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Source: Florida Entomologist, 101(3) : 498-504

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.101.0308

Ecological niche difference associated with varied ethanol tolerance between *Drosophila suzukii* and *Drosophila melanogaster* (Diptera: Drosophilidae)

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Abstract

Drosophila suzukii (Matsumura) (Diptera: Drosphilidae) is an important pest that causes damage to fruits of over 60 plant species. *Drosophila suzukii* oviposits on ripe fruit, while *D. melanogaster* oviposits on decaying fruit. Therefore, these species occupy separate ecological niches. To provide a better understanding of the alcohol tolerance between these 2 species and explore the relationship of ecological niche differences and alcohol tolerance, ethanol and acetaldehyde content was examined in red grapes infested by *D. melanogaster* and *D. suzukii*. We assessed mortality and alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activity levels for 2 *Drosophila* species exposed to ethanol. The study results showed that ethanol content gradually increased as the fruit decayed while being infested by *Drosophila*. The ethanol content was higher in the presence of *D. melanogaster* than in the presence of *D. suzukii*. In the mortality experiment, the LC_{50} of *D. melanogaster* adults was approximately 8.0% following exposure to ethanol for more than 6 h, while it was only 2.7% in *D. suzukii*. Moreover, *D. melanogaster* adults and larvae all had higher ADH and ALDH activity than *D. suzukii* exposed to ethanol. Our results suggest that *D. melanogaster* and *D. suzukii* may occupy different ecological niches due to their discrepancy in tolerance to environmental ethanol, which is mainly regulated by ADH and ALDH.

Key Words: niches, *Drosophila*, alcohol, ADH, ALDH

Resumo

Drosophila suzukii (Matsumura) es una plaga importante que causa daño a las frutas de más de 60 especies de plantas que oviposita en fruta madura, mientras que *D. melanogaste*r oviposita en fruta en descomposición. Por lo tanto, estas especies ocupan nichos ecológicos separados. Con el fin de proveer una mejor comprensión de la tolerancia al alcohol entre estas dos especies y explorar la relación de las diferencias del nicho ecológico y la tolerancia al alcohol, se examinó el etanol y el contenido de acetaldehído en uvas tintas infestadas por *D. melanogaster* y *D. suzukii*. Se evaluaron los niveles de actividad de la mortalidad y alcohol deshidrogenasa (ADH) y acetaldehído deshidrogenasa (ALDH) para dos especies de *Drosophila* expuestas al etanol. Los resultados del estudio mostraron que el contenido de etanol aumentó gradualmente a medida que el fruto decayó mientras se infestaba con *Drosophila*. El contenido de etanol fue mayor en presencia de *D. melanogaster* que en presencia de *D. suzukii*. En el experimento de mortalidad, la CL_{50} de adultos de *D. melanogaster* fue de aproximadamente el 8,0% después de la exposición al etanol durante más de 6,0 horas, mientras que fue de sólo el 2,7% en *D. suzukii*. Además, los adultos y las larvas de *D. melanogaster* tuvieron una actividad ADH y ALDH más alta que *D. suzukii* expuestas al etanol. Nuestros resultados sugieren que *D. melanogaster* y *D. suzukii* pueden ocupar diferentes nichos ecológicos debido a su discrepancia en la tolerancia al etanol ambiental, el cual está regulado principalmente por ADH y ALDH.

Palabras Claves: nichos, *Drosophila*, alcohol, ADH, ALD

Drosophila suzukii (Diptera: Drosophilidae) is one of the few *Drosophila* species that is able to lay eggs and feed on healthy ripening fruit. More than 60 plant species have been identified as its primary host (Kenis et al. 2016; Lee et al. 2015), many of which are commercial fruit crops widely grown across the world. *Drosophila suzukii* larvae feed on the fresh fruit and have caused 40 to 80% loss of fruit yield in America (Sasaki & Sato 1995; Mitsui et al. 2006). In contrast, *D. melanogaster* (Diptera: Drosophilidae) and many other drosophilids prefer to lay eggs and feed on rotten fruit (Milan et al. 2012). Decaying fruits contain carbohydrates that are decomposed into short carbon chain alcohols, such as methyl alcohol, ethanol, propyl alcohol and butanol, which can attract the saprophagous *Drosophila* species to lay eggs. For instance, ethanol levels in natural *D. melanogaster* habitats range up to 6% ethanol by volume (Gibson et al. 1981; McKechnie & Morgan 1982). *Drosophila melanogaster* possesses many adaptations that allow it to survive and thrive in ethanol-rich environments (Merçot et al. 1994; Milan et al. 2012; Devineni & Heberlein 2013).

