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Derived esterase activity in *Drosophila sechellia* contributes to evolved octanoic acid resistance

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Abstract

The dietary specialist fruit fly *Drosophila sechellia* has evolved resistance to the secondary defence compounds produced by the fruit of its host plant, *Morinda citrifolia*. The primary chemicals that contribute to lethality of *M. citrifolia* are the medium-chain fatty acids octanoic acid (OA) and hexanoic acid. At least five genomic regions contribute to this adaptation in *D. sechellia* and whereas the fine-mapped major effect locus for OA resistance on chromosome 3R has been thoroughly analysed, the remaining four genomic regions that contribute to toxin resistance remain uncharacterized. To begin to identify the genetic basis of toxin resistance in this species, we removed the function of well-known detoxification gene families to determine whether they contribute to toxin resistance. Previous work found that evolution of cytochrome P450 enzymatic activity or expression is not responsible for the OA resistance in *D. sechellia*. Here, we tested the role of the two other major detoxification gene families in resistance to *Morinda* fruit toxins – glutathione-S-transferases and esterases – through the use of the pesticide synergists diethyl maleate and tribufos that inhibit the function of these gene families. This work suggests that one or more esterase(s) contribute to evolved OA resistance in *D. sechellia*.

Keywords: insecticide resistance, ecological genetics, evolutionary genetics, host specialization.

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Introduction

A recurring challenge amongst phytophagous insects is to overcome the chemical defences produced by their host plants. Most plant-feeding insects are dietary specialists that feed on a small number of closely related plant species and the evolution of plant–insect associations has been directed to a large extent by differences in plant chemistry (Jaenike, 1990; Bernays and Chapman, 1994). The fruit fly *Drosophila sechellia* is an exceptional model system to study the genetic basis of evolved toxin resistance. First described in 1981, *D. sechellia* is a dietary specialist that has evolved to feed exclusively on the toxic fruit of *Morinda citrifolia* (Tsacas and Bachli, 1981; Louis and David, 1986; Matute and Ayroles, 2014). This specialization is interesting because the ripe fruit is toxic to other drosophilids including the closely related dietary generalist sister species *Drosophila melanogaster* and *Drosophila simulans* (R'Kha *et al.*, 1991; Legal *et al.*, 1992; Farine *et al.*, 1996). Toxicity of *Morinda* fruit is mainly due to the high concentration of carboxylic acids produced by the host plant as secondary defence compounds. Octanoic acid (OA) is primarily responsible for lethality whereas the less potent, but still toxic, hexanoic acid (HA) is also abundant in ripe *Morinda* fruit (Legal *et al.*, 1994; Farine *et al.*, 1996). Despite extensive research into the genetic basis of OA and HA resistance in *D. sechellia*, the specific genes or gene families involved in this case of evolved toxin resistance remain elusive.

Quantitative trait loci (QTLs) for OA resistance have been developed through hybridization between OA-resistant *D. sechellia* and susceptible sister species *D. simulans* in both adult and larval stages (Jones, 1998, 2001; Huang and Erezyilmaz, 2015). The resolutions of these QTLs vary amongst genomic region and life stage. For example, a locus on chromosome 3R has been shown to be responsible for the greatest variation in OA resistance between adult *D. sechellia* and *D. simulans* and has been narrowed to a 168 kb region containing 18 genes using an introgression based approach (Hungate *et al.*, 2013). Utilizing RNA interference in *D. melanogaster*, this fine-mapped region was further refined using functional tests of the 18 genes residing in the QTL (Andrade-Lopez

et al., 2017). This analysis revealed three genes in the *Osiris* family, *Osiris 6* (*Osi6*), *Osi7* and *Osi8*, that led to a decrease in OA resistance when gene expression was ubiquitously knocked down in *D. melanogaster* (Andrade López *et al*., 2017). A subsequent study revealed knockdown of *Osi8* in the larval stage of *D. melanogaster* also increased susceptibility to OA (Lanno *et al*., 2019). Outside of the fine-mapped region on chromosome 3R, the remaining adult QTL studies yielded confidence intervals that were too large to implicate individual genes (for example, all of chromosome 2 is under one QTL; Jones, 1998, 2005). Whereas the genetic and molecular basis of *Osiris* gene function in toxin resistance are beginning to be revealed, at least four other loci contribute to OA resistance in *D. sechellia* adult flies (Jones, 1998).

