Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

______________________________ ____________

Neil Milan Date

Ecological and Evolutionary Interactions between Fruitflies and Their Parasitic Wasps

By

Neil F. Milan
Doctor of Philosophy

Graduate Division of Biological and Biomedical Science
Population Biology, Ecology, and Evolution

____
Todd A. Schlenke
Advisor

____
Jacobus C. de Roode
Committee Member

____
Nicole M. Gerardo
Committee Member

____
Michael A. Goodisman
Committee Member

____
Yun Tao
Committee Member

Accepted:

____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

____
Date

Ecological and Evolutionary Interactions between Fruitflies and Their Parasitic Wasps

By

Neil F. Milan
B.S., University of California, Davis, 2005

Advisor: Todd A. Schlenke, Ph.D.

An abstract of
A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Population Biology, Ecology, and Evolution
2012

Abstract

Ecological and Evolutionary Interactions between Fruitflies and Their Parasitic Wasps
By Neil F. Milan

My dissertation project is focused on the evolution and ecology of the Drosophila-wasp parasitoid system, particularly the fruitfly *D. melanogaster* and wasps of the genus *Leptopilina*. The parasitoid wasps use an ovipositor (modified stinger) to inject eggs into fruitfly larvae or pupae. At that point, there is a competitive, with-in host interaction between the fruitfly's immune response and the wasp egg invader. The immune response attempts to surround and kill the egg so that the fruitfly can complete its development; the wasp egg invader, in contrast, attempts to develop and emerge as a larva quickly enough to evade the immune response, in order to consume the host from the inside-out and develop into an adult wasp. The first research area is the effect of ethanol on the interaction between the fruitfly host and wasp parasitoid. While so much of the host-parasite interaction can be (and is) affected by the genotypes of the fruitfly and wasp, the surrounding environment in which the two interact may also crucially affect attack rates, immune system evasion and wasp development. I have been particularly interested in how the host-parasite dynamic changes when wasp parasitoids encounter fruitflies that feed on food plants that contain toxins to which the flies are resistant, but which may be toxic to the parasitoid. My work indicates that the presence of ethanol does limit wasp attack, hinders wasp infection success, and induces a "self-medication" behavior in parasitized fly larvae. The second research area is the horizontal gene transfer of transposable elements between fruitflies and their wasp parasitoids. Although the traditional view once held that genes are passed on only from parents to offspring, the last few decades have seen numerous reports of horizontal gene transfer between higher eukaryotes, including multicellular animals. Since hosts and parasites have very strong intimate associations, it can be hypothesized that there should be high rates of gene transfer between these organisms. Indeed, using the Drosophila-wasp parasitoid system I have found evidence for high rates of horizontal gene transfer, which is an exciting development for evolutionary biologists in particular because it may shed light on how novelty evolves within genomes and populations. Additionally, this host-parasite interaction may become a fertile system for deeper explorations of gene transfer in animals, which severely lack study models for probing and refining hypotheses.

Ecological and Evolutionary Interactions between Fruitflies and Their Parasitic Wasps

By

Neil F. Milan
B.S., University of California, Davis, 2005

Advisor: Todd A. Schlenke, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of Doctor of Philosophy in
Population Biology, Ecology, and Evolution
2012

Acknowledgements

I would like to express my heartfelt gratitude to my parents and family for their unfailing support and encouragement since day one of this long journey; to my friends for providing perspective through humor and "real world" updates throughout grad school; to Todd Schlenke for his incredible patience, generosity, and guidance as my advisor; and to all the Schlenke Lab members over the years for their camaraderie and the many fond memories.
Ad maiorem Dei gloriam.

Contents

1. Dissertation Introduction 1

2. "Alcohol Consumption As Self-Medication Against Parasites In The Fruitfly" 12

Figure 1 34

Figure 2 36

Figure 3 38

Figure 4 40

Figure 5 42

Figure 6 44

Figure 7 46

Manuscript Acknowledgements 49

3. "Extensive horizontal gene transfer between Drosophila melanogaster and its endoparasitoid wasps" 50

Table 1 67

Table 2 68

Table 3 69

Figure 1 70

Figure 2 72

Figure 3 74

Figure 4 76

Figure 5 78

Figure 6 80

Supplemental Figure S1 82

Supplemental Figure S2 84

Supplemental Figure S3 86

Manuscript Acknowledgements 92

4. “Phylogenetic Analyses of Two TEs” 93

Figure 1 102

Figure 2 104

Figure 3 106

Figure 4 108

5. Dissertation Discussion 110

6. Bibliography 125

Dissertation Introduction

Parasites are organisms that live on or in another organism (their host), from which they draw the nutrients necessary for growth and subsistence. Parasites are capable of causing huge morbidity and mortality burdens on their hosts and can be highly adept at regulating host population sizes [1,2,3,4]. For example, in the United States, parasites were the causative agent for the first three out of the top ten leading causes of death in 1900AD, and their subsequent control over the course of the century is credited in part with increasing average life span by almost 30 years and dramatically reducing infant and child mortality [5]. While the negative effects on human health understandably have driven much of their study, host-parasite interactions have also become a model system for studying evolution and coevolution [6,7]. Because hosts and parasites must dedicate tremendous resources to gaining any advantage over one another (often at the risk of death before being able to reproduce), there is strong pressure driving adaptation in both host and parasite, leading to an "evolutionary arms race" in host-parasite systems [8,9]. Considering that host-parasite interactions can often involve organisms with rapid generation times and high numbers of offspring (e.g., bacteria, fungi, arthropods), the potential for witnessing and studying evolutionary processes on a short timescale has been part of the draw toward host-parasite model systems.

Parasitism has arisen independently in hundreds of diverse taxonomic lineages (reviewed in [10]). Thus, parasites represent an amazing degree of biological

diversity. Virulence, or a parasite's ability to harm hosts, can also affect their ability to infect and adequately exploit them. It can take one of two general forms, immune evasive or immune suppressive. Virulence can take an "evasive" form where the parasite passively hides from host defenses to avoid recognition and neutralization [11,12]. Virulence can also take a "suppressive" form where a parasite actively blocks the immune response, preventing immunity from functioning altogether and potentially making the host susceptible to other infections. The active suppression of immune responses has been shown to facilitate long-term parasitic infection with high morbidity over that span of time, as well as susceptibility to other pathogens [13].

Virulence is often correlated with how parasites transmit between hosts [14]. In fact, a trade-off between the two can exist: a parasite must be able to exploit its host enough to escape defenses, survive and even reproduce (virulence), but cannot exact too heavy a toll too quickly such that its host dies before the parasite or its offspring can infect another host (transmission) [15,16,17]. This general theory can be used to explain why pathogens that specialize on a particular host may have a more optimal virulence-transmission balance than pathogens with wider host ranges: specialists' virulence may be better suited to combating a host's immune response without causing an excessive fitness burden on the host and potentially limiting successful transmission, whereas pathogens with wider host ranges may have poor transmission rates because of virulence strategies that may not be effective enough for successful infection or cause excessive host mortality.

Host organisms combat the virulence mechanisms and strategies of pathogens through a suite of physiological structures and responses broadly called immunity or the immune response. Immunity is an organism's set of defenses against invasion and infection by pathogens [18,19,20]. These responses can be subdivided into further categories, such as behavioral, physical, and physiological responses. Behavioral immunity broadly encompasses actions that organisms perform to limit or cure themselves of infection, often heavily involving the nervous system. In humans, one of the best studied forms of behavioral immunity is the induction of fevers after infection [21]. One particularly intriguing form of behavioral immunity is self-medication, whereby an infected animal will seek out foods containing compounds capable of combating the infection [22]. Physical immunity has to do with physical barriers hosts use to prevent infections, such as skin, hair, and mucous membranes. Physiological immunity encompasses structures and mechanisms that are based in particular organ systems, and can be further divided into the innate and adaptive immune responses. It is the innate immune response in particular that only recently has been given tremendous attention due to its role as a first response to infection and prelude to the more specific adaptive response [23].

Innate immunity encompasses the physiological responses of an organism against pathogens that are "non-specific, non-anticipatory, and non-clonal" and is quite different from the more widely-studied adaptive immune response of vertebrates, which includes B and T blood cells, antibodies, long-term pathogen memory, and

specificity of response [24]. Innate immunity is common to all organisms and has been well-studied in the model insect *Drosophila melanogaster*. Other models, particularly the mouse, are commonly used for immunity studies due to their similarity to humans and their (relative) ease of genetic and organ manipulation [25]. However, the use of mouse models for innate immunity studies have been somewhat limited because their innate and adaptive immune responses cannot be easily isolated from one another during infections. *D. melanogaster*, though more evolutionarily distant from humans, has been a particularly good study model: innate immune responses can be easily isolated since they do not have an adaptive response, many key components in fruitflies (such as *Toll*) have mammalian orthologs, a wealth of genetic tools and methods for further genetic dissection of the immune system are available, and organisms can be reared in very high numbers allowing for high replicability in experiments [26,27,28]. Additionally, fruitfly immunity functions as an ideal model for other insects that are important to agriculture and human disease, such as bees and mosquitoes respectively.

The innate immune response of *D. melanogaster* can also be divided further into two broad functional arms: humoral immunity and cellular immunity. Humoral immunity is governed by the fruitfly's fat body (an organ analogous to the mammalian liver) and involves the release of active immune molecules, such as antimicrobial peptides (AMPs), into the hemolymph to neutralize bacterial and fungal pathogens, though larger parasites (macroparasites) may also be targets [27]. The Toll and Imd pathways are two of the major regulators of the humoral immune

response, with the JAK/STAT and JNK pathways believed to play smaller complementary roles in the response [29]. It is thought that each pathway is activated by particular pathogens (e.g., Toll in response to Gram-positive bacteria and fungi), but it is likely that these pathways may be acting in a more synergistic and nuanced manner [30].

D. melanogaster cellular immunity is governed by the lymph gland (the hematopoietic organ) and involves the action of hemocytes (blood cells), which can be classified into three groups [31]. Plasmatocytes make up the vast majority (upwards of 95%) of standing hemocytes, patrol the body for infections, and perform phagocytosis. The remaining standing hemocytes are the crystal cells, which are key components of encapsulation, coagulation, and wound repair. The third class of hemocytes, lamellocytes, are large flattened cells produced in response to infection and encapsulate macroparasites that are too large for phagocytosis, such as wasp eggs. Lamellocytes can be mobilized from the lymph gland or differentiate from circulating plasmatocytes [32]. Much of the cellular immune response appears to be regulated by the Toll pathway, which functions in hematopoiesis [33]. Specific cell classes, such as the lamellocytes, and their roles in the response may be regulated further by the JAK/STAT pathway [31,34]. Parasitoid wasps can be particularly useful model pathogens for studying cellular immunity because they strongly elicit the cellular immune response, which is less characterized than its humoral counterpart [27,31,35,36,37]. It is also extremely interesting how the fruitfly cellular immune response might recognize wasp eggs as

foreign in the first place, given they are both insects and fruitflies do not produce anticipatory receptors such as the immunoglobulins of vertebrates.

Parasitoid wasps infect a wide range of insects. They were initially studied for their potential as biological control agents of agricultural pests because they can have very limited host ranges that include only the target pest species, can persist in a particular area without re-releases, and can effectively regulate host populations [1,38,39]. They are remarkable in the incredibly diverse morphologies, ranges, and life cycles that they exhibit. Even among those that parasitize Drosophila, the life cycles and infection strategies can drastically differ [40,41]. Drosophila immunity studies can benefit from the use of parasitoid wasps as an infection model for several reasons. Unlike many other pathogens and infection models of Drosophila, parasitoid wasps are natural pathogens requiring no experimenter input such as the needle poke often used in bacterial models [27,35,40]. Furthermore, parasitoid wasps of Drosophila attack the larval and pupal life stages, whose immune responses have largely been ignored in studies, and do so using a wide variety of strategies to counter host defenses [27,35,41].

The females of these obligate, solitary parasitoid wasps seek out fruitfly larvae and use a modified ovipositor to pierce the cuticle, then inject an egg into the host. Venom is injected along with the egg and is composed of virulence factors that allow the egg to counter the host's immune response to infection, though only a small subset of virulence genes and their exact functions having been identified so far

[42,43]. For one wasp species (*Leptopilina heterotoma*), venom acts as part of an "immune suppressive" strategy within hosts—that is, host immune functions are shut down or severely limited—while another wasp species (*L. boulardi*) seems to use an "immune evasive" strategy—that is, the host's immune response generally remains active yet unable to encapsulate the wasp egg [41,44]. A functional host immune response will attempt to melanotically encapsulate the wasp egg in a cellular response driven heavily by the action of lamellocytes (reviewed in [35]). These specialized, induced blood cells will attach to the egg, form layers around it, and release cytotoxic agents in an attempt to kill it. The result is a dead egg—and controlled infection—inside a melanized mass of cells. If, however, the encapsulation response is unsuccessful, a wasp larva will emerge from the egg, gradually consume its host, and emerge as an adult after the host fruitfly has pupated.

In addition to their different approaches to combating the host immune response, parasitoid species can also have different host ranges. *D. melanogaster* is parasitized by many wasp species, some that specialize on *D. melanogaster* and its close relatives, and others that are generalists which attack multiple species groups in the genus Drosophila [40]. One aspect of specialist and generalist host ranges that is particularly intriguing concerns how specialists may be well-adapted to success against the immune responses of specific hosts, whereas generalists may exchange lower success rates for a broader array of hosts. Other tradeoffs caused by adapting

specialist or generalist strategies are likely to exist and may manifest themselves when the infection occurs under particular circumstances or habitats.

The outcome of the interaction between fruitflies and parasitoid wasps—namely, the success of one over the other—is not determined solely by each organism's immune response and virulence strategies, or the genotype-by-genotype compatibility between host and parasite [1,45]. The environmental context in which the two encounter each other can alter the dynamics of attack, infection, and outcome. The connection between environment and organisms may appear indirect, such as when temperature and season alter the mixture of available host species and, in turn, the attack rates and success of parasitoids, especially those with more specialized host ranges [1]. Environment can also have more direct effects, such as when the nutrient quality of food substrates on which Drosophila feed affects the growth and survival of organisms that consume them [46]. For example, work in a Drosophila-nematode host-parasite system showed that some species were likely driven toward specialization on a fungi, in spite of the nutritional fitness costs, because one of its toxic secondary compounds helped provide a parasite-free space [47,48]. Thus, environmental context may play an important role in how *D. melanogaster* and its parasitoid wasps interact with one another. My dissertation focuses on two environmental factors, one abiotic and one biotic, that potentially play extremely important roles in fruitfly-wasp interactions.

First, fruitflies of the genus Drosophila utilize a wide variety of host substrates (e.g., fruits, fungi, leaves) for habitats on which to complete their life cycles, beginning with an egg laid on or near the food source and ending with an adult emerging from a pupal case after it has spent the larval stage feeding in the substrate. However, some Drosophila species that can subsist on a variety of food substrates are known to preferentially exploit certain substrates [49,50,51]. This specialization on food substrates is likely due to a need to escape competition by utilizing an open niche or to avoid predators and parasites that could readily access the previous ecological niche [47,52]. Specialization can be strong enough and have persisted long enough for species to have adapted traits that allow full utility of particular food substrates [49,50,51]. Much of the need for specialized adaptations is that these food sources often can be toxic [53]. They may contain secondary metabolites or compounds that are harmful to organisms that cannot breakdown or otherwise nullify their effects [49,50,51,54,55,56].

D. melanogaster is commonly considered a food generalist; that is, these fruitflies can complete their life cycles and subsist on nearly any rotting food substrate that can support the growth of yeast (and other microorganisms) that they actually consume as their food source [56]. *D. melanogaster* is also one of the most ethanol-tolerant fruitflies, which is not surprising given that it prefers rotting fruits with high sugar content that (along with the right microorganisms) can lead to high levels of alcohol through fermentation [55,57,58,59]. Its parasitoid wasp enemies, on the other hand, show variable levels of resistance to alcohols, which in the case of

L. boulardi seems to correspond to the frequency it attacks *D. melanogaster* on fermenting food substrates in nature [60]. So, considering *D. melanogaster*'s remarkably high tolerance to alcohol and its parasitoid wasps' variable levels of tolerance, I sought to investigate how ethanol, an abiotic component of the *D. melanogaster* environment, might influence the fruitfly-parasitoid wasp interaction. I focused on four particular topics: (1) comparing the ethanol resistance levels of a specialist (*L. boulardi*) and a generalist (*L. heterotoma*) wasp relative to *D. melanogaster*, (2) assaying the effect ethanol in food has on wasps' rate of attack on fruitfly larvae, (3) testing for any effect ethanol may have on wasp survival within hosts, and (4) determining if parasitoid wasp attack is driving fruitflies to choose food substrates with ethanol. The results of that work are covered in Chapter 2.

Second, other organisms that closely associate with the fruitflies and wasps can also affect fruitfly-wasp interactions. For example, parasitoid wasps are only one parasite among a myriad that Drosophila must interact with and defend against. Just within their feeding substrates alone, Drosophila face a variety of viral pathogens, bacterial pathogens, fungal pathogens, protozoan pathogens such as trypanosomes, and animal pathogens such as nematode worms and ectoparasitic mites [61,62,63,64,65,66]. Some of these parasites are already known to interact with both the fruifly host and parasitoid wasp. For example, the bacterial pathogen *Wolbachia* and the unicellular microsporidian pathogen *Tubulinosema kingi* were both shown to be capable of horizontal transmission from fruitflies to the parasitoid wasps infecting them, which is surprising given that both pathogens are obligate

intracellular parasites of host cells and rely on closely coupled interactions with those hosts [67,68]. Given the strong effects these microparasites have on their primary fruitfly hosts, it is likely that they act as a serious selection pressure on parasitoid wasps developing within the fruitflies.

Given a report that a genomic parasite—a transposable element—could be horizontally transmitted from a moth to its parasitoid wasp [69], I decided to explore the possibility that a similar horizontal transmission of genomic parasites could be occurring in the Drosophila-parasitoid wasp system. If horizontal transfer is occurring, the influx of genetic material from fruitflies into their parasitoid wasps could have metabolic effects on wasp genomes and also alter the evolutionary potential of the wasps, continually reshaping the fruitfly-parasitoid wasp interaction [70,71,72]. I also sought to test theories about the conditions that would promote horizontal gene transfer and make inferences about the mechanisms of transfer in my particular study system. That work is covered in Chapter 3. In an extension of the horizontal transfer project, I focused on two transposable elements that succeeded in transferring to parasitoid wasp genomes and examined whether these TEs had been able to reproduce and thrive in their novel wasp hosts. That work is covered in Chapter 4.

Alcohol Consumption As Self-Medication Against Blood-Borne Parasites In The Fruitfly

Neil F. Milan*, Balint Z. Kacsoh*, and Todd A. Schlenke

* these authors contributed equally to this work

Department of Biology, Emory University, 1510 Clifton Road NE, Atlanta, GA 30322, USA

Summary

Background

Organisms frequently utilize food resources that contain compounds toxic to other organisms. The ability to consume such toxins not only allows access to potentially underutilized resources, but can also provide protection against non-resistant predators and parasites. Given that larvae of the fruitfly *Drosophila melanogaster* live within rotting fruit and have evolved resistance to high levels of ethanol and other products of fermentation, we decided to test whether ethanol protects fruitflies from otherwise lethal parasites.

Results

Here, we show that environmental ethanol causes reduced infection of fruitfly larvae by endoparasitoid wasps. Furthermore, if infected, ethanol consumption by fruitfly larvae results in developmental retardation and death of wasps growing in the fly hemocoel, without need of the stereotypical anti-wasp immune response.

This double protection afforded to fly larvae by ethanol is significantly more effective against a generalist wasp than a wasp that has evolved to specialize on *D. melanogaster*. Finally, fly larvae actively seek out ethanol-containing food when infected, showing they use alcohol as an anti-wasp medicine and self-medicate accordingly.

Conclusions

Our data uncover a novel form of behavioral immunity, whereby fruitflies protect themselves against blood-borne parasites by consuming alcohol. 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.

Highlights

- environmental ethanol protects *D. melanogaster* from being parasitized by wasps
- consumption of ethanol by *D. melanogaster* also kills internal wasp parasites
- *D. melanogaster* choose high ethanol content food when infected by parasitic wasps
- protection afforded to fly hosts by ethanol is stronger against a generalist parasite

Introduction

Plants and fungi often produce toxic secondary metabolites that limit their consumption by herbivores and fungivores [73,74,75,76]. However, natural

selection can favor toxin resistance because resistant herbivores and fungivores gain access to food resources for which there is little competition. Resistant species can also benefit if the toxins they consume make them more resistant to natural enemies such as predators and parasites [48,75,77,78,79]. A classic example of the use of plant secondary metabolites for protection against enemies involves monarch butterflies, which sequester toxic cardenolides from milkweed hosts, making them unpalatable to and actively avoided by a bird predator [80]. These same toxins were also shown to limit monarch butterfly infection by a protozoan parasite [81].

Use of toxic secondary metabolites in defense against enemies is often passive and preventative, i.e. organisms consume a toxic food source as part of their normal diet, which causes a buildup of toxin in their bodies, which prevents predation and establishes internal host conditions that limit subsequent infection. However, parasitized organisms can also therapeutically self-medicate, whereby they actively seek out plant and fungal secondary metabolites that help cure them of infection once they are infected [82]. The original examples of self-medication came predominantly from mammals [83,84], but recently insects have also been shown to detect infection and preferentially utilize food sources that palliate pre-existing infections [85,86,87]. The protective effects of toxins against natural enemies can be due to passive or active behaviors by the organism being preyed upon or infected. For example, toxic compounds may be simply passing through an organism's gut, they may be taken up into the blood due to normal absorption of nutrients across the gut wall, they may be sequestered into particular tissues or cell

compartments, or they may be stored and then secreted during a defensive response [75].

Ethanol is a potentially toxic secondary metabolite of yeast generated by the fermentation of sugars. Any role ethanol might play in protecting hosts from their parasites is of special interest, because ethanol is a simple and relatively ubiquitous compound that is consumed by a number of organisms. The fruitfly *D. melanogaster* eats yeasts growing on rotting fruits, uses rotting fruits as mating and oviposition sites, and develops in rotting fruits until pupation. Consequently, *D. melanogaster* frequently comes into contact with ethanol and other alcohols derived from fruit sugar fermentation and has evolved a greater resistance to ethanol than almost all other animals, including other *Drosophila* species. *D. melanogaster* can grow in artificial media with ethanol concentrations upwards of 10% by volume [59,88]. This roughly corresponds to ethanol levels found in the natural habitats of *D. melanogaster*, which range up to 6% ethanol by volume in rotting fruits, and 11% ethanol by volume in fermented grape extract and wine seepages found at wineries, which often support large populations of fruitflies [89,90]. The genetic basis for ethanol detoxification in *D. melanogaster*, e.g. involving induction of alcohol dehydrogenase and other enzymes by the gut and fat body (analogous to the mammalian liver), has been intensely studied for more than 40 years [91,92,93].

D. melanogaster larvae and adults derive benefit from consumption of ethanol in their food when the concentration of ethanol remains in the lower ranges (i.e. less

than 4%). At these concentrations, flies convert ethanol into increased energy stores, develop more quickly from larva to adult, and have significantly increased longevity [94,95,96]. However, at higher ethanol concentrations (i.e. greater than 4%), *D. melanogaster* larval development is protracted and flies suffer increasing mortality [96,97,98]. Because ethanol content of any rotting fruit varies micro-spatially within the fruit, and overall fruit ethanol content varies temporally during the rotting process, it is not surprising that *D. melanogaster* are highly attuned to ethanol concentration and choose to oviposit on and live in food resources with ethanol concentrations that maximize fitness [99,100,101].

Among the most common natural parasites of *D. melanogaster* are endoparasitoid wasps, which have been found to infect more than 50% of fly larvae in natural populations [102,103,104]. These wasps lay their eggs in fruitfly larvae and pupae, which - if allowed to hatch - begin to devour the hosts from the inside out, eventually eclosing from fly pupal cases as adults. Fruitflies use behavioral immunity to avoid oviposition sites infested by wasps, as well as physiological immunity to combat wasp eggs once infected [105,106]. The physiological immune response is thought to involve several steps [105,107]: it begins when circulating, constitutively produced plasmatocytes (small circular blood cells) recognize the wasp egg as foreign and signal to induce differentiation of lamellocytes (large, flattened blood cells), either directly from circulating plasmatocytes or from prohemocytes in the lymph gland (the fly hematopoietic organ). The newly derived lamellocytes migrate towards, attach to, and spread around the wasp egg in a multi-

layered capsule. In the final step, the inner cells of the capsule surrounding the wasp egg lyse, releasing reactive oxygen species and creating an impermeable layer of melanin, resulting in death of the wasp egg. During oviposition, female wasps can anchor their eggs to fly tissues to help them evade fly immunity, and they can inject venom with their egg to actively suppress fly immunity [108,109]. The outcome of any fly-wasp interaction is dependent on the genotypes of both host and parasite [110].

We tested whether *D. melanogaster* consumption of ethanol could protect the flies from infection by endoparasitoid wasps, as host secondary metabolites have been shown to harm endoparasitoid wasps in other systems [75,78,111,112]. It was also previously shown that a wasp that specializes on *D. melanogaster* hosts (*Leptopilina boulardi*) was more ethanol resistant than other *Drosophila* endoparasitoids, and that adult female wasps, which must attack flies on ethanol-impregnated substrates, were more ethanol resistant than male wasps of the same species [60,113]. Thus, it appears that adaptation of *D. melanogaster* to ethanol-rich habitats imparts selection pressure for ethanol resistance on endoparasitoids that specialize in infecting *D. melanogaster* in nature, and that ethanol might act as a protective toxin in fly interactions with wasps.

We first compared ethanol resistance in two *Drosophila* endoparasitoids: *L. boulardi* is a specialist parasite of *D. melanogaster* and its close relatives, while *L. heterotoma* is a generalist parasite that infects multiple species groups in the *Drosophila* genus

[40,114,115]. Both wasp species regularly infect *D. melanogaster* in nature, and have extremely high infection success when reared on *D. melanogaster* lab strains in the lab [114,115]. We then tested whether food resources containing ethanol protected *D. melanogaster* from being infected by either wasp, and whether juvenile wasps suffered greater mortality when living inside *D. melanogaster* hosts grown on ethanol food. We hypothesized that ethanol resistance would be lower in the generalist endoparasitoid than in the specialist, and that the generalist would suffer greater effects of any protective properties of dietary ethanol in *D. melanogaster* hosts. Finally, we tested whether infected *D. melanogaster* larvae actively seek out ethanol food as self-medication against wasp infection, which would represent a form of behavioral immunity.

Results

Ethanol knockdown resistance of adult female flies and wasps was measured over a 24 hr period using four concentrations of ethanol mixed with *Drosophila* food (Figure 1). There was no death of any flies or wasps in food without ethanol (data not shown). At the lowest ethanol concentration (4%) most flies and wasps survived, while at the highest concentration (10%) most flies and wasps died. Statistical comparison of survival curves between species was performed using the Kaplan-Meir survival analysis assuming constant hazard. *D. melanogaster* showed significantly greater survival than *L. boulardi* after exposure to 4 and 8% ethanol, and significantly greater survival than *L. heterotoma* after exposure to 4, 6, and 8%

ethanol. Furthermore, the *D. melanogaster* specialist *L. boulardi* showed significantly greater survival than the generalist *L. heterotoma* at 6 and 8% ethanol.

To determine whether flies alter oviposition rates in response to ethanol, groups of ten adult female *D. melanogaster* were allowed to lay eggs for 24 hrs on food containing varying concentrations of ethanol (Figure 2A). The statistical association between food ethanol concentration and fly egg lay counts was evaluated using a general linear model (GLM) with a Poisson probability distribution. The flies laid significantly fewer (approximately one third fewer) eggs on food containing 4, 6, or 8% ethanol than on control food, although there was no difference in oviposition across these three ethanol concentrations. At 10% ethanol, where adult fly knockdown was high (Figure 1G), substantially fewer eggs were laid. To determine the impact of ethanol on wasp oviposition rates, groups of thirty 72 hrs old fly larvae were placed on control food or food containing 6% ethanol, and were immediately exposed to ten female wasps for two hours before being dissected to count wasp eggs (Figure 2B). A GLM with a Poisson probability distribution was used to test the effects of ethanol, wasp species, and their interaction on wasp egg lay counts. There was a statistically significant effect of ethanol, as ethanol treatments were associated with significant egg lay reductions in both wasp species. There was no statistical difference in egg lay counts between *L. boulardi* and *L. heterotoma*, but a significant ethanol by wasp interaction effect indicates that ethanol had a significantly stronger effect in reducing oviposition by the generalist

L. heterotoma than the specialist *L. boulardi*. This difference is not explained by wasp mortality, as there was no wasp death over the course of the two-hour trial.

Next, the hemolymph ethanol concentration of fly larvae grown in 6% ethanol food was measured in order to assess the possibility that ethanol in fly hemolymph might limit the survival of infecting wasp larvae (Figure 3A). Using a one-tailed t-test with the Satterthwaite correction for unequal variances, fly hemolymph ethanol concentration was found to be significantly higher in flies grown on food containing ethanol, with concentrations reaching approximately 0.01% hemolymph ethanol content by volume. To determine how fast hemolymph ethanol is degraded, as well as to assess the possibility that fly larvae might sequester ethanol and release it into the hemolymph following wasp infection, hemolymph ethanol levels were measured in attacked and non-attacked fly larvae, as well as in larvae grown continuously in ethanol food versus removed from ethanol food for 24 hrs (Figure 3B, 3C). Statistical analysis with ordinary least squares regression showed that ethanol treatment was significantly associated with fly larvae hemolymph ethanol concentration, which declined to nearly un-measurable levels in flies removed from ethanol food for 24 hours. However, there was no association between wasp infection or wasp species on fly larvae hemolymph ethanol concentration, and no significant effect of ethanol and wasp treatment interactions.

To determine whether host ethanol consumption affects wasp larvae growing within *D. melanogaster* larvae, fly larvae raised in food containing 6% ethanol were

briefly removed from the food for attack by wasps before being returned to the food. Mortality of wasp larvae was compared 60 hrs post-attack between host flies grown on control food and on food containing ethanol using a GLM model with a binomial outcome distribution. The model showed a significant effect of host ethanol consumption on wasp larval mortality (Figure 4A). 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 if wasp larval mortality was an effect of ethanol experienced by the host fly larvae before or after attack, a similar experiment was performed in which food treatments were switched after the fly larvae were attacked (Figure 4B). A GLM with a binomial outcome distribution showed there was no overall effect of ethanol treatment on wasp larval mortality, i.e. host consumption of ethanol pre- or post-infection resulted in similar rates of wasp death. However, limiting the GLM analysis to one wasp species at a time showed a significant increase in death of *L. boulardi* larvae in hosts grown on ethanol food post-attack compared to pre-attack ($p = 0.003$), whereas *L. heterotoma* larvae showed no such difference ($p = 0.623$). *L. heterotoma* larval mortality was similar whether ethanol treatments occurred pre-attack, post-attack, or pre- and post-attack (Figure 4A, 4B). There were also overall significant effects of wasp species and the interaction between ethanol treatment and wasp species on wasp larval mortality, indicating once again that *L. boulardi* larvae were more resistant to the effects of host ethanol consumption than *L. heterotoma* larvae (Figure 4B).

