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Report

Alcohol Consumption as Self-Medication against Blood-Borne Parasites in the Fruit Fly

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Summary

Plants and fungi often produce toxic secondary metabolites that limit their consumption [1–4], but herbivores and fungivores that evolve resistance gain access to these resources and can also gain protection against nonresistant predators and parasites [3, 5–8]. Given that *Drosophila melanogaster* fruit fly larvae consume yeasts growing on rotting fruit and have evolved resistance to fermentation products [9, 10], we decided to test whether alcohol protects flies from one of their common natural parasites, endoparasitoid wasps [11–13]. Here, we show that exposure to ethanol reduces wasp oviposition into fruit fly larvae. Furthermore, if infected, ethanol consumption by fruit fly larvae causes increased death of wasp larvae growing in the hemocoel and increased fly survival without need of the stereotypical antiwasp immune response. This multifaceted protection afforded to fly larvae by ethanol is significantly more effective against a generalist wasp than a wasp that specializes on *D. melanogaster*. Finally, fly larvae seek out ethanol-containing food when infected, indicating that they use alcohol as an antiwasp medicine. Although the high resistance of *D. melanogaster* may make it uniquely suited to exploit curative properties of alcohol, it is possible that alcohol consumption may have similar protective effects in other organisms.

Results and Discussion

Ethanol levels found in natural *D. melanogaster* habitats range up to 6% ethanol by volume in rotting fruits, and 11% in wine seepages found at wineries [14, 15]. Fly consumption of food with moderate levels of ethanol (i.e., less than 4% by volume) results in increased fitness [16–18], but consumption of higher ethanol concentrations (i.e., greater than 4%) causes increasing fly mortality [18–20]. Given that secondary metabolites were shown to harm endoparasitoid wasps in other systems [3, 7, 21, 22], and the suggestion that *D. melanogaster* living in fruits with high ethanol concentrations might experience less wasp parasitism [23], we decided to test whether natural levels of ethanol could act as a protective toxin in fly interactions with two wasp species: *Leptopilina boulardi* is a specialist parasite of *D. melanogaster* and its close relatives that was previously shown to have relatively high ethanol knockdown resistance, whereas *L. heterotoma* is a generalist parasite that infects a diversity of *Drosophila* species living in fermenting fruits, decaying plant materials, and sap fluxes [24–26]. Both wasp species are attracted to the odor of fermentation products such as ethanol, presumably as a means to locate hosts [25, 27], and they are each highly infectious in *D. melanogaster* lab strains [28]. We compared ethanol knockdown resistance of adult female flies and wasps over a 24 hr period using *Drosophila* food mixed with concentrations of ethanol ranging from 4% to 10% by volume (Figure 1A; see also Figure S1 available online). At 6% ethanol, *D. melanogaster* adults and adults of the specialist wasp *L. boulardi* both showed significantly greater knockdown survival than adults of the generalist wasp *L. heterotoma* (Figure 1A). Considering all ethanol concentrations used, *D. melanogaster* is most ethanol resistant, followed by the specialist wasp *L. boulardi*, followed by the generalist wasp *L. heterotoma* (Figure S1).

Given that wasps suffer knockdown by natural levels of environmental ethanol, we tested whether wasps also show a reduction in oviposition when presented with host fly larvae grown in 6% ethanol food (Figure 1B). There was a significant effect of ethanol in reducing oviposition of both wasp species. A significant ethanol-by-wasp interaction effect also indicated that ethanol had a stronger effect in reducing oviposition by the generalist *L. heterotoma* than the specialist *L. boulardi*. This difference is not explained by a difference in wasp mortality, because there was no wasp death over the course of the 2 hr trial. Wasps may lay fewer eggs because they are sickened by ethanol fumes and attack less, but it is also possible that they insert their ovipositors into fly larvae growing on ethanol food at a normal level and limit oviposition because they detect a hostile environment for their offspring. Given that wasp oviposition was not reduced in fly larvae briefly removed from ethanol (data not shown), we favor the former hypothesis. Thus, ethanol can provide protection to fly larvae from being attacked by endoparasitoid wasps.