Toxin resistance in insects is often gained through the enzymatic activity of cytochrome P450 (CYP) genes. The CYP monooxygenase enzymatic system is involved in Phase I metabolism of a large number of endogenous and exogenous compounds and often results in detoxification of substrates, although activation is also possible (Feyereisen, 1995; Scott and Wen, 2001; Ffrench-Constant *et al*., 2004; Berenbaum and Johnson, 2015). Studies have shown that toxin resistance can be gained through regulatory changes in expression of a single CYP gene, such as *Cyp6g1*, whose overexpression in *D. melanogaster* is sufficient for dichlorodiphenyltrichoroethane resistance (Daborn *et al*., 2002). Peyser *et al.* (2017) used piperonyl butoxide to simultaneously remove the function of all CYPs and tested *D. sechellia* and closely related species *D. melanogaster* and *D. simulans* flies for changes in OA and HA resistance upon loss of gene function. This study concluded CYP enzymatic activity contributed to basal resistance to the toxins in *Morinda* fruit, but are ultimately not responsible for the derived toxin resistance observed in *D. sechellia* (Peyser *et al*., 2017). Our current study attempts to further narrow the list of possible candidate toxin resistance genes by functionally testing the same fly species with and without gene function of two other major detoxification gene superfamilies: glutathione-S-transferases (GSTs) and esterases (ESTs).

Insect GSTs and ESTs are multifunctional enzymes that primarily act to detoxify a wide range of endogenous compounds and xenobiotics (for full review see Oakeshott *et al*., 1993; Hayes and Pulford, 1995; Montella *et al*., 2012). Diethyl maleate (DEM) and S,S,S-tributyl phosphorotrithioate (also known as tribufos) are often coformulated with various pesticides as synergists to counteract metabolic resistance by targeting GSTs and ESTs, respectively (Bernard and Philogène, 1993; Pasay *et al*., 2009). DEM works by conjugating reduced glutathione, making it unavailable in cells. This leaves the GSTs nothing to conjugate to toxins, rendering them nonfunctional in toxin resistance (Boyland and Chasseaud, 1970; Welling and De Vries, 1985; Fujioka and Casida, 2007; Snoeck *et al*., 2017). Tribufos is an organophosphorus compound that covalently bonds to esterase active sites and upon enzymatic cleavage of the synergist leaves the esterase inactivated by phosphorylation (Plapp *et al*., 1963; Snoeck *et al*., 2017). Insect toxin resistance mediated by GSTs (Chiang and Sun, 1993; Kostaropoulos *et al*., 2001; Enayati *et al*., 2005; Lumjuan *et al*., 2011; Pavlidi *et al*., 2015) and ESTs (Campbell *et al*., 1998; Farnsworth *et al*., 2010; Zhang *et al*., 2013; Feng *et al*., 2018) is well documented, including examples in other species of *Drosophila*, such as the cactophilic species *Drosophila mettleri* and *Drosophila mojavensis* (Matzkin *et al*., 2006; Bono *et al*., 2008; Hoang *et al*., 2015).

Because insects have been shown to evolve toxin resistance through changes in GST and EST function or expression, we hypothesize that these gene families may underlie a QTL for toxin resistance in *D. sechellia*. Here, we present a study functionally testing *D. sechellia*, *D. simulans* and *D. melanogaster* flies with and without pesticide synergist exposure in the presence of OA or HA to determine if derived GST or EST activity may contribute to the evolved toxin resistance observed in *D. sechellia*.

Results

Investigating the role of GST genes in OA resistance

In order to test the contribution of GST activity to OA resistance in *Drosophila* species, we used DEM, a chemical synergist, to inhibit the function of all members of the GST gene family simultaneously. Combination of DEM treatment with exposure to 1.2% OA (Andrade López *et al*., 2017; Peyser *et al*., 2017) allowed for tests of differences in mortality associated with reduction of GST activity in the presence of OA. Any observed differences between DEM + OA and OA alone would demonstrate the function of GST(s) in OA resistance. To test for evolved function of GST(s) in OA resistance we measured OA resistance in the derived OA-resistant species *D. sechellia* as well as in the sensitive sister species *D. melanogaster* and *D. simulans* (Fig. 1A–C). As expected, exposure to OA alone resulted in a large difference in relative survival of adult flies ($-\beta$ from Cox proportional hazards test) with *D. sechellia* significantly more resistant to 1.2% OA than *D. melanogaster* ($P < 2 \times 10^{-16}$) and *D. simulans* ($P < 2 \times 10^{-16}$), and *D. simulans* significantly more resistant than *D. melanogaster* ($P = 0.0085$).

