Ecological Niche Difference Associated with Varied Ethanol Tolerance between Drosophila suzukii and Drosophila melanogaster (Diptera: Drosophilidae)

Authors: Huan-Huan Gao, Yi-Fan Zhai, Hao Chen, Yong-Mei Wang, Qian Liu, et. al.
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Ecological niche difference associated with varied ethanol tolerance between Drosophila suzukii and Drosophila melanogaster (Diptera: Drosophilidae)

Huan-Huan Gao¹, Yi-Fan Zhai², Hao Chen², Yong-Mei Wang¹, Qian Liu², Qing-Ling Hu³, Feng-Shan Ren¹*, and Yi Yu²*

Abstract
Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) 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 L50 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) é uma plaga importante que causa dano às frutas de mais de 60 espécies de plantas que oviposita em fruta madura, enquanto que D. melanogaster oviposita em fruta em decomposição. Por isso, essas espécies ocupam 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 CL50 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, ALDH

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; Devisenzi & Heberlein 2013).

¹Shandong Academy of Grape, Jinan, 250100, China; E-mails: gaohuanhuan368@126.com (H. H. G.); wangym228@126.com (Y. M. W.); renasd65@163.com (F. S. R.)
²Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Jinan, 250100, China; E-mails: zyifan@saas.ac.cn (Y. F. Z.); cha.active@163.com (H. C.); 1149364986@qq.com (Q. L. H.); robertyuyi@126.com (Y. Y.)
³College of Chemistry and Environment, Weinan Normal University, Weinan, 714000, China; E-mails: 493375971@qq.com (Q. L. H.)
Corresponding authors; E-mails: renasd65@163.com (F. S. R.); robertyuyi@126.com (Y. Y.)
Varied ethanol tolerance between two Drosophila species

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 (Klie thermes 2015). Female D. melanogaster preferentially oviposits 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 (Mercot et al. 1994; Wiens et al. 2008), but little is known about the physiological basis of this resistance.

Mercot 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 (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-LAYING 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 larva 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 (α = 0.05) and Student-Newman-Keuls multiple comparisons using the SPSS 17.0 statistical analysis package (IBM, www.ibm.com). Two-way ANOVA (α = 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 LC50 (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 acetaldelyde content in the grapes of the 3 treatments. The acetaldelyde content in grapes infested by D. melanogaster and the control showed similar patterns as ethanol content throughout different time points. Interestingly, the acetaldelyde 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 LC50 of D. melanogaster and D. suzukii adults decreased steadily; however, the LC50 of D. melanogaster adults was consistently higher than that of D. suzukii adults at the same concentration. The LC50 was 3.9% for D. suzukii adults and 10.8% for D. melanogaster adults at 1.5 h. The LC50 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.

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 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 (Mercot et al. 1994; Lebreton et al. 2014). The abundance of yeast species was lower in uninfested fruit juice samples com-
Varied ethanol tolerance between two *Drosophila* species