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Many studies have reported the resistance mechanism of Drosophilidae species to alcohol. Behavior, metabolic rate, body mass, and development times of *D. melanogaster* are sensitive to ethanol (Castaneda & Nespolo 2013). Stimulant response is a conserved behavioral response to ethanol among arthropod species, seen in 69.2% of Drosophilidae species (Kliethermes 2015). Female *D. melanogaster* preferentially oviposit on food substrates containing high concentration of ethanol, a process that is regulated by dopaminergic neural circuits (Azanchia et al. 2013). Ethanol can rapidly penetrate the cell membranes of insects, therefore increasing excretion and reducing absorption of ethanol are not viable mechanisms for ethanol resistance in insects (Harris et al. 2008). Adaptations to high levels of ethanol are often achieved by modifications in the metabolic detoxification system in *D. melanogaster* (Fry 2014). During ethanol detoxification metabolism, ethanol is converted into acetaldehyde by alcohol dehydrogenase (ADH) and into acetic acid by acetaldehyde dehydrogenase (ALDH), then into acetyl CoA. Two products of ethanol catabolism, acetaldehyde and acetate, have deleterious effects on the animal's fitness (Deitrich 2004). Acetyl CoA is the final product of both carbohydrate and fat metabolism. Two well-studied enzymes, ADH and ALDH, are related to the detoxification of ethanol in *D. melanogaster* (Fry 2014). *Adh* and *Aldh* mutant lines show significant decrease in alcohol tolerance compared to wild type individuals (Fry & Saweikis, 2006). Natural *D. melanogaster* populations maintained on ethanol-supplemented media evolve higher activity of ALDH and ADH (Fry et al. 2004). Therefore, ethanol could modify the process of energy allocation, which could result in the evolutionary response of *D. melanogaster* (Castaneda & Nespolo 2013). Moreover, many animals, including *Drosophila* species and mammalian species, have evolved resistance to ethanol toxicity (Merçot et al. 1994; Wiens et al. 2008), but little is known about the physiological basis of this resistance.

Merçot et al. (1994) reported that the ecological niches of *Drosophila* species are closely associated with their alcohol tolerance and ADH activity. Unlike *D. melanogaster*, *D. suzukii* feeds on healthy ripening fruit with little alcohol, therefore, research on the difference in alcohol resistance between *D. melanogaster* and *D. suzukii* to alcohol or its metabolite products may elucidate factors contributing to occupation of different niches. The goals of this study were to determine: (1) if there are differences in the content of alcohol and acetaldehyde in 2 natural *Drosophila* species habitats, (2) whether there are differences in the effect of ethanol on mortality of *D. melanogaster* and *D. suzukii*, and (3) if there are differences in ADH and ALDH activity between *D. melanogaster* and *D. suzukii* exposed to ethanol.

Materials and Methods

INSECTS

Drosophila melanogaster and *D. suzukii* adults were collected in Jun 2015 in a grape orchard in Jinan (1.2833°E, 36.6600°N), Shandong Province, China. They were reared on grapes (Red Globe grape) and on an artificial diet for 5 to 6 consecutive generations at 25 ± 0.5 °C, 70 ± 0.5% relative humidity (RH), and a photoperiod of 16:8 h (L:D) in a climate-controlled growth chamber. The artificial diet was composed of mashed banana and apple, corn flour, sucrose, yeast extract, sorbitol, and agar as described in Zhai et al. (2014).

EGG-LAYIING ESTIMATE IN THE FIELD

Grapes (Red Globe grape) were collected from a commercial vineyard in Jinan (1.2833°E, 36.6600°N), using the 5-point sampling method with 30 grapes in each point. The midpoint of the diagonal was selected randomly as the center of the sampling point along the diagonal, and the 4 points that were 5 m equidistant from the center were chosen as the other sample points. The samples were collected in May 2015 when grapes were first ripening, and in Jun 2015 when most grapes were rotten. The grapes that were collected were then individually placed into 1 tissue-culture bottle (5.5 cm diam × 9 cm ht) under the laboratory conditions described above. After 5 days, the numbers of 3rd instar larvae or pupae in fruit were recorded, which served as a proxy measurement of the reproductive success of adults in the vineyard. The proportion of grapes containing larvae for each sample point was calculated and considered as the crop damage rate.