Exposure to DEM alone caused no mortality in any control experiments. When DEM was combined with 1.2% OA, we found no effect on relative survival across all species as compared to exposure to OA alone ($P = 0.64$). When we tested for DEM effects on OA resistance in individual species, we found a significant increase in *D. melanogaster* OA resistance ($-\beta = 0.51$, $P = 0.006$, Fig. 1A), but no effects were observed for *D. simulans* ($-\beta = 0.0057$ $P = 0.98$, Fig. 1B) or *D. sechellia* ($-\beta = -0.45$, $P = 0.11$, Fig. 1C) when DEM + OA was compared to OA.

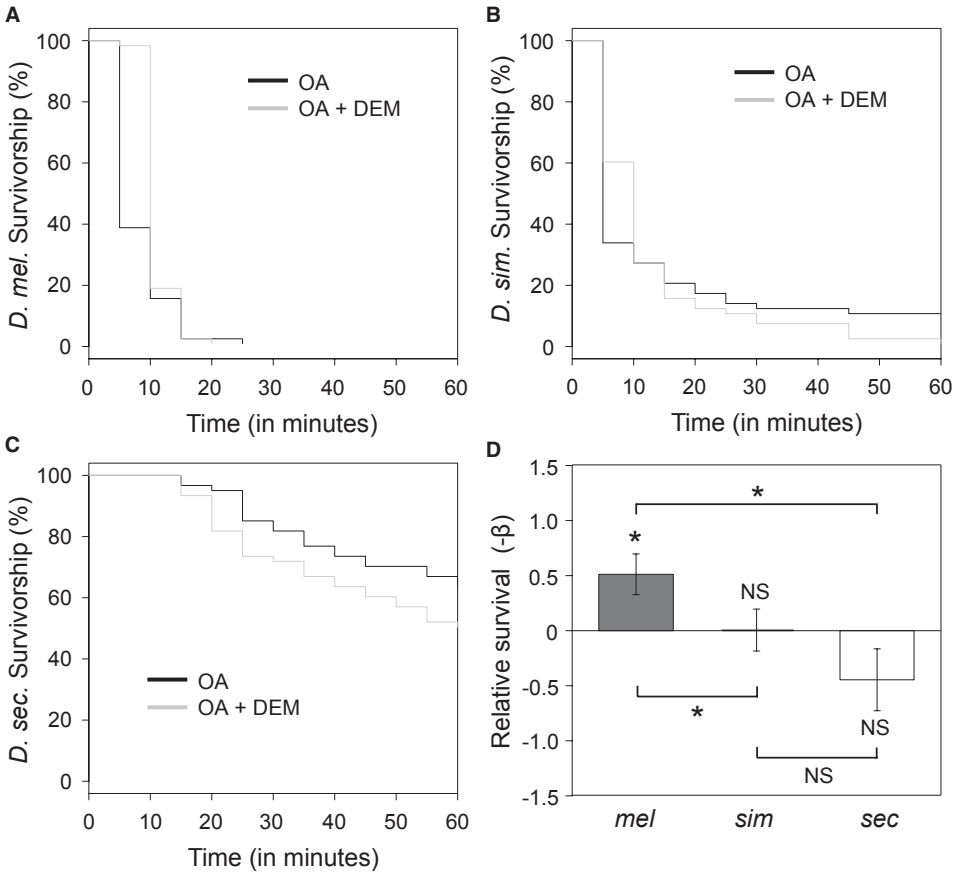

Figure 1. Glutathione-S-transferase enzymatic activity alters octanoic acid (OA) resistance in *Drosophila melanogaster*. Differences in survival are shown for adult flies in 1.2% OA (control) compared to 1.2% OA with diethyl maleate (DEM). (A) *D. melanogaster*, (B) *Drosophila simulans*, (C) *Drosophila sechellia*. (D) Relative survival (–β) of each species in OA + DEM compared to OA alone is shown with *D. melanogaster* in dark grey, *D. simulans* in light grey and *D. sechellia* in white. Error bars indicate standard error. Significant differences between species are indicated with asterisks ($P > 0.05$, Cox proportional hazards test). NS, nonsignificant.

To test for changes in GST function that may have occurred during evolution in these species we made pairwise comparisons between the three species and looked for differences in the effect of DEM on OA resistance (Fig. 1D). We found significant differences in the effect of DEM on OA resistance between *D. melanogaster* and both *D. simulans* ($P = 0.042$) and *D. sechellia* ($P = 0.0083$) with DEM only having an effect on *D. melanogaster* as compared to both *D. simulans* and *D. sechellia* where no effect was observed. Notably, we found no significant difference in DEM effect on OA resistance between *D. simulans* and *D. sechellia* ($P = 0.43$).

