protein function in the above mentioned group of genes using SIFT4G (Vaser *et al.*, 2016). Nonsynonymous SNVs in *Cyp12d1-d*, *Cyp9b2*, *Cyp6d5* (DS10_00010146), *Cyp4d1*, *Est-6*, *Ugt36Bb*, *Ugt86Dd*, *Mdr49*, and *Cpr49Ac* genes in Clarke population were predicted to have deleterious effects on protein function (Table 4). In contrast, only 6 genes (*Cyp9b2*, *Cyp4p1*, *Cyp4d1*, *Ugt86Dd*, and *Ugt*) were predicted to have nonsynonymous SNVs leading to deleterious effects in the Pierce population flies (Table 4). Among them, *Cyp9b2*, *Cyp4d1*, and *Ugt86Dd* genes have common nonsynonymous SNVs in Pierce and Clarke population. Furthermore, *Cyp9b2* has additional predicted deleterious SNVs in Clarke population (Table 4). *Mdr49* gene has 2 nonsynonymous SNVs with predicted deleterious effect in the Clarke population but none were predicted in the Pierce population (Table 4). Coincidently, V1225I of the nonsynonymous SNVs predicted to have deleterious effect on protein function is present in the highly conserved Walker B domain of MDR49 protein (based on NCBI conserved domain database search).

Indels (insertions and deletions) were predicted for the above-mentioned group of genes in untranslated (UTR) and coding sequence (CDS) regions, which could lead to deleterious effects on expression pattern or protein function. *Cyp4s3* gene has 2 insertions and 2 deletions in 3′-UTR region of Clarke population. In contrast only 1

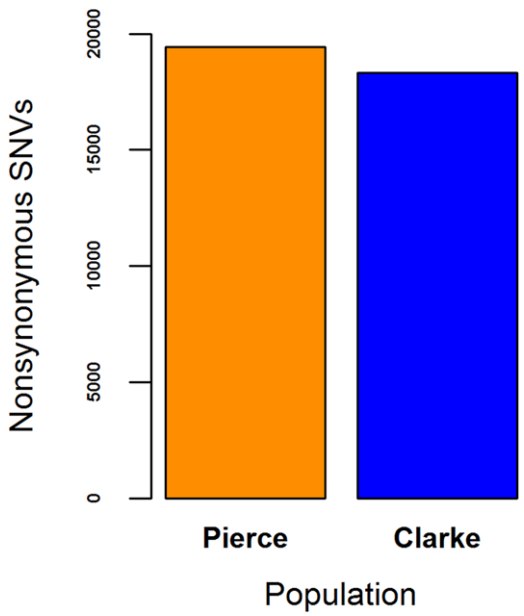

Fig. 4 Predicted nonsynonymous SNPs in Pierce and Clarke populations. Total number of nonsynonymous SNPs predicted in all expressed genes in Pierce and Clarke populations. SNPs were identified using VarScan v2.3.4 with a $P \leq 0.01$). SnpEff was used to annotate and predict the effects of statistically significant SNPs ($P \leq 0.01$). SnpEff and SIFT4G output files are available at the spottedwingflybase (http://spottedwingflybase.org/downloads) along with additional statistical information. The figure was generated using R v3.2.3.