DETERMINATION OF ETHANOL AND ACETALDEHYDE CONTENT IN GRAPES

Fifteen male-female pairs of *D. melanogaster* and 15 male-female pairs of *D. suzukii* adults, 3 days post-eclosion, were placed into 1 tissue-culture bottle (5.5 cm diam × 9 cm ht) containing 1 fresh grape, with 5 replicate bottles for each species. *Drosophila melanogaster* and *D. suzukii* cannot pierce grape fruits with their mouthparts to feed on juice; flies were fed honey-water (60%) in a plastic disc (1 cm diam) placed in each bottle. Moreover, to keep flies from drowning in the honey solution, a filter paper was placed in the bottom of each bottle. Fresh grapes without flies were designed as the control. After 2, 4, 6, 8, 10, and 12 d, the ethanol and acetaldehyde content of the grapes were determined using K-ETOH Ethanol and K-ACHYD Acetaldehyde Assay Kits following the manufacturer's instructions (Megazyme, Bray, Ireland).

EFFECT ON ADULTS AND LARVAE

Fifty female *D. melanogaster* flies at 3 d post-eclosion, and 50 larvae that were 2 d post-hatching, respectively, were placed into artificial diet with different concentration of ethanol in 5 tissue-culture bottle (5.5 cm diam × 9 cm ht) replicates. The percentages of ethanol by weight in the artificial diet were 0, 2.5, 5, 7.5, 10, 12.5, and 15%. The dead adults were recorded after 1.5, 3, 6, 12, and 24 h. The individuals were counted after eclosion, as surviving larvae were needed in larvae experiments.

The *D. melanogaster* and *D. suzukii* adults and larvae surviving exposure to ethanol after 24 h were collected and assayed for the activity of ADH and ALDH using Alcohol Dehydrogenase Activity Assay and Aldehyde Dehydrogenase Activity Colorimetric Assay Kits (Sigma-Aldrich, Munich, Germany). ADH and ALDH activity was calculated using alpha-naphthol standard curve and expressed as U. 1U is the amount of enzyme required to synthesize 1 micromole alpha-naphthol per minute.

DATA ANALYSIS

The ethanol and acetaldehyde content in grapes with different degrees of rotting, the mortality and ADH and ALDH activity levels of *D. melanogaster* and *D. suzukii* at different concentrations of ethanol, were analyzed using a 1-way ANOVA ($\alpha = 0.05$) and Student-Newman-Keuls multiple comparisons using the SPSS 17.0 statistical analysis package (IBM, www.ibm.com). Two-way ANOVA ($\alpha = 0.05$) was used to test the significance of the ethanol and acetaldehyde content with species and time as factors, the significance of mortality of *D. melanogaster* and *D. suzukii* adults with ethanol concentration and time as factors, and the significance of mortality of *D. melanogaster* and *D. suzukii* larvae with species and ethanol concentration as factors. Moreover, the LC_{50} (lethal concentration for 50% of flies) of *D. melanogaster*

and *D. suzukii* exposed to ethanol at different concentrations was estimated through probit regression analysis with SPSS 17.0.

Results

REPRODUCTIVE SUCCESS ON GRAPE IN THE FIELD

Table 1 indicates the egg-laying estimate of *D. melanogaster* and *D. suzukii* in the field, and the damage rate of grapes for Jun 2015 and Aug 2015. The rate of damage caused by *D. melanogaster* and *D. suzukii* in fresh grape was lower than that observed in rotten grape. In Jun, most grapes were ripe and *D. suzukii* laid eggs in less than 10% grapes. However, all rotten grapes remaining in the orchard until Aug contained *Drosophila* eggs or larvae. About 68.3% of eggs were *D. melanogaster*; 9.9% and 23.3% were *D. suzukii* and other *Drosophila* species, respectively.

ETHANOL AND ACETALDEHYDE CONTENT IN GRAPES

The time, species, as well as the interaction of species and time showed significantly impact on ethanol and acetaldehyde content in grapes (Table 2). Ethanol content in grapes increased in the presence of *D. melanogaster* and decreased in the presence of *D. suzukii* compared to grapes that were placed in a container without flies (Fig. 1A). With increasing time for the fruit to decay, the ethanol content increased gradually in grapes infested by *D. melanogaster*, which reached 4.0 ± 0.1 g per L on the 12th d. The highest content of ethanol in the control was 0.891 ± 0.043 g per L on the 12th d, while the ethanol content in grapes infested by *D. suzukii* increased for the first 6 d (0.5 ± 0.0 g per L) and then decreased in the following 6 d. Figure 1B shows the acetaldehyde content in the grapes of the 3 treatments. The acetaldehyde content in grapes infested by *D. melanogaster* and the control showed similar patterns as ethanol content throughout different time points. Interestingly, the acetaldehyde in grapes infested by *D. suzukii* was higher than grapes infested by *D. melanogaster* or the control before the 10th d, but was lower on the 12th d.