Investigating the role of GST genes in HA resistance

The second most abundant toxic compound produced by *M. citrifolia* is HA. To investigate whether GST(s) play a role in *Drosophila* resistance to HA, we again used DEM to reduce the activity of GSTs and tested for differences in HA resistance. We compared DEM + 1.2% HA (Peyser *et al.*, 2017) to 1.2% HA alone, which allowed for tests of differences in mortality associated with reduction of GST activity in the presence of HA. Similar to the experiments described above for OA, any differences observed between DEM + HA and HA alone would demonstrate the function of GST(s) in HA resistance. When *Drosophila* species were fed 1.2% HA alone we found significant differences between species ($P = 2.7 \times 10^{-10}$) agreeing with previous reports (Amlou *et al.*, 1997; Legal *et al.*, 1999; Peyser *et al.*, 2017). We found that *D. sechellia* had significantly greater resistance to HA than both *D. simulans* ($P = 2.7 \times 10^{-9}$) and *D. melanogaster* ($P = 0.007$) and that *D. melanogaster* was significantly more resistant to HA than *D. simulans* ($P = 1.9 \times 10^{-5}$) (Fig. 2).

When we tested for an effect of GST activity reduction across all species we found no significant effect of DEM on HA resistance ($P = 0.46$). When we tested for DEM effects on HA resistance in individual species, we found no effect in *D. melanogaster* ($-\beta = -0.3$, $P = 0.14$, Fig. 2A), *D. simulans* ($-\beta = -0.16$, $P = 0.42$, Fig. 2B) and *D. sechellia*

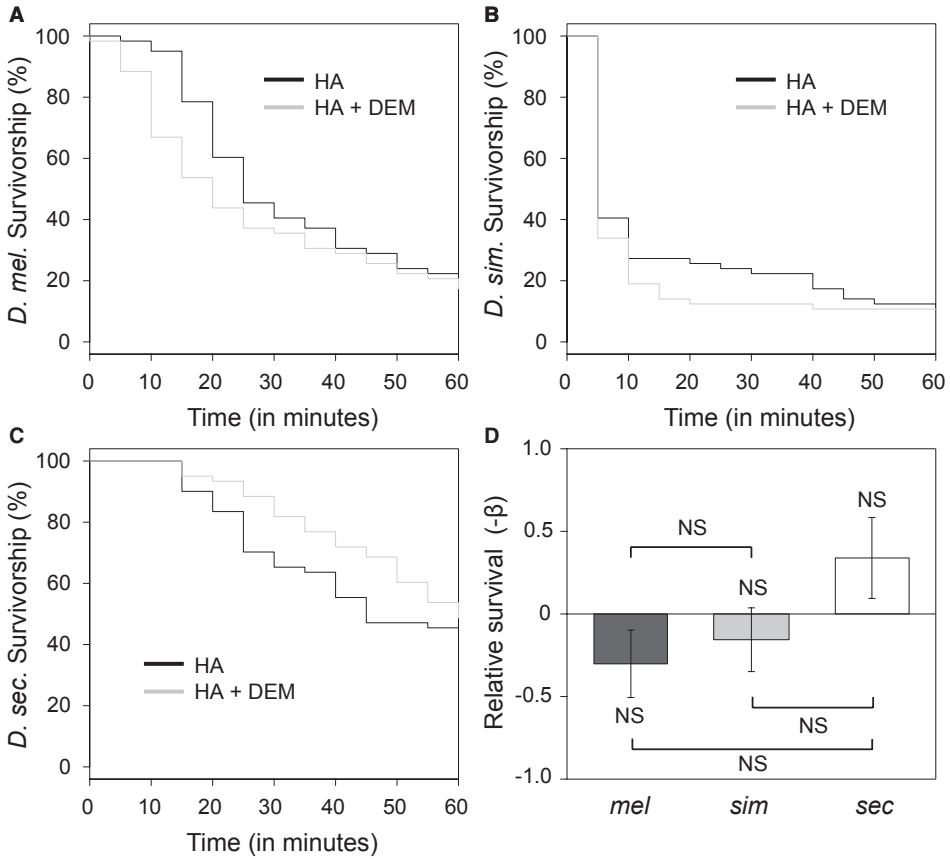

Figure 2. Glutathione-S-transferase enzymatic activity does not contribute to differences in hexanoic acid (HA) resistance. Differences in survival are shown for adult flies in 1.2% HA (control) compared to 1.2% HA with diethyl maleate (DEM). (A) *Drosophila melanogaster*, (B) *Drosophila simulans*, (C) *Drosophila sechellia*. (D) Relative survival (–β) of each species in HA + DEM compared to HA alone is shown with *D. melanogaster* in dark grey, *D. simulans* in light grey and *D. sechellia* in white. Error bars indicate standard error. Significant differences between species are indicated with asterisks ($P > 0.05$, Cox proportional hazards test). NS, nonsignificant.