Host ethanol consumption appeared to have a range of effects on developing wasps. Although most wasps dissected from fly larvae 60 hrs post-attack had hatched into larvae, some were not yet hatched. Wasp eggs found in singly infected hosts grown on control food (Figure 5A) were significantly larger and further developed than those from hosts grown on 6% ethanol food (Figure 5D). Wasp larvae dissected from singly infected fly larvae grown on control food had defined internal organs and moved vigorously (Figure 5B, 5C). However, many *L. boulardi* and *L. heterotoma* larvae dissected from fly larvae grown on 6% ethanol food did not move, showed amorphous internal organ structure, and often had everted tissues, in many cases in close proximity to their anuses (Figure 5E, 5F). In no case were wasp eggs or larvae dissected from hosts grown on ethanol food found to be encapsulated by *Drosophila* hemocytes in the stereotypical melanotic encapsulation immune response.

In order to understand the lack of melanotic encapsulation of dead wasps in fly larvae grown on ethanol food, plasmatocyte and lamellocyte counts were performed. Poisson regression models were used to investigate the effects of ethanol consumption and wasp infection on hemocyte numbers. Ethanol consumption was associated with a significant increase in plasmatocyte numbers across wasp treatments (Figure 6A). There were also significant differences in plasmatocyte counts across all three wasp treatments, with non-infected fly larvae having the greatest number of plasmatocytes, followed by flies infected by *L.*

heterotoma and *L. boulardi*. As expected, non-infected flies did not produce lamellocytes (Figure 6B). Nevertheless, there was a significant effect of ethanol consumption on fly larvae lamellocyte counts, due to a near complete lack of lamellocytes in infected flies grown on ethanol food. There were also significant differences in lamellocyte counts across all three wasp treatments, with flies infected by the generalist *L. heterotoma* having a greater number of lamellocytes than flies infected by the specialist *L. boulardi*.

To determine whether fruitflies self-medicate using ethanol, infected and uninfected fly larvae were placed in bisected petri dishes containing half control food and half 6% ethanol food. Preference for ethanol food was measured by counting the number of fly larvae that moved to (or remained on) the ethanol food side of the dish, using starting conditions where fly larvae were initially placed on the control food side (Figure 7A, 7B) or ethanol food side (Figure 7C, 7D). For fly larvae initially placed on control food, a GLM model with a binomial outcome distribution showed a significant effects of wasp treatment at 24 hrs, with fly larvae infected by the generalist *L. heterotoma* significantly more likely to be on the ethanol food side of the dishes than fly larvae infected by the specialist *L. boulardi*, and fly larvae infected by *L. boulardi* significantly more likely to be on the ethanol food side of the dishes than uninfected fly larvae (Figure 7B). For fly larvae initially placed on ethanol food, infected larvae moved off the ethanol food faster than uninfected fly larvae, but returned to the ethanol food in greater numbers than uninfected fly larvae by 24 hrs. A GLM model with a binomial outcome distribution also showed

there were significant effects of wasp treatment, with fly larvae infected by the generalist *L. heterotoma* once again significantly more likely to be on the ethanol food side of the dishes than fly larvae infected by the specialist *L. boulardi*, and fly larvae infected by *L. boulardi* significantly more likely to be on the ethanol food side of the dishes than uninfected fly larvae (Figure 7D).

Discussion

Although *D. melanogaster* appears to benefit from consumption of lower concentrations of ethanol, significant fitness costs accrue as food ethanol concentration increases past approximately 4% [94,95,96,97,98], and *D. melanogaster* prefer to oviposit on control food rather than on food containing 4% or greater concentrations of ethanol (Figure 2). Nevertheless, we have shown that *D. melanogaster* larvae specifically prefer to live in food containing 6% ethanol by volume when they are infected by endoparasitoid wasps (Figure 7), likely due to the fact that ethanol helps them cure themselves of infection (Figure 4). The curative properties of ingested ethanol presumably stem from increased ethanol concentration in fly hemolymph (Figure 3), where the wasp larvae live. There is an especially dramatic effect of ethanol on *L. heterotoma* growing in *D. melanogaster* larvae, as this wasp has greater than 90% infection success under "normal" lab conditions [115], but showed a 60% increase in mortality when host flies consumed ethanol (Figure 4). Interestingly, infected flies showed greater movement in food choice trials even when they began the trial on ethanol food, suggesting they actively sample their environment before settling on the most suitable food source

for fighting off infection. Larval choice of ethanol food not only can cure them of wasp infections, but could also act as a preventative measure to limit attack in the first place (Figure 2B). Altogether, our data show that *D. melanogaster* larvae assess food ethanol content and choose to live, at least temporarily, in relatively toxic, high ethanol content food in order to rid themselves of potentially lethal parasites. Such a choice would be relevant in nature, as ethanol content varies spatially within rotting fruit.

Previous work has suggested that generalist parasitoids suffer more from secondary metabolites than specialist parasitoids [111]. *L. heterotoma* is a relative generalist that competes for *D. melanogaster* hosts in nature with the specialist *L. boulardi*, but also infects a diversity of other *Drosophila* species living in fermenting fruits, as well as in decaying plant materials and sap fluxes [114]. Thus, *L. heterotoma* is not expected to be under the same strong selection pressure for ethanol resistance as *L. boulardi*. The observed variation in the relative abundance of natural *L. boulardi* and *L. heterotoma* populations over space and time [103] might be explained, at least in part, by geographic and seasonal variation in fruit ethanol levels. Surprisingly, infected fly larvae were significantly more likely to seek out ethanol food when infected by the generalist *L. heterotoma* than when infected by the specialist *L. boulardi* (Figure 7). These data suggest that fly larvae can distinguish between endoparasitoids with different levels of ethanol resistance, or that *L. boulardi* can better manipulate the ethanol seeking behavioral immune response of *D. melanogaster*.

We indeed found the specialist *L. boulardi* to be better adapted to infection of *D. melanogaster* hosts grown on ethanol food in multiple ways. *L. heterotoma* adult females were significantly less resistant to knockdown by ethanol than *L. boulardi* at food ethanol concentrations between 6-8% (Figure 1), and showed a significantly greater reduction in oviposition when allowed to attack *D. melanogaster* larvae growing in food with 6% ethanol (Figure 2). Wasps may lay fewer eggs because they are repelled by ethanol fumes and attack less, but it is also possible they insert their ovipositors into fly larvae growing on ethanol food at normal frequency, but choose to lay eggs less often because they detect a hostile host environment for their offspring. Given that wasp oviposition was not reduced in fly larvae briefly removed from ethanol for the wasp larvae survival experiment (data not shown), we favor the former hypothesis. *L. heterotoma* larvae were also significantly more likely to die in host flies grown on ethanol food than *L. boulardi* larvae, and, unlike *L. boulardi* larvae, were negatively affected by host ethanol consumption both pre- and post-attack (Figure 4). It is noteworthy that *L. boulardi* eggs are typically attached to host tissues such as the gut and fat body, which may offer some protection from hemolymph-borne toxins, whereas *L. heterotoma* eggs are always found floating freely in the hemolymph [116].

D. melanogaster larvae growing on food containing 6% ethanol were found to have an approximately 0.013% (3 mM) ethanol concentration in the hemolymph (Figure 3). Although this level was significantly higher than that of larvae grown on control

food, it is relatively low compared to the ethanol concentration of the food, suggesting that ethanol does not easily pass across the larval gut wall, and/or that *D. melanogaster* has very efficient ethanol detoxification mechanisms. In other studies, ethanol vapor inebriation of adult *D. melanogaster* resulted in up to 300 mM ethanol concentrations in whole body extracts [117,118], while feeding adult honeybees 5% ethanol food resulted in 50 mM hemolymph concentrations [119]. However, these higher ethanol concentrations were measured from different tissue samples, life stages, species, ethanol delivery techniques, and ethanol measurement techniques, and may not be meaningful comparisons for our data. Regardless, we do not yet know whether the protection from parasitism afforded to *D. melanogaster* larvae by ethanol consumption is mediated by the relatively modest observed increase in hemolymph ethanol levels (Figure 3) or by a correlated change such as a potentially increased level of hemolymph acetaldehyde, the toxic major breakdown product of ethanol.

Hemolymph ethanol levels were found to decline precipitously after *D. melanogaster* larvae were removed from ethanol food for 24 hours, and did not increase upon infection by endoparasitoid wasps (Figure 3). These data suggest that hemolymph ethanol levels are maintained only by continuous ethanol uptake from the gut, and that ethanol is not preserved in the hemolymph or sequestered in other tissues and released into the hemolymph during infection. Surprisingly, larval mortality of the generalist wasp *L. heterotoma* was significantly increased even when hosts were moved off ethanol food post-attack (Figure 4), suggesting that a small window of

ethanol (or related toxin) exposure to the wasp egg is sufficient to cause death of wasp larvae two days later. However, host consumption of ethanol prior to attack was not sufficient to harm *L. boulardi* larvae, indicating that *L. boulardi* eggs are better able to withstand short-term exposure to ethanol than *L. heterotoma* eggs. On the other hand, post-attack consumption of ethanol by *D. melanogaster* larvae was sufficient to limit survival of eggs and larvae from both wasp species, proving there is a benefit to fly larvae that seek out ethanol food after being infected. Although developmentally arrested wasp eggs were occasionally found in host flies grown on ethanol food (Figure 4D), the most common phenotype associated with wasp death was a lack of internal organ structure in hatched wasp larvae along with the eversion of tissues outside the larval cuticle (Figure 4E, 4F). It is unclear at this point how ethanol or associated products might cause this phenotype.

D. melanogaster is adept at melanotically encapsulating foreign objects found in its hemocoel, including wasp eggs and inert objects such as paraffin oil droplets [105,120]. Though consumption of ethanol was associated with the death of a significant fraction of wasp larvae, these dead foreign tissues were never melanotically encapsulated by the host immune response. At least two hypotheses may explain this result. First, the wasp strains used in this experiment are highly virulent in *D. melanogaster* hosts, and utilize venoms that suppress the melanotic encapsulation response [109,115,121]. It is possible that the wasp venoms injected into hosts with wasp eggs suppress the host encapsulation response even after the developing wasp egg or larva has died. However, we found that ethanol

consumption by infected host larvae increases the number of fly plasmatocytes, and dramatically reduces the number of lamellocytes, the dominant cell type involved in encapsulation (Figure 6). These data suggest the lack of melanotic encapsulation is caused by an ethanol-mediated mis-regulation of hemocyte numbers, potentially due to suppression of the differentiation of lamellocytes from plasmatocytes, or lamellocyte death. It could be adaptive for hosts to purposefully suppress induction of an immune response that is un-needed in the presence of an anti-parasite toxin, given the presumed energetic cost of mounting an immune response [122].

To our knowledge, our data are the first to show that alcohol consumption can have a protective effect against infectious disease, and in particular against blood-borne parasites. Many studies in humans have documented decreases in blood cell function and decreases in immunity against viral and bacterial infections in chronic consumers of alcohol [123,124], but little attempt has been made to assay any medicinal benefit of alcohol use in moderate doses or in short bursts. Although the high alcohol resistance of *D. melanogaster* may make it uniquely suited to exploit curative properties of alcohol, it remains possible that alcohol consumption could have similar protective effects in other organisms, including humans.

Experimental Procedures

Insect rearing

D. melanogaster strain Oregon R was used for all experiments. *L. boulardi* strain Lb17 and *L. heterotoma* strain Lh14 originated from single females collected in

Winters, California in 2002 [115], and have been continuously maintained in the lab on *D. melanogaster* strain Canton S. Both wasp strains are highly infectious in *D. melanogaster* lab strains and are rarely melanotically encapsulated and killed by the fly immune system [115].

Instant *Drosophila* medium (Formula 4-24, Carolina Biological Supply) in 0.25 g aliquots per 35 mm diameter petri dish was used for all experiments, supplemented with approximately 20 granules of active baker's yeast and concentrations of ethanol ranging between 0 and 10% by volume. For standard experimental infections, Oregon R flies were allowed to lay eggs overnight; 48 hrs later, second-instar larvae were moved into petri dishes containing the experimental medium in groups of forty per dish. At 72 hrs, early third-instar fly larvae were moved into new, non-ethanol food dishes to be attacked by groups of ten female wasps for two hrs, after which they were returned to the experimental food conditions. Insects were kept in a 25 degrees C incubator with 12 hr light-dark cycle for all experiments.

Adult ethanol resistance

We used ACS/USP grade 95% ethanol (111000190, Pharmco-AAPER), which contains less than 0.001% methanol, for this and all other experiments. Batches of ten female flies and wasps were collected 3-5 days post-eclosion and aspirated into food dishes containing 0, 4, 6, 8, and 10% ethanol in five replicates. Counts for dead flies and wasps were made 1.5, 3, 6, 12, and 24 hrs later.

<u>Oviposition rate</u>

Fly oviposition rates were measured at 24 hrs by counting the total number of eggs laid in the petri dishes used in the adult ethanol resistance experiments. For wasp oviposition rates, 72 hrs old fly larvae grown on control food were placed in batches of thirty in new dishes containing either control food or 6% ethanol food, in five replicates, and immediately exposed to 10 female wasps for 2 hrs. Fly larvae were then dissected to count the number of wasp eggs found inside. The 6% ethanol concentration was chosen for this and following experiments because this is the upper limit of ethanol concentrations found in naturally rotting fruits [89], the upper limit that adult *L. boulardi* and *L. heterotoma* can withstand for 2 hrs with minimal death (Figure 1), and a concentration at which fly larvae experience moderate mortality (between 10 and 40%) during development [98]. Thus, it is an ecologically relevant ethanol concentration that should maximize patterns observed in our experiments.

<u>Hemolymph ethanol content</u>

D. melanogaster hemolymph (blood) was collected by bleeding groups of approximately thirty larvae onto a cold glass slide and drawing up 2 uL of hemolymph with a micropipette. The level of ethanol found in fly hemolymph following various treatments was measured using a colorimetric assay kit (#K620, BioVision) following the manufacturer's recommended protocol. Briefly, alcohol oxidase was used to oxidize ethanol and generate hydrogen peroxide, which reacted

with a probe to generate colored product with an absorption maximum of 570 nm. The amount of ethanol present in the experimental samples was calculated by comparing experimental spectrophotometric readings to a standard curve established with known concentrations of ethanol. Comparison of hemolymph ethanol content from flies grown on control versus ethanol food was made with five replicates, whereas experiments in which the food treatment was switched after wasp attack were run in three replicates.

Wasp larvae survival

Fly larvae grown on control and 6% ethanol food were dissected 60 hrs post-attack, a time by which the majority of *L. boulardi* and *L. heterotoma* wasp eggs should have hatched [125,126]. Dead wasp larvae were scored as those that did not move and that did not have defined internal organ structure. All wasp larvae survival experiments were run in five replicates.

Hemocyte counts

Fly larvae were reared under different food and wasp exposure conditions in three replicates. Batches of five larvae from each replicate food dish were cleaned, dried, and bled onto a glass side into 20 uL of 1X PBS solution containing 0.01% phenylthiourea to prevent hemolymph melanization [127]. This liquid was applied to a hemocytometer; the hemocytes were allowed to settle for 30 minutes before counting. Hemocytes were classified as plasmatocytes (small round cells with

obvious nuclei) or lamellocytes (large, clear flattened cells) [128]. Hemocyte numbers are approximately one fortieth of the number of cells per fly larva.

Ethanol food preference assay

Divided 100 mm diameter petri dishes (#08-757-150, Fisher Scientific) were used to make two distinct food compartments in each petri dish, across which ethanol cannot diffuse. Each side was filled with 1 g of instant *Drosophila* medium, with one side containing no ethanol and the other side containing 6% ethanol by volume. Batches of 100 uninfected or infected 72 hrs old fly larvae were placed in either the control food side or the ethanol food side of the dish, for three replicates of each treatment. Fly larvae were free to crawl over the divider and into either compartment, and counts for the number of larvae in each compartment were made 1.5, 3, 6, 12, and 24 hrs later.

Statistical analysis

Survival analyses were performed in R version 2.10.1. Generalized linear models (GLMs) and all other statistical analyses were performed in SAS version 9.2.

Figure 1

Differences in ethanol knockdown resistance between adult flies and wasps. Survival curves (A,B,C,D) show a decrease in insect survival over time and across ethanol concentration levels. Statistical comparisons between species using Kaplan-Meir survival analyses are shown. Error bars indicate 95% confidence intervals. Dm = *D. melanogaster*, Lb = *L. boulardi*, Lh = *L. heterotoma*.

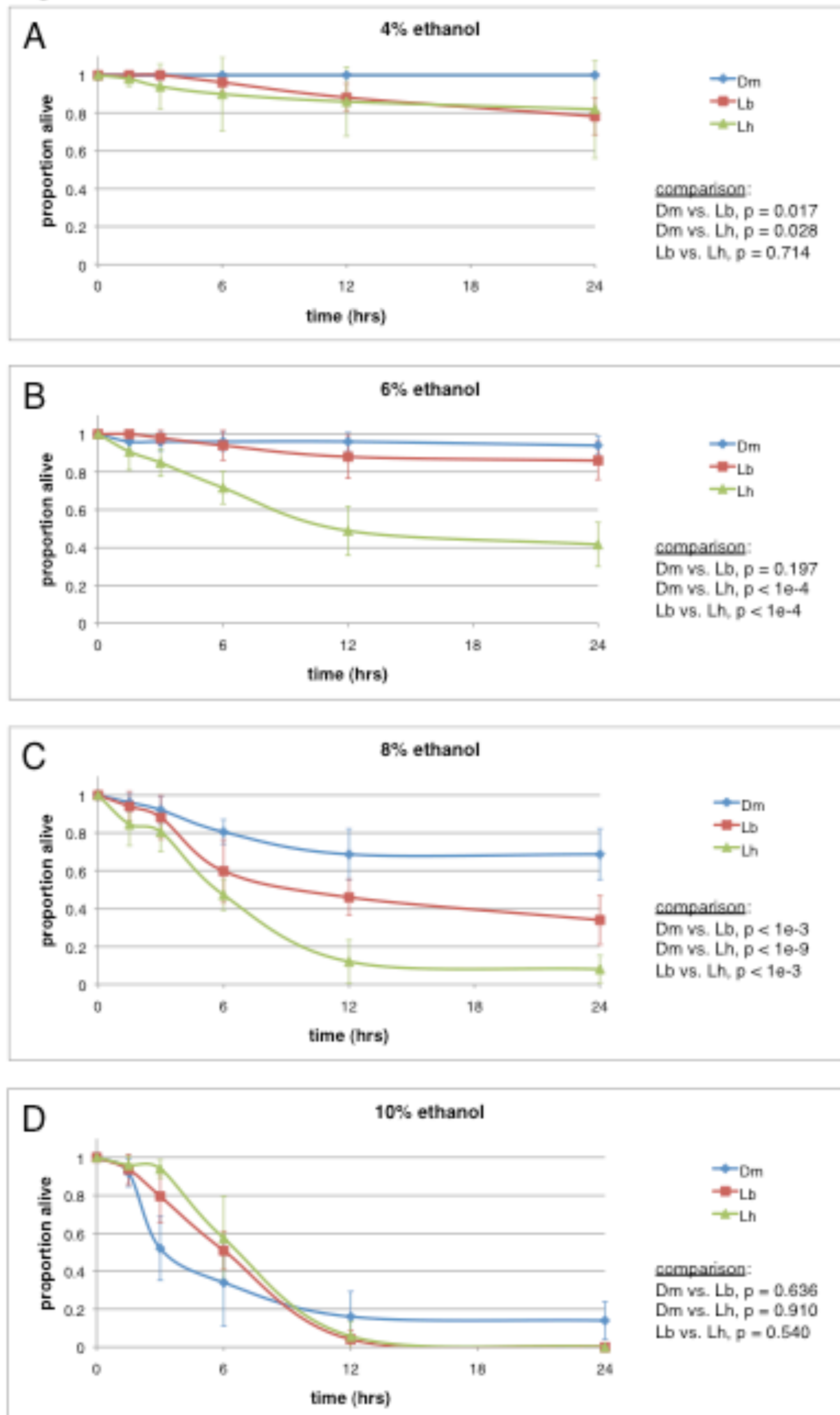


Figure 1
A
4% ethanol
proportion alive
time (hrs)
Dm
Lb
Lh
comparison:
Dm vs. Lb, p = 0.017
Dm vs. Lh, p = 0.028
Lb vs. Lh, p = 0.714
B
6% ethanol
proportion alive
time (hrs)
Dm
Lb
Lh
comparison:
Dm vs. Lb, p = 0.197
Dm vs. Lh, p < 1e-4
Lb vs. Lh, p < 1e-4
C
8% ethanol
proportion alive
time (hrs)
Dm
Lb
Lh
comparison:
Dm vs. Lb, p < 1e-3
Dm vs. Lh, p < 1e-9
Lb vs. Lh, p < 1e-3
D
10% ethanol
proportion alive
time (hrs)
Dm
Lb
Lh
comparison:
Dm vs. Lb, p = 0.636
Dm vs. Lh, p = 0.910
Lb vs. Lh, p = 0.540

Figure 2

The effect of ethanol on fly and wasp oviposition rates. The number of eggs laid per *D. melanogaster* female was measured in food with varying levels of ethanol (A), and a GLM with Poisson probability distribution was used to determine statistical differences across treatments. Significance groups using significance threshold $p < 1e-4$ are indicated by lower case letters. The number of wasp eggs laid per host was measured by dissecting fly larvae placed on food with 0 or 6% ethanol and exposed to female wasps (B); the effects of ethanol, wasp species, and their interaction were measured using a GLM with Poisson probability distribution. Error bars indicate standard deviation.

Figure 2

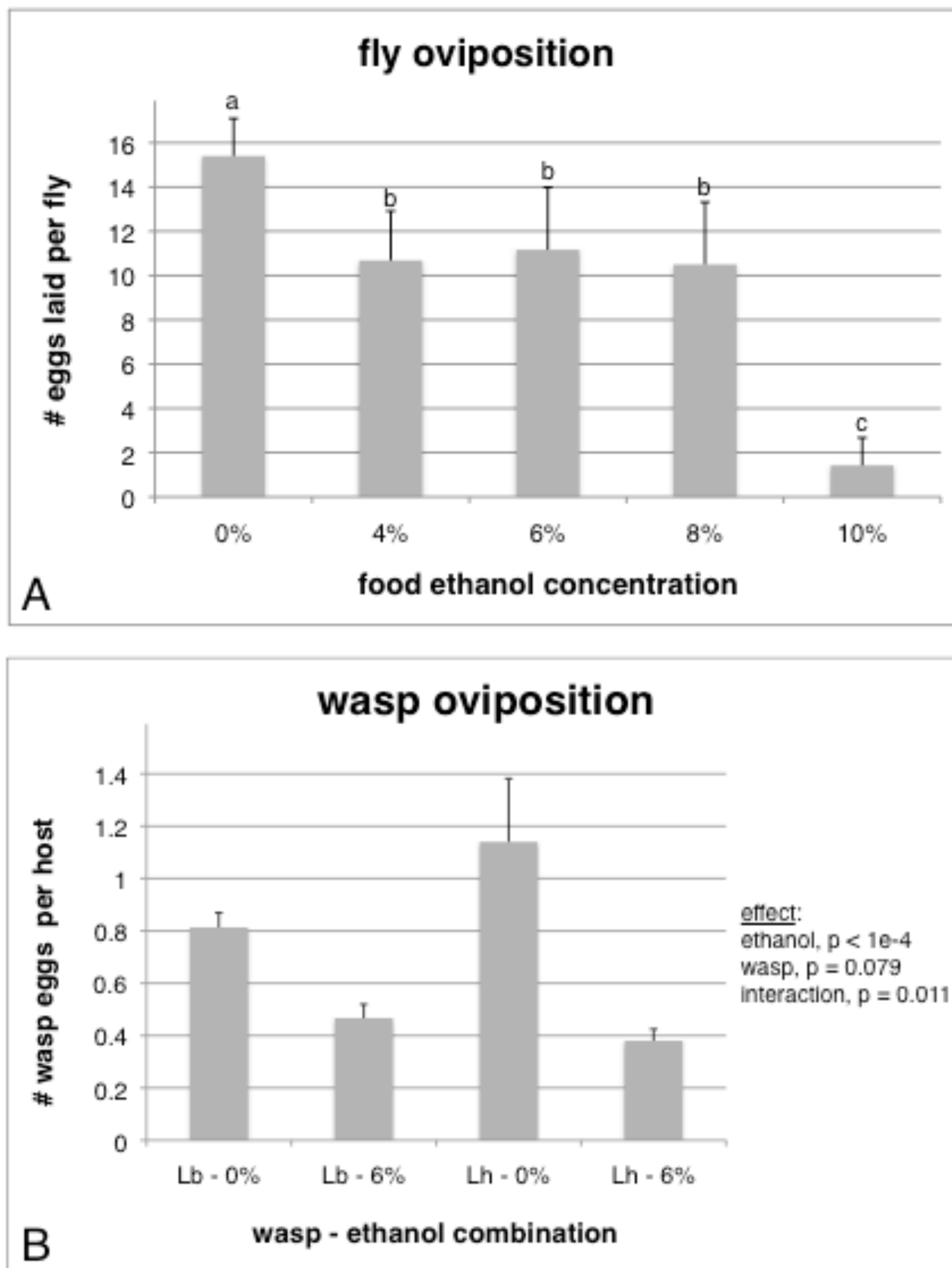
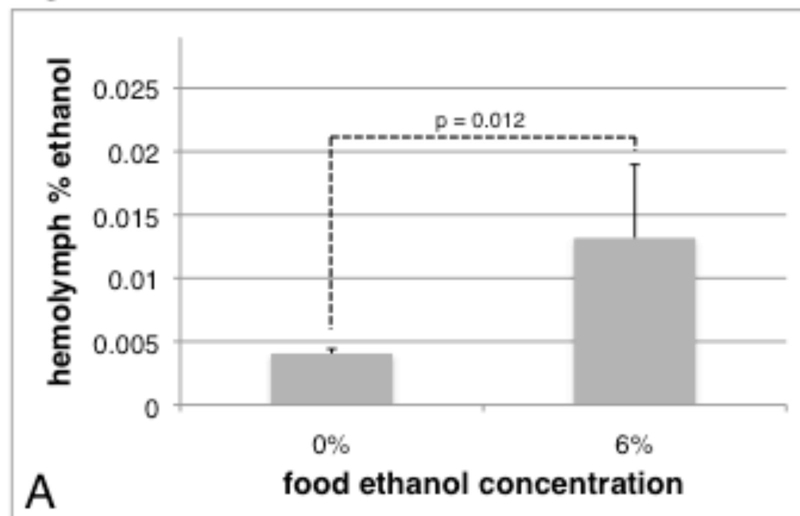
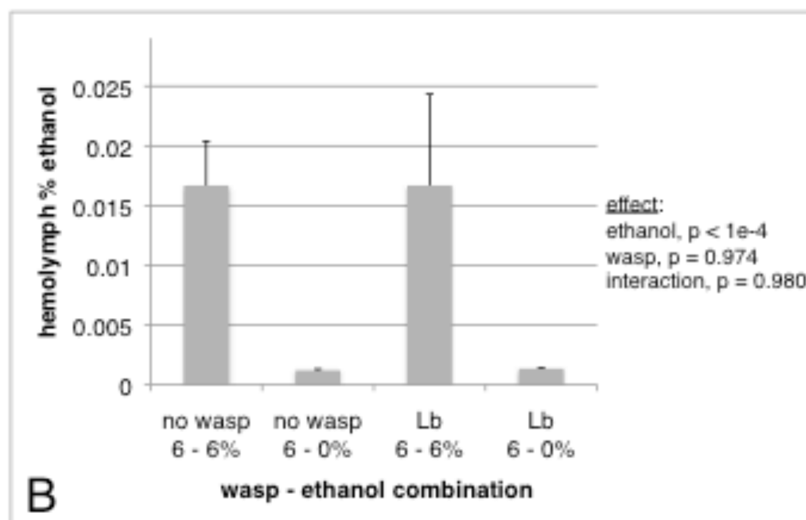


Figure 3

The relationship between ethanol content in food and in fly hemolymph. Hemolymph ethanol concentration was compared between 72 hrs old flies grown on control food and food containing 6% ethanol using a one-tailed t-test (A). The speed of hemolymph ethanol degradation was assayed by comparing 96 hrs old fly larvae grown on ethanol food to larvae removed from ethanol food for 24 hrs, and this effect was compared between control, *L. boulardi*-attacked (B), and *L. heterotoma*-attacked (C) flies. In these trials, the statistical effect of ethanol treatment, wasp infection, and their interaction was measured using ordinary least squares regression. Error bars indicate standard deviation.

Figure 3





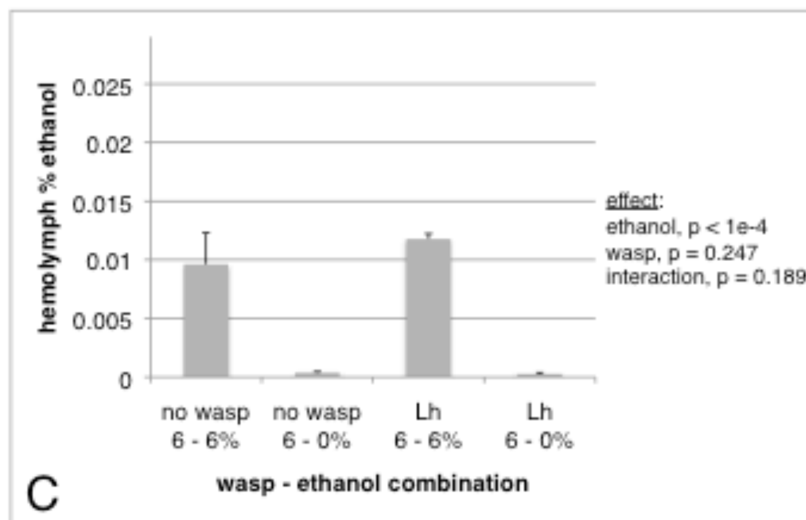


Figure 4

The effect of ethanol on wasps growing within fly larvae. Fly larvae growing on control or ethanol food were exposed to female wasps and then dissected to determine the viability of wasp larvae growing within them (A). Fly larvae were also switched between control and ethanol food after wasp attack to compare the effects of hosts grown on ethanol pre- versus post-attack (B). GLMs with binomial outcome distributions were used to determine the effects of ethanol, wasp species, and their interaction on the proportion of wasp larvae found dead in both experiments. Error bars indicate 95% confidence intervals.