EFFECT OF ETHANOL ON ADULTS AND LARVAE

Drosophila melanogaster and *D. suzukii* adult mortality was significantly affected by the concentration of ethanol, exposure time, and the interaction of ethanol concentration and exposure time (Table 3). *Drosophila melanogaster* mortality was not affected when exposed to ethanol with the concentrations of 2.5 and 5% (Fig. 2A). However, the mortality of *D. suzukii* increased when the ethanol concentration exceeded 3% (Fig. 2B). For both *D. melanogaster* and *D. suzukii*, mortality increased gradually with increasing ethanol concentration and exposure time (Fig. 2; Table 3). With increasing exposure time, the LC_{50} of *D. melanogaster* and *D. suzukii* adults decreased steadily; however, the LC_{50} of *D. melanogaster* adults was consistently higher than that of *D. suzukii* adults at the same concentration. The LC_{50} was 3.9% for *D. suzukii* adults and 10.8% for *D. melanogaster* adults at 1.5 h. The LC_{50} of *D. melanogaster* adults was around 8.0% after being exposed to ethanol for 6 h, which was significantly higher than that of *D. suzukii*, which was around 2.7%.

The mortality rate of *D. melanogaster* and *D. suzukii* larvae also increased with increasing ethanol concentration (Fig. 3). Larvae were significantly affected by the concentration of ethanol, time, and the interaction of ethanol concentration and time (Table 3). For *D. melanogaster* and *D. suzukii*, the mortalities of larvae were all higher than those of adults, and all *D. suzukii* larvae died when the media contained 5% ethanol or above.

Table 1. Reproductive success of *Drosophila melanogaster* and *Drosophila suzukii* in the field, and corresponding damage on host grapes.

Time	Reproductive success (Number of 3rd instar larvae and pupae per sample point)			Damage rate (%)
	D. melanogaster	*D. suzukii*	Other flies	
Jun-2015	0	20.4 ± 4.5	0	9.5 ± 2.1
Aug-2015	114.3 ± 19.3	14.8 ± 5.7	38.3 ± 8.4	100%

ADH AND ALDH ACTIVITY

We measured the ADH and ALDH activity levels in *D. melanogaster* and *D. suzukii* flies that were exposed to ethanol for 24 h. The mortality of *D. melanogaster* adults and larvae reached 100% when they were exposed to 10% ethanol for 24 h (Fig. 2A); therefore, individuals exposed to 2.5, 5, and 7.5% ethanol were selected for ADH and ALDH activity assays. High mortality occurred also in *D. suzukii* in response to concentrations above 5% ethanol; therefore, individuals were exposed to 2.5% ethanol in enzyme activity assays (Fig. 2B).

The ADH and ALDH activity of *D. melanogaster* adults was markedly higher than the larvae (Fig. 4A, B; ADH: $F = 29.0$, df = 7, $P < 0.001$; ALDH: $F = 14.1$, df = 7, $P < 0.001$). Exposure to 5% ethanol significantly increased ADH activity in *D. melanogaster* adults by 23.21%. However, exposure to 7.5% ethanol resulted in a 30.79% decrease in activity levels compared to unexposed adult controls (21.5 ± 1.6 U per mg) (Fig. 4A). *Drosophila melanogaster* adults exposed to 7.5% ethanol showed 20.54% decrease in ALDH activity compared to adult controls (1.7 ± 0.1 U per mg) (Fig. 4B) . In contrast, ethanol positively affected ADH activity of *D. melanogaster* larvae, resulting in more than 50% increase in activity level compared to larval controls (8.4 ± 1.4 U per mg) (Fig. 4A). ALDH activity of *D. melanogaster* larvae exposed to ethanol with the concentration of 2.5, 5, and 7.5% also showed significant increases in ALDH activity levels compared to the control (1.2 ± 0.0 U per mg) (Fig. 4B).

The activity level of ADH and ALDH in *D. suzukii* adults and larvae exposed to ethanol (Fig. 4C, D) were consistently lower than that of *D. melanogaster* adults and larvae (Fig. 4A, B). Meanwhile, the adults had higher ADH and ALDH activity levels than those of larvae (ADH: $F = 114.3$, df = 3, $P < 0.001$; ALDH: $F = 5.2$, df = 3, $P = 0.027$). Exposure to 2.5% ethanol increased ADH activity of *D. suzukii* adults and larvae by 14.23 and 38.70%, respectively, compared to controls (adult: 13.7 ± 0.5 U per mg; larvae: 6.7 ± 0.3 U per mg) (Fig. 4C). There were no significant differences in ALDH activity between the control and *D. suzukii* adults or larvae exposed to 2.5% ethanol.