(–β = 0.34 , $P = 0.17$, Fig. 2C) when DEM + HA was compared to HA.

To test for changes in GST function that may have occurred during evolution in these species we made pairwise comparisons between the three species and looked for differences in the effect of DEM on HA resistance (Fig. 2D). We found no significant differences in the effect of DEM on HA resistance between *D. melanogaster* and both *D. simulans* ($P = 0.94$) and *D. sechellia* ($P = 0.06$) or between *D. simulans* and *D. sechellia* ($P = 0.10$).

Investigating the role of EST genes in OA resistance

To test the contribution of EST gene activity to OA resistance in *Drosophila* species, we used tribufos to inactivate all the ESTs and measure OA resistance. Using a combination of tribufos treatment with exposure to 1.2% OA allowed for tests of differences in OA resistance upon reduction of EST activity where any observed differences between tribufos + OA and OA alone would suggest the function of EST(s) in OA resistance.

Similar to that reported above for DEM, exposure to tribufos alone caused no mortality in any control experiments. When tribufos was combined with 1.2% OA, we found no effect on relative survival across all species as compared to exposure to OA alone ($P = 0.16$). When we tested for tribufos effects on OA resistance in individual species, we found a pattern very similar to the effect of DEM on OA resistance. We found a significant increase in *D. melanogaster* OA resistance upon exposure to tribufos (–β = 0.74, $P = 0.00016$, Fig. 3A). No effect of tribufos was observed for *D. simulans* OA resistance (–β = –0.039 $P = 0.83$, Fig. 3B); however, tribufos was found to significantly decrease *D. sechellia* OA resistance (–β = –0.57, $P = 0.011$, Fig. 3C).

To test for changes in EST function that may have occurred during evolution in these species we made pairwise comparisons between the three species and looked for differences in the effect of tribufos on OA resistance (Fig. 3D). We found significant differences in the effect of tribufos on OA resistance between *D. melanogaster* and *D. simulans* ($P = 0.03$) and between *D. sechellia*