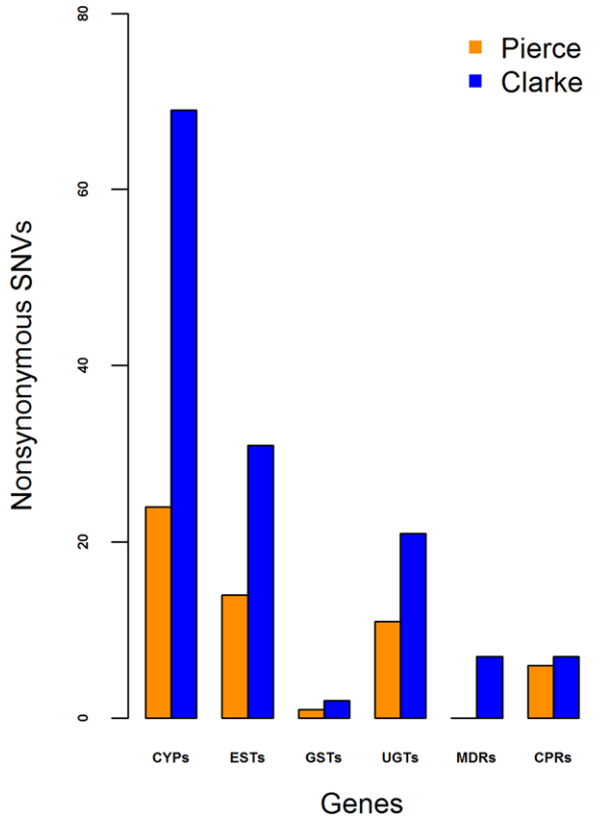

Fig. 5 Number of predicted nonsynonymous SNPs in detoxification, insecticide target and cuticular protein encoding genes in Pierce and Clarke populations. Significantly differentially expressed genes in the above mentioned gene groups in Pierce and Clarke populations were selected for analyses. Statistical analyses and availability of output files are described in Fig. 4 legend. The figure was generated using R v3.2.3.

insertion was predicted in 3′-UTR region of *Cyp4s3* gene Pierce population (Table 5). *Cpr92F* gene has a predicted frameshift deletion in the CDS region in Clarke population but not in Pierce population (Table 5).

Discussion

Drosophila suzukii is a recent invasive species in the United States that is already a serious agricultural pest. To detect early evidence of resistance to chemical pesticides, populations of insects were collected in 2 different locations, Pierce and Clarke counties, of Georgia for bioassays and RNAseq analyses. Pierce population flies were collected from a field heavily sprayed with pesticides since their invasion and Clarke population flies were collected from an unsprayed field. LC_{50} values were determined for zeta-cypermethrin, spinosad and malathion using a contact mortality bioassay similar to Haddi *et al.* (2017) with pesticide exposure times of 2 h for zeta-cypermethrin and malathion and 6 h for spinosad. While the Clarke population was about 3 fold more susceptible than the Pierce population to each pesticide, this difference would not affect chemical control in the field.

We identified differentially expressed genes (DEGs) in Clarke and Pierce *D. suzukii* population flies surviving LC_{50}-level treatments with zeta-cypermethrin, spinosad, or malathion insecticide. Live flies were analyzed to reveal transcriptomic changes (i.e., DEGs) that could reflect a defensive response to the tested toxicants. Some observed DEGs could also reflect differences in transcript abundance between surviving flies and the untreated control population. Since treatments were based on LC_{50} values, Pierce flies were exposed to a 3 fold higher concentration of each insecticide than Clarke flies; although these were equivalent selective concentrations, the amounts of pesticide that flies contacted differed between pesticides and insect strains. Also there was a time difference between treatments, as flies exposed to spinosad were treated for 6 h versus 2-h treatments for zeta-cypermethrin and malathion. Differences between treatment pesticide, the concentration of each pesticide, and time after treatment when transcripts were harvested in surviving flies each could have had quantitative effects on expressed transcripts.

We used Gene Ontology (GO) enrichment analysis to identify gene classes that are overrepresented among the DEGs. We focused our analyses on detoxification and cuticle related genes due to their presence in the identified GO gene classes and known involvement in insecticide resistance (Li *et al.*, 2007; Rivero *et al.*, 2010; Silva *et al.*, 2012), and found that many of these genes were significantly upregulated in flies surviving insecticide treatments. Pierce population flies, more so than Clarke flies, were notable for an abundance of enriched gene categories including a number of upregulated detoxification genes. The higher number of significantly DEGs identified in Pierce population when compared to Clarke population flies correlates with the higher LC_{50} values calculated for Pierce county flies. DEGs could reflect a combination of a selective effect yielding gene transcripts enriched in flies surviving the insecticide treatment, and an inductive effect, referred to as the defensome which occurs in animals during the treatment period in response to toxicants (Niu *et al.*, 2012; De Marco *et al.*, 2017).

Cytochrome P450 monooxygenases have primary roles in the detoxification process of many xenobiotics so it is not surprising that they were commonly up regulated in Pierce flies surviving zeta-cypermethrin, spinosad, and

Table 4 Predicted effect of nonsynonymous (missense) SNPs on protein function in Pierce and Clarke county population.