Discussion

Drosophila melanogaster prefers to lay eggs and feed on rotten fruit, which often accumulates higher levels of ethanol as the fruit continues to decay. David and Vanherrewege (1983) reported that *D. melanogaster* fruit fly larvae consume yeasts growing on rotting fruit and have evolved resistance to products of fermentation, such as ethanol and acetaldehyde. *Drosophila melanogaster* can tolerate as much as 6 to 7% ethanol in its breeding sites (Gibson et al. 1981). In this study, *D. suzukii* can tolerate as much as 2.0 to 2.5% ethanol in its breeding sites. Yeasts growing on overripe fruit provide nutrients for adults and larvae of saprophagous Drosophila species (Merçot et al. 1994; Lebreton et al. 2014). The abundance of yeast species was lower in uninfested fruit juice samples com-

Table 2. Probit regression analyses of the effect of ethanol on adults of *Drosophila suzukii* and *Drosophila melanogaster.*

Insect	Time (h)	Regression equation	LC_{50} (%)	Confidence interval (95%)	χ^2	*P*	df
D. suzukii	1.5	Probit(P) = 59.456x − 2.326	3.9	0.029 – 0.046	2015.4	< 0.001	32
	3	Probit(P) = 98.163x − 2.980	3.0	0.027 – 0.034	620.3	< 0.001	32
	6	Probit(P) = 114.945x − 3.160	2.7	0.023 – 0.031	848.2	< 0.001	32
	12	Probit(P) = 121.083x − 3.324	2.7	0.021 – 0.031	1171.9	< 0.001	32
	24	Probit(P) = 123.622x − 3.174	2.6	0.020 – 0.029	907.7	< 0.001	32
D. melanogaster	1.5	Probit(P) = 37.284x − 4.023	10.8	0.093 – 0.125	1597.3	< 0.001	30
	3	Probit(P) = 36.553x − 3.500	9.6	0.088 – 0.104	466.4	< 0.001	30
	6	Probit(P) = 55.290x − 4.496	8.1	0.071 – 0.097	6321.0	< 0.001	30
	12	Probit(P) = 58.007x − 4.577	7.9	0.067 – 0.094	101942.7	< 0.001	30
	24	Probit(P) = 29.602x − 2.796	9.4	0.078 – 0.149	256.3	< 0.001	30

pared to infested fruit juice samples (Hamby et al. 2012). Therefore, the increase of ethanol content in *D. melanogaster* infested grapes may be due to the presence of beneficial microorganisms in rotting fruits. In contrast, we found that ethanol content increased in decaying grapes infested by *D. suzukii* until 6 d after infestation, and ethanol content decreased afterwards. By the 12th d of culture, the ethanol content of *D. suzukii*-infested grapes was significantly lower than grapes infested by *D. melanogaster*. This may be due to the difference in microorganisms that thrive in grapes infested by *D. suzukii* and *D. melanogaster*; however, further investigation is needed to understand this process.

Moreover, previous studies have shown that *D. suzukii* is attracted by traps containing bait with relatively high alcohol content, such as wine (Lee et al. 2011; Cini et al. 2012; Cha et al. 2013). We found that *D. melanogaster* had significantly higher alcohol tolerance compared to that of *D. suzukii* (Fig. 2). *Drosophila melanogaster* adults are more tolerant to environmental alcohol compared to its sister species, *D. simulans*, in both laboratory and field conditions (McKenzie & Parsons, 1972). In wild-type *D. melanogaster*, more than 90% of ethanol is metabolized via the ADH system, and ADH and ALDH activities regulated by dietary ethanol, suggesting that ADH activity reflects the capacity for ethanol tolerance (Geer et al. 1985). We found that *D. melanogaster* adults and larvae had higher ADH activity than *D. suzukii* when exposed to ethanol, which is consistent with previous findings that *D. melanogaster* is more tolerant to alcohol than *D. suzukii* (Sampson et al. 2016). Therefore, the lower resistance to ethanol may underlie the preference of *D. suzukii*to oviposit on healthy ripening fruit with a lower concentration of ethanol (Sampson et al. 2016).