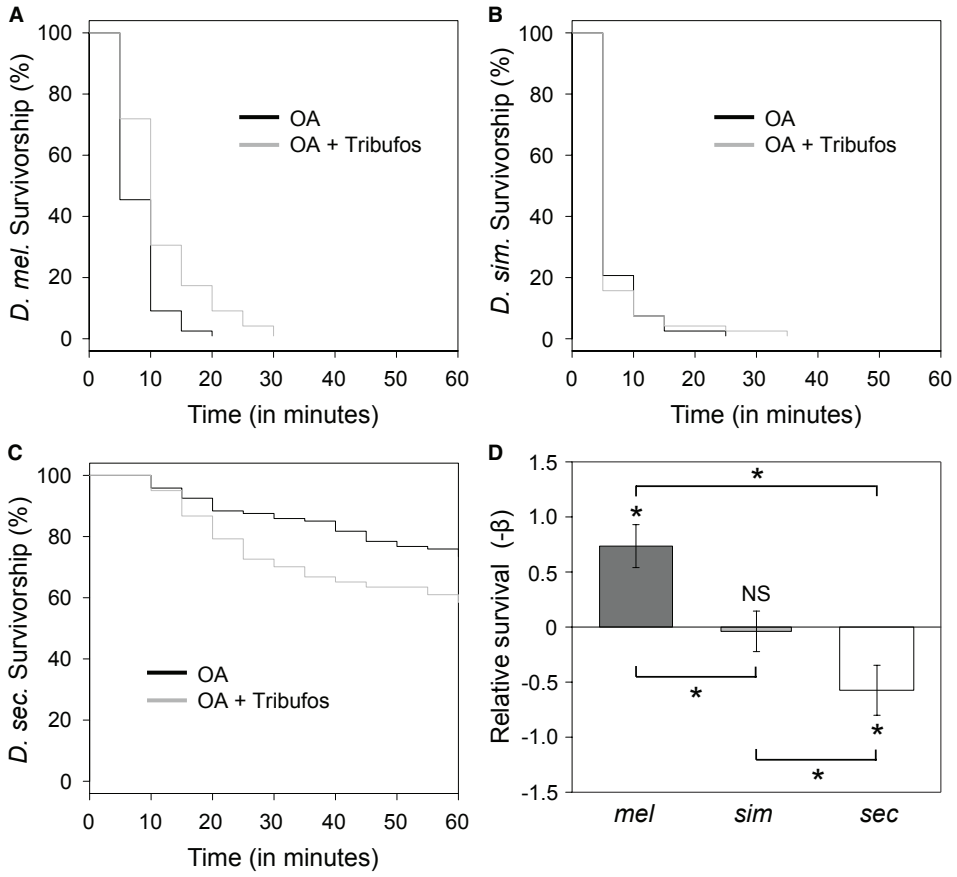

Figure 3. Enzymatic activity of esterases contribute to octanoic acid (OA) resistance in *Drosophila sechellia*. Differences in survival are shown for adult flies in 1.2% OA + cyclohexane (control) compared to 1.2% OA with tribufos. (A) *Drosophila melanogaster*, (B) *Drosophila simulans*, (C) *D. sechellia*. (D) Relative survival (–β) of each species in OA + tribufos compared to OA alone is shown with *D. melanogaster* in dark grey, *D. simulans* in light grey and *D. sechellia* in white. Error bars indicate standard error. Significant differences between species are indicated with asterisks ($P > 0.05$, Cox proportional hazards test). NS, nonsignificant.

and both *D. simulans* ($P = 0.048$) and *D. melanogaster* ($P = 4.7 \times 10^{-6}$).

Investigating the role of EST genes in HA resistance

To investigate whether EST(s) play a role in *Drosophila* resistance to HA, we again used tribufos to inactivate all the ESTs and tested for differences in HA resistance. We compared tribufos + 1.2% HA to 1.2% HA alone, which allowed for tests of differences in mortality associated with EST inactivation in the presence of HA. Similar to the experiments described above for tribufos and OA, any differences observed between tribufos + HA and HA alone would demonstrate the function of EST(s) in HA resistance.

When we tested for an effect of EST inactivation across all species, we found a significant effect of tribufos on HA resistance ($P = 0.0007$). When we tested for DEM effects on HA resistance in individual species, we found a significant reduction of HA resistance in *D. melanogaster* upon tribufos exposure ($-\beta = -0.69$, $P = 0.0012$, Fig. 4A). We found no significant effect of tribufos on HA resistance in *D. simulans* ($-\beta = -0.095$, $P = 0.64$, Fig. 4B) and similar to that observed for *D. melanogaster*, we found a significant reduction in HA resistance in *D. sechellia* after tribufos exposure ($-\beta = -1.26$, $P = 0.0019$, Fig. 4C) when DEM + HA was compared to HA.

To test for changes in EST function that occurred during evolution in these species, we made pairwise comparisons between the three species and looked for differences in the effect of tribufos on HA resistance (Fig. 4D). We found no significant differences in the effect of tribufos on HA resistance between *D. melanogaster* and both *D. simulans* ($P = 0.14$) and *D. sechellia* ($P = 0.22$) but we did observe a significant difference between *D. sechellia* and *D. simulans* ($P = 0.02$).

Discussion

To investigate the possible role of common detoxification gene families in *D. sechellia*'s evolved resistance to toxins produced by its preferred host plant *M. citrifolia*, we used