Figure 4

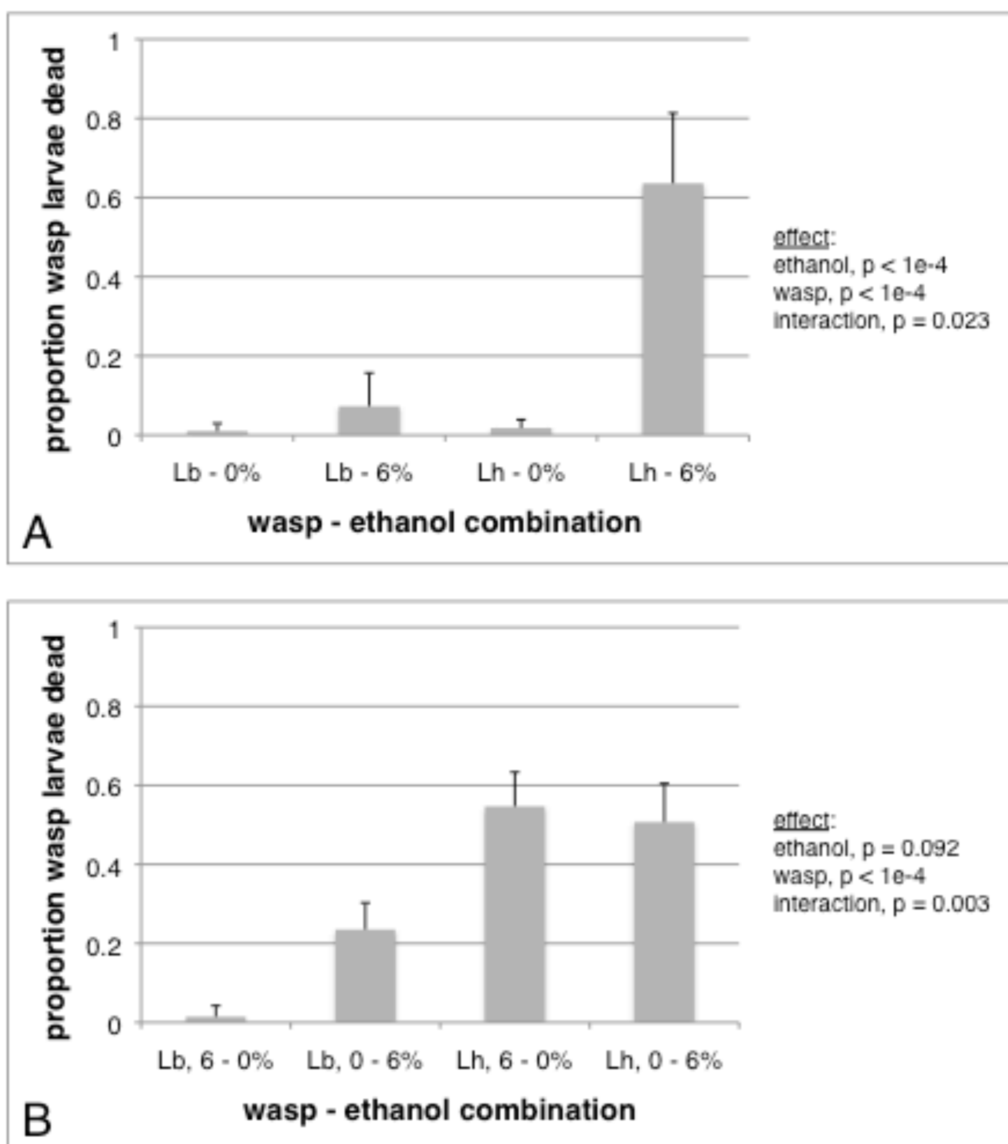


Figure 5

Developmental retardation and death of *L. heterotoma* in hosts fed ethanol. Wasp eggs dissected from control fly larvae (A) were compared to wasp eggs dissected from host larvae grown on 6% ethanol (D). Wasp larvae dissected from control fly larvae (B,C) were compared to wasp larvae dissected from fly larvae grown on 6% ethanol (E,F). Images were taken at 200X.

Figure 5

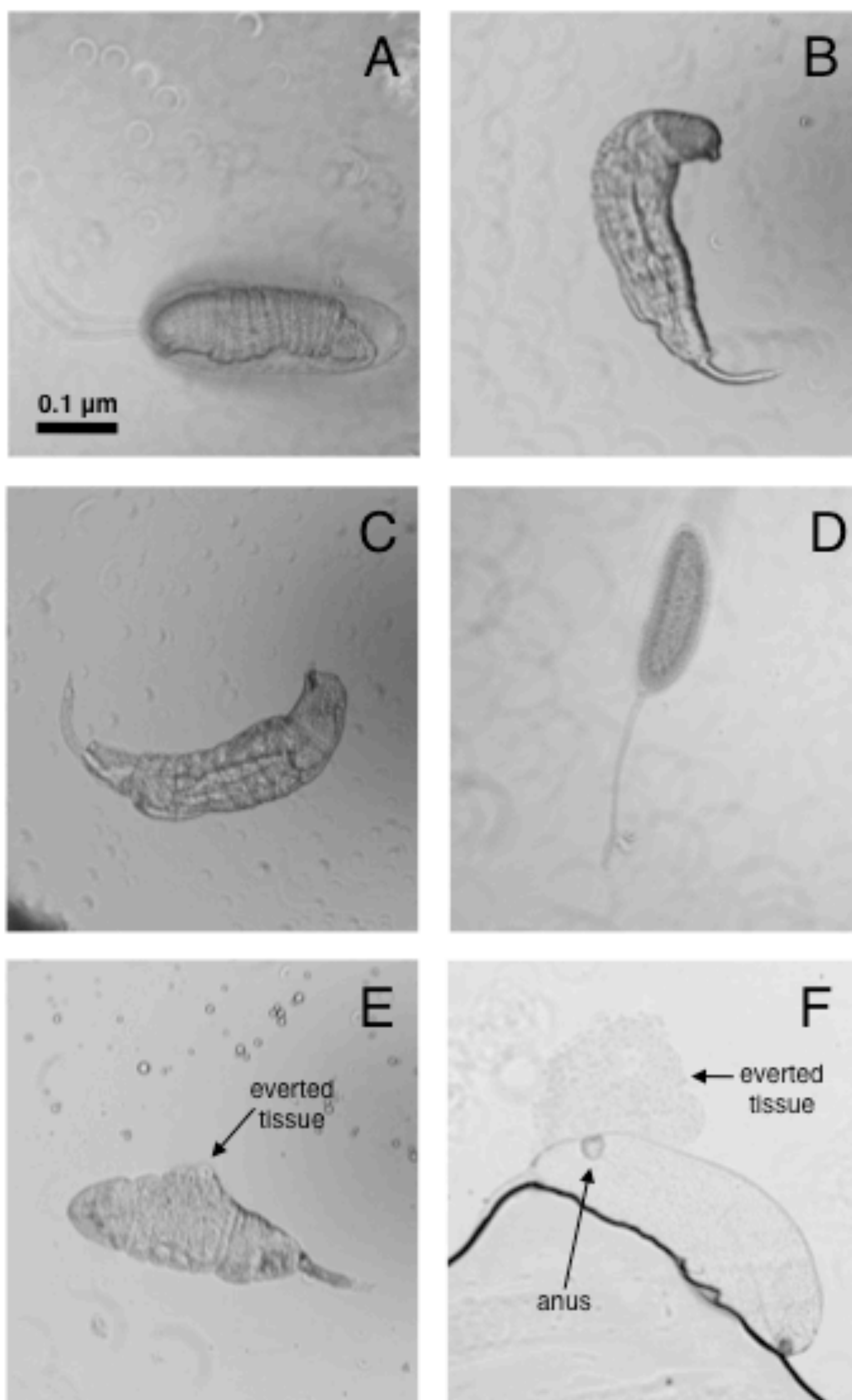


Figure 6

Fly larval hemocyte counts under different conditions. Plasmatocytes (A) and lamellocytes (B) were counted in 96 hrs old larvae that were grown continuously on control or 6% ethanol food, and that were exposed or not to wasps at 72 hrs. GLMs with Poisson probability distribution were used to determine the statistical effects of ethanol and wasp treatment on hemocyte counts, with individual significance comparisons made between wasp treatments. Error bars indicate standard deviation. EtOH = ethanol.

Figure 6

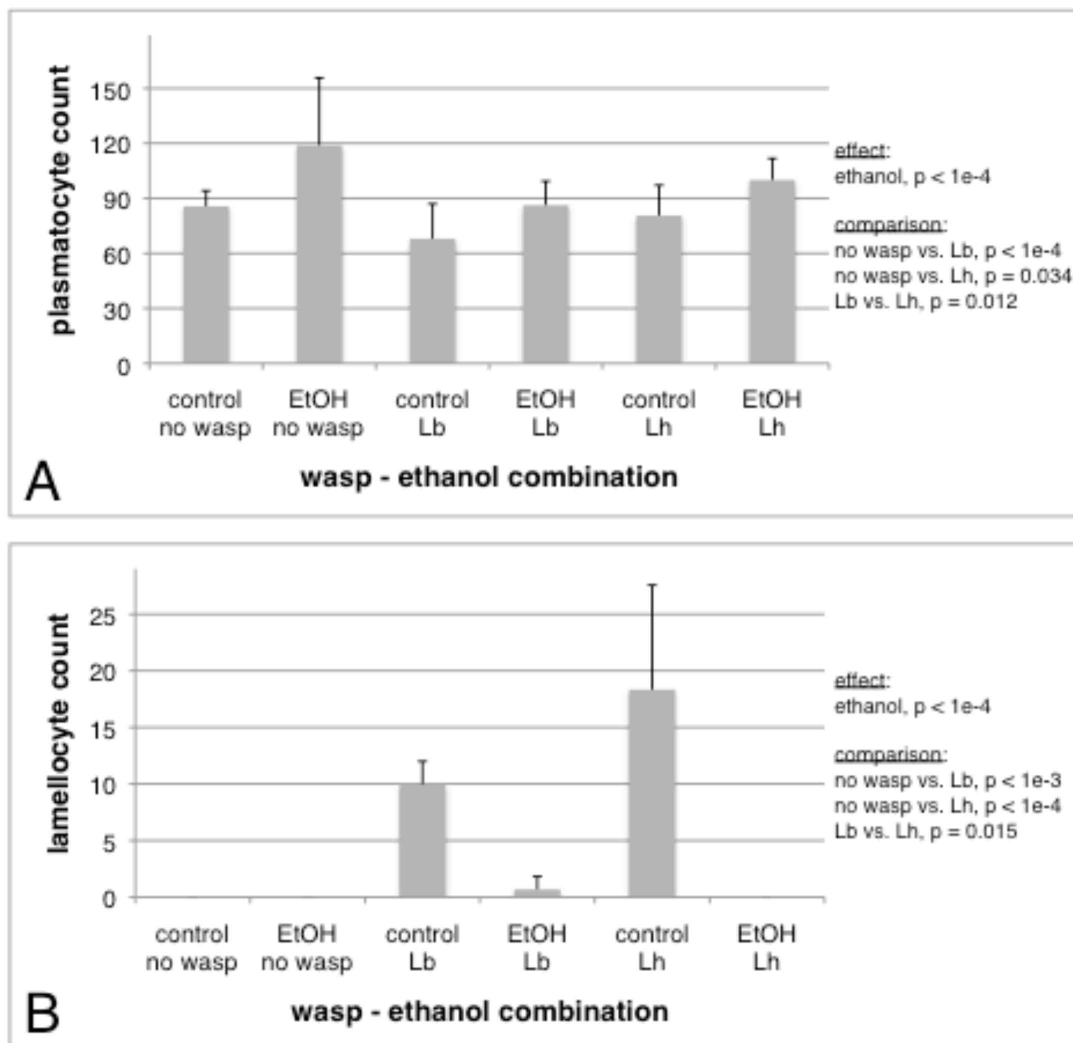
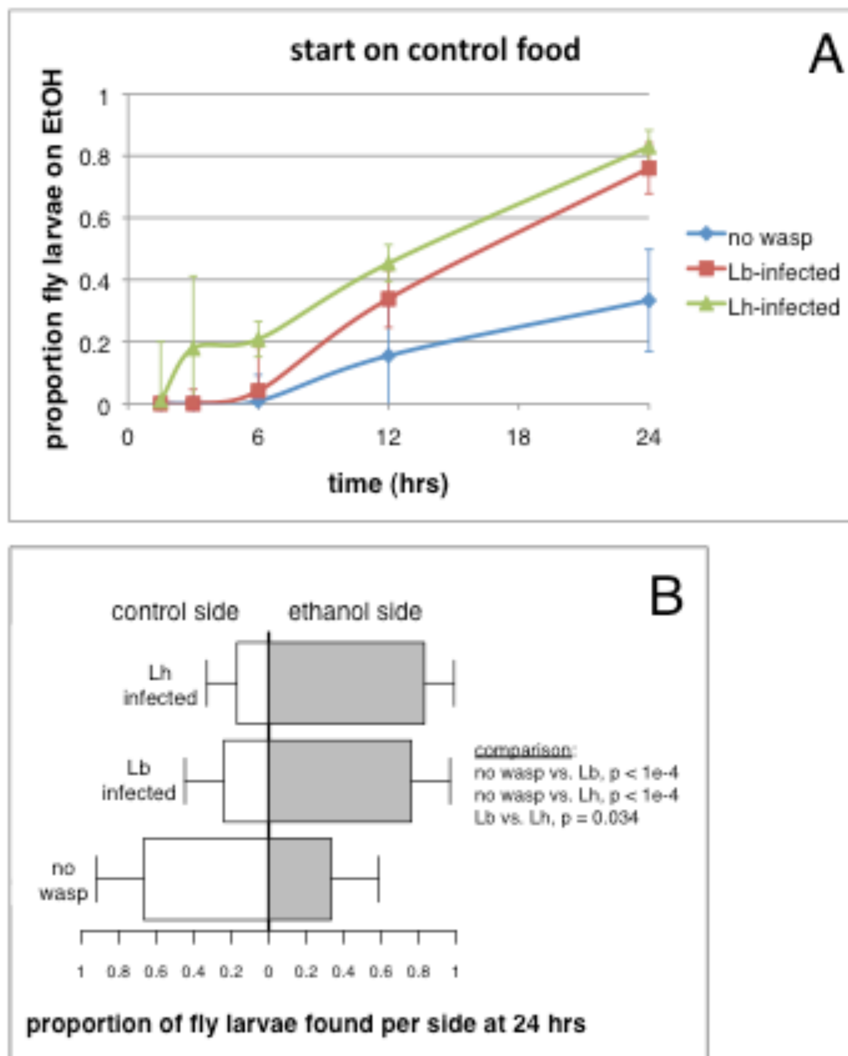
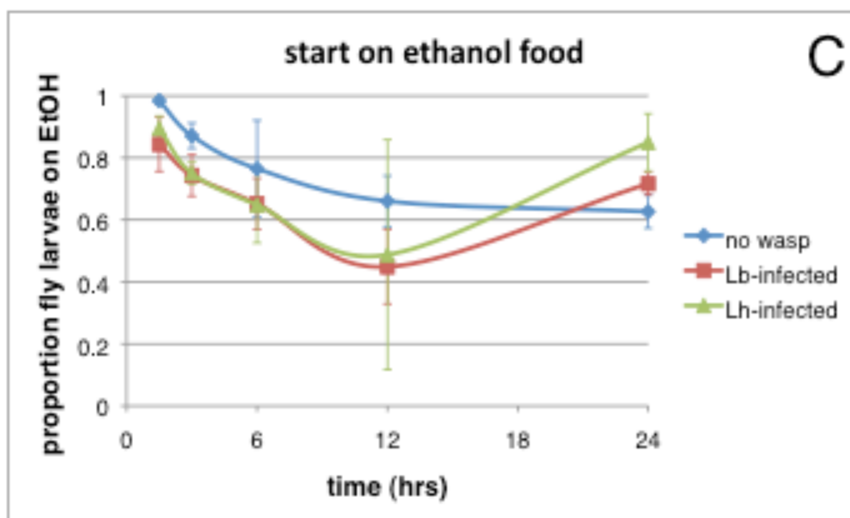


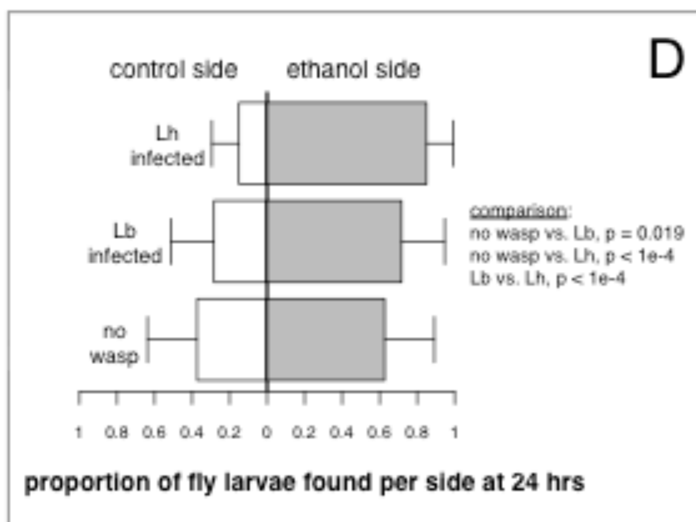
Figure 7

Choice of ethanol food by wasp-infected fly larvae. Preference for food containing 6% ethanol was compared between infected and uninfected flies over time using bisected petri dishes, with fly larvae initially placed on the control food side (A,B) or ethanol food side (C,D). GLMs with binomial outcome distribution were used to determine the effect of wasp treatment on the proportion of fly larvae on the ethanol food side of the dishes at 24 hrs (B,D). Error bars indicate 95% confidence intervals.

Figure 7




start on ethanol food
C
proportion fly larvae on EtOH
1
0.8
0.6
0.4
0.2
0
0
6
12
18
24
time (hrs)
no wasp
Lb-infected
Lh-infected


control side
ethanol side
D
Lh infected
Lb infected
no wasp
comparison:
no wasp vs. Lb, p = 0.019
no wasp vs. Lh, p < 1e-4
Lb vs. Lh, p < 1e-4
1 0.8 0.6 0.4 0.2 0 0.2 0.4 0.6 0.8 1
proportion of fly larvae found per side at 24 hrs

Acknowledgements

We thank Thierry Lefevre, Kate Schlenke, and Erin Keebaugh for helpful comments on the manuscript. This work was supported by NIH grant AI081879 to TAS.

Extensive horizontal gene transfer between *Drosophila melanogaster* and its endoparasitoid wasps

Neil F. Milan and Todd A. Schlenke

Department of Biology, Emory University, 1510 Clifton Road NE, Atlanta, GA 30322, USA

Abstract

In eukaryotes, genomes are thought to be inherited relatively faithfully from parents to offspring, unlike prokaryotes which frequently obtain new genomic information from their environments. However, a lack of genome sequences from closely interacting eukaryotes presumably limits our ability to detect eukaryote gene swapping. Some of the most intimate ecological relationships in nature occur between arthropod hosts and endoparasitoid wasps, which lay their eggs within the bodies of their hosts where they can potentially be transformed by surrounding host tissues. Given that several wasp species use the model fruitfly *Drosophila melanogaster* as a primary host in nature, we hypothesized that *D. melanogaster* genes might horizontally transfer into these wasp genomes. Using the canonical transposable element sequences from the *D. melanogaster* genome, we found evidence of up to 63 cases of horizontal transfer in *D. melanogaster* specialists, involving 35 of the 43 transposable element families tested, but virtually no evidence of horizontal transfer using other *Drosophila* endoparasitoids. The disjunct distribution of horizontally transferred transposable elements across wasp

lineages confirms the direction of transfer is from flies to wasps. Thus, endoparasitoid wasps regularly incorporate genetic information from their hosts into their genomes, despite being more than 300 million years diverged. Our findings demonstrate that the content and evolutionary potential of eukaryotic genomes can be strongly influenced by the closely interacting eukaryotic species around them.

Introduction

Horizontal transfer (HT) is the movement of genetic material between individuals outside of typical parent-offspring inheritance, and can be an important source of evolutionary novelty. For example, HT is common in bacteria and has led to the rapid evolution of virulence, antibiotic resistance, ability to exploit new ecological niches and many other important bacterial adaptations [129]. HT in eukaryotes is thought to be rare relative to prokaryotes, because eukaryotes protect their genomes behind nuclear membranes and, in multicellular eukaryotes, germ cells make up only a small portion of the organism and are often segregated from somatic cells (most highly so in animals). Consequently, the most numerous examples of HT into multicellular eukaryote nuclear genomes involve transfer of genes from intracellular prokaryotic symbionts that are closely associated with the germ cells of their eukaryotic hosts, such as mitochondria, plastids, and Wolbachia [130,131]. Conversely, examples of HT between eukaryotic nuclear genomes are relatively uncommon [132,133,134]. However, a handful of recent studies show that eukaryotes sharing intimate ecological relationships in nature, such as hosts and

parasites, can occasionally swap genetic information [135,136,137,138,139,140]. But because genome sequencing efforts have been focused on a diversity of individual eukaryotes rather than on groups of eukaryotes that closely interact in nature, interacting eukaryotes with presumably the greatest potential for HT have not been rigorously tested. The possibility exists that HT between eukaryotes may be much more common than currently appreciated.

Some of the tightest ecological relationships between eukaryotes that exist in nature are those between obligate endoparasitoid wasps and their arthropod hosts. These wasps spend a large fraction of their lives within the bodies of their hosts, and develop in concert with their hosts by actively manipulating host physiology and behavior [141]. For example, endoparasitoid wasps of *Drosophila* lay their eggs inside fly larvae and pupae. If these eggs are not killed by the *Drosophila* immune system, wasp larvae develop within and slowly consume the flies until they pupate and eclose from fly pupal cases 3-4 weeks later [40]. The frequency of infection in this system is high, as endoparasitoid wasps have been found to infect more than 50% of *Drosophila* larvae in natural populations [142]. Given this tight ecological bond, we hypothesized that HT between wasps and their *Drosophila* hosts might occur, and might even be common. Furthermore, the natural history of endoparasitoid wasps suggests a potentially simple transfer mechanism, whereby wasp embryonic germ cells could be directly transformed by the fly somatic tissue they are surrounded by. Thus, we hypothesized that any HT between flies and wasps would occur in the direction of flies to wasps.

We tested these hypotheses using transposable element (TE) families from the *D. melanogaster* genome. TEs are good candidates to test for HT because they typically exist in multiple copies in genomes (giving them a higher chance of transferring, all else being equal), they are naturally selected to be mobile, and because they potentially have a persistence advantage in new genomes due to their ability to reproduce independently of host genome replication [143]. A majority of the previously documented cases of eukaryote-to-eukaryote HT involve TEs [144,145,146,147,148]. Many factors may influence the propensity of a TE to horizontally transfer from one organism to another. For example, besides the copy number of full-length, active TEs in a host genome, the mechanism of transposition of different types and classes of TEs could also be important for the potential for horizontal transfer, *e.g.* retrotransposons are thought to be less likely to horizontally transfer because of their more fragile RNA stage, and because they usually require multiple enzymes to duplicate [149].

We attempted to PCR amplify 43 of the 95 annotated *D. melanogaster* TE families from six *Drosophila* endoparasitoid wasps (Table 1). The three main goals of this study were to determine the extent to which TEs horizontally transfer between fruitflies and their endoparasitoid wasps, to determine the general direction of transfer, and to test whether specific TE attributes affect the likelihood of HT. We found evidence for extensive TE HT between flies and wasps, in the direction from

flies to wasps, but there appeared to be little effect of host TE copy number, or TE type or class on the propensity to transfer.

Materials and Methods

BLASTs

BLASTs were run on the FlyBase website (http://flybase.org/blast/) in April 2011 using the blastn algorithm to search for sequences homologous to the 95 full-length canonical *D. melanogaster* TE sequences [150].

Insects

The *D. melanogaster* (14021-0231.36), *D. ananassae* (14024-0371.13), *D. pseudoobscura* (14011-0121.94), and *D. virilis* (15010-1051.87) genome strains were acquired from the *Drosophila* Species Stock Center. The wasp strains (Table 1) were collected by our lab with the exception of strain Gx, which was kindly provided by Shubha Govind. Wasps were reared on the *D. melanogaster* strain Oregon R. Three of these wasp species (*L. boulardi*, *L. heterotoma*, and *A. tabida*) have been shown to utilize *D. melanogaster* as a primary host in nature [151]. Although the wasp species *L. victoria*, *G. xanthopoda*, and *Trichopria sp.* can be grown on *D. melanogaster* in the lab, very little information exists about what host species they actually use in nature. *L. victoria* has been said to utilize the ananassae subgroup (melanogaster group) fly *D. malerkotliana* as host in nature [40], and we collected the *L. victoria* strain used in this study in the Philippines where it appeared to use ananassae subgroup flies as primary hosts. We also found that *L. victoria* had

significantly higher infection success in the lab using *D. ananassae* as a host rather than *D. melanogaster* (unpublished data). Thus, we believe *L. victoria* is an ananassae subgroup specialist. *G. xanthopoda* has been said to utilize *D. melanogaster* and the saltans group fly *D. sturtevanti* as hosts [40]. Provenance information for the *G. xanthopoda* strain used in this study has been lost, but it is thought to have been collected in the Caribbean. We found that our *G. xanthopoda* strain had high infection success in the lab using melanogaster group fly species, including *D. ananassae*, as hosts (unpublished data), but the natural hosts of *G. xanthopoda* are still obscure. The Trichopria genus is poorly characterized, but members of the genus Trichopria have been found to successfully infect flies from a variety of *Drosophila* species groups in nature [40], and we found the strain used in this study to have high infection success in members of the melanogaster, obscura, immigrans, melanica, repleta, and virilis species groups (unpublished data). Therefore, *Trichopria sp.* may act as a generalist of the genus *Drosophila* in nature.

DNA Extraction

High molecular weight genomic DNA from fruitflies and wasps was isolated using a modified phenol-chloroform extraction protocol. Approximately 20 insects per sample were ground in 1X CTAB buffer (0.7M NaCl, 0.1M Tris, 10mM EDTA, 1% CTAB, 1% β-mercaptoethanol) with 1ug/uL proteinase K, and incubated for 30 minutes at 65 C. KOAc was added to a final concentration of 1M, and the solutions were incubated on ice for 10 minutes before cellular debris was spun down. The supernatants were then run through a standard phenol chloroform extraction with

ethanol precipitation, after which the DNA pellets were re-suspended in a 7M guanidinium-hydrochloride solution with 0.5% (by weight) RNase A for 1 hour. Finally, the purified DNAs were re-precipitated and re-suspended in low EDTA 0.5X TE buffer (5mM Tris-HCl, 50uM EDTA). DNA was quantified and checked for purity using a Nanodrop spectrophotometer.

PCR

PCR primers were designed in the DNAStar program PrimerSelect (Supplemental Material S2). PCR reactions to amplify TEs or control genes from flies and wasps were prepared using Qiagen Taq Master Mix PCR Kit and run according to the following cycle program: initial denaturation at 94 C for 3:00, 35 cycles of 94 C denaturation for 1:00, primer annealing using the primer-specific recommended temperatures for 1:00, and 72 C extension for 1:30, with a final 72 C extension for 7:00.

Sequencing

PCR bands of the expected size were gel extracted and purified using the Qiagen QIAquick Gel Extraction Kit. PCR products were then cloned using either the invitrogen TOPO TA or Stratagene StrataClone PCR cloning kits, and inserts from positive colonies were PCR amplified using vector M13 primers. These products were sent to Beckman Coulter Genomics for sequencing using the original gene specific primers. All wasp transposable element sequences are deposited in

GenBank under accession numbers XXX-XXX. Transposable element sequence alignments are available upon request.

Southern blots

Genomic DNA was digested using appropriate restriction enzymes to leave the targeted TE intact (MspI for *Doc*, EcoRI for *jockey*). Digested DNA was run on 0.75% agarose gels for 19 hours at 45 V, after which the gels were soaked with gentle agitation in both denaturation solution (0.5M NaOH, 1.5M NaCl) and neutralizing solution (1M Tris at pH 7.4, 1.5M NaCl) for 45 minutes at room temperature. Gels were then placed into an upward capillary action transfer stack in 10X SSC buffer (1.5M NaCl, 150mM sodium citrate) using sponges and paper towels to allow the DNA to transfer onto a charged nylon membrane (Millipore Immobilon-Ny+) overnight. After transfer, membranes were rinsed in 5X SSC buffer and stored at –20 C until use. To hybridize, membranes were first rotated in Blotto hybridization solution (5X SSPE buffer (0.375M NaCl, 50mM sodium phosphate, 5mM EDTA) supplemented with 10% formamide, 6% PEG, 1% SDS, 0.5% dry instant milk, and 0.013% sheered salmon sperm DNA) at 65 C for 1 hour. Radio-labeled TE probes were constructed using TE PCR primers (2516f-4122r for *Doc*, 764f-2332r for *jockey*; Supplemental Material S2), a purified and diluted *D. melanogaster* TE PCR product as the template, and (α-32P) dCTP in a standard PCR reaction. The "hot" PCR products were then purified using the Qiagen QIAquick Nucleotide Removal Kit, boiled for 5 minutes, and iced briefly before being added into the hybridization solutions and exposed to the membranes. The membranes were rotated in a

hybridization oven at 50 C overnight, washed with dilute SSC and SDS solutions of progressively lower concentrations (2x SSC, 0.5% SDS; 2x SSC, 0.1% SDS; 0.1x SSC, 0.1% SDS; 0.1x SSC), and then wrapped in plastic and exposed to a Phosphorimager screen. After one week at room temperature, the Phosphorimager screen was used to develop a digital image of the blot. Southern blots for both TEs were run multiple times and were consistent with results shown in Figure 4.

Phylogenies

TE phylogenies were generated in Phylip version 3.69 using the parsimony criteria as implemented by the program dnapars, using default settings. For each set of TE sequences, 100 bootstrapped datasets were generated using the program seqboot to generate bootstrap values for all nodes in the inferred phylogenies.

Analysis

All statistical analyses were run in the JMP statistical package.

Results and Discussion

We first used BLAST to determine whether the 95 canonical *D. melanogaster* TE families [150] are normally phylogenetically limited by scanning 11 other sequenced insect genomes, including several Dipterans (six *Drosophila* species and *Anopheles gambiae*) and single representatives of the Lepidoptera, Coleoptera, Hymenoptera, and Hemiptera. The BLAST searches revealed a highly consistent pattern, with little sequence conservation of *D. melanogaster* TEs outside of the

melanogaster subgroup of the genus *Drosophila* (*i.e.*, *D. melanogaster*, *D. simulans*, *D. yakuba*) (Figure 1, Supplemental Material S1). BLAST scores greater than $1e^{-75}$ were considered poor matches because such scores reflect a combination of relatively low sequence homology and sequence coverage (Figure 2). As expected, the *D. melanogaster P* element did not BLAST to the *D. melanogaster* genome because the strain was chosen to be *P* element free, yet a nearly identical match was found within the genome of *D. willistoni*, where the *P* element is thought to have originated before being horizontally transferred into *D. melanogaster* (Supplemental Material S1) [152,153]. In general, the BLAST results are highly consistent with earlier *in situ* hybridization and Southern blot experiments that also showed lack of *D. melanogaster* TE homology outside the melanogaster subgroup [154]. Thus, *D. melanogaster* TE lineages are highly specific to *D. melanogaster* and its close relatives and it is unreasonable to assume that Diptera and Hymenoptera, which diverged more than 300 MYA [155], might share TE sequences due to common ancestry.