Population	Gene_Id/name	SNPs[1]	Ref AA/Alt AA[2]	AA position[3]	SIFT score[4]	SIFT median	SIFT prediction
Clarke	DS10_00002643/*Cyp12d1*	scaffold2: 6068291_T/A	I/F	981	0.007	4.32	Deleterious (low conf)[5]
	DS10_00005250/*Cyp4d1*	Scaffold5:1948347_C/G	A/G	15	0.040	4.32	Deleterious (low conf)[5]
	DS10_00005633/*Cyp9b2*	scaffold6: 2068271_G/T	F/L	450	0.027	2.68	Deleterious
		scaffold6: 2068759_T/G	I/L	358	0.045	2.68	Deleterious
	DS10_00010146/*Cyp6d5*	scaffold99: 37973_C/A	A/S	159	0.043	4.32	Deleterious (low conf)[5]
	DS10_00004134/*Est-6*	scaffold3: 6208049_C/A	D/Y	389	0.005	3.39	Deleterious
	DS10_00008658/*Ugt86Dd*	Scaffold123: 382551_A/C	Q/P	840	0.015	4.32	Deleterious (low conf)[5]
	DS10_00012705/*Ugt36Bb*	Scaffold1724: 69775_C/T	T/I	72	0.009	2.74	Deleterious
		Scaffold1724: 70315_G/T	V/F	232	0.011	2.74	Deleterious
	DS10_00005769/*Mdr49*	scaffold6: 3398679_G/A	V/I	1225	0.027	3.56	Deleterious (low conf)[5]
		scaffold6: 3407477_G/A	V/I	2522	0.007	4.32	Deleterious (low conf)[5]
	DS10_00003821/*Rdl*	scaffold3: 1855481_T/A	D/V	309	0.008	2.41	Deleterious
	DS10_00008225/*para*	scaffold23: 585370_A/G	K/R	426	0.026	2.55	Deleterious
	DS10_00010216/*rpk*	scaffold305: 184502_C/G	S/R	379	0.033	2.66	Deleterious
	DS10_00002609/*Cpr49Ac*	scaffold2: 5702262_G/A	E/K	310	0.037	4.32	Deleterious (low conf)[5]
Pierce	DS10_00005633/*Cyp9b2*	scaffold6: 2068759_T/G	I/L	358	0.045	2.68	Deleterious
	DS10_00003335/*Cyp4p1*	scaffold2: 11817769_C/T	A/V	489	0.013	4.32	Deleterious (low conf)[5]
	DS10_00005250/*Cyp4d1*	scaffold5:1948347_C/G	A/G	15	0.040	4.32	Deleterious
	DS10_00008658/*Ugt86Dd*	scaffold123: 382551_A/C	Q/P	840	0.015	4.32	Deleterious (low conf)[5]
	DS10_00008811/*Ugt*	Scaffold97: 25366_C/G	T/R	112	0.001	2.65	Deleterious
	DS10_00010216/*rpk*	scaffold305: 184502_C/G	S/R	379	0.033	2.66	Deleterious

SNPs[1]: a description of single nucleotide polymorphism Scaffold2 and 6068291 represent chromosome and position at which substitution occurred, respectively and T/A represents (T replaced by A). Ref AA/Alt AA[2]: I/F (reference amino acid/alternate amino acid, that is, I substituted by F). AA position[3]: amino acid position where substitution occurred. SIFT score[4]: amino acid substitution predicted deleterious when score is ≤0.05. (low conf)[5]: a warning low confidence occurs when SIFT median score is greater than 3.5 because this indicates that the prediction is based on closely related sequences. SIFT4G was used to predict the effect of nonsynonymous SNPs on protein function.

Table 5 Predicted insertions and deletions in UTR and CDS regions of detoxification, insecticide receptor, and cuticular proteins encoding genes in Pierce and Clarke county population.