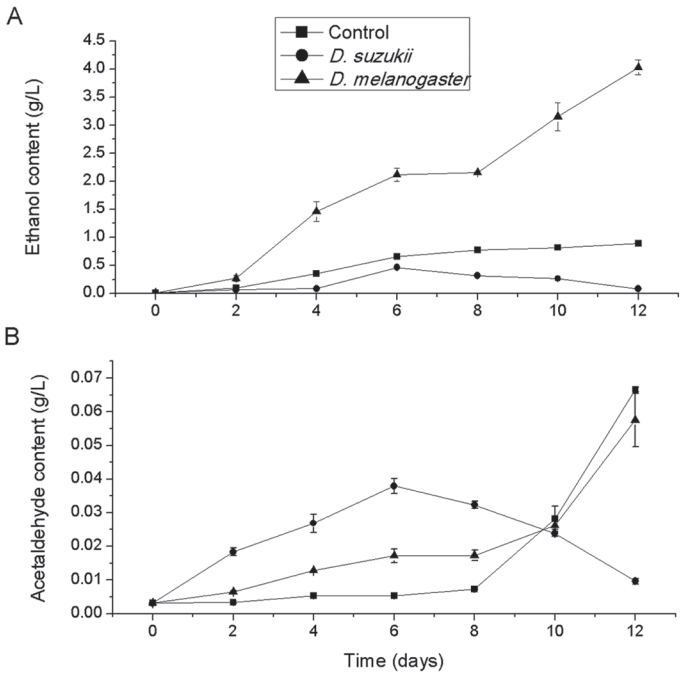

Fig. 1. Ethanol (A) and acetaldehyde (B) contents of grapes infested by *Drosophila melanogaster* and *Drosophila suzukii*.

Acetaldehyde is converted into acetic acid by ALDH (Deitrich 2004). Grapes infested by *D. suzukii* had higher acetaldehyde levels compared to grapes infested by *D. melanogaster*, which may be due to the lower ALDH activity levels of *D. suzukii* adults and larvae when exposed to ethanol. The higher levels of acetaldehyde in grapes infested by *D. melanogaster* were likely detoxified by ALDH, which is one of the characteristics of higher ethanol tolerance. Heinstra et al. (1982) reported that ADH in *Drosophila* not only catalyzes the oxidation of ethanol to acetaldehyde, but additionally catalyzes the conversion of this highly toxic product into acetate. In this study, the ethanol content in grapes infested by 2 *Drosophila* species was more than 10-fold higher than the acetaldehyde content (Fig. 1), which was in accordance with the pharmacokinetic models for ethanol metabolism. The reaction of converting acetaldehyde back into ethanol is essential and keeps acetaldehyde levels approximately 10-fold lower than if the reaction were irreversible (Umulis et al. 2005). It is far more likely that the main metabolic course of alcohol is accomplished by ADH for *Drosophila* when acetaldehyde content is limited. However, the regulation mechanism of enzyme activity by ethanol and acetaldehyde content needs further study.

Aside from differences in protein coding sequences, transcriptional regulation and post-translational modifications also can regulate ADH and ALDH activity (Dannenberg et al. 2005). For instance, Sha et al. (2014) reported that the neuropeptide corazonin (Crz) and its receptor (CrzR), involved in the neuroendocrine system, are important physiological regulators of ethanol metabolism in *Drosophila*, and the CrzR-associated signaling pathway is critical for ethanol detoxification. Moreover, acetyl CoA, the metabolic product of alcohol, can participate in carbohydrate and fat metabolism (Deitrich 2004). *Drosophila* can increase response to oxidative stress through abnormal fat metabolism which results in reduced production of insulin-like peptides (dILPs) and their receptor (Logan-Garbisch et al. 2014). Insulin signaling responds indirectly to ethanol through the phosphoinositide 3-kinase (PI3K) and phosphoinositide-dependent kinase (Pdk) pathways. Ethanol also increases immune response by inhibiting lipid peroxidation (LPO), and promoting the activity of superoxide dismutase (SOD) and Catalase (CAT) (Jahromi et al.

Table 3. Results of two-way ANOVA analysis of alcohol content of grapes, and the effects of alcohol concentration on adult or larval mortality.

Parameters	Source	Type III Sum of Squares	df	Mean Square	*F*	*P*	R Squared (%)
Ethanol content	Corrected Model	115.9	20	5.8	146.1	< 0.001	98.6
	Time	43.2	6	7.28	181.7	< 0.001	
	Species of flies	42.0	2	21.1	530.1	< 0.001	
	Time * Species of flies	30.6	12	2.6	64.3	< 0.001	
Acetaldehyde content	Corrected Model	0.0	20	0.0	64.4	< 0.001	96.8
	Time	0.0	6	0.0	108.6	< 0.001	
	Species of flies	0.0	2	0.0	8.2	0.001	
	Time * Species of flies	0.0	12	0.0	51.7	< 0.001	
Mortality of *D. suzukii* adults (%)	Corrected Model	242103.3	27	8966. 8	165.6	< 0.001	97.7
	Concentration	230138.2	6	38356.4	708.3	< 0.001	
	Time	8083.4	4	2020.9	37.3	< 0.001	
	Concentration * Time	8080.9	17	475.3	8.8	< 0.001	
Mortality of *D. melanogaster* adults (%)	Corrected Model	226866.6	30	7562.2	151.8	< 0.001	97.6
	Concentration	205574.0	6	34262.3	687.9	< 0.001	
	Time	5499.6	4	1374.9	27.6	< 0.001	
	Concentration * Time	6238.6	20	311.9	6.	< 0.001	
Mortality of larvae (%)	Corrected Model	3.0	9	0.3	130.1	< 0.001	96.7
	Species of flies	0.0	1	0.0	1.2	0.3	
	Concentration	3.0	4	0.8	289.6	< 0.001	
	Species of flies * Concentration	0.0	4	0.0	2.8	0.0	