To test for HT of TEs between *Drosophila* and *Drosophila* endoparasitoid wasps, two sets of PCR primer pairs each for 43 of the 95 canonical *D. melanogaster* TE families were designed. These primers were used in an attempt to amplify TE sequences from the genomic DNA of six wasp species (Table 1), using four *Drosophila* species as controls. Although a single TE PCR primer pair may occasionally yield a PCR band of the expected size spuriously, it would be highly unlikely for this to occur for both primer pairs. The PCR primers were designed to be unique to specific TE

families, to avoid the terminal repeat sequences found in various TE classes, and to amplify internal TE sequences of ~ 500-1500 bp in length. The primer pairs were highly successful at amplifying high quality PCR bands of the expected size from *D. melanogaster* DNA, with 42 of 43 TEs being amplified by at least one primer pair, and 36 of 43 TEs being amplified by both primer pairs (Table 2, Figure 3, Supplemental Material S2). As expected from the BLAST results, PCR amplification was much less successful for the other three *Drosophila* species. Only 44 of 129 of these fly-TE combinations showed successful amplification by at least one primer pair, and only 12 of 129 fly-TE combinations showed successful amplification by both primer pairs. The ratio of successful versus unsuccessful TE PCR amplification was significantly greater for *D. melanogaster* than for the other three flies (2-tail Fisher exact test, one primer pair, $p = 1.59e^{-14}$; both primer pairs, $p = 7.53e^{-20}$).

There was an *a priori* expectation of which TE PCRs should have worked in the three fly species *D. ananassae*, *D. pseudoobscura*, and *D. virilis*, given that the genome-sequenced strains of these flies were used in the PCR experiments. In the majority of cases, PCRs expected to fail failed, and PCRs expected to work worked (*e.g.*, the *Bari1*, *17.6*, *springer*, and *Transpac* elements for *D. ananassae*, the *Stalker* element for *D. pseudoobscura*, and the *412* element for *D. virilis*). Although several fly-TE combinations that yielded highly significant BLAST hits did not show PCR amplification, close inspection of multiple of these cases revealed that the actual primer binding sites were not conserved. There were also a handful of "successful" PCRs from fly-TE combinations that would not have been expected to work based on

overall BLAST scores, but close inspection revealed that many of these cases could be explained by primer binding site homology, even though there was little homology to other parts of the canonical *D. melanogaster* TE sequence. Finally, there remained a few instances where successful amplification was unexpected based on the *D. ananassae*, *D. pseudoobscura*, and *D. virilis* genome sequences, and where sequencing of the PCR bands yielded sequences similar to the canonical *D. melanogaster* TE sequences (*e.g.*, the *G2* element in *D. ananassae*, the *Bari1* and *springer* elements in *D. pseudoobscura*, and the *Bari1* element in *D. virilis*). Contamination of fly DNA preps cannot explain these cases, as multiple independent fly DNA extractions yielded identical results. Instead, we believe that these *D. melanogaster*-like TE sequences truly exist in the *D. ananassae*, *D. pseudoobscura*, and *D. virilis* genomes, but are located in heterochromatic or other regions that were not fully sequenced or assembled.

Although all six *Drosophila* parasitic wasp species were reared on *D. melanogaster* in the lab, only *L. boulardi*, *L. heterotoma*, and *A. tabida* have been shown to utilize *D. melanogaster* as a primary host in nature [151]. In TE PCR assays for the other *Drosophila* endoparasitoids (*L. victoria*, *G. xanthopoda*, *Trichopria sp.*), only 10 of 129 wasp-TE combinations showed successful amplification by at least one primer pair, and only 1 of 129 wasp-TE combinations showed successful amplification by both primer pairs (Table 2, Figure 3, Supplemental Material S2). However, for the three known *D. melanogaster* endoparasitoids, 63 of 129 wasp-TE combinations showed successful amplification by at least one primer pair, involving 35 of the 43

TEs tested. 21 of 129 wasp-TE combinations showed successful amplification by both primer pairs. The ratio of successful versus unsuccessful TE PCR amplification was significantly greater for known *D. melanogaster* endoparasitoids than for the other *Drosophila* endoparasitoids (2-tail Fisher exact test, one primer pair, p = 8.03e^{-14}; both primer pairs, p = 4.89e^{-6}), indicating that TE HT is specific to naturally interacting host-parasite pairs. This opens the possibility that the historical hosts of poorly characterized endoparasitoids might be identified by matching endoparasitoid genome TE content with that of candidate host species.

The overall lack of TE amplification success in the *Drosophila* endoparasitoids not known to naturally infect *D. melanogaster* suggests that spurious PCR amplification was rare. However, to confirm that the "successful" TE PCRs from known *D. melanogaster* endoparasitoids amplified genuine TE sequences, PCR bands from 24 of the 63 successful wasp-TE combinations (representing 15 TEs total) were cloned and sequenced and all 24 sequences were found to be close matches to the *D. melanogaster* TE sequences from which the primers were designed (Table 2). Phylogenetic trees including representative homologous TE sequences from all fly and wasp species available showed that wasp TE sequences were often most closely related to *D. melanogaster* sequences, although cases in which wasp sequences grouped more closely with TE sequences from the other melanogaster subgroup species *D. simulans* and *D. yakuba* also occurred (Supplemental Material S3).

The large difference in TE amplification success between known *D. melanogaster* endoparasitoids and other *Drosophila* endoparasitoids also suggests that contamination of wasp genomic DNA preps with fly DNA, *e.g.* due to consumed fly tissue left over in wasp guts, does not explain wasp TE amplification success. Nevertheless, to test whether wasp DNA preps were contaminated with fly DNA, two additional PCR-based experiments were run. First, a second round of TE PCRs was performed using new DNA extractions from wasp wings and legs. Seven of eight TE PCRs that yielded a correctly sized band in the original analysis (*Bari1, blastopia, Doc, HMS-Beagle, hopper, jockey, opus*) were also successful in this analysis. Second, if contamination exists, other fly genes should also amplify from the wasp DNA preps. PCR primers for nine *D. melanogaster* nuclear genes (*BG4*, *Cyp6a8*, Cyp6w1, *GNBP1*, *Myd88*, *psh*, *PGRP-SA*, *PGRP-SD*, and *tub*) all successfully amplified high quality correctly sized bands from *D. melanogaster* DNA preps, but all failed to amplify bands from wasp DNA preps. Thus, the possibility that some wasp DNA preps were contaminated with *D. melanogaster* DNA was ruled out.

Finally, to confirm that *D. melanogaster*-like TEs are actually inserted into wasp genomes, Southern blot hybridizations were run using wasp DNA preps probed with *D. melanogaster* TE sequences. Blots for two TE probes showed clear banding patterns in each wasp, which differed between wasp species and between the wasps and *D. melanogaster* (Figure 4). Furthermore, the TE probes hybridized to multiple wasp genomic fragments, indicating that fly-like TEs occur at appreciable copy number in wasp genomes, either due to frequent HT or to natural reproduction of

founder TEs once inserted in wasp genomes. In sum, the PCR and confirmatory experiments show that HT between *D. melanogaster* and its endoparasitoid wasps occurs at an unprecedented scale, and opens the possibility that other, non-TE loci might also horizontally transfer between flies and wasps.

Several pieces of evidence demonstrate that the direction of TE HT is from flies to wasps. First, most *Drosophila* endoparasitoid wasps infect multiple *Drosophila* species groups in nature [40]. If wasps transfer TEs to flies, they would likely transfer those TEs to multiple Drosophilid species groups, upsetting the limited phylogenetic distribution of TEs observed in Figure 1. Second, there is a significant difference in the number of *D. melanogaster* TEs amplified from the wasp sister species *L. heterotoma* and *L. victoria*, which seems to correspond with the relative propensity of these wasp species to infect *D. melanogaster* in nature (Methods, Table 2). Such a difference would be unexpected if the TE families originated in a common Leptopilina ancestor before being moved into *Drosophila*. Third, some *D. melanogaster*-like TEs are found in disjunct, highly diverged wasp families, making it unlikely those TEs are shared due to common ancestry. For example, the *transpac* element is found in a Braconid (*A. tabida*) and some Figitids (*e.g.*, *L boulardi* but not *G. xanthopoda*), but not in the genomes of a Diapriid (*Trichopria sp.*) or Pteromalid (*Nasonia vitripennis*), which are more closely related to the Figitids than Braconids (Figure 5) [156]. This direction of exchange from fly to wasp makes sense in light of the natural history of endoparasitoids, which develop from embryos completely surrounded by host tissues.

Many factors may influence the propensity of a TE to horizontally transfer from one organism to another. For example, TE copy number in the host genome and TE type should both presumably correlate with the propensity to horizontally transfer into new genomes. However, no significant correlation was found between full-length TE copy number in the *D. melanogaster* genome and number of wasp species from which a TE could be amplified ($r^2 = 0.030$, $p = 0.263$; Figure 6). The lack of even a trend may be due to differences in the current versus historical load of different TE classes in the *D. melanogaster* genome, differences between the genome sequenced *D. melanogaster* strain and other strains, or to lack of a connection between TE copy number and TE activity. Furthermore, there was no evidence that DNA transposons were better able to horizontally transfer than retrotransposons, as has been hypothesized [149]. In fact, the proportion of retrotransposons amplified using at least one TE primer pair was marginally significantly greater than that of DNA transposons (2-tail Fisher's Exact Test $p = 0.045$), although this difference was not significant for the proportion of TEs amplified by both primer pairs (Table 3). Thus, it appears that unique characteristics of particular TEs may be the determining factors in efficiency of HT, rather than host copy number or TE type and class. Once TEs gain a foothold in a novel genome, other TE characteristics, such as self-regulatory mechanisms and the necessity of host proteins for excision, will control whether TEs survive and reproduce in the new host [149].

Regardless of the ability of TEs to duplicate once they reach a new genome, the sheer number of loci being transferred between flies and wasps indicates that these interacting species can have a substantial impact on the evolutionary potential of one another. TE insertions into functional elements of the host genome usually have deleterious effects [157,158,159] but can occasionally be adaptive [160,161]. Furthermore, TEs frequently hop linked fragments of host genome with them to their new location, which can result in novel phenotypes [71,161,162]. Genome sequencing of *Drosophila* endoparasitoid wasps could determine whether, *e.g.*, fly nuclear genes have been moved into wasp genomes and co-opted for use by the wasps. Altogether, our findings suggest that future genome sequencing projects focused on species that share intimate ecological relationships may uncover many more instances of HT between eukaryotes. It will be interesting to determine whether endoparasitism, or parasitism in general, provides unique opportunities for eukaryote HT, or whether other ecological relationships between eukaryotes can foster HT as well. Nevertheless, these data show that at least some multicellular eukaryotic genomes are more like the labile prokaryotic genomes than is currently appreciated.

Table 1

Provenance of wasp strains used in this study.

Wasp Species	Family	Description	Strain Name	Place Collected	Date Collected
Leptopilina boulardi	Figitidae	larval parasitoid	Lb17	Winters, California	2002
Leptopilina heterotoma	Figitidae	larval parasitoid	Lh14	Winters, California	2002
Leptopilina victoria	Figitidae	larval parasitoid	LvPhil	Consolacion, Cebu, Philippines	2007
Ganaspis xanthopoda	Figitidae	larval parasitoid	Gx	unknown	unknown
Trichopria sp.	Diapriidae	pupal parasitoid	TriCal	Winters, California	2002
Asobara tabida	Braconidae	larval parasitoid	AtSw	Uppsala, Sweden	2007

Table 2

Number of *D. melanogaster* TEs successfully amplified from fly and wasp strains.

Species	# Insect-TE Combinations	# TEs Amplified, One Primer Pair	# TEs Amplified, Both Primer Pairs	# TEs Confirmed By Sequence
D. melanogaster	43	42	36	0
Other Drosophila Species	**129**	**45**	**12**	**12**
D. ananassae	43	18	6	7
D. pseudoobscura	43	11	4	4
D. virilis	43	16	2	1
Other Drosophila Parasitoids	**129**	**10**	**1**	**0**
L. victoria	43	5	0	0
G. xanthopoda	43	3	1	0
Trichopria sp.	43	2	0	0
Known *D. melanogaster* Parasitoids	**129**	**63**	**21**	**22**
L. boulardi	43	23	5	7
L. heterotoma	43	26	10	14
A. tabida	43	14	6	1

Amplification Proportion Comparison	2-tail Fisher's Exact Test *p*-value
One Primer Pair	
D. melanogaster versus other Drosophila	1.93E-14
Known *D. mel*. parasitoids versus other Drosophila	0.032
Known *D. mel*. parasitoids versus other wasps	8.03E-14
L. victoria versus *L. heterotoma*	3.79E-06
Both Primer Pairs	
D. melanogaster versus other Drosophila	7.53E-20
Known *D. mel*. parasitoids versus other Drosophila	0.135
Known *D. mel*. parasitoids versus other wasps	4.89E-06
L. victoria versus *L. heterotoma*	0.001

Table 3

Relationship between TE type and class and TE HT success in *D. melanogaster* specialist wasps.

Single PCR hits

TE Type	TE Class	# Wasp/TE Combinations Tested	# Combinations Showing Amplification	% Combinations Showing Amplification
DNA	FB	3	2	66.7
DNA	TIR	30	9	30.0
Total DNA		33	11	33.3
RNA	LINE	36	21	58.3
RNA	LTR	60	31	51.7
Total RNA		96	52	54.2

	2-tail Fisher's Exact Test *p*-value
Row 1 vs 2	0.534
Row 4 vs 5	0.673
Row 3 vs 6	0.045

Double PCR hits

TE Type	TE Class	# Wasp/TE Combinations Tested	# Combinations Showing Amplification	% Combinations Showing Amplification
DNA	FB	3	0	0.0
DNA	TIR	30	3	10.0
Total DNA		33	3	9.1
RNA	LINE	36	6	16.7
RNA	LTR	60	12	20.0
Total RNA		96	18	18.8

	2-tail Fisher's Exact Test *p*-value
Row 1 vs 2	1
Row 4 vs 5	0.791
Row 3 vs 6	0.276

Figure 1

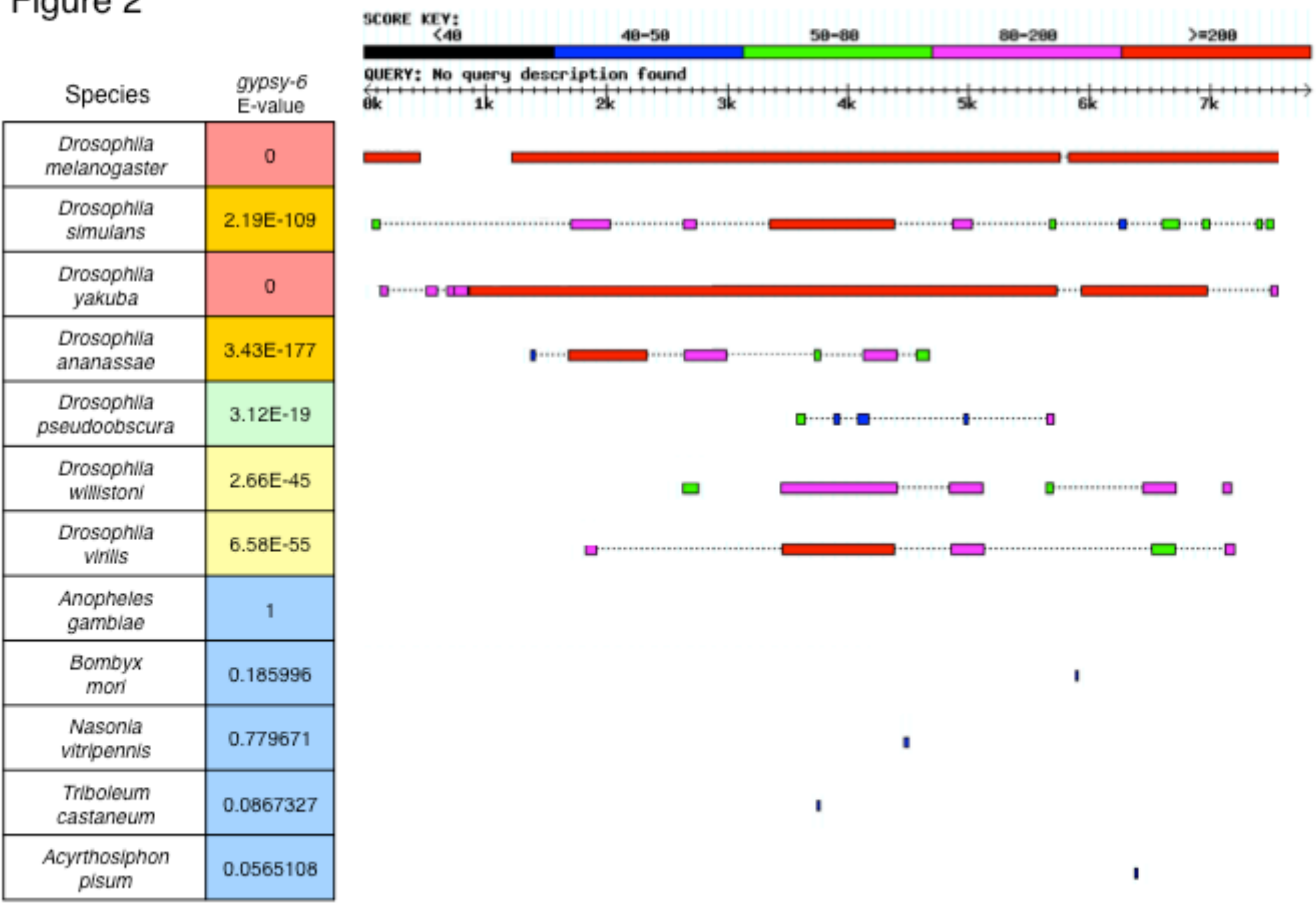
TE BLAST results across insect lineages. Color-coded E-value scores were generated for 43 of the 95 canonical *D. melanogaster* TE family sequences. Lower scores represent higher homology found.

Figure 1

Figure 2

BLAST results and alignments for the *D. melanogaster gypsy6* TE sequence in twelve insect genomes. E-value color code matches that of Figure 1. Note there is not a perfect match to the *D. melanogaster* genome, due to the lack of any one *gypsy6* TE insert with the exact structure of the canonical sequence.

Figure 2



Species	*gypsy-6* E-value
Drosophila melanogaster	0
Drosophila simulans	2.19E-109
Drosophila yakuba	0
Drosophila ananassae	3.43E-177
Drosophila pseudoobscura	3.12E-19
Drosophila willistoni	2.66E-45
Drosophila virilis	6.58E-55
Anopheles gambiae	1
Bombyx mori	0.185996
Nasonia vitripennis	0.779671
Triboleum castaneum	0.0867327
Acyrthosiphon pisum	0.0565108

Figure 3

Representative PCR gels showing amplification of fly TEs in wasps. Each gel shows a 100 bp DNA ladder and PCR products from four fly species and four wasp species for (A) the control gene *cytochrome oxidase I* and (B-D) the TEs *F*, *hopper,* and *transpac*. Boxes indicate the expected size of PCR products. Numbers represent fragment size in kb. Dm = *D. melanogaster*, Da = *D. ananassae*, Dp = *D. pseudoobscura*, Dv = *D. virilis*, Lb = *L. boulardi*, Lh = *L. heterotoma*, Gx = *G. xanthopoda*, At = *A. tabida.*

Figure 3

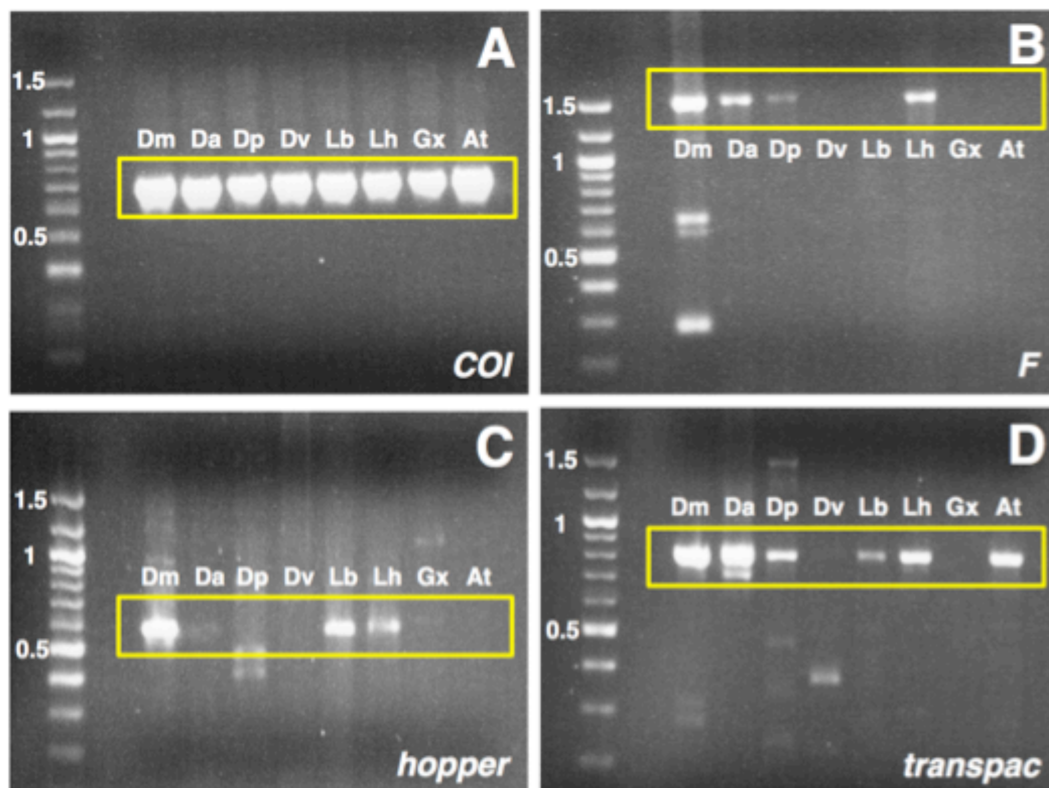


Figure 4

Southern blots showing fly TE probe hybridization in wasp genomes. Membranes show hybridization of *D. melanogaster* probes for the TEs (A) *Doc* and (B) *jockey* against digested genomic DNA from *D. melanogaster* and the wasps *L. boulardi* and *L. heterotoma*. Numbers represent fragment size.

Figure 4

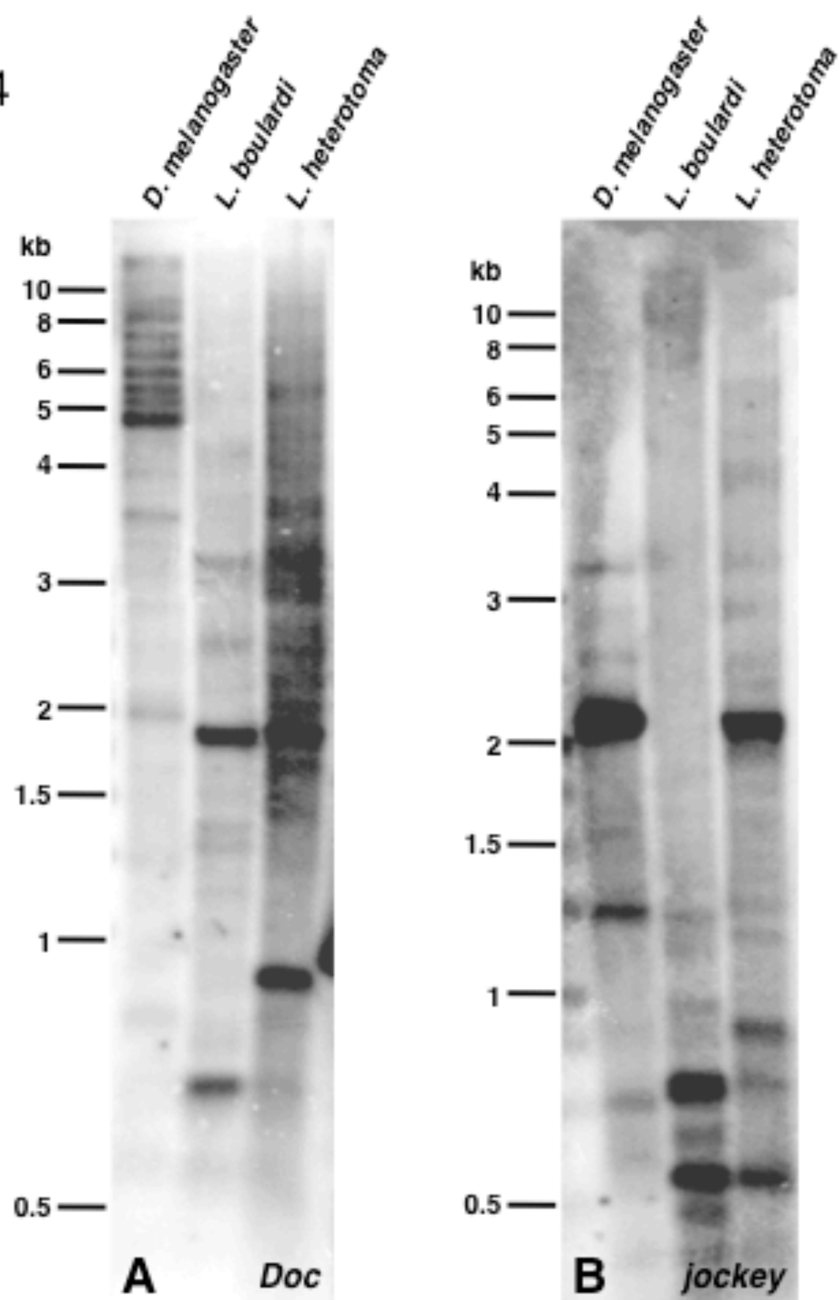


Figure 5

Disjunct distribution of the *Transpac* element across the insect phylogeny. Phylogenetic relationships are a consensus of multiple studies and branch lengths are approximate [155,156].

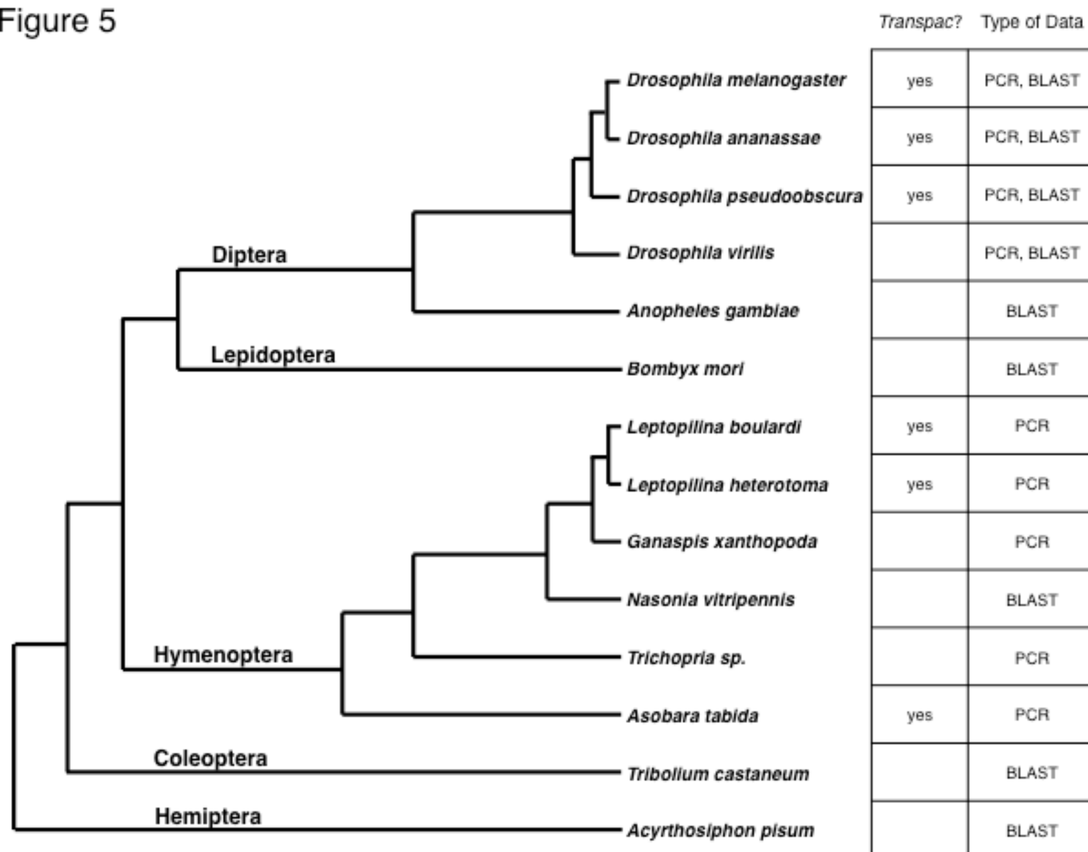


Figure 5

Figure 6

Correlation between TE copy number and TE HT success. For each of the 43 TEs, the number of full-length TE copies in the *D. melanogaster* genome sequence was compared to the number of *D. melanogaster* specialist wasp (*L. boulardi*, *L. heterotoma*, *A. tabida*) DNAs successfully amplified by at least one of the two TE primer pairs.

Figure 6

TE Family	Total Copies in *D. mel.* Genome	Full-length Copies in *D. mel.* Genome	# Wasps Amplified, One Primer Pair	# Wasps Amplified, Both Primer Pairs
FB	32	13	2	0
1360	105	10	1	0
Bari1	5	5	2	2
H	24	1	0	0
HB	32	5	0	0
hopper	15	11	2	1
mariner2	17	4	1	0
pogo	44	5	1	0
S	51	14	0	0
Tc1	21	1	1	0
transib2	12	0	0	0
baggins	14	0	1	0
BS	29	6	1	1
Cr1a	56	1	1	0
Doc	55	30	2	1
F	42	16	2	1
G2	14	2	3	2
I	28	8	0	0
jockey	69	12	2	0
Juan	9	6	1	0
R1	10	2	2	0
Rt1b	37	5	3	1
X	25	6	3	0
17.6	12	7	1	1
297	57	18	0	0
412	31	24	1	0
blastopia	17	13	2	1
blood	22	22	1	0
Burdock	13	7	0	0
copia	30	26	2	1
Dm88	32	0	3	2
GATE	20	0	2	0
gypsy	2	1	2	0
HMS-Beagle	13	9	2	0
invader1	26	1	2	2
mdg1	25	13	1	0
opus	24	16	1	0
Quasimodo	14	5	1	0
roo	146	58	1	0
springer	11	5	3	2
Stalker	12	3	3	1
Tirant	20	15	0	0
Transpac	5	5	3	2

	r^2	ANOVA *p*-value
Column 1 vs 3	0.024	0.324
Column 1 vs 4	0.038	0.211
Column 2 vs 3	0.030	0.263
Column 2 vs 4	0.017	0.407

Supplemental Figure S1

TE BLAST results across insect lineages. E-value scores were generated for the 95 canonical *D. melanogaster* TE family sequences BLASTed against 12 insect genomes.