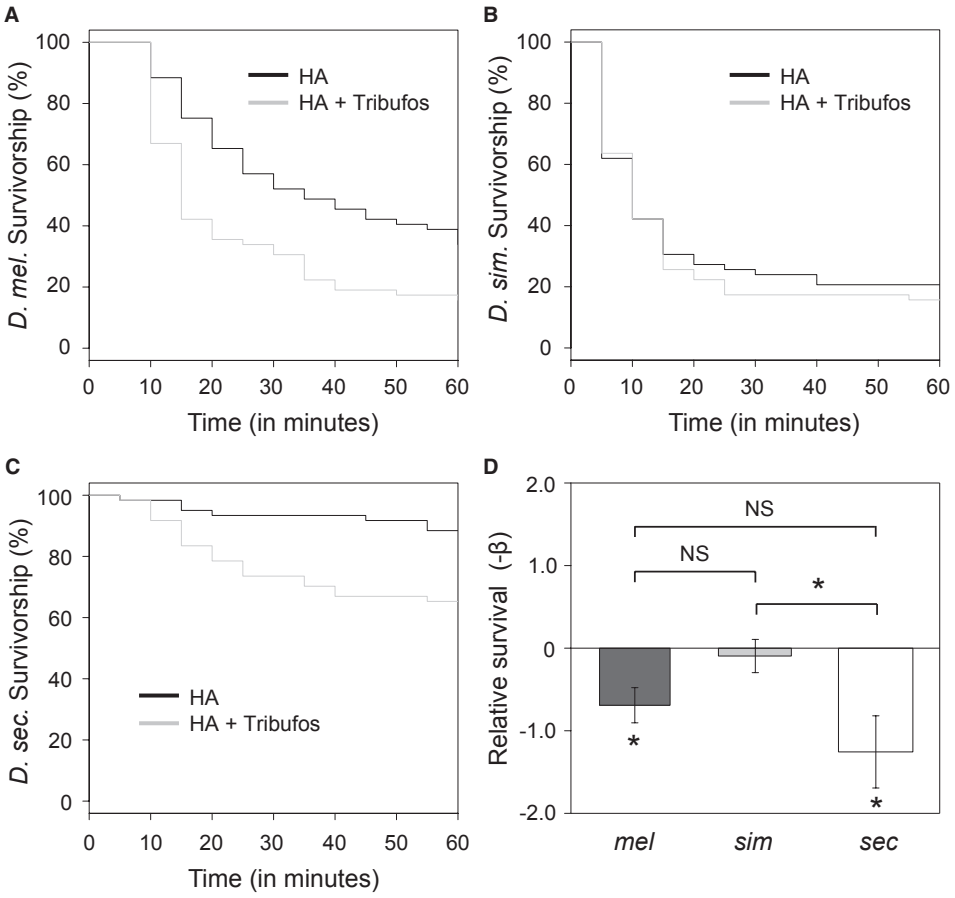

Figure 4. Enzymatic activity of esterases alter hexanoic acid (HA) resistance in *Drosophila melanogaster* and *Drosophila sechellia*. Differences in survival are shown for adult flies in 1.2% HA + cyclohexane (control) compared to 1.2% HA with tribufos. (A) *D. melanogaster*, (B) *Drosophila simulans*, (C) *D. sechellia*. (D) Relative survival (−β) of each species in HA + tribufos compared to HA alone is shown with *D. melanogaster* in dark grey, *D. simulans* in light grey and *D. sechellia* in white. Error bars indicate standard error. Significant differences between species are indicated with asterisks ($P > 0.05$, Cox proportional hazards test). NS, nonsignificant.

chemical synergists to inactivate the functions of these gene families and determined if this inactivation altered resistance to the plant toxins OA and HA. We used the pesticide synergist DEM to eliminate the function of GST gene family members and tribufos to inactivate members of the EST gene family (Plapp *et al.*, 1963; Boyland and Chasseaud, 1970; Snoeck *et al.*, 2017). Prior research showed that members of the common detoxification gene family, the CYP genes, provide basal resistance to both OA and HA in *Drosophila* species but that they are not the source of *D. sechellia*'s derived resistance to both plant toxins (Peyser *et al.*, 2017). The research presented herein aimed to investigate the remaining major detoxification gene families (GSTs, ESTs) to determine if they play a role in *D. sechellia*'s evolved resistance to OA and/or HA.

Consistent with the findings from a similar study of CYPs in these species (Peyser *et al.*, 2017), we found that GSTs are not the source of evolved OA or HA resistance in *D. sechellia*. Interestingly, one or more GST genes may actually serve to increase susceptibility of *D. melanogaster* to the toxicity of OA. This was evidenced by the increase in resistance of *D. melanogaster* to OA when they are concurrently treated with the GST inactivating synergist DEM. Despite the lack of involvement in *D. sechellia*'s evolved resistance, identification of the genetic basis of *D. melanogaster*'s increased resistance upon GST inactivation may allow for a better understanding of the exact biochemical nature of OA toxicity, which remains poorly understood. Furthermore, DEM had no effect on HA associated toxicity in any of the species tested, highlighting the fact that the molecular and biochemical basis of response and resistance to OA and HA are genetically distinct.

Similar to the finding for DEM, *D. melanogaster* has increased resistance to OA when given the EST inactivating synergist tribufos. This indicates that the function of one or more EST genes in *D. melanogaster* increases susceptibility of this species to OA. If, or how, this might be related to the similar pattern observed for GST function

in *D. melanogaster* remains unknown. Further molecular and genetic dissection of increased resistance upon EST inactivation in this species may also increase our understanding of the mechanism of OA toxicity.

Esterases were found to play a role in HA resistance in both *D. melanogaster* and *D. sechellia* but not in *D. simulans*. The phylogenetic relationship amongst these species suggests that the lack of a role of ESTs in *D. simulans*' HA resistance is derived and specific to this lineage. This would predict that ancestrally, ESTs were involved in HA resistance but that this was lost in the lineage leading to *D. simulans.* Although the molecular basis of the esterase function in HA resistance is interesting, the analyses show that ESTs are not the mechanism by which *D. sechellia*'s evolved increased resistance to HA occurred and is instead providing basal resistance to this plant toxin.