We next considered whether ethanol can help flies kill wasp parasites in the hemocoel once flies are infected. First, we measured the hemolymph ethanol concentration of *D. melanogaster* larvae grown in 6% ethanol food and found that fly hemolymph ethanol concentration was significantly higher in flies grown on food containing ethanol, with concentrations reaching approximately 6 mM (0.02% hemolymph ethanol content by volume) (Figure 2A). This ethanol concentration is low relative to those found in adult flies and honeybees [29–32], suggesting that *D. melanogaster* larvae may be particularly resistant to passage of ethanol across the gut wall or cuticle into the hemolymph and/or may have very efficient ethanol detoxification mechanisms. Fly hemolymph ethanol content returned to baseline level within 24 hr after larvae were removed from ethanol food, and wasp infection did not result in increased fly hemolymph ethanol concentration or prolong the presence of ethanol in the hemolymph (Figures S2A and S2B). Altogether, these data show that wasp eggs and larvae living in fly hemolymph are exposed to a moderate level of ethanol (and presumably to ethanol breakdown products such as acetaldehyde) when flies live in or consume ethanol. Any protective effect ethanol might have for infected flies is likely passive, because infected flies do not appear to purposefully increase hemolymph ethanol levels, for example by downregulating ethanol breakdown enzymes.

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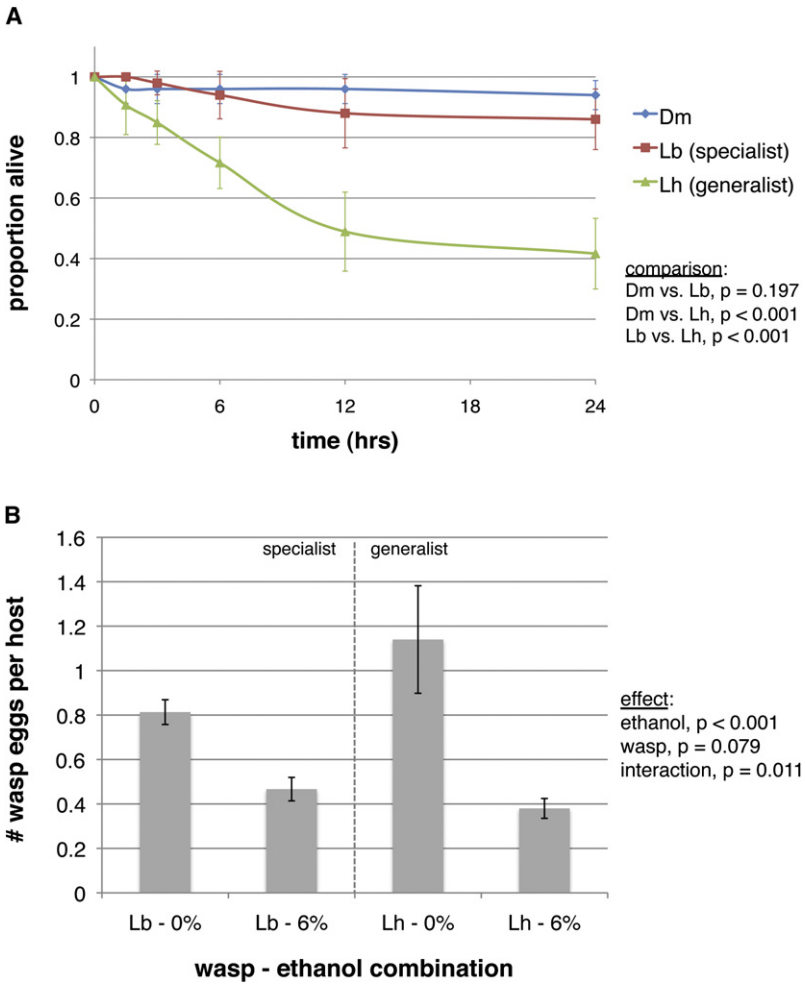

Figure 1. The Effect of Ethanol on Wasp Knockdown and Oviposition

Survival curves were generated for adult insects living in petri dishes with 6% ethanol food (A). Error bars indicate 95% confidence intervals. The number of wasp eggs laid per host (B) was measured by dissecting fly larvae grown on food containing 0% or 6% ethanol and exposed to wasps for 2 hr. Error bars indicate SD. Dm, *D. melanogaster*; Lb, *L. boulardi*; Lh, *L. heterotoma*. There were five dish replicates for all treatments. See also Figure S1.