Figure S1

0 - $1E^{-200}$ | $1E^{-200}$ - $1E^{-75}$ | $1E^{-75}$ - $1E^{-25}$ | $1E^{-25}$ - $1E^{-5}$ | $1E^{-5}$ - 1

TE Type	TE Class	TE Family	*Drosophila melanogaster*	*Drosophila simulans*	*Drosophila yakuba*	*Drosophila ananassae*	*Drosophila pseudoobscura*	*Drosophila willistoni*	*Drosophila virilis*	*Anopheles gambiae*	*Bombyx mori*	*Nasonia vitripennis*	*Tribolium castaneum*	*Acyrthosiphon pisum*
DNA	FB	*FB*	0	2.26E-123	1.38E-32	0.00335551	0.00221992	0.211056	0.7295	0.0523306	0.102261	0.428665	0.188601	0.00199003
DNA	TIR	*1360*	0	0	0	4.45E-08	4.78E-10	1.11E-05	1.90E-59	0.0104496	0.0807373	0.0856513	0.0376605	0.00620803
DNA	TIR	*Bari 1*	0	0	2.52E-07	0	3.76E-09	0.000344261	1.24E-06	0.0822269	0.0103006	0.674181	0.0749981	0.012367
DNA	TIR	*Bari2*	0	7.98E-117	2.01E-102	0.000206609	1.42E-07	0.00328881	4.34E-36	0.198788	0.0983062	0.10429	0.0458863	0.118025
DNA	TIR	*H*	0	0	0	0.141644	0.00600196	1.68E-13	0.12632	0.55908	0.0750136	0.074275	0.0326598	0.00538347
DNA	TIR	*HB*	0	0	2.24E-38	8.48E-08	0.0521021	2.11E-05	0.277335	0.00503519	0.0389151	0.644577	0.0717049	0.184612
DNA	TIR	*hopper*	0	0	5.48E-11	4.89E-12	0.0451509	0.0175954	0.0608643	0.0681212	0.0021597	0.14135	0.00100783	0.0132455
DNA	TIR	*hopper2*	0	0	1.86E-26	3.61E-16	0.783618	1	0.0676082	0.299228	0.217189	0.620894	0.858184	0.0214394
DNA	TIR	*looper1*	0	0	1.18E-123	1.17E-22	0.234602	3.37E-32	1.96E-36	0.0226738	0.175237	0.186903	0.0817164	0.0532442
DNA	TIR	*mariner2*	0	8.90E-175	3.35E-82	0.170069	0.444581	4.56E-05	1	1	0.0839934	1	1	0.398462
DNA	TIR	*NOF*	0	4.15E-171	0	1.44E-08	0.0349163	0.00344615	0.0119114	0.208311	0.0260897	0.432141	0.012169	0.00752678
DNA	TIR	*P*	0.00539714	0.32796	6.64E-06	0.0352126	3.03E-23	0	0.490304	0.138987	0.0687744	0.288294	0.415016	0.0825563
DNA	TIR	*pogo*	0	0.238802	0.0726482	0.101257	0.264706	0.103233	6.72E-15	0.101147	0.0500678	0.0531152	0.00149536	0.0601107
DNA	TIR	*S*	0	4.65E-144	6.16E-05	0.000339254	8.26E-35	9.79E-14	5.29E-12	0.00133906	0.0408931	0.171419	0.0190692	0.012425
DNA	TIR	*S2*	0	2.64E-62	0.234469	0.00133974	2.71E-19	1.59E-15	8.56E-14	0.00528803	0.010343	0.0433567	0.29756	0.0124177
DNA	TIR	*Tc1*	0	0	0	2.16E-08	2.24E-07	2.66E-23	4.88E-09	0.313115	0.0392249	0.0135311	0.228234	0.186082
DNA	TIR	*transib1*	0	0	0	2.72E-05	0.0684576	7.28E-09	9.58E-05	0.408419	0.051164	0.0542781	0.0942746	0.00393425
DNA	TIR	*transib2*	0	0	0	1.77E-22	6.72E-52	3.03E-27	0.121377	0.135954	0.265821	0.0713877	0.124002	0.0807639
DNA	TIR	*transib3*	0	0	0	0.00883741	4.04E-10	5.37E-35	0.00788127	0.137831	0.269493	0.018311	0.0318153	0.0207218
DNA	TIR	*transib4*	0	0	1.03E-121	0.00205921	1.47E-09	3.53E-08	0.0286727	0.126603	0.062793	0.26322	0.00741331	0.00482826
RNA	LINE	*baggins*	0	0	8.95E-16	7.70E-14	8.57E-19	0.0675423	1.29E-27	0.261509	0.511491	0.542623	0.238563	0.614065
RNA	LINE	*BS*	0	0	5.28E-162	1.05E-06	1.14E-119	1.15E-12	2.56E-13	0.246536	0.122035	0.129463	0.343324	0.0370777
RNA	LINE	*Cr1a*	0	0	0	1.10E-21	4.02E-11	3.82E-09	1.37E-11	0.0034725	0.0268315	0.0284446	0.195399	0.00815216
RNA	LINE	*Doc*	0	0	0	1.21E-24	2.62E-09	2.49E-07	0.00327783	0.226509	0.112098	0.469901	0.0522846	8.95E-06
RNA	LINE	*Doc2*	0	0	0	1.65E-11	6.48E-07	9.98E-07	5.39E-05	0.0581039	0.448967	1	0.827443	0.00873668
RNA	LINE	*Doc3*	0	0	0	3.97E-09	1.00E-05	2.53E-07	0.0513411	0.0575069	0.00720252	0.1193	0.0524513	0.0086469
RNA	LINE	*F*	0	0	0	5.13E-30	2.53E-06	2.58E-10	0.00326599	0.057117	0.44134	0.118491	0.813389	0.00858826
RNA	LINE	*G*	0	0	0	1.94E-32	3.76E-08	2.76E-22	3.92E-24	0.0527066	0.00042279	0.920727	0.048073	0.233015
RNA	LINE	*G2*	0	0	0	5.89E-38	0.00159286	1.17E-17	1.72E-22	0.586282	0.0734213	0.307773	0.13533	0.00564549
RNA	LINE	*G3*	0	2.70E-132	5.47E-137	0.000229403	6.23E-07	4.95E-64	8.39E-07	0.0558621	0.431642	0.015818	0.201327	4.16E-06
RNA	LINE	*G4*	0	0	0	5.72E-57	0.000498801	1.34E-11	2.11E-22	0.183767	2.24E-06	0.205605	0.526907	0.000842617
RNA	LINE	*G5*	0	0	1.11E-05	6.12E-05	1.03E-05	0.000246682	0.000215783	0.232816	0.687579	1	1	0.0042224
RNA	LINE	*G6*	0	0	4.17E-34	0.000101167	1	0.0251434	2.28E-05	0.384641	1	0.108912	1	1
RNA	LINE	*HeT-A*	0	0	1.56E-14	0.00119839	0.19332	0.0190759	0.0166864	0.0186916	0.14446	0.00248406	0.0673637	0.00281111
RNA	LINE	*I*	0	0	0	1.67E-113	0.000699894	1.81E-08	9.79E-07	0.0041748	0.0322653	1	6.17E-05	0.153059
RNA	LINE	*Ivk*	0	0	0	1.79E-08	4.51E-05	1.52E-30	0.0148156	0.0655603	0.0324523	0.136036	0.015136	0.0389602
RNA	LINE	*jockey*	0	0	1.20E-82	3.65E-71	0.0102109	0.015735	9.50E-10	0.0609183	0.00193094	0.00809427	0.21955	0.00915996
RNA	LINE	*jockey2*	0	0	0	2.98E-12	0.0275005	0.0423783	2.66E-12	0.648297	0.0076249	0.725485	1	0.0117594
RNA	LINE	*Juan*	0	0	0	0.203189	0.00860982	1.38E-05	0.0458589	0.0513664	0.100445	0.106559	0.0468506	0.120588
RNA	LINE	*R1*	0	0	0	0	8.74E-22	1.95E-14	1.91E-23	0.0164499	0.502358	0.00863831	0.0592967	0.00977583
RNA	LINE	*R2*	0	0	0	4.41E-76	4.21E-32	5.63E-94	1.06E-08	0.172686	0.337667	1	0.157505	1
RNA	LINE	*Rt1a*	0	0	6.33E-177	0.245215	0.0410571	0.00102526	0.0140063	0.0619905	0.702373	0.0011243	1	0.274078
RNA	LINE	*Rt1b*	0	0	2.61E-105	6.77E-08	0.648977	0.000262683	1.47E-05	1.06E-06	0.484938	0.000135163	0.893721	1
RNA	LINE	*Rt1c*	0	0	9.67E-59	4.92E-15	7.36E-07	1.18E-09	0.00377604	0.261027	0.748627	0.0187103	0.940915	0.0739314
RNA	LINE	*TART*	0	0	0	0.000531973	0.0217138	0.0334599	0.00187459	0.129549	0.37151	0.268784	0.0940204	1
RNA	LINE	*X*	0	0	0	0.0145695	0.000617361	0.000240765	0.00328628	0.0145537	0.444353	0.471399	0.207255	1
RNA	LTR	*17.6*	0	0	1.44E-138	1.10E-53	0.000245581	4.08E-10	6.97E-27	0.00146494	0.0447373	0.741012	2.17E-05	0.0135924
RNA	LTR	*297*	0	0	0	0	2.40E-07	4.29E-19	6.80E-30	0.0849689	0.0136443	0.000183034	0.0774983	0.0504941
RNA	LTR	*412*	0	0	0	0	0	3.08E-177	0	0.000343654	0.00265597	0.0439925	2.01E-05	0.0125993
RNA	LTR	*1731*	0	0	1.72E-07	2.40E-07	1.14E-17	1.01E-09	0.01274	0.01427	0.027905	0.116974	0.203215	0.132375
RNA	LTR	*3S18*	0	0	0	1.30E-09	0.0124695	3.23E-07	0.0664018	0.0188242	0.00931795	0.0390596	0.00109964	0.000181323
RNA	LTR	*accord*	0	0	6.51E-76	6.50E-86	4.11E-09	2.41E-05	8.65E-08	9.34E-05	0.0028518	0.000785652	3.63E-10	0.0534554
RNA	LTR	*aurora*	0	0	0	0	1.14E-134	0.00337925	1.52E-23	8.70E-07	0.101088	1	1	1
RNA	LTR	*blastopia*	0	0	0.000179988	1.03E-06	7.34E-13	1.26E-21	5.66E-05	0.241385	0.000124019	0.032073	0.014101	0.000588318
RNA	LTR	*blood*	0	0	2.90E-158	4.00E-47	2.85E-53	1.55E-43	7.80E-23	0.000369293	0.176083	0.798115	0.0821099	0.0135393
RNA	LTR	*Burdock*	0	0	0	0	2.46E-16	2.34E-14	1.90E-08	0.0778536	0.152276	0.0408831	0.108418	0.0462657
RNA	LTR	*Circe*	0	0	0	3.29E-07	0.0129392	0.000323125	4.58E-06	0.304981	0.59652	0.632827	0.0178159	0.18124
RNA	LTR	*copia*	0	0	0	5.08E-169	0	8.03E-94	2.86E-22	0.0624048	0.122059	8.94E-09	0.0144076	0.037065
RNA	LTR	*diver*	0	0	0	0.0742873	0.0491588	0.0191671	0.261777	0.018781	0.145151	0.0389701	0.267453	0.0441006
RNA	LTR	*diver2*	0	0	0	4.28E-12	3.17E-21	2.59E-07	1.51E-11	1.57E-05	0.00747267	0.0313245	0.678871	0.00897122
RNA	LTR	*Dm88*	0	0	3.43E-27	1.51E-08	0.036619	3.75E-08	0.195045	0.883254	0.00692474	0.114699	0.199263	0.0328494
RNA	LTR	*frogger*	0	0	3.91E-13	6.33E-25	2.31E-14	2.04E-06	1.85E-09	0.000123098	0.231924	0.0622413	0.10815	0.278319
RNA	LTR	*GATE*	0	0	0	1.74E-06	2.01E-14	7.02E-06	2.61E-11	0.00167586	0.000829548	0.00347736	0.000386831	0.0155469
RNA	LTR	*gtwin*	0	0	0	7.23E-21	3.69E-37	3.14E-26	1.31E-40	0.355771	0.695862	0.738215	0.324491	0.211422
RNA	LTR	*gypsy*	0	0	0	7.28E-21	3.00E-93	8.31E-30	2.40E-88	0.358563	0.177489	0.188291	1	0.053926
RNA	LTR	*gypsy2*	0	0	0	2.95E-29	6.16E-11	2.15E-39	7.45E-39	1	1	0.681276	1	0.0124967
RNA	LTR	*gypsy3*	0	0	0	4.23E-93	2.39E-07	3.43E-38	5.05E-43	0.0847009	0.0106107	0.175752	0.0772538	0.0503348
RNA	LTR	*gypsy4*	0	0	3.32E-65	2.45E-14	2.53E-13	9.90E-51	3.30E-47	1.40E-06	0.162785	0.682375	0.299946	0.0494587
RNA	LTR	*gypsy5*	0	0	0	1.51E-06	0.00379816	2.49E-08	3.40E-07	2.35E-05	0.00283828	0.185763	0.000334956	0.0134642
RNA	LTR	*gypsy6*	0	2.19E-109	0	3.43E-177	3.12E-19	2.66E-45	6.58E-55	1	0.185996	0.779671	0.0867327	0.0565108
RNA	LTR	*HMS-Beagle*	0	0	0	3.93E-09	2.51E-10	2.39E-08	3.40E-47	3.94E-13	0.0424836	0.00288523	0.309167	0.201438
RNA	LTR	*Idefix*	0	0	0	2.12E-33	8.05E-26	1.39E-34	4.45E-28	2.37E-05	2.96E-06	4.91E-05	0.00525967	0.0135411
RNA	LTR	*invader1*	0	0	0	1.30E-116	0.00819321	8.72E-10	3.25E-15	0.763194	6.36E-06	0.400674	0.176167	0.453427
RNA	LTR	*invader2*	0	0	0	0	7.19E-10	1.24E-18	1.01E-12	0.000255092	0.0307757	0.509756	0.0036327	6.22E-07
RNA	LTR	*invader3*	0	0	0	1.12E-06	1.88E-35	1.49E-27	6.41E-08	1.75E-05	0.0329468	0.00884557	0.0807191	0.00253334
RNA	LTR	*invader4*	0	0	0	2.42E-40	0.00630012	2.55E-06	4.23E-94	0.0375866	0.0185993	0.0779659	0.135462	0.00565099
RNA	LTR	*invader5*	0	5.59E-164	2.27E-77	6.61E-26	0.00820547	0.000809859	0.000179284	0.048954	0.0957266	1	4.64E-05	0.114923
RNA	LTR	*McClintock*	0	0	2.09E-106	2.78E-29	1.36E-05	8.94E-11	9.43E-26	0.00126963	0.000828466	0.0411326	0.00457757	4.83E-05
RNA	LTR	*mdg1*	0	0	0	9.84E-45	2.89E-90	1.46E-74	3.47E-44	9.43E-25	0.177751	0.188589	0.327521	0.0540795
RNA	LTR	*mdg3*	0	0	0	6.96E-05	1.96E-10	1.94E-11	1.01E-06	0.00108577	0.0331579	0.549216	0.00391383	0.00254957
RNA	LTR	*micropia*	0	0	0	3.04E-13	0.000179974	4.84E-12	9.95E-07	0.261701	0.00829686	0.543023	0.238738	0.0393584
RNA	LTR	*opus*	0	0	0	1.23E-25	0.0605286	6.44E-09	2.31E-11	0.00985253	0.0452318	0.000196839	0.0833434	0.214569
RNA	LTR	*qbert*	0	0	0	0	6.39E-05	4.04E-07	0.000340385	1.08E-13	0.181802	0.00312618	8.80E-05	0.0552365
RNA	LTR	*Quasimodo*	0	3.88E-123	1.58E-36	7.79E-64	1.81E-17	3.37E-32	8.04E-39	3.97E-10	0.0444237	0.00301841	1.43E-09	0.000864462
RNA	LTR	*roo*	0	0	0	3.78E-26	4.05E-28	5.58E-19	4.38E-10	0.0279695	0.0547063	0.000940705	0.00645608	6.82E-05
RNA	LTR	*rooA*	0	0	0	0.0231399	4.01E-06	2.44E-05	0.00132012	0.0913949	0.178762	0.749795	1	0.21461
RNA	LTR	*rover*	0	0	0	1.87E-24	0.0149038	1.65E-12	3.03E-35	1.50E-06	0.0111373	0.000191511	0.00519348	0.0528327
RNA	LTR	*springer*	0	0	0	0	4.19E-09	4.82E-59	2.51E-54	0.0916821	0.179323	1	0.330419	0.0544835
RNA	LTR	*Stalker*	0	0	0	2.54E-159	0	0	0	9.15E-05	0.00279463	0.0117148	0.122755	0.0523838
RNA	LTR	*Stalker2*	0	0	0	7.32E-166	0	0	0	0.00151096	0.0461425	5.08E-05	0.0850214	0.0553958
RNA	LTR	*Stalker4*	0	0	0	0	0	0	0	9.28E-05	0.00283442	0.0171017	0.267016	0.0531295
RNA	LTR	*Tabor*	0	0	0	1.42E-148	2.64E-121	7.90E-64	0	9.62E-08	0.00282901	1	0.0813876	0.0063965
RNA	LTR	*Tirant*	0	0	0	0.000425508	7.12E-05	2.78E-05	6.15E-06	2.83E-08	0.202677	0.215013	9.81E-05	0.061569
RNA	LTR	*Transpac*	0	0	0	0	0.658804	1.71E-05	9.57E-07	0.0636962	0.0315296	0.00214231	0.0147057	0.0378524
RNA	LTR	*ZAM*	0	0	0	2.70E-05	2.89E-07	2.75E-05	1.60E-09	0.000106424	0.050744	0.000119113	0.0296627	0.029037

Supplemental Figure S2

PCR results in flies and wasps using *D. melanogaster* TE primer pairs. "X" represents cases where a strong, individual PCR band of the expected size was obtained. Boxes represent cases where both primer pairs successfully amplified the TE from a particular species. Gray cells represent cases where the PCR product was confirmed by sequencing.

Figure S2

TE Type	TE Class	TE Family	Forward Primer	Sequence	Reverse Primer	Sequence	Annealing Temperature		Other Drosophila Species			Other Drosophila Parasitoids			Parasitoids of *D. melanogaster*		
								Drosophila melanogaster	*Drosophila ananassae*	*Drosophila pseudoobscura*	*Drosophila virilis*	*Leptopilina victoria*	*Ganaspis xanthopoda*	*Trichopria sp.*	*Leptopilina boulardi*	*Leptopilina heterotoma*	*Asobara tabida*
DNA	FB	*FB*	219f	CCACGTTTGCCCACCCTTTAG	1039r	TGAATTGGCGCCACATCTTACA	52.6	x								x	
DNA	FB	*FB*	457f	TCGTTAGCCCACCTCTTCAAAATA	1034r	TGGCGCCACATCTTACAAAAC	51.5								x	x	
DNA	TIR	*1360*	150f	TACGCATACAGTGATAGCAGACAA	1136r	TAAATACCCCGAACAACAAATACG	51.2	x			x					x	
DNA	TIR	*1360*	2400f	TCGGGAAATTAAAGGCACCATCT	3325r	GCTGTCTTTCCGCTCGCTCAC	52	x	x								
DNA	TIR	*Bari1*	371f	TAAATAAAATGCCCAAAACAAAAG	946r	TGCCCCCACCAAATCTAT	52.3	x	x	x	x				x	x	
DNA	TIR	*Bari1*	408f	GAGGCCCGGGCTGGTATTGTT	1279r	TGATGGCTCCCTCTCGTTTTCTAT	55.1	x	x	x					x	x	
DNA	TIR	*H*	98f	GCTACTCGCCACGCACAT	921r	ATCCGATTTGGCCTTCCGACTTA	54	x					x				
DNA	TIR	*H*	1759f	CCACCCGCAGCACATCTTCAG	2948r	AGCCCGCCACTCGCACTCTAC	54.8	x	x								
DNA	TIR	*HB*	142f	ATCCGGTCAGTTTTTCTTGTTATC	750r	CTGGCCAGTTTAGGTAGGTTTTAG	52.4	x									
DNA	TIR	*HB*	497f	CGAAAACCGTGGTACCAAACATAA	1209r	CGGGGACTTCTTCGACACAAAC	55	x									
DNA	TIR	*hopper*	213f	TAATTCATAACCGTCTTCCTCTTC	799r	AGTGGGCATTTTTCTGATAGC	51.6	x	x						x	x	
DNA	TIR	*hopper*	419f	CCGTGGCTGGCTTCAACA	1107r	GTGCGCCTATTTCTAACAA	50.3	x			x					x	
DNA	TIR	*mariner2*	38f	CGCGCCATTCAGTTAGTT	455r	AAGTGTCCCCCAAATCCTCAAGTC	55.3	x									
DNA	TIR	*mariner2*	179f	TTTTCAAGCGGATGTTCGGTTATT	731r	TGGCGTTTGTCGTGACTCTTGTTA	57.2	x		x						x	
DNA	TIR	*pogo*	259f	AGTATCTGCGCATGTCCGTTTTT	1093r	TTTTTCGCCAGAGATCCTTAGTCA	54.7	x									x
DNA	TIR	*pogo*	504f	TGTGGCCGCGTCAGGTTTA	1555r	TACGCATTGTTTGTCTTTTTCAGC	54								x		
DNA	TIR	*S*	281f	CATTTTTCCGTTATTTTTGTCTTA	921r	AACGGTTTGCGCCATACTCTG	52.6	x									
DNA	TIR	*S*	497f	TCCGTATCCCGTAAAACCGTCTAT	1328r	TTATCGCAGCCATGAGTTGTTGTC	55.5	x									
DNA	TIR	*Tc1*	204f	AGGCGGTAACAAAAATAGCACTGA	844r	ACGCCGAACATAAGACCGAGAG	55.3	x				x			x		
DNA	TIR	*Tc1*	831f	CTTATGTTCGGCGTCCACCAC	1622r	TTGTCCCAGCGTTTCTCTATTTTC	54.3										
DNA	TIR	*transib2*	501f	TTGCCCATTTTTGCTCGTTTTC	1525r	GACTGCGGTGCTCTGTTTATCCA	54.6				x						
DNA	TIR	*transib2*	1758f	GGAGTCAGCCCAACACAAATG	2610r	AATAATCGCCCTCTTCTTCTTCTA	52.5										
RNA	LINE	*baggins*	810f	CTTCGAAGCGGGCAAACTCCAC	1718r	CATCCGCCGGTACCGCTTCTTC	60.4	x									
RNA	LINE	*baggins*	4307f	CGCCGCTTCATATCGTCATT	5148r	CCCCGTAAGAAGGCCAGTCAAA	56.6	x								x	
RNA	LINE	*BS*	1549f	CACCCGACCGATAGACGCACTG	2459r	AACACCGCTACTCCGCCAACACTA	57.3	x		x				x		x	
RNA	LINE	*BS*	3203f	ACCCCTGGGACCGACTTTTATGG	4151r	ACGCGGGGAATCGAGTAGGAGTT	57.9	x	x							x	
RNA	LINE	*Cr1a*	555f	AATGGCCGTACAAGTGATGACCTA	1392r	CCACGGGCCCCTTTTTCCTATTT	56.2	x			x				x		
RNA	LINE	*Cr1a*	3614f	GCCAACCCAATACGCACGACTT	4446r	CCACACCTCCCGCCCAACC	55.8	x									
RNA	LINE	*Doc*	760f	CCAACAACGGCAATGAAAACTCTG	2314r	ACCCGCTGCGATGAACCTGTC	56.6	x		x						x	
RNA	LINE	*Doc*	2516f	ACCGCCGAAGCACTAGCAGATTTA	4122r	TATGTTACCTCGTCGGCTTGTGGA	57	x	x	x						x	
RNA	LINE	*F*	805f	CAATATACATCCGCGAGAAGAGCA	2324r	GTCGCGATCCCCAGTGAGTATGTT	56.6	x	x	x					x	x	
RNA	LINE	*F*	2532f	TACTAATTCACCTCCGCCGATACG	4136r	TGCGCCATGTGAGTCTTCTGTCTA	57.1	x							x	x	
RNA	LINE	*G2*	138f	TGAATCTCCGCAACCCAGTAAC	1225r	TTGCGCGAGAAGAACCAGAT	57.9	x	x						x	x	x
RNA	LINE	*G2*	1998f	TCGGCCGTATTCCTGGATGTCAAG	2944r	AGGTCAAGCGGGTGCGTTCTCTG	60.5	x	x			x			x		x
RNA	LINE	*I*	1149f	CACACCCAAATCAGCAGCACAA	2738r	GTCGCCGGAGAGGTCAGAGAA	55.5	x			x						
RNA	LINE	*I*	3062f	ACCCATCTCCCTCAACTGCTGTAT	4664r	AATTTTAGGTGCGTGTTGCGTTAT	54.4	x			x						
RNA	LINE	*jockey*	764f	TCAGCCCCCATTACCTATCCTCAC	2332r	CACTCTCGCTATCTGCCTGTCATT	56.5	x							x	x	
RNA	LINE	*jockey*	2886f	AGCTTGCTGCACCGAGAAATC	4400r	TGAGCCAAGACATCCGAGCAACC	57.1	x	x		x						
RNA	LINE	*Juan*	555f	CCGGGCTGGATAAACTACC	1550r	AACGCCGCCTCCCCTACTT	52.3	x									
RNA	LINE	*Juan*	1793f	TTTTCTAATTTGCGGGGACTTC	2827r	ATATGATTAATGGCGCTTGTAGGA	52.9	x							x		
RNA	LINE	*R1*	938f	CCGCCAATGCTCCCCAGATGC	1908r	TCCCTCGCCGGTCGGTGAAAAC	62.4	x	x	x	x	x	x	x	x		x
RNA	LINE	*R1*	3987f	CTTCGCCAGCGGATGACAGGA	4831r	GTATTCTCGCCCGCTAACCACACG	60.7										
RNA	LINE	*R1b*	1017f	TGGGTGGCAACGGTGAAGAAAA	2091r	CCCGCATAGGTAAAGCAGCCAGTT	60.6	x	x								x
RNA	LINE	*R1b*	2963f	CCCGGGGGTCCTGGCTAACATA	4064r	GTGGCTCGCGTGGTCCTTGAA	60.3	x	x						x	x	x
RNA	LINE	*X*	473f	CCATCGGCAAACAAGGGGAAAAC	1377r	GGATCGCTGCAGGAACGCTTGTA	59.7	x		x					x	x	x
RNA	LINE	*X*	3140f	CTGCGAGGTAGCCGAAGTCATTG	4176r	TGCGCGAAGAAAGTAGCCAGTAGT	59.2										
RNA	LTR	*17.6*	3476f	CACCTGCAATCGCTCGGACTA	4516r	TTTATGTGCGGCTTGAATGACTT	53.4	x	x							x	
RNA	LTR	*17.6*	5896f	ACAAACGCCCATAACTCGGTAAA	6901r	CTTGGGCTGGGATTGATGGATA	51.7	x	x							x	
RNA	LTR	*297*	2048f	AAATAGTATACAAAGGGCGTTCAT	3650r	AATTCTTTTTCGATAGCACTGTAA	51.1	x									
RNA	LTR	*297*	3353f	CTCGGGGCTGTCCTTTCTCAA	5385r	GTGCATGGTAATCGGTTCGTAGTC	53.2	x									
RNA	LTR	*412*	776f	ACTGCCTGGGGACTTGTGA	2504r	AATTGCCTCTTTGGTGCTTAG	51.6	x			x						
RNA	LTR	*412*	3216f	TCCCACACGATTTTCATTTAGTAG	5056r	ATGATTGTCCTTGCCCTTTAGATA	52.3	x		x	x					x	
RNA	LTR	*blastopia*	277f	GGGGGCTCGTCCAGTCC	1115r	GCCTCGGCATTTTTCTCAA	53.3	x							x	x	
RNA	LTR	*blastopia*	2699f	TATATGCCGCTACCCCAGAAGA	3901r	GCCCCAAATGATCGATGTGTAGT	55.2	x			x					x	
RNA	LTR	*blood*	1273f	GCGGCGGGCTGCGTTAT	3096r	GGGTCTGGCTGTTTTCCGATTGT	54.9	x									
RNA	LTR	*blood*	4729f	CCACTTAACGAGGCTTTGT	6219r	AGTTCGGTGGCTTCTTTCTA	50.7	x				x				x	
RNA	LTR	*Burdock*	2101f	CGGGGCTTCGAAAAATTACATACA	2941r	CGGCAAACGGCAAAACTCG	55.1	x									
RNA	LTR	*Burdock*	4253f	AGAGCGCATAGGGCAGCACAG	5154r	TGGCGATGGTCACAAAAGGAA	55.4	x									
RNA	LTR	*copia*	1288f	ACTGCGGGTTTGTCCTT	2910r	CATTCGGGTTGCCTGAT	50.9	x					x			x	x
RNA	LTR	*copia*	3148f	GGGAAGAAGCCATCAATAC	4744r	GGAAAACCCAGAAAAACC	49.7	x					x			x	
RNA	LTR	*Dm88*	935f	TCTGCGAAACAAAAAGCGAAAAG	1818r	AAATAGCGTGCACCTCCAACAGA	55.2	x							x	x	x
RNA	LTR	*Dm88*	3012f	TGGCACGCTACTCATCAATCAAAT	3852r	GCCCAATCCGCGTCAACATA	56.5	x	x						x		x
RNA	LTR	*GATE*	1047f	CCCGTCGGCAAATCCAACCA	2122r	CTGCCGCTCGCCTGCTGAC	56.8	x									
RNA	LTR	*GATE*	6009f	GCCGGTCCGCGATATAAAGAACTA	7005r	GACTACGCGCACATGGCTCCTC	59.2								x		x
RNA	LTR	*gypsy*	1161f	GCATTAGGTCGGCCGTTAGATAGT	2071r	TTGGGGCGTTTGGTGAAGTGG	57.9	x							x	x	
RNA	LTR	*gypsy*	4458f	TATTCACTGCGACCTGCCCACTCT	5342r	ACACGCGTTCTCCCACCTCAAA	58.2	x	x		x						
RNA	LTR	*HMS-Beagle*	568f	AGCCCAGGCAAAAAGGACAACATT	1514r	TTCGGACAACACCTGCACAAAACA	53.7	x			x						
RNA	LTR	*HMS-Beagle*	5868f	CAACCGGACAAGAAAGCCCATAAA	6776r	TTGTCCTCGAGCGGTCATCATAAG	54.2	x	x						x	x	
RNA	LTR	*invader1*	1240f	AACTGCGGAAAAGCTGGACACATC	2176r	GTATTTTGCCAGACGGAGGTTTTG	55.5	x							x		x
RNA	LTR	*invader1*	2504f	ACAATGGCCAAGTGGAGCGAGTC	3317r	ACCGGCCAGTCCATCTTTTGTTAG	56.3	x							x		x
RNA	LTR	*mdg1*	853f	ACGACTGCGAGGGACCACTGTT	1873r	AATATCTGCGCATGCTTGTTGAG	53.2	x									
RNA	LTR	*mdg1*	4953f	TTCACTGGGCAATAAATCACTTCA	5851r	TGCTGTTGCTGGCGTTTCTGTTAT	52.6	x									x
RNA	LTR	*opus*	1741f	GCGCACGCACCGAGTATGAG	3564r	ATGGGGTAAGTGTCGGGTATGGTG	58.2	x								x	
RNA	LTR	*opus*	3978f	TTAAGGCAGATCCGAAAAAGGTCA	5744r	TCTGCCGCCGGAAATCTGTCA	57	x									
RNA	LTR	*Quasimodo*	1129f	GCTTTGGGCCAGATTCGTC	1964r	TTTTTGGGGCTGGTTAGTAGT	51.8	x			x						
RNA	LTR	*Quasimodo*	3317f	CCCGCAAAAATTAAAGCCGTAGT	4250r	TGTAAGCGTTTTGAAATGTAAGGA	51.6	x	x								x
RNA	LTR	*roo*	1429f	TTGTTAAAGATCGGCGTTGTCG	3153r	TAAAATCGGGGTCGGCTAAT	52.7	x								x	
RNA	LTR	*roo*	5789f	AGACGCATTTTTAGCAGCACTCA	7784r	AATTCCAGGGCCAACTTCC	52.7	x			x						
RNA	LTR	*springer*	2504f	GCCCGGTGCAGTCCCCATTTAC	3556r	CAGTCGCCGCCCCATTGTTG	58.8	x	x	x						x	x
RNA	LTR	*springer*	5727f	CATCGGCCCACATTACTGACTATT	6843r	CCCGGCGCCCTATTCTGGAC	56.8	x	x	x					x	x	x
RNA	LTR	*Stalker*	1008f	ATGCCCCAATTTGCTTTAGA	2043r	GCGCCCGCTTGATTGAAC	50.8	x		x							x
RNA	LTR	*Stalker*	3859f	ATCGTCGAACCGTCTGTATCTGAG	4829r	GCCGCTAGTTCTTGTTCCGTTGTA	54.2	x		x					x	x	x
RNA	LTR	*Tirant*	2047f	GCCCCACTCCATCCCCTCTG	3838r	GTCGTCCGTGGTTTTAATCGTGTG	57	x									
RNA	LTR	*Tirant*	5445f	TCCTCCCCGAAATTACTGATAG	7193r	TTTTCGCCAATAGCATACTTTCTG	52.5	x			x						
RNA	LTR	*Transpac*	1685f	ACCCGAAGTGGAAAACAGACAT	2510r	CCGAAAGGCATACGAAGGAACT	52.7	x	x	x		x			x	x	x
RNA	LTR	*Transpac*	2858f	GGCGCTACATAAAAGACTACTCCA	3733r	TTTCCTGATTTCCGCTTGATTTA	51.6	x	x						x	x	

Supplemental Figure S3

Phylogenetic trees of fly and wasp TE sequences. Trees (A-O) show the unrooted relationships between *D. melanogaster* canonical TE sequences, single homologous TE sequences from any other *Drosophila* genomes with significant matches, and TE sequences cloned from the endoparasitoid wasp species *L. boulardi, L. heterotoma*, and *A. tabida*. Bootstrap values greater than 50% are shown. Branch lengths represent inferred number of mutations per site.