Population	Gene_Id/name	Indels[1]	Region[2]	Var type[3]	AA_indel[4]
Clarke	DS10_00006248/ *Cyp4s3*	scaffold11: 772157_C/CA	UTR_3	Insertion	N/A
		scaffold11: 772668_A/ATT	UTR_3	Insertion	N/A
		scaffold11: 772718_AT/A	UTR_3	Deletion	N/A
		Scaffold11: 773151_GC/G	UTR_3	Deletion	N/A
	DS10_00012547/ *GSTZ2*	scaffold459: 69561_ GT/G	UTR_3	Deletion	N/A
	DS10_00002615/ *Cpr49Ae*	scaffold2: 5731703_CT/C	UTR_3	Deletion	N/A
	DS10_00003845/ *Cpr67B*	scaffold3: 2134602_C/CA	UTR_3	Insertion	N/A
	DS10_00004445/ *Cpr62Bb*	scaffold3: 9885475_AT/A	UTR_5	Deletion	N/A
	DS10_00009142/ *Cpr100A*	scaffold290: 138696_ T/TA	UTR_3	Insertion	N/A
	DS10_00012013/ *Cpr92F*	scaffold282: 132664_ TA/T	CDS	Frameshift deletion	p.Asp382fs[5]
Pierce	DS10_00006248/ *Cyp4s3*	scaffold11: 772157_C/CA	UTR_3	Insertion	N/A
	DS10_00002615/ *Cpr49Ae*	scaffold2: 5731982_ AT/A	UTR_3	Deletion	N/A
	DS10_00003845/ *Cpr67B*	scaffold3: 2134602_C/CA	UTR_3	Deletion	N/A

Indels[1]: a description of Insertions and deletions, Scaffold11 and 772157 represent chromosome and position after which insertion occurred, respectively and C/CA represents an A is inserted after C. Region[2]: The region where insertion or deletion occurred, UTR_3: 3′prime untranslated region, UTR5: 5′ untranslated region and CDS: coding sequence. Var type[3]: Variant type. AA_indel[4]: amino acid position where insertion or deletion occurred. p.Asp382fs[5]: p.Asp382fs (HGVS nomenclature) denotes frameshifting change deleting Asp382 amino acid.

malathion treatments. Interestingly, while overexpression of P450 genes is a feature of metabolic detoxification, P450 overexpression is also associated with modified cuticle and insecticide resistance in *A. gambiae* (Balanbanidou *et al.*, 2016). Other families of detoxification enzymes were also significantly upregulated, such as ESTs, UGTs, and GSTs. The relative significance of cytochrome P450 genes could be experimentally tested by inhibiting the enzymes with pipronyl butoxide (PBO) and comparing LC_{50} values of insecticides.

In *D. suzukii* Pierce population treatments, 7 *Cpr* genes were overexpressed with a fold change ≥ 5 and none of the *Cpr* genes were overexpressed with fold change ≥ 2 in Clarke population treatments. Cuticular protein encoding genes have previously been shown to be overexpressed in pyrethroid resistant *Anopheles* species (Awolola *et al.*, 2009; Gregory *et al.*, 2011; Bonizzoni *et al.*, 2012; Nkya *et al.*, 2014). Cuticle thickening is hypothesized to function as an auxiliary mechanism for pyrethroid tolerance in *Anopheles* (Wood *et al.*, 2010). Relationships between *Cpr* genes, cuticle thickening and insecticide resistance make *Cpr* genes candidates to track for insecticide resistance development in *D. suzukii*.

Many of the DEGs were regulated in the same manner regardless of the specific insecticide used in the treatment, suggesting these genes are involved in mechanisms that are induced to counteract and cope with the negative effects of different classes of insecticides. G-protein coupled receptor (GPCR) signaling pathway term was enriched in Pierce flies, and in a recent study GPCR related genes were found to be upregulated in permethrin resistant *Culex quinquefaciatus* strain (Li *et al.*, 2014). The GPCR-related genes were shown to regulate the expression of P450 insecticide resistance genes (Li *et al.*, 2014). Similarly, in *Aedes* mosquitoes coregulation of genes such as P450s that are involved in metabolic detoxification occurs through induction of signaling transduction cascades (Li *et al.*, 2013). Induction of the GPCR signaling pathway could account for coordinate regulation of differentially expressed detoxification genes observed in Pierce flies.

Metabolic-based resistance has major importance in some dipteran species; in permethrin-resistant *Anopheles* species, metabolic detoxification by increased levels of P450 monooxgenases can account for a high level of resistance (Muller *et al.*, 2008; Zhong *et al.*, 2013). In the event that genetic changes occur resulting in altered expression of genes associated with metabolic resistance, for example, upregulation of a gene that is normally induced only upon insecticide treatment, the outcome could be an increased basal level of tolerance to more than one class of insecticides.