To determine whether host ethanol consumption affects wasp larval development, we briefly removed *D. melanogaster* larvae from the 6% ethanol food for attack by wasps before being returned to the food. There was a significant effect of host ethanol consumption on wasp larval mortality (Figure 2B). There was also a significant effect of wasp species and a significant interaction between ethanol treatment and wasp species, indicating that the increase in wasp larval mortality due to host consumption of ethanol was significantly greater for the generalist *L. heterotoma* than the specialist *L. boulardi.* To determine whether wasp larval mortality was an effect of ethanol experienced by the host fly larvae before or after attack, we performed a similar infection experiment in which food treatments were switched after the fly larvae were attacked (Figure S2C). Although there was no overall effect of different ethanol treatments on wasp larval mortality, in a regression analysis stratified by wasp type there was a significant increase in death of *L. boulardi* larvae in hosts grown on ethanol food postinfection compared to preinfection ($p = 0.003$), whereas *L. heterotoma* larvae suffered high mortality regardless of ethanol consumption timing ($p = 0.623$). Larval wasp death resulted in a decreased proportion of wasps surviving through eclosion and a significant increase in the proportion of flies that eclosed, despite an overall increase in ethanol-mediated fly mortality (Figure 2C). There were significant ethanol-by-wasp interaction effects on the proportion of flies and wasps eclosed, again indicating that ethanol has a stronger protective effect in flies infected by the generalist *L. heterotoma*. Altogether, these results indicate that ethanol consumption enhances fitness of wasp-infected flies and that flies can receive maximal therapeutic benefit by consuming ethanol postinfection.

Wasp larvae dissected from singly infected control hosts invariably had defined internal organs and moved vigorously (Figure S2D). However, wasp larvae dissected from fly larvae grown on 6% ethanol food often did not move, showed amorphous internal organ structure, and had everted tissues, in many cases in close proximity to their anuses (Figure S2E), suggesting that ethanol causes defects in wasp organ development or maintenance. Normally, flies attempt to kill wasps in a process termed encapsulation, and the increased mortality of wasps growing in ethanol-fed host flies might be the result of a heightened fly encapsulation response. Encapsulation involves constitutively produced plasmatocytes recognizing a wasp egg or larva as foreign and signaling to induce differentiation of lamellocytes, which spread over the wasp in a multilayered capsule, leading to wasp death [33]. The wasp strains used here are highly virulent in *D. melanogaster* hosts and normally completely suppress the encapsulation response, but no wasp eggs or larvae dissected from ethanol-consuming fly larvae were found to be encapsulated by host hemocytes either. Although ethanol consumption was associated with a significant increase in fly plasmatocyte numbers, ethanol consumption was associated with a significant decrease in the number of lamellocytes, the hemocyte type specifically induced to mount the encapsulation response (Figures S2F and S2G). Lack of induction and/or death of host lamellocytes could be the result of ethanol toxicity, but it may be adaptive for hosts to purposefully suppress induction of an immune response that is unneeded in the presence of an antiparasite toxin, given the presumed energetic cost of mounting an immune response [34].

Use of toxic secondary metabolites in defense against enemies is usually preventative, i.e., organisms consume a toxic food source as part of their normal diet and the presence of toxin in their bodies results in internal host conditions that limit subsequent predation and infection. However, parasitized organisms can also therapeutically self-medicate, whereby they actively seek out compounds that help cure preexisting infections [35, 36]. The fact that fly consumption of