Figure S3-A

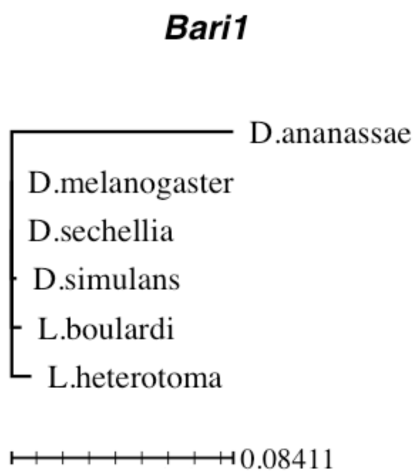


Figure S3-B

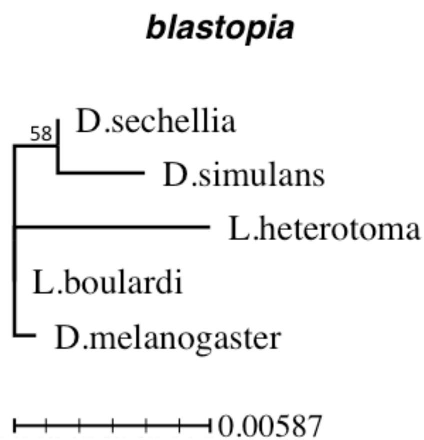


Figure S3-C

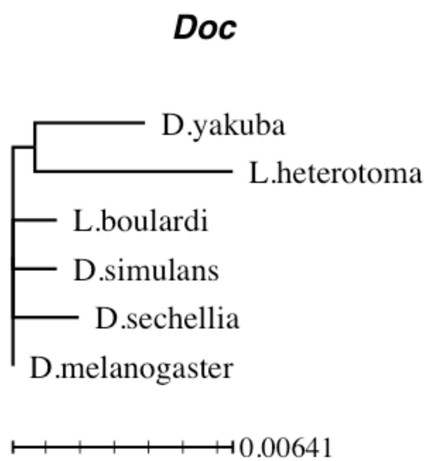


Figure S3-D

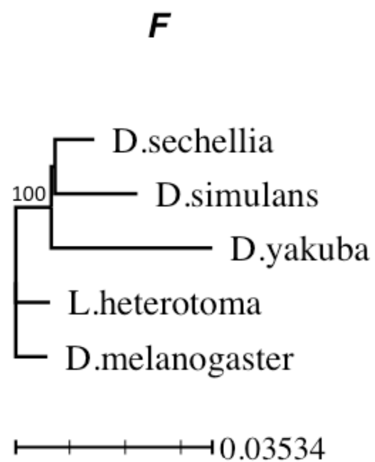


Figure S3-E

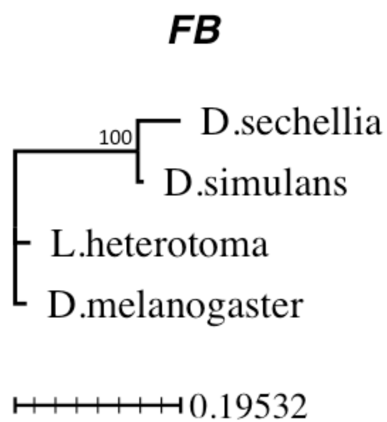


Figure S3-F

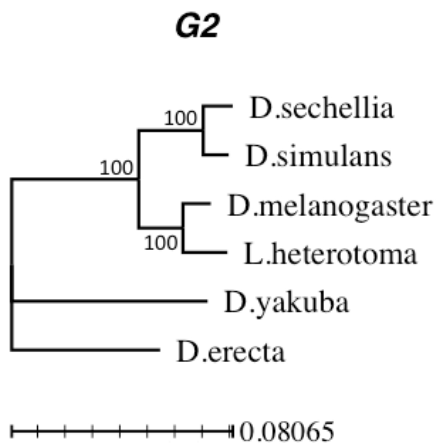


Figure S3-G

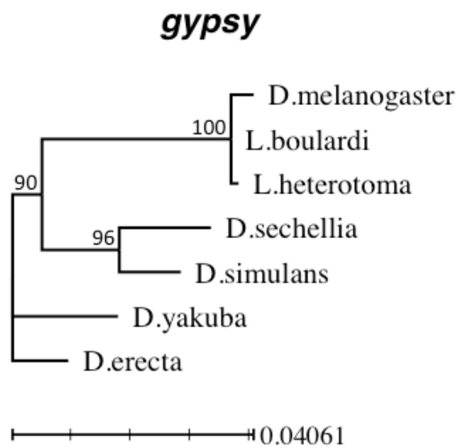


Figure S3-H

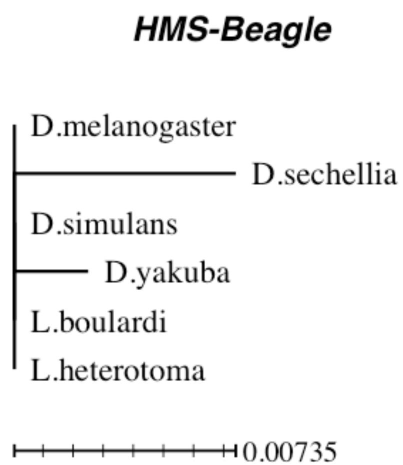


Figure S3-I

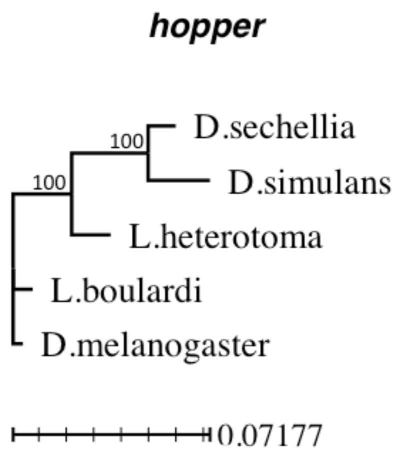


Figure S3-J

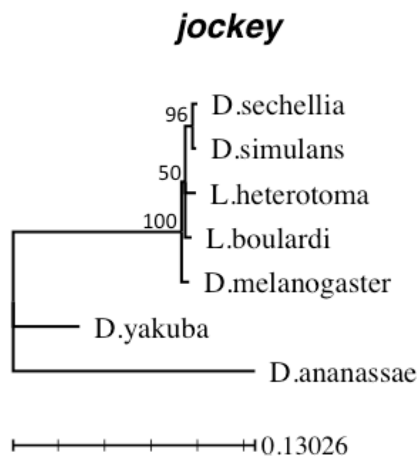


Figure S3-K

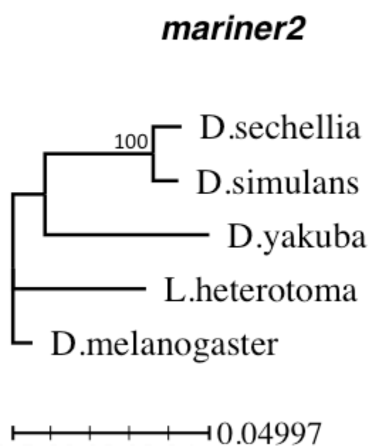


Figure S3-L

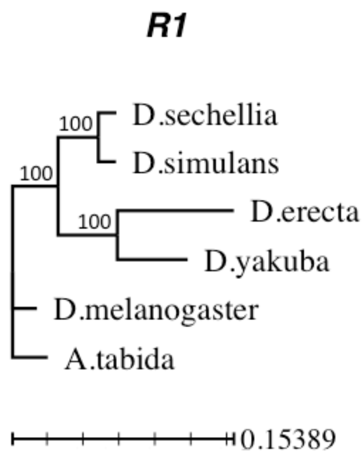


Figure S3-M

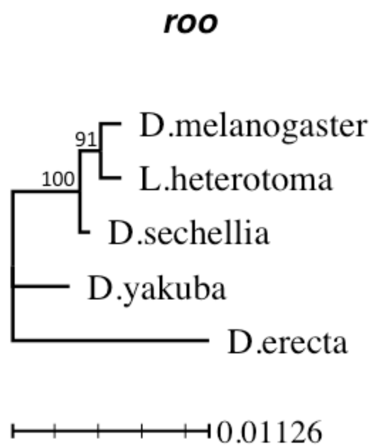


Figure S3-N

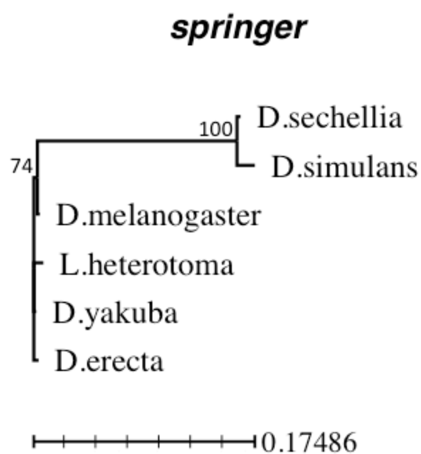
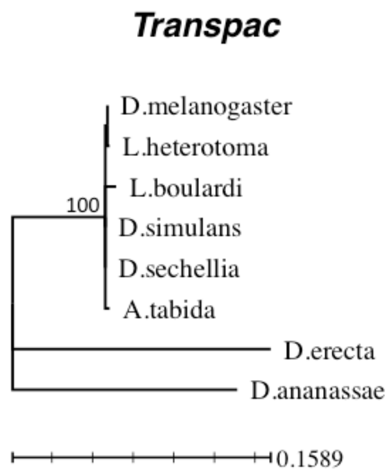


Figure S3-O



Funding

This work was supported by the National Institutes of Health (AI081879 to T.A.S.) and the National Science Foundation (DEB-1011556).

Acknowledgements

We thank the Schlenke lab for helpful discussions about the manuscript.

Phylogenetic Analysis of Two TEs in Drosophila and Their Wasp Parasitoids Reveals an Interaction Highly Permissive to Horizontal Transfer But Unlikely TE Adaptation in Novel Hosts

Neil F. Milan and Todd A. Schlenke

Department of Biology, Emory University, 1510 Clifton Road NE, Atlanta, GA 30322, USA

Abstract

Transposable elements (TEs) are candidates for horizontal gene transfer in animal systems and their invasion of novel host species can present the opportunity for rapid evolution, such as through the movement of genes from previous hosts or the domestication of the element itself. Previous work has shown that the *Drosophila*-wasp parasitoid interaction contains many instances of likely horizontal transfer from multiple TEs families. Here, we present phylogenetic analyses of two TEs believed to have horizontally transferred from the fruitfly host to its wasp parasitoid. Our data demonstrate that, while the fruitflies and wasp parasitoids have very strong host-parasite associations that are highly permissive to horizontal transfer, fruifly TEs that horizontally transfer to the wasps have not adapted to their new wasp hosts' genomes. These findings also suggest the possibility that horizontally transferred TEs can be used to identify previously unknown host-parasite associations or confirm suspected associations.

Background

Transposable elements (TEs) are DNA or RNA elements that can excise and insert autonomously within host genomes. TEs are also believed to be the most likely candidates for horizontal transfer between genomes, particularly those of multicellular eukaryotes like animals [163]. Recent work in our lab (Chapter 3) demonstrated that TEs can and do horizontally transfer between animals on a large scale, and lent support to the idea that close association due to a host-parasite relationship may aid the occurrence of horizontal transfer events [69,164,165]. In particular, our work focused on TEs derived from the hosts and parasites of the *Drosophila*-wasp system, which has been used as a model for studies on immunity [35,40,41]. The previous results also showed that the TEs amplified out of wasps which infect *Drosophila melanogaster* in nature tended to be very similar in sequence to TEs from *D. melanogaster* or a closely related species. In that analysis, only one sequence per wasp and fruitfly species was used to construct the trees in the analysis. The sequence used to represent each host was the TE copy that most closely matched the canonical *D. melanogaster* TE sequence in BLAST searches, so the analysis had a very limited sample size and was biased toward the most similar TEs. In this work, we expanded on the analysis by focusing on two TEs that we were able to obtain more sequences of from the wasps. Multiple sequences of TEs from different population strains of two wasps (*L. boulardi* and *L. heterotoma*) were cloned and sequenced. TE sequences from published Drosophila species genomes that met minimum homology criteria were also gathered from FlyBase and combined with the wasp TE data.

Given that fruitfly TEs are transferring into wasp genomes, it is interesting to consider whether these TEs are able to adapt to their novel hosts and reproduce in wasps independent of fruitflies. By comparing all sequences available for these two TE families from FlyBase and from the wasp strains, we sought to determine if TEs that invaded wasp genomes had thrived and evolved into distinct lineages within their new hosts. If wasp TEs had successfully proliferated, phylogenetic analyses would be expected to show some wasp sequences clustering together, with relatively long branch lengths separating wasp TE sequences from fruitfly sequences. On the other hand, poor invasion success would result in wasp TEs distributed across the tree, always within close proximity of fruitfly TE lineages. Additionally, these data can help determine whether wasp TEs likely originated from *D. melanogaster* or from another fruitfly host. Both *L. boulardi* and *L. heterotoma* infect *D. melanogaster* and its close relatives in nature, although *L. heterotoma* is a generalist of the entire genus Drosophila. Tight clustering of wasp TE sequences with TE sequences from a particular fruitfly species would strongly implicate that fruitfly species as a TE's original host and could act as a simple assay for determining wasp host species in nature.

Methods

The two TEs used in this study were *hopper* and *mariner2.* Canonical *D. melanogaster* TE sequences, which represent the full length of an active copy of a TE, were used for construction of primers in PCR assays and for the query sequence in

BLAST searches [166]. The analysis was limited to ~600bp internal regions used for the TE PCR assays in a previous study (Chapter 3). Both TEs produced strongly amplified bands in wasp PCR assays.

hopper is a transposable element flanked by short terminally-inverted repeat structures, it transposes using a DNA intermediate, and has a canonical length of 1435 bases in *D. melanogaster* [166]. In the genome-sequenced fruitfly strain, it is known to have 11 full-length copies which presumably means the element is capable of excising and inserting itself. *hopper* is considered part of the *hobo-Activator-Tam3* (hAT) family of transposons based on sequence and coding structure [167]. While the *hAT* family has a wide range of hosts that even includes plants, previous work (Chapter 3) has shown *hopper* in *D. melanogaster* is divergent and unique enough such that it does not show high homology to sequences from species outside of the melanogaster subgroup when using BLAST searches of published genomes.

mariner2 is a transposable element flanked by short terminally-inverted repeat structures, it transposes using a DNA intermediate, and has a canonical length of 912 bases in *D. melanogaster* [166]. It has only four full-length copies in the genome-sequenced strain of the fruitfly. *mariner2* is part of the evolutionarily successful mariner/Tc1 superfamily of transposable elements, which are almost ubiquitous in insect genomes and even known to be found in vertebrates such as humans [168]. Though highly successful in many different host species, the mariner/Tc1

superfamily is composed of individual TEs that have limited host ranges—that is, mariner/Tc1 TEs from one species differ in sequence from those in another. mariner elements have been studied repeatedly in natural populations of different Drosophila species though, at one point, they were thought to be missing from *D. melanogaster* [169,170]. In previous work (Chapter 3), PCR assays confirmed *mariner2*'s presence in *D. melanogaster* and its limited range (based on BLAST searches) beyond that host species.

TEs from the wasps *L. boulardi* and *L. heterotoma* were selected because those wasps were two of the three species with the highest rates of *D. melanogaster* TE transfer, and because purified DNA extractions from different populations of each species were available for use. For *L. boulardi*, a strain from Winters, California (LB17) and a strain from Nairobi, Kenya (LBKen) were used. For *L. heterotoma*, a strain from Winters, California (LH14) and a strain from Uppsala, Sweden (LHSw) were used. The wasp strains LB17 and LH14 were used in a previously published study [41]. The parasitoid wasps were reared on the Oregon R strain of *D. melanogaster* at 25C with a 16:8 light-dark cycle.

High molecular weight genomic DNA from fruitflies and wasps was extracted using a modified phenol-chloroform extraction protocol. Approximately 20 insects per sample were ground in 1X CTAB buffer (0.7M NaCl, 0.1M Tris, 10mM EDTA, 1% CTAB, 1% β-mercaptoethanol) with 1ug/uL proteinase K, and incubated for 30 minutes at 65 C. KOAc was added to a final concentration of 1M, and the solutions

were incubated on ice for 10 minutes before cellular debris was spun down. The supernatants were then run through a standard phenol chloroform extraction with ethanol precipitation, after which the DNA pellets were re-suspended in a 7M guanidinium-hydrochloride solution with 0.5% (by weight) RNase A for 1 hour. Finally, the purified DNAs were re-precipitated and re-suspended in low EDTA 0.5X TE buffer (5mM Tris-HCl, 50uM EDTA). DNA was quantified and checked for purity using a Nanodrop spectrophotometer.

The Qiagen *taq* PCR Master Mix kit (Hilden, Germany) was used to PCR amplify TE sequences according to the following thermocycler program: initial denaturation at 94 C for 3:00, 35 cycles of 94 C denaturation for 1:00, primer annealing using the primer-specific recommended temperatures for 1:00, and 72 C extension for 1:30, with a final 72 C extension for 7:00. Primers "hopper 213f" (5'-TAATTCATAACCGTCTTCCTCTTC-3'), "hopper 799r" (5'-AGTGGGCATTTTTCTGATAGC-3'), "mariner2 179f" (5'-TTTTCAAGCGGATGTTCGGTTATT-3'), and "mariner 731r" (5'-TGGCGTTTGTCGTGACTCTTGTTA-3') were used for their respective TEs.

Gel electrophoresis was used to check for PCR products with the expected sizes, which were then cloned using the Stratagene Strataclone kit (Agilent Technologies, Santa Clara, California). Successful clones were checked using blue-white X-gal (5-bromo-4-chloro-indolyl-β-D-galactopyranoside) selection on ampicillin-LB agar plates, confirmed through PCR using the standard M13 f/r primer set, and checked

again with gel electrophoresis before being submitted to Beckman Coulter Genomics (Brea, California) for sequencing. Sequences were assembled and edited using Lasergene SeqMan and EditSeq software (DNASTAR, Madison, Wisconsin). Fruitfly sequences were taken from the published Drosophila genomes available through FlyBase BLASTn searches. Drosophila TE sequences that spanned at least 50% of the queried canonical *D. melanogaster* TE sequence and matched with moderate-to-high sequence similarity (an E-value of less than $1e^{-50}$) were added to the analysis.

The cloned and sequenced wasp TEs and fruitfly TEs from published genomes were combined and used to reconstruct phylogenetic relationships using the software package Phylip 3.69 (Joe Felsenstein, University of Washington) for both neighbor-joining and parsimony. Neighbor-joining and parsimony with bootstrap support (for 100 bootstrapped data sets) were used with default parameters for analyses on both TEs. The software package Mr. Bayes 3.2 (Fredrik Ronquist, John Huelsenbeck and Maxim Teslenko; University of California at San Diego) was used for the Bayesian analyses. A general time reversible model for a haploid DNA dataset was run for one million generations under default parameters. All trees were constructed using the drawing program FigTree 1.3 (Andrew Rambaut, University of Edinburgh).

Results and Discussion

For the TE *hopper*, a total of 79 sequences spanning 574 bases were used to construct the phylogenetic trees, including the canonical *D. melanogaster hopper*

sequence. A total of 55 *hopper* sequences from three Drosophila species (*D. melanogaster*, *D. sechellia*, and *D. simulans*) were identified by BLAST searches, and 23 *hopper* sequences were cloned and sequenced from the two wasp species. In the neighbor-joining, parsimony, and Bayesian analyses, wasp TE sequences tended to cluster as closely with or more closely with Drosophila sequences compared to other wasp sequences, and branch lengths separating fruitfly and wasp TE sequences tended to be very short (Figures 1-3). Additionally, in all three analyses, almost all wasp TE sequences had highest homology to TE sequences from *D. melanogaster*. The Bayesian analysis, in contrast to both neighbor-joining and parsimony, did produce a distinct clade composed only of TEs from non-*D. melanogaster* fruitflies. This difference in tree topologies could be due to the Bayesian models' use of a posterior probability distribution.

For the TE *mariner2*, a total of 26 sequences spanning 576 bases were used to construct the phylogenetic trees, including the canonical *D. melanogaster mariner2* sequence. A total of 18 *mariner2* sequences from four Drosophila species (*D. melanogaster*, *D. sechellia*, *D. simulans*, and *D. yakuba*) were identified by BLAST searches, and 7 *mariner2* sequences were cloned and sequenced from the two wasp species. In the neighbor-joining tree analysis, wasp TE sequences did not cluster together but were instead most closely related to TE sequences from different fruitfly species (Figure 4). The parsimony analysis yielded a tree with a substantially different topology. In it, a monophyletic group with a bootstrap value of 100 is entirely defined by wasp TE sequences (two from LH14 and one from LBKen)

(Figure 5). Finally, the Bayesian analysis yielded a tree with where wasp TE sequences clustered more closely with each other than with fruitfly TE sequences , though the overall topology was similar to the neighbor-joining analysis. It is curious that the analysis methods produced phylogenetic trees with different topologies, particularly parsimony compared to neighbor-joining and Bayesian analyses. Parsimony is generally considered superior to neighbor-joining because it searches all tree space for the topology requiring the shortest branches, whereas neighbor-joining is a greedy algorithm that is channeled into particular topologies based on an initial pairing decision. However, parsimony can suffer from long-branch attraction and other problems. In all three analyses, wasp TE sequences tended to be more closely related to TE sequences from *D. sechellia* and *D. yakuba* than to *D. melanogaster*.

Based on the short lengths of wasp TE branches and their general lack of clustering with other wasp TE sequences (both from the same population and from the same species), the analyses support the conclusion that only the most recently transferred TEs have been sequenced from the wasps and that older TEs likely have been deleted away after failing to reproduce in the wasps' genomes. If TEs had established distinct lineages in their new host species, the trees would have shown longer branch lengths between the fruitfly and wasp TEs, and the wasp TEs would have clustered together more distinctly. The alternative hypothesis that the fruitfly TEs have only begun to horizontally transfer into wasp genomes in the recent evolutionary past seems unlikely given the length and geographic breadth of their

host-parasite association [40]. Therefore, we believe TE HGT has been common but that TEs rarely adapt to the new wasp genomes in which they occasionally find themselves. However, this does not negate the possibility that the fruitfly TEs hop flanking fruitfly genomic sequences into wasp genomes, or that TEs otherwise alter the evolutionary trajectory of wasp genomes, as has been documented in mammalian systems [171,172,173].

There is one possible complicating factor in the experimental design. The primers used to amplify TEs in wasps were constructed from the canonical sequence in *D. melanogaster* [166]. This method of primer construction may have biased the experiment toward finding wasp TEs that have high sequence similarity to canonical *D. melanogaster* TEs. However, primers were located in the TE coding regions where sequence evolution may be functionally constrained. Furthermore, at least some relatively divergent wasp TE sequences were amplified using these primers, and yet they were still most closely related to other fruitfly sequences (e.g., the *mariner2* sequences LH14c3 and LBKena) (Figure 4). Thus, we find it unlikely that there are a large number of divergent wasp TE lineages that failed to amplify.

These analyses show poor evidence that fruitfly TEs in the wasps have evolved unique lineages and thrived in their new wasp hosts. TEs sequenced from wasps are almost all closely related to fruitfly TE sequences, suggesting they were recently inserted. Presumably horizontal transfer of TEs between fruitflies and wasps has been occurring for a long time, but earlier TE insertions failed to proliferate and

were deleted from wasp genomes. Together, these data support the idea that the fruitfly-parasitoid wasp interaction is highly permissive to horizontal transfer but that fruitfly TEs have not been capable of adapting to their new hosts' genomes. This host-parasite system's permissiveness to horizontal transfer ought to spur further investigation into possible mechanisms of TE transfer and the likelihood of TEs invading new host genomes, and help establish it as a model system for HGT studies.

One practical application of HGT studies developed using this model system is the determination of host species for parasitoid wasps. The wasp strains used for the experiments in Chapter 3 and 4 were reared on *D. melanogaster* but most of the wasps' preferred or common hosts in nature are not well known. Given the correlation between preference for attacking *D. melanogaster* in nature and the number of *D. melanogaster* TEs found in wasps discussed in an earlier work (Chapter 3), it seems plausible that wasps will have more TEs from hosts they commonly attack in nature compared to hosts they rarely attack. Phylogenetic analyses using TE sequences from a wasp and from any suspected hosts might reveal close sequence homology between TEs from the wasp and a particular host, which in turn would indicate TE HGT through a host-parasitoid association. For example, in the analyses of the *mariner2* TE, the wasps' TE sequences were much more closely related to the TE sequences from *D. sechellia* and *D. yakuba* than from *D. melanogaster*, which may indicate that those particular wasp populations attacked *D. sechellia* and *D. yakuba* more often in nature than *D. melanogaster* (Figure 5). With a similar approach, it may be possible to use TE sequence data to

determine the hosts of poorly characterized wasp parasitoids and would be especially beneficial for wasps to be used as biological control, where host specialization is desirable in order to prevent unintended damage to other fauna.

Figure 1

Phylogenetic tree constructed for *hopper* using a neighbor-joining algorithm

A neighbor-joining tree showed that nearly all of the *hopper* TE sequences taken from both wasp species (22 out of 23 sequences) clustered as part of a very closely-related group with TE sequences taken primarily from *D. melanogaster*. The branches more distant from this cluster are almost exclusively TEs taken from *D. sechellia* and *D. simulans*. (Scale on the bottom. Labels: *D. melanogaster* = "*Dmel*…", *D. sechellia* = "*Dsec*…", *D. simulans* = "*Dsim*…", *L. boulardi* = "*LB*…", *L. heterotoma* = "*LH*…".)

Figure 1 – *hopper*, neighbor-joining

Figure 2

Phylogenetic tree constructed for *hopper* using a parsimony algorithm with bootstrap support

A parsimony tree for *hopper* showed a few well-supported clusters of *D. melanogaster*/wasp TE sequences. (Bootstrap values are marked for their respective nodes. Labels: *D. melanogaster* = "*Dmel*…", *D. sechellia* = "*Dsec*…", *D. simulans* = "*Dsim*…", *L. boulardi* = "*LB*…", *L. heterotoma* = "*LH*…".)

Figure 2 – *hopper*, parsimony with bootstrap values

Figure 3

Phylogenetic tree constructed for *hopper* using a Bayesian algorithm

A Bayesian tree showed wasp TE sequences to be scattered among fruitfly TE sequences, although there was one clade composed solely of TEs taken from non-*D. melanogaster* fruitflies. (Scale on the bottom. Labels: *D. melanogaster* = "*Dmel*…", *D. sechellia* = "*Dsec*…", *D. simulans* = "*Dsim*…", *L. boulardi* = "*LB*…", *L. heterotoma* = "*LH*…".)

Figure 3 – *hopper*, Bayesian

Figure 4

Phylogenetic tree constructed for *mariner2* using a neighbor-joining algorithm

A neighbor-joining tree for *mariner2* showed that TE sequences taken from the wasps were more closely related to sequences taken from *D. sechellia*, *D. simulans* and *D. yakuba*. Sequences from those fruitfly species also tended to differ from *D. melanogaster* sequences. (Scale on the bottom. Labels: *D. melanogaster* = "*Dmel*…", *D. sechellia* = "*Dsec*…", *D. simulans* = "*Dsim*…", *D. yakuba* = "*Dyak*...", *L. boulardi* = "*LB*…", *L. heterotoma* = "*LH*…".)