By inactivating the ESTs with tribufos in this study, we show that derived EST expression or function in *D. sechellia* contributes to evolved increased OA resistance. When ESTs were inhibited in *D. sechellia* there was a significant reduction in resistance to OA. This response was unique to *D. sechellia* amongst the species tested. There was no effect of EST inhibition on OA resistance in *D. simulans* and there was the opposite effect of EST inhibition in *D. melanogaster* with increased resistance observed. The reduction in resistance observed in *D. sechellia* was significantly different than the responses observed for the two other tested species and strongly suggests that one or more EST gene(s) play a role in evolved OA resistance in *D. sechellia*. Previous research has shown that tribufos acts primarily on ESTs, but can also inhibit CYP enzymatic activity, although to a smaller extent (Sanchez-Arroyo *et al*., 2001). Thus, it is important to also rule out CYP enzymatic activity to ensure the specificity of EST contribution when interpreting results from studies using tribufos (Snoeck *et al*., 2017). Importantly, in our prior study we showed that CYP enzymatic activity was not responsible for the evolved OA resistance observed in *D. sechellia* (Peyser *et al*., 2017), strengthening the conclusion that evolved EST(s) expression or function contributes to derived OA resistance in *D. sechellia*. Prior quantitative trait locus mapping studies in *D. sechellia* suggest that five loci contribute to evolved differences in OA resistance and our previous studies identified the gene(s) underlying one of these QTLs (Jones, 1998; Andrade López *et al*., 2017; Lanno *et al*., 2019). The work presented here suggests that either one or more EST genes or a regulator of one or more EST genes underlies at least one of the other four QTLs that contribute to OA resistance. There are 34 esterase or esterase-related genes identified in the *D. melanogaster* genome that have orthologues in *D. sechellia* (Attrill *et al*., 2016). Of these, 28 have genomic annotation, and 24 of these fall within the QTLs for adult OA resistance generated by Jones (1998). However, if we consider the fine-mapped QTL on chromosome 3R to be fully resolved (Hungate *et al*., 2013), this number drops to nine genes (Supporting Information Table S1). Future studies, including investigations of EST gene expression and function, will be required to determine which gene(s) in the EST family, and/or their regulators, are responsible for the derived OA resistance observed in *D. sechellia*.

Experimental procedures

Fly strains and maintenance

Strains of three species of *Drosophila* were used in this study: *D. sechellia* (14021-0428.25), *D. simulans* (14021-0251.195) and *D. melanogaster* (14021-0231.36). All flies were reared on cornmeal medium at 20 °C using a 16:8 h light : dark cycle.

Pesticide synergist pre-exposure & OA and HA resistance bioassays

Flies were reared in bottles containing cornmeal medium at a common density. Adult flies aged 0–4 days were separated by sex under light CO_2 anaesthesia and allowed to revive in empty vials (Genesee Scientific, San Diego, CA, USA) for 1 h. Female flies were then transferred to bottles containing either control food (H_2O for DEM control, H_2O + cyclohexane for tribufos control) or food mixed with a pesticide synergist (0.3% DEM or 0.6% tribufos) for a pre-exposure period of 24 h to allow for inactivation of GSTs or ESTs prior to the start of each resistance assay. Following the pre-exposure period flies were transferred to experimental vials in a full factorial design containing 0.75 g *Drosophila* instant medium (Carolina Biological Supply Co. Formula 4-24, Burlington, NC, USA) supplemented with either 1.2% OA or 1.2% HA and either control, 0.3% DEM or 0.6% tribufos. Flies were 1–5 days old at the time of resistance assays. Each experimental condition was replicated six times with 10 flies per biological replicate (n = 60 for each treatment type). Toxin resistance was measured by calculating the number of flies 'knocked down' (a fly was determined to be knocked down when it was no longer able to walk or fly) every 5 min for a toxin exposure period of 60 min (Andrade López *et al*., 2017; Lanno *et al*., 2017; Peyser *et al*., 2017). Data generated for this article have been deposited in DRYAD.

Cox proportional hazards regression statistical analysis

A Cox proportional hazards statistical model was used to test the effect of DEM or tribufos exposure compared to OA or HA alone using the *coxph* command in the *survival* package in R (Cox, 1972; Therneau and Grambsch, 2000; Fox, 2008; Therneau, 2015; Andrade López *et al*., 2017; Peyser *et al*., 2017; R Core Development Team, 2017). We report relative survival as the regression coefficient ($-\beta$) for each treatment group compared to its respective control group.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table S1. Table of esterase genes using orthologous annotations and chromosomal positions in D. melanogaster downloaded from FlyBase. Genes with chromosomal positions residing within QTL peaks generated by Jones (1998) or updated to reflect analysis by Hungate (2013) are indicated by a 1 in their respective column.