Rather surprisingly, the baseline expression level of many genes, including those known to be involved in insecticide detoxification, are higher in Clarke population samples when compared to the Pierce population untreated samples. These results could be considered

puzzling as the LC_{50} values of the Clarke population are lower than the Pierce population. Possibly the higher expression levels of detoxification genes achieved in Pierce population flies surviving insecticide treatment could have been more important than baseline gene expression levels in generating protein products to counteract the effects of insecticides.

Our analysis identified nonsynonymous SNVs and indels expressed in genes of Pierce and Clarke population flies and many are in genes associated with insecticide detoxification. It is possible that some can have negative or positive effects on metabolic resistance. For example, nonsynonymous SNVs present in both SWD populations include mutations in *Cyp9b2*, *Cyp4p1*, *Cyp4d1*, *Ugt86Dd*, and *Ugt* (Table 4) that are predicted to have deleterious effects on protein function. Should encoded enzymes be functionally impaired reduced pesticide detoxification could occur. Positive effects of nonsynonomous SNVs on metabolic resistance to pyrethroids are supported by a recent investigation into the genomic basis of metabolic resistance in *A. aegypti* (Faucon *et al.*, 2017). Those authors report nonsynonymous variations within the substrate recognition site of P450s that are more abundant in resistant strains of *Aedes*. The suggestion being that binding site changes in P450s, which confer enhanced pesticide binding, are selected as pesticide resistance is acquired. It is possible that some nonsynonomous SNVs identified in this study will similarly become 'fixed' upon continued exposure of *D. suzukii* populations to chemical insecticides.

A nonsynonymous mutation K426R in *para* gene with predicted deleterious effects on protein function was identified in Clarke population flies (Table 4). The same, K426R mutation was also identified in a permethrin resistant *kdr–his1* housefly strain, although there was uncertainty expressed by the authors over the role of the K426R mutation in resistance development (Rinkevich *et al.*, 2006).

The results from our combined gene expression and SNV analysis in Clarke and Pierce population flies illustrate complexity of *D. suzukii* responses to insecticide and the challenges in identifying the most relevant candidate genes involved in the response. The insecticide response genes that lack nonsynonymous mutations with deleterious effects and are significantly differentially expressed in the Pierce population could be considered most relevant to insect survival. We anticipate that high throughput sequencing can be used as an effective tool to assess the risk of insecticide resistance development and lead to strategies to delay the development of resistance in devastating pests such as *D. suzukii*.

Availability of RNA sequencing data

The data discussed in the submission have been submitted to NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO series accession number GSE73595 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73595). BioProject Id: PRJNA297377 and SRA Id: SRP064328. SnpEff and SIFT4G output data available from following link (http://spottedwingflybase.org/downloads).

Note: Field and Laboratory populations in the NCBI GEO are renamed as Pierce and Clarke, respectively in the manuscript.

Acknowledgments

We thank the Georgia Genomic Facility (GGF) at the University of Georgia for performing the RNA sequencing. This project was funded by a University of Georgia Seed Grant awarded to A.A.S. and M.J.A. This research was partially supported by grants from Georgia Blueberry Growers Association, Georgia Agricultural Commodity Commission for Blueberries, and the U.S. Department of Agriculture, National Institute of Food and Agriculture under Extension Implementation Program Award No. 2014-70006-22533 to A.A.S.; the U.S. Department of Agriculture under National Research Initiative Award No. 2010-65105-20590 to M.J.A; and support from Clarence and Estelle Albaugh Endowment to JCC. We thank Dr. Pablo Cingolani for building a *D. suzukii* database in SnpEff v4.1i.

Disclosure

The authors declare that they have no competing interests.

Authors' contribution

AAS, RM, MJA, and JC designed research; AAS performed the experiments to produce the animals under different conditions; GH extracted RNA pools and prepared materials for sequencing; RM and JCC conducted bioinformatic analyses; RM, JCC, MJA, and AAS cowrote the manuscript. NRT built *D. suzukii* database for SIFT4G analysis. All authors read and approved the manuscript.

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Manuscript received March 28, 2017
Final version received May 30, 2017
Accepted June 18, 2017

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Squared coefficient of Variation plot to assess cross-replicate variability between control and treated SWD Pierce and Clarke population. Pierce population: cypermethrin (A), spinosad (B), and malathion (C) treatment. Clarke population: cypermethrin (D), spinosad (E), and malathion (F). CummeRbund was used for statistical analyses and generating plots.