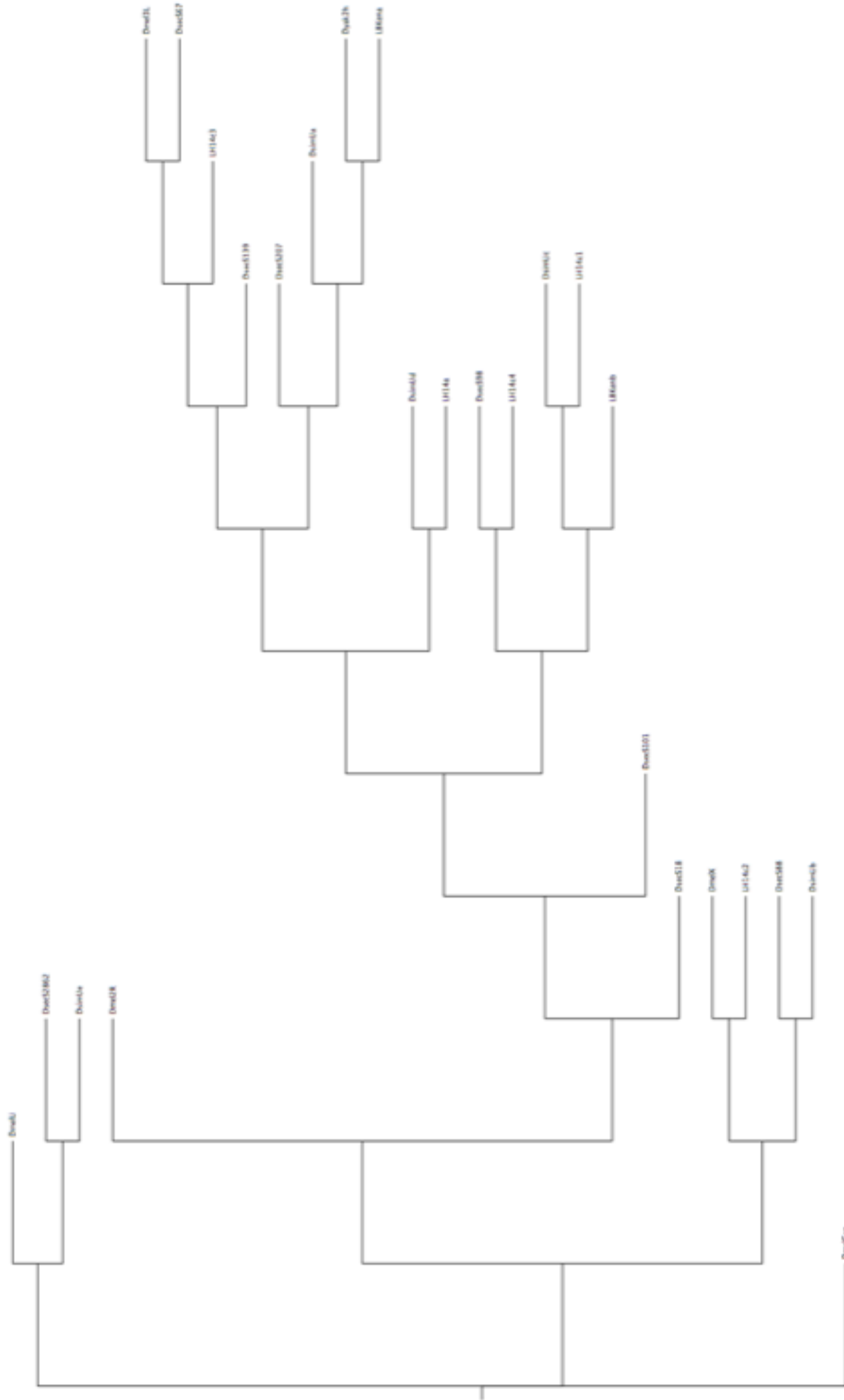


Figure 4 – *mariner2*, neighbor-joining

Figure 5

Phylogenetic tree constructed for *mariner2* using a parsimony algorithm with bootstrap support

A parsimony tree shows the left half is composed of all seven TEs taken from wasps and some sequences taken from *D. sechellia* and *D. yakuba*. The right half of the tree is composed solely of sequences taken from fruitflies. (Bootstrap values are marked for their respective nodes. Labels: *D. melanogaster* = "*Dmel*…", *D. sechellia* = "*Dsec*…", *D. simulans* = "*Dsim*…", *D. yakuba* = "*Dyak*...", *L. boulardi* = "*LB*…", *L. heterotoma* = "*LH*…".)

Figure 5 – *mariner2*, parsimony with bootstrap values

Figure 6

Phylogenetic tree constructed for *mariner2* using a Bayesian algorithm

A Bayesian tree shows that some wasp sequences cluster close together, but they still are part of a clade interspersed with TEs from fruitflies. (Scale on bottom. Labels: *D. melanogaster* = "*Dmel*…", *D. sechellia* = "*Dsec*…", *D. simulans* = "*Dsim*…", *D. yakuba* = "*Dyak*...", *L. boulardi* = "*LB*…", *L. heterotoma* = "*LH*…".)

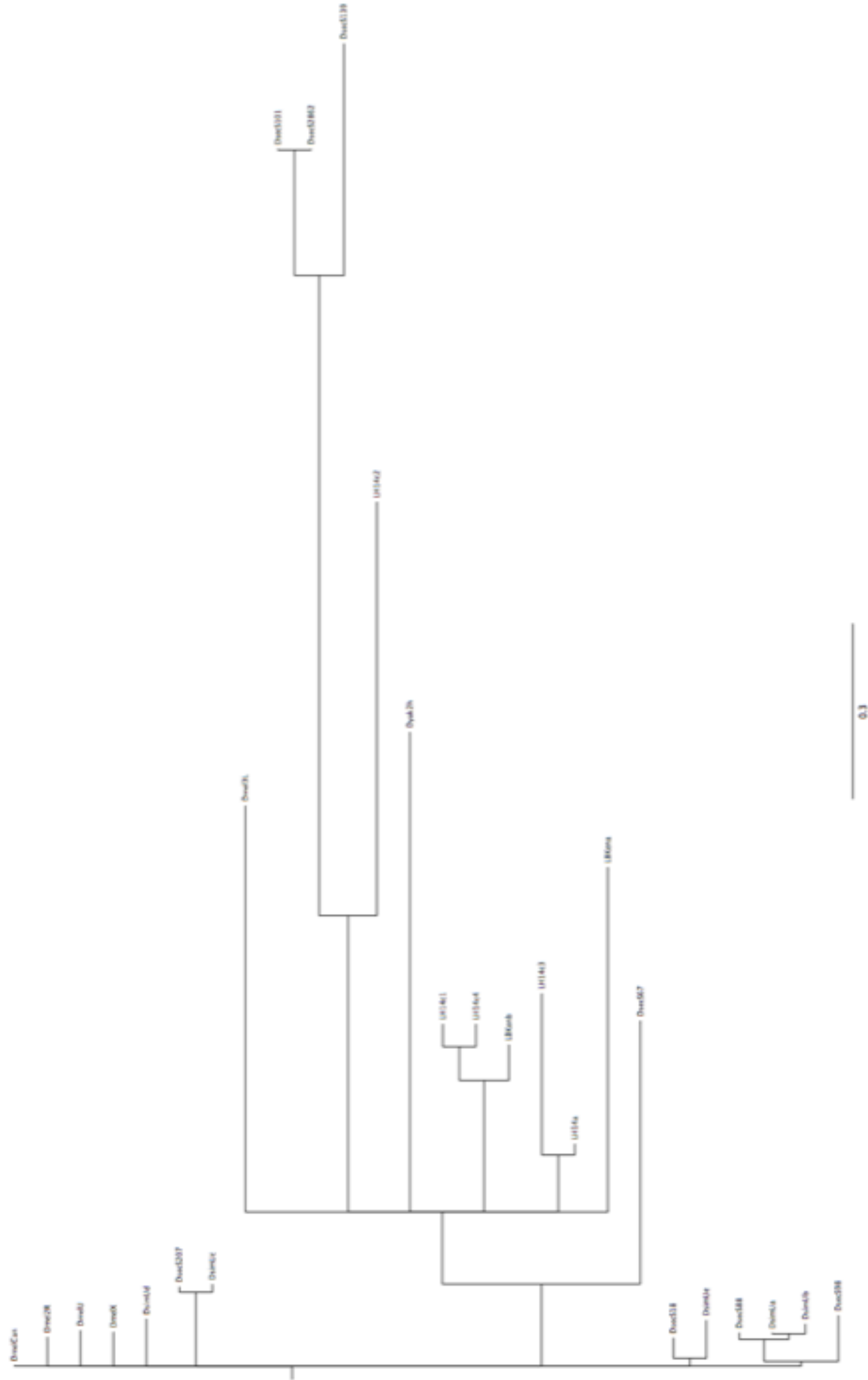


Figure 6 – *mariner2*, Bayesian

Dissertation Discussion

Through my work on ethanol in food and on horizontal transposon transfer, I showed that the *Drosophila*-wasp interaction can be affected by the environmental context in which it takes place and that the interaction itself is a more complex picture than one host and one parasite. In Chapter 2, I have shown that environmental alcohol (specifically ethanol) can dramatically alter the nature of the interaction between *D. melanogaster* and parasitoid wasps that infect them. In particular, ethanol in food substrates alters the interaction with four key effects: (1) wasps have a lower ethanol resistance level relative to *D. melanogaster*, with a specialist more resistant than a generalist; (2) wasps' attack rates are lower when larvae are feeding on substrates with ethanol; (3) feeding on ethanol in food substrates increased fruitflies' ability to kill wasps infecting them, apparently without the assistance of their immune response; and (4) fruitflies infected by wasps seek out food substrates with ethanol at higher rates than uninfected larvae. When a host can limit a pathogen's ability to infect it and also limit its success should infection occur, the dynamic of the host-parasite interaction has been altered toward the host's advantage and there is likely strong selective force to maintain that advantage. Many questions remain as to the generality of the use of alcohol as the medicine, as well as the particular mechanisms responsible for the behavioral shift towards ethanol affinity.

As expected, the specialist (*L. boulardi*) and generalist (*L. heterotoma*) wasp parasitoids of *D. melanogaster* showed different levels of resistance to the effects of ethanol, with the former being more resistant. My project was limited to two wasps of a particular genus, but there are other wasps that are specialists and generalists of *D. melanogaster* as well (see [40] for a review of *Drosophila* parasitoid wasps). Expanding the study to such wasps would allow us to draw more inferences about variation in susceptibility between specialists and generalists and, more broadly, if ethanol has similar effects across all parasitoid wasps of *D. melanogaster*. Additionally, with so little known about the natural history of a number of wasps that can survive on *D. melanogaster* as hosts, strong resistance to ethanol may even support the possibility that *D. melanogaster* and/or other fungivorous fruitflies are preferred hosts in nature.

Another aspect of the ethanol project experimental design that could be broadened is what host stage is tested, as I focused only on the fruitfly larval stage. A number of wasps parasitize the pupal stage of *D. melanogaster*. Given that ethanol levels in hemolymph drop precipitously shortly after consumption and that fruitfly pupae no longer eat, it may be likely that ethanol consumption would have little effect on the survival of wasp eggs and young larvae feeding on or in fruitfly pupae. However, the fact that ethanol in food substrates can act as a protective barrier from wasps and limit attack rates does present some interesting possibilities for larvae selecting pupation sites. *D. melanogaster* larvae, in particular, often move out of and away from food to pupate—possibly as a way to distance themselves from heterospecific

fruitfly competitors or limiting humidity during pupation [174,175,176]. It may be worth testing if larvae still exhibit this behavior when ethanol is present in the food and wasps are in close proximity. That is, will larvae choose to pupate closer to food with high levels of ethanol (or, perhaps, regions of the food likely to have high levels as fermentation continues) because the vapors will act to limit the possibility of wasp attack, even if the possibility of burial/suffocation is higher? Given that larval parasitoids search substrates where larvae feed, it seems reasonable that larvae can sense the presence of wasps [22]. What is unclear, however, is how larvae would sense the presence of pupal parasites if such wasps were located at a distance from the food substrate.

Another consideration on generalizing the effects of ethanol on parasites of *D. melanogaster* is that the levels of ethanol in the hemolymph of larvae was considerably lower than that of adults reported in the literature [177,178]. It would seem reasonable to assume that higher hemolymph ethanol levels would correlate with an even greater success against parasitoid infection. Unfortunately, there are no known wasp parasitoids that attack and infect the adult life stage of *Drosophila*. There are, however, other macroparasites—such as trypanosomes, mites, and nematodes—that do parasitize *D. melanogaster* adults and may be subject to the same immune responses (or lack thereof) that parasitoid wasps face. Microparasites, such as bacteria, can be more sensitive to their environments than larger organisms and elicit different responses from host immune systems, so investigating them may help further characterize the suite of effects that ethanol

consumption has on a host's immune response and infection outcomes. While each of these parasites interact with *Drosophila* in unique ways, any effects ethanol has on their success would certainly help in determining how general my findings are—both for *D. melanogaster* and possibly for other organisms capable of high alcohol consumption, such as humans.

The choice of using 6% ethanol in food for the majority of the study was determined based on pilot data and the resistance experiments, but it was in many respects serendipitous that this ethanol level showed as many effects as it did. Likewise, the timeframes we chose for the various experiments were based on limited data from related studies and were chosen mainly for experimental/procedural practicality and feasibility, so the fact that I detected strong effects was fortuitous. This "first pass" at understanding *Drosophila* use of ethanol during infection begs for follow-up experiments that systemically and thoroughly test the effects of other ethanol levels over more timeframes, though ideally kept within what is known from natural surveys [179]. In natural environments, such as within a rotting fruit, ethanol levels are known to vary spatially (i.e., some areas have more ethanol and more active fermentation) and temporally (i.e., ethanol evaporates rather quickly and at different rates depending on the substrate) as well.

In an effort to control variance and properly test specific experimental questions, I tried to exclude the possibility of spatial and temporal variation in ethanol content as much as possible in my experimental designs. To adequately account for how

ethanol is present in many of the natural substrates *D. melanogaster* feeds on, it may be appropriate to deliberately design tests that introduce this spatial and temporal variability of ethanol content. For example, given that high levels of ethanol are toxic but that higher hemolymph ethanol levels can mean greater success against wasp infection, it would be interesting to see how larvae find a balance in this trade-off when the food substrate (or petri dish) holds a continuum of ethanol concentrations. Would larvae remain in the low ethanol patch and consume more? Or would they periodically venture into the high ethanol patch, quickly consume food, and leave before succumbing to its effects themselves? Would larvae slowly migrate to patches of increasing ethanol levels as the toxin evaporated over time? These are exciting questions to explore and could lend more insight into the behavioral aspects of self-medication and ecological immunity in *Drosophila*.

A key finding in my study was that larvae appeared to be self-medicating in response to wasp infection: when given a choice between food containing no ethanol versus food with 6% ethanol, larvae infected by either wasp species migrated toward and stayed in ethanol food at higher frequencies than uninfected larvae. Although *D. melanogaster* has an affinity for alcohol in general, this dramatic preference for food containing ethanol shows that the fruitfly has evolved the use of ethanol as part of its defense strategy. As mentioned in Chapter 2's discussion, the strategy of consuming a substance for its curative effect ("self-medication") can be seen in other animals and, indeed, there is evidence that *Drosophila* species have evolved to utilize food resources based on how well it helps combat infection [48].

But there had not been any previously published studies on the use of ethanol or other alcohols for self-medication, whether by fruitflies or other animals. While there are numerous human studies on the potential health benefits of moderate alcoholic beverage consumption, such work has limited relevance for two major reasons: first, the health benefits are usually long-term and directed at cardiovascular disease prevention and, second, the effect is obtained through consumption of very small amounts of alcohol over time (see [180,181] for short reviews of alcohol consumption studies). This is in contrast to the fruitflies' consumption of high amounts of ethanol (with tangible risk of death, as seen in the resistance trials) in quick response to infection, and with almost immediate curative effects. Thus, it may be worthwhile to test for potential curative properties of short bursts of ethanol intake in other animals, including humans.

One potentially interesting piece of evidence that ethanol consumption has evolved as part of *D. melanogaster*'s defense strategy (at least against parasitoid wasps) is that infected larvae feeding on ethanol had almost no lamellocytes, which are the main hemocyte responsible for the wasp encapsulation response. Yet the fruitflies that consumed ethanol managed to have significantly higher success rates killing wasp eggs and larvae, compared to fruitflies that fed on plain food and managed to mobilize lamellocytes. During cell counts of larvae hemolymph, there were no signs of hemocyte debris or irregular cell morphologies that would have suggested the death of lamellocytes by a toxin such as alcohol. The other possible explanation for the lack of lamellocytes is that larvae simply did not mobilize or differentiate those

cells. The mechanistic basis for how dietary ethanol could have prevented lamellocyte mobilization or differentiation in the larvae is unclear. Lamellocytes are regulated by the Toll and JAK/STAT pathways, but these pathways do not show any clear crossover with known alcohol detoxification pathways and responses [31,33,34]. One possible mechanism is that alcohol exposure triggers the downregulation of signal transduction genes that may be associated with general defense responses [182]. From a metabolic perspective, it would be beneficial to omit a costly immune response that may or may not be successful when there is another less costly means of adequately defending against the infection. It would be interesting to see if this same phenomenon of no lamellocytes after ethanol consumption occurs in response to infection by other parasites that fruitflies also attempt to encapsulate. Entomopathogenic nematodes, for example, would be especially useful parasites to test because of their ability to actively prevent and break through encapsulations [183].

In a series of pilot experiments, I sought to determine if other toxins that fruitflies are resistant to also conferred advantages over the same two species of parasitoid wasps. Some experiments showed promise and others failed to show clear results. Overall, they likely deserve some follow up with revised designs to limit the variance between replicates, which was a serious problem. The key finding from the pilots was that the toxins had protective effects, but there was also variation in the mechanism behind the protection. A short summary of those pilot experiments follows.

Octanoic acid, a fatty acid found at high concentrations in the fruit of *Morinda citrifolia* [184], was tested using the fruitfly *D. sechellia* and the same two wasps. *D. sechellia*, like the fruit, is native to the Seychelles and not only preferentially feeds on *M. citrifolia* fruit, but also shows a remarkable resistance to octanoic acid—a toxin that paralyzes and kills other insects that come in close proximity to it [50,51]. Consumption of octanoic acid by the fruitfly host seemed to limit wasp attack rates but did not limit successful development of the wasp within its host. The effect on attack rate was not surprising given the high vapor toxicity of octanoic acid. The lack of toxicity within fruitfly hosts may simply be a function of *D. sechellia*'s ability to efficiently metabolize octanoic acid (an energy rich fatty acid). Alpha-amanitin is a protein found in *Amanita phalloides* and other poisonous mushrooms. The toxicity is caused by its high affinity for binding to the crucial and ubiquitous enzyme RNA polymerase II and interfering with key cell functions [53]. Due to problems rearing *Drosophila* species that naturally use fungi as oviposition sites, I used a resistant *D. melanogaster* strain in pilot experiments. These pilots seemed to indicate that wasp attack rate was not affected but that wasp success within the host was negatively affected, though I had not determined a possible mechanism.

I also conducted a small series of pilots using the insecticides DDT (dichlorodiphenyltrichloroethane) and malathion, two *D. melanogaster* strains bred for resistance to them, and the wasps *L. boulardi* and *L. heterotoma*. The primary research question was whether or not man-made toxins like insecticides, which *D.*

melanogaster had rapidly developed resistance to in nature [53,185,186], would affect the interaction with wasps. The attack rate assays did not yield a clear effect of insecticides—the only instances in which wasps failed to attack larvae were when the insecticide concentration in the food was high enough to harm the larvae as well. However, there was an effect of insecticides on developing parasites once the fruitflies were infected. Wasp eggs in fruitfly larvae feeding on insecticide-supplemented food usually arrested at an early stage. What this finding suggests is that *D. melanogaster*'s rapid evolution of insecticide resistance (DDT and malathion were introduced in the middle of the 20th century) has given it an advantage over the parasitoid wasps. This advantage may be temporary if wasps also can rapidly evolve insecticide resistance, which coincidentally has been facilitated by insertions of transposable elements [72,186].

In Chapters 3 and 4, I focused on a different way in which environmental context could affect fruitfly-wasp interactions. Whereas ethanol is an abiotic part of the environment, I now shift to the biotic components of the environment, particularly on microparasites that may be transferred between fruitflies and their parasitoids. Microparasites can have the potential to change the nature of the interaction. Transposable elements, perhaps more than other microparasites, may alter the evolution of a host-parasite interaction and do so on a relatively rapid time scale. For example, TEs may alter the evolutionary potential of a parasitoid wasp by providing it the raw genetic material of fruitfly genes through imprecisely excising and carrying fragments from flanking genes [72]. On the other hand, TEs could alter

the evolutionary potential of a parasitoid wasp by proliferating in the parasitoid's genome and increasing the danger of deleterious mutations (insertions) in coding regions [70,159,187]. As a first step toward understanding such complex interactions, my goals were to determine whether horizontal transfer between fruitflies and wasps occurs, and to determine the frequency at which it occurs. My experiments on possible horizontal transfer of transposable elements (TEs) within the *Drosophila*-wasp system found three key results: (1) TEs from fruitflies are present in high numbers in wasps, with those that attack *D. melanogaster* in nature containing the most TEs; (2) RNA-based transposons were just as likely as DNA-based transposons to be found in wasps, contrary to some theories; and (3) transposons found in wasps appear to be the result of common, recent, and short-lived transfer events.

Based on reviews of studies on horizontal transfer of transposable elements and the excise-insertion mechanisms of known TEs, the initial working hypothesis was that, if any TE transfer was detected in the wasps, it would be from the Class II (DNA) transposons. This is because DNA transposons use a more stable DNA-based intermediate when hopping and they required fewer host factors to successfully insert [163]. So, it was rather surprising that there was no significant difference in the rates at which Class I (RNA) and Class II transposons were successfully found in wasps. Rather than proposing novel ideas on how RNA-based transposons can be more stable outside of cells than previously thought, a simpler possibility is that a host-parasite system (especially when it involves an endoparasitoid) is more

permissive to nucleic acid transfer between organisms. Part of the permissiveness may be due to proximity. When a wasp egg is laid within its host, a cellular immune response consisting of thousands of cells attempts to defeat the wasp invader by forming a capsule around it, with cells near the egg lysing to release highly toxic agents. Given this set of events that occurs with every wasp infection, it seems possible that fruitfly cell lysis is exposing a developing wasp embryo to large amounts of nucleic acids (and TEs) that, in a process analogous to transformation in bacteria, can readily cross into the embryo and invade a new host genome.

While the success rate of such a horizontal transfer event is likely incredibly low, the number of cells lysing as part of every encapsulation attempt, the amount of nucleic acids being released as part of that lysis, and the fact that wasp embryonic germ cells are completely surrounded by foreign tissue all may increase the likelihood of horizontal transfer. Additionally, the high burden of wasp parasitism in nature (some estimates go as high as 90% in some sites [1]) and the high population sizes coupled with short generation times of these insects suggests abundant contact—and ample opportunity for horizontal transfer—between fruitflies and parasitoid wasps over evolutionary time scales. Supporting this idea is the fact that parasitoid wasps known to infect *D. melanogaster* in nature were also the same species with the highest positive hits for *D. melanogaster* TEs. Thus, more than anything else, the high rates of TE transfers from both classes seem to indicate that the *Drosophila*-parasitoid wasp interaction is particularly amenable to horizontal transposon transfer. Although not as simple as direct transfer of TEs between fruitflies and

wasps, viruses, bacteria, and even mites have been implicated as vectors for horizontal transfer of TEs in other systems and all three are present within the *Drosophila*-parasitoid wasp system[188]. However, it is quite possible that a vector is not needed in this particular system because of the biology of wasp infection and the evidence that transfer always occurs in the direction of fruitflies to wasps.

A number of experiments may act as proof-of-concept tests for the idea that TEs can directly transfer from fruitfly cells to wasp embryos. First, it is relatively easy to dissect and isolate encapsulated wasp eggs. It might be possible to open the encapsulation and try to isolate any nucleic acids residing in the space between the capsule and the wasp egg. Then, primers for TEs could be used for PCR assays of this isolate, confirming the presence or absence of TEs near the wasp egg. Second, since fertilized wasp eggs can be grown in a culture of *Drosophila* cells until at least hatching into larvae, it may be possible to create tagged TEs sequences and inoculate them into a culture containing a wasp egg. Some time after exposure, the wasp embryo or larva can be assayed for the presence of the tagged sequence. If positive, the results would demonstrate the possibility that wasp eggs can be transformed from nucleic acids in close proximity to them. Third, one can attempt to induce a horizontal transfer episode directly in live fruitflies and wasps. I attempted such a project as a pilot, where I first created a *D. melanogaster* strain that combined a GFP-tagged *p* transposable element regulated by either histone, ubiquitin, or actin promoters with a non-mobile source of transposase. These three strains all had *p*-elements actively hopping within their cells and this could be seen when fruitflies

were examined under fluorescence. I then attacked the larvae from these strains with *LB17* and *LH14* wasps. I assumed that using fruitfly strains with constitutively active TEs would increase the likelihood of horizontal transfer to a detectable level. However, after examining thousands of fruitflies under fluorescent light and PCR assaying them for GFP sequence, I did not detect any clear sign of a successful GFP *p*-element transfer event. It is likely that TE horizontal transfers are so rare that my experimental set-up did not have high enough numbers of actively moving tagged TEs or high enough numbers of wasp infections to guarantee observing even one instance of horizontal transfer.

The data from Chapter 3 demonstrated that TEs had transferred from fruitflies to their wasp parasitoids, but it was unclear whether these TE could survive and thrive in their new hosts as well as what the ramifications of the transfers would be. To address these questions, I collected more sequence data from particular TEs that had invaded the wasps and conducted the phylogenetic analyses in Chapter 4. The limited sequencing of many fruitfly TEs from wasps (Chapter 3), plus the expanded sequencing of two TEs (Chapter 4), seems to indicate that transfers frequently occur between *Drosophila* and parasitoid wasps, but that once inserted into wasp genomes fruitfly TEs do not last long over evolutionary time. This hypothesis is based on the fact that numerous wasp versions of fruitfly TEs were cloned from particular wasp strains, but they generally had high sequence identity to TEs from the *D. melanogaster* genome and did not frequently phylogenetically cluster with each other. Lack of clustering among the wasp TEs suggests they are purged quickly

before they can evolve and reproduce independently in wasp genomes. Altogether, the evidence supports a model of recurrent TE invasion with only the most recent round of invaders detected in sequencing.

The repercussions of horizontal transfer in this particular system are still difficult to infer. Inverse PCR could provide the sequences of regions flanking the TEs and, for example, help determine whether or not a TE inserted into heterochromatin or within a gene. The latter can lead to negative consequences for a host and such an insertion would likely be selected out very rapidly. Another potential result from inverse PCR is sequence data showing TE imprecise excision. Imprecise excision occurs when TE machinery does not correctly recognize the boundaries of a TE sequence and instead excises the TE with some of the sequence flanking it. The TE-induced movement of TE flanking sequences can result in adaptive changes in host genomes [72,186], so the possibility that imprecisely excised TEs can hop fruitfly genomic sequence into wasp genomes opens interesting avenues for future research. For example, such transfer might allow wasps access to fruitfly-specific antigens with which they could coat their eggs to avoid the host immune response. Given the relative accessibility of genome sequencing technology today and the possibility that a large number of fruitfly TEs have inserted in the wasps' genomes, it likely would be much more cost-effective to sequence the wasps' genomes rather than rely on inverse PCR. Whole genome sequencing also likely would be able to provide TE insertion site data, sequences of TE repeat regions and any flanking sequence, and some estimation of copy number in hosts.

The *Drosophila*-wasp system has been a particularly useful model for studying the genetic basis for innate immunity, but my dissertation has shown that its uses may be expanded into other inquiries. My results add further evidence that the environment can have important effects on host-pathogen interactions. In Chapter 2, my experiments explored the effects of abiotic factors in the environment, specifically the ethanol produced in the food substrates that fruitflies consume. The effects of ethanol in this system showed that there is tremendous potential for it as a model of ecological and behavioral immunity, neither of which has been deeply studied in a model system such as *Drosophila*. In Chapters 3 and 4, my experiments explored the effects of biotic factors, namely TEs that have invaded wasp genomes from their fruitfly hosts. The data showed that TEs jumped into the wasps' genomes repeatedly. With many questions still remaining from my work, this interaction may be a model for horizontal transfer, which is lacking among animal systems.

Bibliography

1. Fleury F, Ris N, Allemand R, Fouillet P, Carton Y, et al. (2004) Ecological and genetic interactions in Drosophila-parasitoids communities: a case study with D-melanogaster, D-simulans and their common Leptopilina parasitoids in south-eastern France. Genetica 120: 181-194.
2. Irvine RJ, Corbishley H, Pilkington JG, Albon SD (2006) Low-level parasitic worm burdens may reduce body condition in free-ranging red deer (Cervus elaphus). Parasitology 133: 465-475.
3. Jaenike J (1998) On the capacity of macroparasites to control insect populations. American Naturalist 151: 84-96.
4. Tompkins DM, Dunn AM, Smith MJ, Telfer S (2011) Wildlife diseases: from individuals to ecosystems. Journal of Animal Ecology 80: 19-38.
5. CDC (1999) Achievements in Public Health, 1900-1999: Control of Infectious Diseases. Washington, DC: Centers for Disease Control and Prevention. 621-629 p.
6. Carton Y, Nappi AJ, Poirie M (2005) Genetics of anti-parasite resistance in invertebrates. Developmental and Comparative Immunology 29: 9-32.
7. Ebert D (2008) Host-parasite coevolution: Insights from the Daphnia-parasite model system. Current Opinion in Microbiology 11: 290-301.
8. Bermudes D, Joiner KA (1993) THE ROLE OF PARASITES IN GENERATING EVOLUTIONARY NOVELTY. Parasitology Today 9: 458-463.
9. Van Valen L (1973) A New Evolutionary Law. Evolutionary Theory 1: 1-30.

10. Poulin R (2011) The Many Roads to Parasitism: A Tale of Convergence. In: Rollinson D, Hay SI, editors. Advances in Parasitology, Vol 74. San Diego: Elsevier Academic Press Inc. pp. 1-40.

11. Damian RT (1997) Parasite immune evasion and exploitation: reflections and projections. Parasitology 115: S169-S175.

12. Schmid-Hempel P (2008) Parasite immune evasion: a momentous molecular war. Trends in Ecology & Evolution 23: 318-326.

13. Hoerauf A, Satoguina J, Saeftel M, Specht S (2005) Immunomodulation by filarial nematodes. Parasite Immunology 27: 417-429.

14. Schmid-Hempel P (2009) Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. Philosophical Transactions of the Royal Society B-Biological Sciences 364: 85-98.

15. Day T (2003) Virulence evolution and the timing of disease life-history events. Trends in Ecology & Evolution 18: 113-118.

16. Frank SA (1996) Models of parasite virulence. Quarterly Review of Biology 71: 37-78.

17. Frank SA, Schmid-Hempel P (2008) Mechanisms of pathogenesis and the evolution of parasite virulence. Journal of Evolutionary Biology 21: 396-404.