2015). However, it is unclear whether *D. suzukii* has similar genetic regulation mechanism of metabolic enzymes and hydrolysis products of alcohol. This study shows that *D. melanogaster* adults are more tolerant of alcohol than *D. suzukii*. Although the evolutionary adaptation to alcohol for *D. melanogaster* is not fully understood, the availability of different niches for laying eggs and feeding may be contributing factors for evolution of higher tolerance to alcohol. Identifying differences between the genetic regulation mechanisms of *D. melanogaster* and *D. suzukii* after exposure to ethanol could further explain the mechanisms underlying niche differences between these species.

Acknowledgments

This work was financially supported through a grant from the Shandong Provincial Natural Science Foundation, China (ZR-2014CQ014) and Agricultural science and technology innovation projects (CXGC2016D01) and (CXGC2016B11). The authors wish to thank Jianlong Bi for the manuscript language revision.

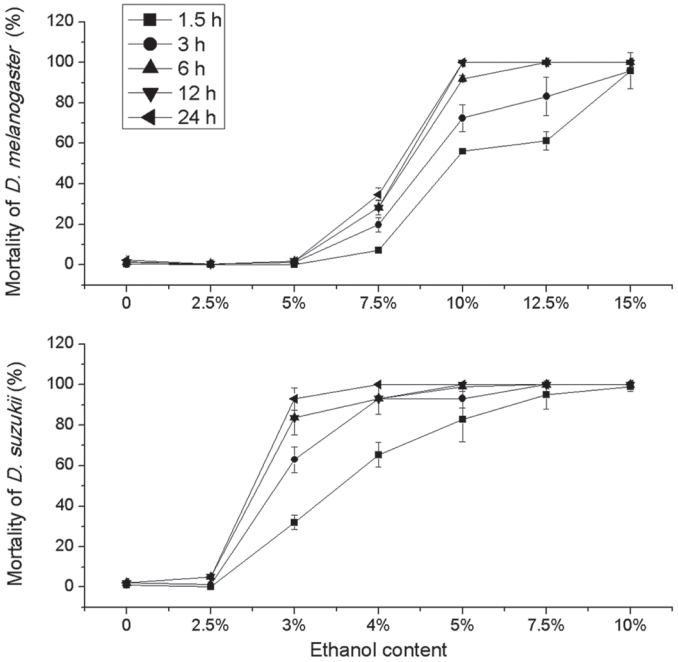

Fig. 2. Mortality of *Drosophila melanogaster* (A) and *Drosophila suzukii* (B) adults exposed to varying concentrations of ethanol.

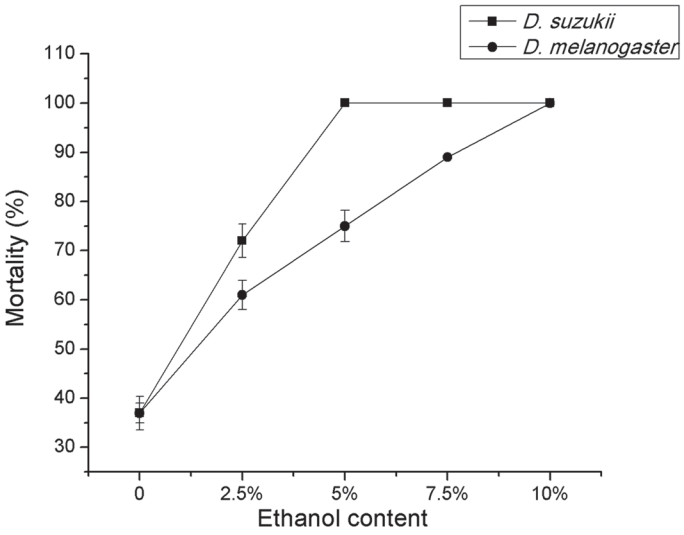

Fig. 3. Mortality of *Drosophila melanogaster* and *Drosophila suzukii* larvae exposed to varying concentrations of ethanol.