Table S1 Alignment summary of SWD Pierce and Clarke population reads. The numbers shown in each row represents the range of number of reads for 3 biological replicates.

Tables S2–S5 Enriched gene ontology (GO) biological processes (BP) and molecular function (MF) terms for significantly up- and downregulated genes in 3 insecticide treatments (zeta-cypermethrin, spinosad, and malathion) of *D. suzukii* Pierce and Clarke population. **Table S2** Enriched BP terms for significantly upregulated genes. **Table S3** Enriched MF terms for significantly upregulated genes. **Table S4** Enriched BP terms for significantly downregulated genes. **Table S5** Enriched MF terms for significantly downregulated genes.

Tables S6–S20 Significantly differentially expressed detoxification genes in *D. suzukii* Pierce population. Up- and downregulated *Est* genes in zeta-cypermethrin (**Table S6**), spinosad (**Table S7**), and malathion (**Table S8**) treated Pierce population. Up- and downregulated *Cyp* genes in zeta-cypermethrin (**Table S9**), spinosad (**Table S10**), and malathion (**Table S11**) treated Pierce population. Up- and downregulated *Gst* genes in zeta-cypermethrin (**Table S12**), spinosad (**Table S13**), and malathion (**Table S14**) treated Pierce population. Up- and downregulated *Ugt* genes in zeta-cypermethrin (**Table S15**), spinosad (**Table S16**), and malathion (**Table S17**) treated Pierce population. Up- and downregulated *Mdr* genes in zeta-cypermethrin (**Table S18**), spinosad (**Table S19**), and malathion (**Table S20**) treated Pierce population. The excel file contains information about the locus, FPKM values, *P* value, and *q* value significance (corrected *P* value [Benjamini]) of the detoxification genes.

Tables S21–S35 Significantly differentially expressed detoxification genes in *D. suzukii* Clarke population. Up- and downregulated *Est* genes in zeta-cypermethrin (**Table S21**), spinosad (**Table S22**), and malathion (**Table S23**) treated Clarke population. Up- and downregulated *Cyp* genes in zeta-cypermethrin (**Table S24**), spinosad (**Table S25**), and malathion (**Table S26**) treated Clarke population. Up- and downregulated *Gst* genes in zeta-cypermethrin (**Table S27**), spinosad (**Table S28**), and malathion (**Table S29**) treated Clarke population. Up- and downregulated *Ugt* genes in zeta-cypermethrin (**Table S30**), spinosad (**Table S31**), and malathion (**Table S32**) treated Clarke population. Up- and downregulated *Mdr* genes in zeta-cypermethrin (**Table S33**), spinosad (**Table S34**), and malathion (**Table S35**) treated Clarke population.The excel file contains information about the locus, FPKM values, *P* value and *q* value significance (corrected *P* value [Benjamini]) of the detoxification genes.

Tables S36–S38 Significantly differentially expressed *Cpr* genes in *D. suzukii* Pierce population. Up- and downregulated *Cpr* genes in zeta-cypermethrin (**Table S36**), spinosad (**Table S37**), and malathion (**Table S38**) treated Pierce population.

Tables S39–S41 Significantly differentially expressed *Cpr* genes in *D. suzukii* Clarke population. Up- and downregulated *Cpr* genes in zeta-cypermethrin (**Table S39**), spinosad (**Table S40**), and malathion (**Table S41**) treated Clarke population.

Table S42 Significantly upregulated genes in Pierce control when compared to Clarke control.

Table S43 Significantly downregulated genes in Pierce control when compared to Clarke control.

Table S44 Comparing the basal expression levels of genes, encoding proteins involved in detoxification process and insecticide target sites, between Pierce and Clarke populations control samples.

Table S45 Comparing the basal expression levels of *Cpr* genes between Pierce and Clarke population control samples.

Tables S46–S48 Predicted effect of nonsynonymous SNPs in genes encoding proteins involved in detoxification process (**Table 46**), insecticide target sites (**Table 47**), and cuticular protein encoding genes (**Table 48**) in Clarke population.

Tables S49–S51 Predicted effect of nonsynonymous SNPs in genes encoding proteins involved in detoxification process (**Table 49**), insecticide target sites (**Table 50**), and cuticular protein encoding genes (**Table 51**) in Pierce population.