18. Cooper EL (2010) Evolution of immune systems from self/not self to danger to artificial immune systems (AIS). Physics of Life Reviews 7: 55-78.

19. Iriti M, Faoro F (2007) Review of innate and specific immunity in plants and animals. Mycopathologia 164: 57-64.

20. Menezes H, Jared C (2002) Immunity in plants and animals: common ends through different means using similar tools. Comparative Biochemistry and Physiology C-Toxicology & Pharmacology 132: 1-7.

21. Moltz H (1993) Fever - Causes and Consequences. Neuroscience and Biobehavioral Reviews 17: 237-269.

22. Lefèvre T, de Roode JC, Kacsoh BZ, Schlenke TA (2011) Defence strategies against a parasitoid wasp in Drosophila: fight or flight? Biology Letters.

23. Medzhitov R, Janeway CA (1997) Innate immunity: Impact on the adaptive immune response. Current Opinion in Immunology 9: 4-9.

24. Cooper EL, Kauschke E, Cossarizza A (2002) Digging for innate immunity since Darwin and Metchnikoff. Bioessays 24: 319-333.

25. Zhang BJ, Duan ZY, Zhao Y (2009) Mouse models with human immunity and their application in biomedical research. Journal of Cellular and Molecular Medicine 13: 1043-1058.

26. Imler JL, Zheng LB (2004) Biology of Toll receptors: lessons from insects and mammals. Journal of Leukocyte Biology 75: 18-26.

27. Lemaitre B, Hoffmann J (2007) The host defense of Drosophila melanogaster. Annual Review of Immunology. Palo Alto: Annual Reviews. pp. 697-743.

28. Martinelli C, Reichhart JM (2005) Evolution and integration of innate immune systems from fruit flies to man: lessons and questions. Journal of Endotoxin Research 11: 243-248.

29. Boutros M, Agaisse H, Perrimon N (2002) Sequential activation of signaling pathways during innate immune responses in Drosophila. Developmental Cell 3: 711-722.

30. Tanji T, Hu XD, Weber ANR, Ip YT (2007) Toll and IMD pathways synergistically activate an innate immune response in Drosophila melanogaster. Molecular and Cellular Biology 27: 4578-4588.

31. Williams MJ (2007) Drosophila hemopoiesis and cellular immunity. Journal of Immunology 178: 4711-4716.

32. Honti V, Csordas G, Markus R, Kurucz E, Jankovics F, et al. (2010) Cell lineage tracing reveals the plasticity of the hemocyte lineages and of the hematopoietic compartments in Drosophila melanogaster. Molecular Immunology 47: 1997-2004.

33. Matova N, Anderson KV (2006) Rel/NF-kappa B double mutants reveal that cellular immunity is central to Drosophila host defense. Proceedings of the National Academy of Sciences of the United States of America 103: 16424-16429.

34. Sorrentino RP, Melk JP, Govind S (2004) Genetic analysis of contributions of dorsal group and JAK-Stat92E pathway genes to larval hemocyte concentration and the egg encapsulation response in drosophila. Genetics 166: 1343-1356.

35. Carton Y, Poirie M, Nappi AJ (2008) Insect immune resistance to parasitoids. Insect Science 15: 67-87.

36. Lavine MD, Strand MR (2002) Insect hemocytes and their role in immunity. Insect Biochemistry and Molecular Biology 32: 1295-1309.

37. Strand MR (2008) The insect cellular immune response. Insect Science 15: 1-14.

38. Henneman ML, Memmott J (2001) Infiltration of a Hawaiian community by introduced biological control agents. Science 293: 1314-1316.

39. Ueno T (1998) Selective host-feeding on parasitized hosts by the parasitoid Itoplectis naranyae (Hymenoptera : Ichneumonidae) and its implication for biological control. Bulletin of Entomological Research 88: 461-466.

40. Carton Y, Bouletreau M, van Alphen JJM, van Lenteren JC (1986) The Drosophila parasitic wasps. In: Ashburner M, Carson L, Thompson JN, editors. The Genetics and Biology of Drosophila. London: Academic Press. pp. 347-394.

41. Schlenke TA, Morales J, Govind S, Clark AG (2007) Contrasting infection strategies in Generalist and specialist wasp parasitoids of Drosophila melanogaster. Plos Pathogens 3: 1486-1501.

42. Colinet D, Dubuffet A, Cazes D, Moreau S, Drezen JM, et al. (2009) A serpin from the parasitoid wasp Leptopilina boulardi targets the Drosophila phenoloxidase cascade. Developmental and Comparative Immunology 33: 681-689.

43. Labrosse C, Staslak K, Lesobre J, Grangeia A, Huguet E, et al. (2005) A RhoGAP protein as a main immune suppressive factor in the Leptopilina boulardi (Hymenoptera, Figitidae) - Drosophila melanogaster interaction. Insect Biochemistry and Molecular Biology 35: 93-103.

44. Lee MJ, Kalamarz ME, Paddibhatla I, Small C, Rajwani R, et al. (2009) Virulence Factors and Strategies of Leptopilina spp.: Selective Responses in Drosophila Hosts. In: Prevost G, editor. Advances in Parasitology, Vol 70: Parasitoids of Drosophila. San Diego: Elsevier Academic Press Inc. pp. 123-+.

45. Delpuech JM, Frey E, Carton Y (1994) Genetic and Epigenetic Variation in Suitability of a Drosophila Host to 3 Parasitoid Species. Canadian Journal of Zoology-Revue Canadienne De Zoologie 72: 1940-1944.

46. Mayntz D, Toft S (2001) Nutrient composition of the prey's diet affects growth and survivorship of a generalist predator. Oecologia 127: 207-213.

47. Bernays E, Graham M (1988) On the Evolution of Host Specificity in Phytophagous Arthropods. Ecology 69: 886-892.

48. Jaenike J (1985) Parasite Pressure and the Evolution of Amanitin Tolerance in Drosophila. Evolution 39: 1295-1301.

49. Jaenike J, Grimaldi D (1983) Genetic-Variation for Host Preference Within and Among Populations of Drosophila-Tripunctata. Evolution 37: 1023-1033.

50. Jones CD (2005) The genetics of adaptation in Drosophila sechellia. Genetica 123: 137-145.

51. Matsuo T, Sugaya S, Yasukawa J, Aigaki T, Fuyama Y (2007) Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in Drosophila sechellia. Plos Biology 5: 985-996.

52. Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-Plant Biology. Insect-Plant Biology. 2nd ed. ed. New York: Oxford University Press Inc. pp. 293-295.

53. Wilson TG (2001) Resistance of Drosophila to toxins. Annual Review of Entomology 46: 545-571.

54. Begun DJ, Whitley P (2000) Genetics of alpha-amanitin resistance in a natural population of Drosophila melanogaster. Heredity 85: 184-190.

55. Geer BW, Heinstra PWH, McKechnie SW (1993) The Biological Basis of Ethanol Tolerance in Drosophila. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 105: 203-229.

56. Starmer WT (1981) A Comparison of Drosophila Habitats According to the Physiological Attributes of the Associated Yeast Communities. Evolution 35: 38-52.

57. David JR, Vanherrewege J (1983) Adaptation to Alcoholic Fermentation in Drosophila Species - Relationship Between Alcohol Tolerance and Larval Habitat. Comparative Biochemistry and Physiology a-Physiology 74: 283-288.

58. McKenzie JA, Parsons PA (1972) Alcohol Tolerance - Ecological Parameter in Relative Success of Drosophila-Melanogaster and Drosophila-Simulans. Oecologia 10: 373-&.

59. Mercot H, Defaye D, Capy P, Pla E, David JR (1994) Alcohol tolerance, ADH activity, and ecological niche of Drosophila species. Evolution 48: 746-757.

60. Bouletreau M, David JR (1981) Sexually dimorphic response to host habitat toxicity in Drosophila parasitic wasps. Evolution 35: 395-399.

61. Boulanger N, Ehret-Sabatier L, Brun R, Zachary D, Bulet P, et al. (2001) Immune response of Drosophila melanogaster to infection with the flagellate parasite Crithidia spp. Insect Biochemistry and Molecular Biology 31: 129-137.

62. Corby-Harris V, Pontaroli AC, Shimkets LJ, Bennetzen JL, Habel KE, et al. (2007) Geographical distribution and diversity of bacteria associated with natural populations of Drosophila melanogaster. Applied and Environmental Microbiology 73: 3470-3479.

63. Ebbert MA, Marlowe JL, Burkholder JJ (2003) Protozoan and intracellular fungal gut endosymbionts in Drosophila: prevalence and fitness effects of single and dual infections. Journal of Invertebrate Pathology 83: 37-45.

64. Huszart T, Imler JL (2008) Drosophila Viruses and the Study of Antiviral Host-Defense. In: Maramorosch K, Shatkin A, Murphy F, editors. Advances in Virus Research, Vol 72. San Diego: Elsevier Academic Press Inc. pp. 227-265.

65. Perlman SJ, Jaenike J (2003) Evolution of multiple components of virulence in Drosophila-nematode associations. Evolution 57: 1543-1551.

66. Trienens M, Keller NP, Rohlfs M (2010) Fruit, flies and filamentous fungi - experimental analysis of animal-microbe competition using Drosophila melanogaster and Aspergillus mould as a model system. Oikos 119: 1765-1775.

67. Futerman PH, Layen SJ, Kotzen ML, Franzen C, Kraaijeveld AR, et al. (2006) Fitness effects and transmission routes of a microsporidian parasite infecting Drosophila and its parasitoids. Parasitology 132: 479-492.

68. Vavre F, Fleury F, Lepetit D, Fouillet P, Bouletreau M (1999) Phylogenetic evidence for horizontal transmission of Wolbachia in host-parasitoid associations. Molecular Biology and Evolution 16: 1711-1723.

69. Yoshiyama M, Tu Z, Kainoh Y, Honda H, Shono T, et al. (2001) Possible horizontal transfer of a transposable element from host to parasitoid. Molecular Biology and Evolution 18: 1952-1958.

70. Casola C, Lawing AM, Betran E, Feschotte C (2007) PIF-like Transposons are common in Drosophila and have been repeatedly domesticated to generate new host genes. Molecular Biology and Evolution 24: 1872-1888.

71. Moran JV, DeBerardinis RJ, Kazazian HH (1999) Exon shuffling by L1 retrotransposition. Science 283: 1530-1534.

72. Schlenke TA, Begun DJ (2004) Strong selective sweep associated with a transposon insertion in Drosophila simulans. Proceedings of the National Academy of Sciences of the United States of America 101: 1626-1631.

73. Berenbaum MR (1995) The chemistry of defense: Theory and practice. Proc Natl Acad Sci USA 92: 2-8.

74. Fraenkel GS (1959) The raison d'etre of secondary plant substances. Science 129: 1466-1470.

75. Price PW, Bouton CE, Gross P, McPheron BA, Thompson JN, et al. (1980) Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. Ann Rev Ecol Syst 11: 41-65.

76. Vining LC (1990) Functions of secondary metabolites. Ann Rev Microbiol 44: 395-427.

77. Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. Ann Rev Entomol 47: 57-92.

78. Ode PJ (2006) Plant chemistry and natural enemy fitness: Effects on herbivore and natural enemy interactions. Ann Rev Entomol 51: 163-185.

79. Rothschild M (1973) Secondary plant substances and warning colouration in insects. In: van Emden HF, editor. Insect/Plant Interactions. Oxford: Blackwell. pp. 59-83.

80. Brower LP, Brower JV, Corvino JM (1967) Plant poisons in a terrestrial food chain. Proc Natl Acad Sci USA 57: 893-898.

81. de Roode JC, Pedersen AB, Hunter MD, Altizer S (2008) Host plant species affects virulence in monarch butterfly parasites. J Anim Ecol 77: 120-126.

82. Clayton DH, Wolfe ND (1993) The adaptive significance of self-medication. Trends Ecol Evol 8: 60-63.

83. Huffman MA, Caton JM (2001) Self-induced increase of gut motility and the control of parasitic infections in wild chimpanzees. Internl J Primatol 22: 329-346.

84. Villalba JJ, Provenza FD, Shaw R (2006) Sheep self-medicate when challenged with illness-inducing foods. Anim Behav 71: 1131-1139.

85. Lefevre T, Oliver L, Hunter MD, de Roode JC (2010) Evidence for trans-generational medication in nature. Ecol Lett 13: 1485-1493.

86. Povey S, Cotter SC, Simpson SJ, Lee KP, Wilson K (2008) Can the protein costs of bacterial resistance be offset by altered feeding behaviour? J Anim Ecol 78: 1-10.

87. Singer MS, Mace KC, Bernays EA (2009) Self-medication as adaptive plasticity: Increased ingestion of plant toxins by parasitized caterpillars. PLoS One 4: e4796.

88. David JR, Vanherrewege J (1983) Adaptation to alcoholic fermentation in Drosophila species: Relationship between alcohol tolerance and larval habitat. Comp Biochem Physiol A Physiol 74: 283-288.

89. Gibson JB, May TW, Wilks AV (1981) Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* in relation to environmental variation: Ethanol levels in breeding sites and allozyme frequencies. Oecologia 51: 191-198.

90. McKechnie SW, Morgan P (1982) Alcohol dehydrogenase polymorphism of *Drosophila melanogaster*: Aspects of alcohol and temperature variation in the larval environment. Austral J Biol Sci 35: 85-93.

91. Fry JD, Saweikis M (2006) Aldehyde dehydrogenase is essential for both adult and larval ethanol resistance in Drosophila melanogaster. Genet Res 87: 87-92.

92. Geer BW, Heinstra PWH, McKechnie SW (1993) The biological basis of ethanol tolerance in Drosophila. Comp Biochem Physiol B Biochem Mol Biol 105: 203-229.

93. Johnson FM, Denniston C (1964) Genetic variation of alcohol dehydrogenase in *Drosophila melanogaster*. Nature 204: 906-907.

94. Chawla SS, Perron JM, Radoucothomas C (1981) Effects of ingested ethanol on adult *Drosophila melanogaster* (Diptera: Drosophilidae). Can Entomol 113: 315-323.

95. Geer BW, Langevin ML, McKechnie SW (1985) Dietary ethanol and lipid synthesis in *Drosophila melanogaster*. Biochem Genet 23: 607-622.

96. Parsons PA, Stanley SM, Spence GE (1979) Environmental ethanol at low concentrations: Longevity and development in the sibling species *Drosophila melanogaster* and *D. simulans*. Austral J Zool 27: 747-754.

97. McKechnie SW, Geer BW (1984) Regulation of alcohol dehydrogenase in *Drosophila melanogaster* by dietary alcohol and carbohydrate. Insect Biochem 14: 231-242.

98. McKenzie JA, Parsons PA (1972) Alcohol tolerance: An ecological parameter in the relative success of *Drosophila melanogaster* and *Drosophila simulans*. Oecologia 10: 373-388.

99. McKenzie JA, McKechnie SW (1979) A comparative study of resource utilization in natural populations of *Drosophila melanogaster* and *D. simulans*. Oecologia 40: 299-309.

100. Parsons PA (1980) Larval responses to environmental ethanol in *Drosophila melanogaster*: Variation within and among populations. Behav Genet 10: 183-190.

101. Richmond RC, Gerking JL (1979) Oviposition site preference in Drosophila. Behav Genet 9: 233-241.

102. Driessen G, Hemerik L, van Alphen JJM (1990) Drosophila species breeding in the stinkhorn (*Phallus impudicus* Pers.) and their larval parasitoids. Neth J Zool 40: 409-427.

103. Fleury F, Ris N, Allemand R, Fouillet P, Carton Y, et al. (2004) Ecological and genetic interactions in Drosophila-parasitoids communities: a case study with *D. melanogaster*, *D. simulans* and their common Leptopilina parasitoids in south-eastern France. Genetica 120: 181-194.

104. Janssen A, Driessen G, de Haan M, Roodbol N (1988) The impact of parasitoids on natural populations of temperate woodland Drosophila. Neth J Zool 38: 61-73.

105. Carton Y, Poirie M, Nappi AJ (2008) Insect immune resistance to parasitoids. Insect Sci 15: 67-87.

106. Lefevre T, de Roode JC, Kacsoh BZ, Schlenke TA (2011) Defence strategies against a parasitoid wasp in Drosophila: fight or flight? Biol Lett in press.

107. Honti V, Csordas G, Markus R, Kurucz E, Jankovics F, et al. (2010) Cell lineage tracing reveals the plasticity of the hemocyte lineages and of the hematopoietic compartments in Drosophila melanogaster. Mol Immunol 47: 1997-2004.

108. Eslin P, Giordanengo P, Fourdrain Y, Prevost G (1996) Avoidance of encapsulation in the absence of VLP by a braconid parasitoid of Drosophila larvae: An ultrastructural study. Can J Zool 74: 2193-2198.

109. Rizki RM, Rizki TM (1984) Selective destruction of a host blood cell type by a parasitoid wasp. Proc Natl Acad Sci USA 81: 6154-6158.

110. Bouletreau M (1986) The genetics and coevolutionary interactions between parasitoids and their hosts. In: Waage JG, Greathead DJ, editors. Insect Parasitoids. London: Academic Press. pp. 169-181.

111. Barbosa P (1988) Natural enemies and herbivore-plant interactions: Influence of plant allelochemicals and host specificity. In: Barbosa P, LeTourneau DK, editors. Novel Aspects of Insect-Plant Interactions. New York: Wiley. pp. 201-229.

112. Flanders SE (1942) Abortive development in parasitic hymenonoptera, induced by the food-plant of the insect hosts. J Econ Entomol 35: 834-835.

113. Carton Y (1977) Attraction de *Cothonaspis sp.* (Hymenoptera: Cynipidae) par le milieu trophique son hote: *Drosophila melanogaster*. Actes Coll Intern Tours 265: 285-303.

114. Fleury F, Gibert P, Ris N, Allemand R (2009) Ecology and life history evolution of frugivorous Drosophila parasitoids. Adv Parasitol 70: 3-44.

115. Schlenke TA, Morales J, Govind S, Clark AG (2007) Contrasting infection strategies in generalist and specialist wasp parasitoids of *Drosophila melanogaster*. PLoS Path 3: 1486-1501.

116. Rizki TM, Rizki RM, Carton Y (1990) *Leptopilina heterotoma* and *L. boulardi*: Strategies to avoid cellular defense responses of *Drosophila melanogaster*. Exp Parasitol 70: 466-475.

117. Cowmeadow RB, Krishnan HR, Atkinson NS (2005) The *slowpoke* gene is necessary for rapid ethanol tolerance in Drosophila. Alcoholism: Clin Exp Res 29: 1777-1786.

118. Scholz H, Ramond J, Singh CM, Heberlein U (2000) Functional ethanol tolerance in Drosophila. Neuron 28: 261-271.

119. Bozic J, DiCesare J, Wells H, Abramson CI (2007) Ethanol levels in honeybee hemolymph resulting from alcohol ingestion. Alcohol 41: 281-284.

120. Moreau SJM, Eslin P, Giordanengo P, Doury G (2003) Comparative study of the strategies evolved by two parasitoids of the genus Asobara to avoid the immune response of the host, *Drosophila melanogaster*. Dev Comp Immunol 27: 273-282.

121. Labrosse C, Staslak K, Lesobre J, Grangeia A, Huguet E, et al. (2005) A RhoGAP protein as a main immune suppressive factor in the *Leptopilina boulardi* (Hymenoptera, Figitidae) - *Drosophila melanogaster* interaction. Insect Biochem Mol Biol 35: 93-103.

122. Lochmiller RL, Deerenberg C (2000) Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos 88: 87-98.

123. Brayton RG, Stokes PE, Schwartz MS, Louria DB (1970) Effect of alcohol and various diseases on leukocyte mobilization, phagocytosis and intracellular bacterial killing. N Eng J Med 282: 123-128.

124. Szabo G (1999) Consequences of alcohol consumption on host defence. Alcohol and Alcoholism 34: 830-841.

125. Eijs IEM (1999) The evolution of resource utilisation in Leptopilina species [Dissertation]. Leiden, Netherlands: Leiden University.

126. Eijsackers HJP, Bakker K (1971) Duration of the egg stage of the sexes in *Pseudeucoila bochei* Weld (Cynipidae), a parasite of Drosophila larvae. Neth J Zool 21: 166-171.

127. Lerner AB, Fitzpatrick TB (1950) Biochemistry of melanin formation. Physiol Revs 30: 91-126.

128. Rizki MTM (1957) Alterations in the haemocyte population of *Drosophila melanogaster*. J Morphol 100: 437-458.

129. Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. Nature 405: 299-304.

130. Hotopp JCD, Clark ME, Oliveira D, Foster JM, Fischer P, et al. (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science 317: 1753-1756.

131. Timmis JN, Ayliffe MA, Huang CY, Martin W (2004) Endosymbiotic gene transfer: Organelle genomes forge eukaryotic chromosomes. Nat Rev Genet 5: 123-135.

132. Andersson JO (2005) Lateral gene transfer in eukaryotes. Cell Mol Life Sci 62: 1182-1197.

133. Gladyshev EA, Meselson M, Arkhipova IR (2008) Massive horizontal gene transfer in bdelloid rotifers. Science 320: 1210-1213.

134. Keeling PJ, Palmer JD (2008) Horizontal gene transfer in eukaryotic evolution. Nature Rev Genet 9: 605-618.

135. Davis CC, Wurdack KJ (2004) Host-to-parasite gene transfer in flowering plants: Phylogenetic evidence from Malpighiales. Science 305: 676-678.

136. Gilbert C, Schaack S, Pace JK, Brindley PJ, Feschotte C (2010) A role for host-parasite interactions in the horizontal transfer of transposons across phyla. Nature 464: 1347-1352.

137. Hecht MM, Nitz N, Araujo PF, Sousa AO, Rosa AD, et al. (2010) Inheritance of DNA transferred from American trypanosomes to human hosts. Plos One 5: e9181.

138. Thomas J, Schaack S, Pritham EJ (2010) Pervasive horizontal transfer of rolling-circle transposons among animals. Genome Biol Evol 2: 656-664.

139. Yoshida S, Maruyama S, Nozaki H, Shirasu K (2010) Horizontal gene transfer by the parasitic plant *Striga hermonthica*. Science 328: 1128-1128.

140. Yoshiyama M, Tu Z, Kainoh Y, Honda H, Shono T, et al. (2001) Possible horizontal transfer of a transposable element from host to parasitoid. Mol Biol Evol 18: 1952-1958.

141. Quicke DLJ (1997) Parasitic wasps. London: Chapman & Hall. 470 p.

142. Fleury F, Ris N, Allemand R, Fouillet P, Carton Y, et al. (2004) Ecological and genetic interactions in Drosophila-parasitoids communities: A case study with *D. melanogaster*, *D. simulans* and their common Leptopilina parasitoids in southeastern France. Genetica 120: 181-194.

143. Schaack S, Gilbert C, Feschotte C (2010) Promiscuous DNA: horizontal transfer of transposable elements and why it matters for eukaryotic evolution. Trends Ecol Evol 25: 537-546.

144. Diao Y, Qi Y, Ma Y, Xia A, Sharakhov I, et al. (2011) Next-generation sequencing reveals recent horizontal transfer of a DNA transposon between divergent mosquitoes. PLoS One 6: e16743.

145. Kidwell MG (1993) Lateral transfer in natural populations of eukaryotes. Ann Rev Genet 27: 235-256.

146. Pace JK, Gilbert C, Clark MS, Feschotte C (2008) Repeated horizontal transfer of a DNA transposon in mammals and other tetrapods. Proc Natl Acad Sci U S A 105: 17023-17028.

147. Robertson HM (1997) Multiple mariner transposons in flatworms and hydras are related to those of insects. J Hered 88: 195-201.

148. Roulin A, Piegu B, Fortune PM, Sabot F, D'Hont A, et al. (2009) Whole genome surveys of rice, maize and sorghum reveal multiple horizontal transfers of the LTR-retrotransposon Route66 in Poaceae. BMC Evol Biol 9: 58.

149. Silva JC, Loreto EL, Clark JB (2004) Factors that affect the horizontal transfer of transposable elements. Curr Iss Mol Biol 6: 57-71.

150. Kaminker JS, Bergman CM, Kronmiller B, Carlson J, Svirskas R, et al. (2002) The transposable elements of the Drosophila melanogaster euchromatin: a genomics perspective. Genome Biol 3: 1-20.

151. Fleury F, Gibert P, Ris N, Allemand R (2009) Ecology and life history evolution of frugivorous Drosophila parasitoids. Adv Parasitol 70: 3-44.

152. Daniels SB, Peterson KR, Strausbaugh LD, Kidwell MG, Chovnick A (1990) Evidence for horizontal transmission of the P-transposable element between Drosophila species. Genetics 124: 339-355.

153. Houck MA, Clark JB, Peterson KR, Kidwell MG (1991) Possible horizontal transfer of Drosophila genes by the mite *Proctolaelaps regalis*. Science 253: 1125-1129.

154. Biemont C, Cizeron G (1999) Distribution of transposable elements in Drosophila species. Genetica 105: 43-62.

155. Grimaldi DA, Engel MS (2005) Evolution of the Insects. Cambridge: Cambridge University Press. 772 p.

156. Dowton M (2001) Simultaneous analysis of 16S, 28S, COI and morphology in the Hymenoptera: Apocrita - evolutionary transitions among parasitic wasps. Biol J Linn Soc 74: 87-111.

157. Finnegan DJ (1989) Eukaryotic transposable elements and genome evolution. Trends Genet 5: 103-107.

158. Mackay TFC (1989) Transposable elements and fitness in *Drosophila melanogaster*. Genome 31: 284-295.

159. Nuzhdin SV (1999) Sure facts, speculations, and open questions about the evolution of transposable element copy number. Genetica 107: 129-137.

160. Casola C, Lawing AM, Betran E, Feschotte C (2007) PIF-like transposons are common in Drosophila and have been repeatedly domesticated to generate new host genes. Mol Biol Evol 24: 1872-1888.

161. Schlenke TA, Begun DJ (2004) Strong selective sweep associated with a transposon insertion in Drosophila simulans. Proc Natl Acad Sci U S A 101: 1626-1631.

162. Ackerman H, Udalova I, Hull J, Kwiatkowski D (2002) Evolution of a polymorphic regulatory element in interferon-gamma through transposition and mutation. Mol Biol Evol 19: 884-890.

163. Schaack S, Gilbert C, Feschotte C (2010) Promiscuous DNA: horizontal transfer of transposable elements and why it matters for eukaryotic evolution. Trends in Ecology & Evolution 25: 537-546.

164. Gilbert C, Schaack S, Pace JK, Brindley PJ, Feschotte C (2010) A role for host-parasite interactions in the horizontal transfer of transposons across phyla. Nature 464: 1347-U1344.

165. Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Reviews Microbiology 3: 711-721.

166. Kaminker JS, Bergman CM, Kronmiller B, Carlson J, Svirskas R, et al. (2002) The transposable elements of the Drosophila melanogaster euchromatin: a genomics perspective. Genome Biology 3: research0084.0081–0084.0020.

167. Handler AM, Gomez SP (1997) A new hobo, Ac, Tam3 transposable element, hopper, from Bactrocera dorsalis is distantly related to hobo and Ac. Gene 185: 133-135.

168. Hart DL, Lohe AR, Lozovskaya ER (1997) Modern thoughts on an ancyent marinere: Function, evolution, regulation. Annual Review of Genetics 31: 337-358.

169. Capy P, David JR, Hartl DL (1992) Evolution of the Transposable Element Mariner in the Drosophila-Melanogaster Species Group. Genetica 86: 37-46.

170. Picot S, Wallau GL, Loreto ELS, Heredia FO, Hua-Van A, et al. (2008) The mariner transposable element in natural populations of Drosophila simulans. Heredity 101: 53-59.

171. Bourque G, Leong B, Vega VB, Chen X, Lee YL, et al. (2008) Evolution of the mammalian transcription factor binding repertoire via transposable elements. Genome Research 18: 1752-1762.

172. Britten RJ (2010) Transposable element insertions have strongly affected human evolution. Proceedings of the National Academy of Sciences of the United States of America 107: 19945-19948.

173. Jordan IK, Rogozin IB, Glazko GV, Koonin EV (2003) Origin of a substantial fraction of human regulatory sequences from transposable elements. Trends in Genetics 19: 68-72.

174. Beltrami M, Medina-Munoz MC, Arce D, Godoy-Herrera R (2010) Drosophila pupation behavior in the wild. Evolutionary Ecology 24: 347-358.

175. Seyahooei MA, Kraaijeveld-Smit FJL, Kraaijeveld K, Crooijmans JBM, Van Dooren TJM, et al. (2009) Closely related parasitoids induce different pupation and foraging responses in Drosophila larvae. Oikos 118: 1148-1157.

176. Vandal NB, Siddalingamurthy GS, Shivanna N (2008) Larval pupation site preference on fruit in different species of Drosophila. Entomological Research 38: 188-194.

177. Cowmeadow RB, Krishnan HR, Atkinson NS (2005) The slowpoke gene is necessary for rapid ethanol tolerance in Drosophila. Alcoholism-Clinical and Experimental Research 29: 1777-1786.

178. Scholz H, Ramond J, Singh CM, Heberlein U (2000) Functional ethanol tolerance in Drosophila. Neuron 28: 261-271.

179. Gibson JB, May TW, Wilks AV (1981) Genetic-Variation at the Alcohol-Dehydrogenase Locus in Drosophila-Melanogaster in Relation to Environmental Variation - Ethanol Levels in Breeding Sites and Allozyme Frequencies. Oecologia 51: 191-198.

180. Lindberg ML, Amsterdam EA (2008) Alcohol, wine and cardiovascular health. Clinical Cardiology 31: 347-351.

181. Romeo J, Warnberg J, Nova E, Diaz LE, Gomez-Martinez S, et al. (2007) Moderate alcohol consumption and the immune system: A review. British Journal of Nutrition 98: S111-S115.

182. Morozova TV, Anholt RRH, Mackay TFC (2006) Transcriptional response to alcohol exposure in Drosophila melanogaster. Genome Biology 7.

183. Hallem EA, Rengarajan M, Ciche TA, Sternberg PW (2007) Nematodes, bacteria, and flies: A tripartite model for nematode parasitism. Current Biology 17: 898-904.

184. Pino JA, Marquez E, Castro D (2009) Volatile and non-volatile acids of noni (Morinda citrifolia L.) fruit. Journal of the Science of Food and Agriculture 89: 1247-1249.

185. Daborn PJ, Yen JL, Bogwitz MR, Le Goff G, Feil E, et al. (2002) A single P450 allele associated with insecticide resistance in Drosophila. Science 297: 2253-2256.

186. Schmidt JM, Good RT, Appleton B, Sherrard J, Raymant GC, et al. (2010) Copy Number Variation and Transposable Elements Feature in Recent, Ongoing Adaptation at the Cyp6g1 Locus. Plos Genetics 6.

187. Mackay TFC (1989) Transposable Elements and Fitness in Drosophila-Melanogaster. Genome 31: 284-295.

188. Houck MA, Clark JB, Peterson KR, Kidwell MG (1991) Possible Horizontal Transfer of Drosophila Genes by the Mite Proctolaelaps-Regalis. Science 253: 1125-1129.