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

<table>
<thead>
<tr>
<th>Insect</th>
<th>Time (h)</th>
<th>Regression equation</th>
<th>LC50 (%)</th>
<th>Confidence interval (95%)</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. suzukii</em></td>
<td>1.5</td>
<td>Probit(P) = 59.456x − 2.326</td>
<td>3.9</td>
<td>0.029 − 0.046</td>
<td>2015.4</td>
<td>&lt; 0.001</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Probit(P) = 98.163x − 2.980</td>
<td>3.0</td>
<td>0.027 − 0.034</td>
<td>620.3</td>
<td>&lt; 0.001</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Probit(P) = 114.945x − 3.160</td>
<td>2.7</td>
<td>0.023 − 0.031</td>
<td>848.2</td>
<td>&lt; 0.001</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Probit(P) = 121.083x − 3.324</td>
<td>2.7</td>
<td>0.021 − 0.031</td>
<td>1171.9</td>
<td>&lt; 0.001</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Probit(P) = 123.622x − 3.174</td>
<td>2.6</td>
<td>0.020 − 0.029</td>
<td>907.7</td>
<td>&lt; 0.001</td>
<td>32</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>1.5</td>
<td>Probit(P) = 37.284x − 4.023</td>
<td>10.8</td>
<td>0.093 − 0.125</td>
<td>1597.3</td>
<td>&lt; 0.001</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Probit(P) = 36.553x − 3.500</td>
<td>9.6</td>
<td>0.088 − 0.104</td>
<td>466.4</td>
<td>&lt; 0.001</td>
<td>30</td>
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<tr>
<td></td>
<td>6</td>
<td>Probit(P) = 55.290x − 4.496</td>
<td>8.1</td>
<td>0.071 − 0.097</td>
<td>6321.0</td>
<td>&lt; 0.001</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Probit(P) = 58.007x − 4.577</td>
<td>7.9</td>
<td>0.067 − 0.094</td>
<td>101942.7</td>
<td>&lt; 0.001</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Probit(P) = 29.602x − 2.796</td>
<td>9.4</td>
<td>0.078 − 0.149</td>
<td>256.3</td>
<td>&lt; 0.001</td>
<td>30</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
<th>R Squared (%)</th>
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</thead>
<tbody>
<tr>
<td>Ethanol content</td>
<td>Corrected Model</td>
<td>115.9</td>
<td>20</td>
<td>5.8</td>
<td>146.1</td>
<td>&lt; 0.001</td>
<td>98.6</td>
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<tr>
<td>Time</td>
<td>43.2</td>
<td>6</td>
<td>7.28</td>
<td>181.7</td>
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</tr>
<tr>
<td>Species of flies</td>
<td>42.0</td>
<td>2</td>
<td>21.1</td>
<td>530.1</td>
<td>&lt; 0.001</td>
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<td></td>
</tr>
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<td>Time * Species of flies</td>
<td>30.6</td>
<td>12</td>
<td>2.6</td>
<td>64.3</td>
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<td></td>
</tr>
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<td>Acetaldehyde content</td>
<td>Corrected Model</td>
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<td>20</td>
<td>0.0</td>
<td>64.4</td>
<td>&lt; 0.001</td>
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<tr>
<td>Time</td>
<td>0.0</td>
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<td>0.0</td>
<td>108.6</td>
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</tr>
<tr>
<td>Species of flies</td>
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<td>2</td>
<td>0.0</td>
<td>8.2</td>
<td>0.001</td>
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<td></td>
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<td>Time * Species of flies</td>
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<td>51.7</td>
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<tr>
<td>Mortality of D. suzukii adults (%)</td>
<td>Corrected Model</td>
<td>242103.3</td>
<td>27</td>
<td>8966.8</td>
<td>165.6</td>
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<td>97.7</td>
</tr>
<tr>
<td>Concentration</td>
<td>230138.2</td>
<td>6</td>
<td>38356.4</td>
<td>708.3</td>
<td>&lt; 0.001</td>
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<tr>
<td>Time</td>
<td>8083.4</td>
<td>4</td>
<td>2020.9</td>
<td>37.3</td>
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<tr>
<td>Concentration * Time</td>
<td>8080.9</td>
<td>17</td>
<td>475.3</td>
<td>8.8</td>
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<tr>
<td>Mortality of D. melanogaster adults (%)</td>
<td>Corrected Model</td>
<td>226866.6</td>
<td>30</td>
<td>7562.2</td>
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<td>&lt; 0.001</td>
<td>97.6</td>
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<tr>
<td>Concentration</td>
<td>205574.0</td>
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<td>34262.3</td>
<td>687.9</td>
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<td>Time</td>
<td>5499.6</td>
<td>4</td>
<td>1374.9</td>
<td>27.6</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
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<tr>
<td>Concentration * Time</td>
<td>6238.6</td>
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<td>Mortality of larvae (%)</td>
<td>Corrected Model</td>
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<td>9</td>
<td>0.3</td>
<td>130.1</td>
<td>&lt; 0.001</td>
<td>96.7</td>
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<tr>
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<td>1</td>
<td>0.0</td>
<td>1.2</td>
<td>0.3</td>
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<tr>
<td>Concentration</td>
<td>3.0</td>
<td>4</td>
<td>0.8</td>
<td>289.6</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
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<td>Species of flies * Concentration</td>
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<td>4</td>
<td>0.0</td>
<td>2.8</td>
<td>0.0</td>
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</tbody>
</table>

**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.
Varied ethanol tolerance between two *Drosophila* species

References Cited


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**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: α = 0.05).