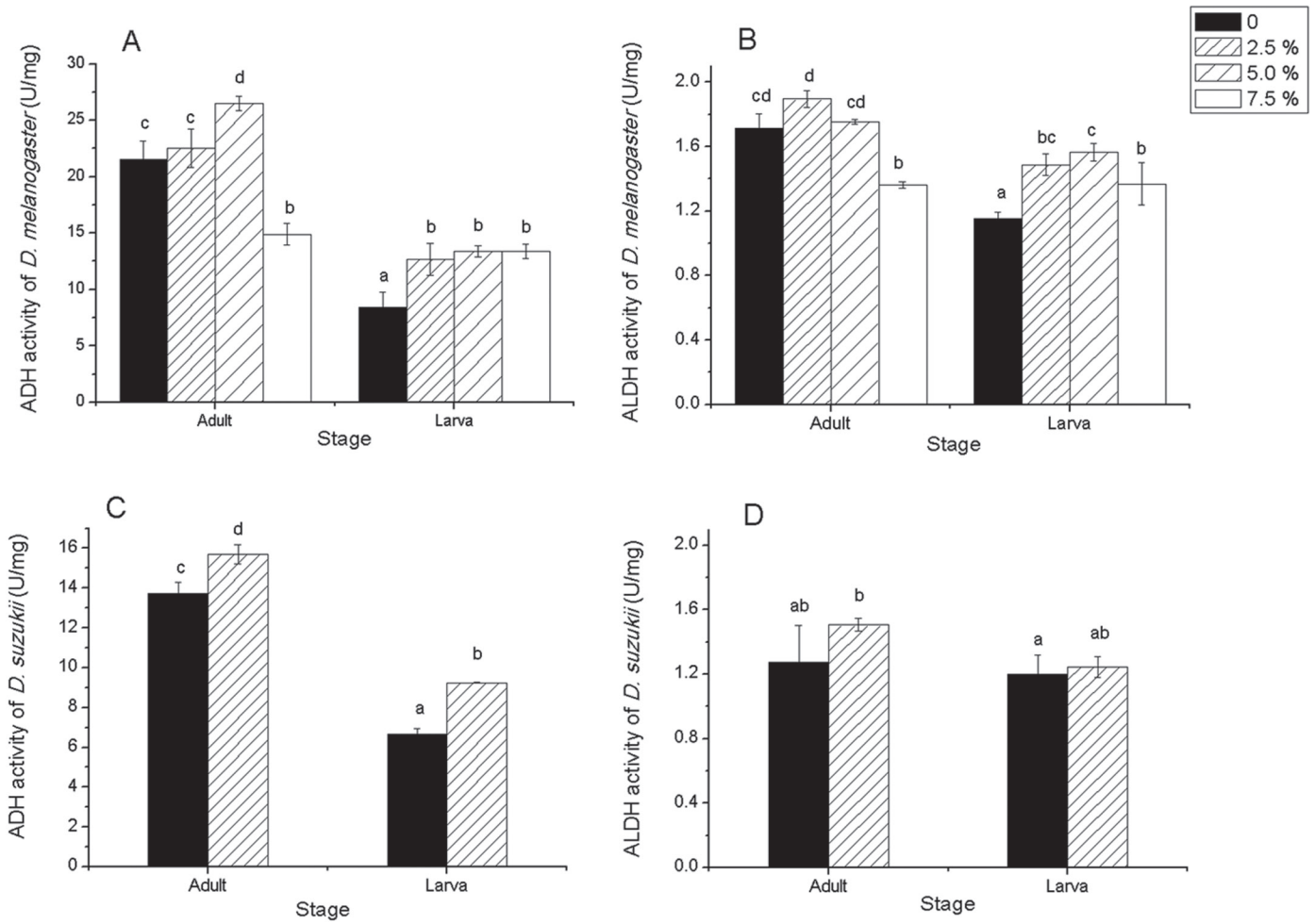

Fig. 4. ADH and ALDH activity levels of *Drosophila melanogaster* and *Drosophila suzukii* exposed to ethanol. (A) ADH activity in *Drosophila melanogaster*; (B) ALDH activity in *Drosophila melanogaster*; (C) ADH activity in *Drosophila suzukii*; (D) ALDH activity in *Drosophila suzukii*. Different letters in each figure (A, B, C, D) indicate a significant difference between adults and larvae (One-way ANOVA: $\alpha = 0.05$).

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