

# The Use of Parasitoid Wasps (*Leptopilina heterotoma*) in Biology Laboratory Courses

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The parasitoid wasp, *Leptopilina heterotoma*, infects the second instar stage larvae of the fruit fly, *Drosophila melanogaster*. This process can be used to enhance student interest in parasitology, genetics, and developmental biology courses. Students can conduct a variety of research studies, including determining the efficacy of infectivity of various mutants of fruit flies. First, students subculture various mutants of fruit flies and wait until second instar larvae have developed. They then remove the adult flies and coat the inside of the plug with honey, which will serve as the food source for the wasps. They then anaesthetize the wasps and add them to the vials with the fruit fly larvae. They can then count surviving adult fruit flies after the normal fly life cycle in both wasp-infected and uninfected flies. The wasps that infect the larvae take 25–30 days to hatch and produce a new source of wasps. Other species of these non-stinging parasitoid wasps have also been sold commercially for pest control. Pros and cons of using parasitoid wasps in this manner can be added to round out the laboratory experience for the student.

**Key words:** parasitoid wasps, *Drosophila*, development

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## Introduction

Parasitoid wasps are an excellent model organism to enhance interest in parasitology, genetics, and developmental biology courses because they can be easily reared in the lab and many experiments using them as a model organism can be completed over a semester. Several parasitoid wasp species (*Leptopilina*, *Ganaspis*, *Asobara*, *Aphaereta Trichopria* and *Pachycrepoideus* spp.) can easily infect laboratory-reared *Drosophila melanogaster* at different life stages (Gokhman et al., 2011; Small et al., 2012). Parasitoid wasps are estimated to be an extremely species-rich group, but it is thought that most species have not yet been identified (Smith et al., 2008). They can be involved in complex ecological community interactions, but that also has not been well-studied (Shaw and Hochberg, 2001). There is therefore a need for student-driven research into parasitoid wasps to help shed light on this poorly-studied group.

Students are interested in parasitoid wasps because they display unique life histories and are used for biological control. Most species fall within the suborder Apocrita of Hymenoptera, and all have larval stages that develop by feeding on the bodies of other Arthropods, usually insects (Godfray, 1994). The endoparasitoid females use ovipositors to inject their eggs in the host larvae or pupae, with infection rates that are sometimes as

high as fifty percent (Lefèvre et al., 2012). Once inside the host, the egg hatches into a larva that resides inside the host until ready to pupate. The parasitoid wasps ultimately kill their hosts as they complete development (Melk and Govind, 1999). Because most of these species are non-stinging to humans, they have been exploited for biological control. For example, several parasitoid wasp species (*Oobius agrili*, *Spathius agrili*, *Tetrastichus planipennisi*, and *Spathius galinae*) are recommended by the US Department of Agriculture for use to combat the emerald ash borer (Gould et al., 2016). Other species including *Muscidifurax raptorellus*, *Muscidifurax zaraptor* and *Spalangia cameroni* are used to combat horse and houseflies (Machtinger et al., 2015), and the tiny *Trichogramma* spp. are commercially available to assail over 200 species of garden and crop pests (Kuhar et al., 2004; Romeis et al., 2005; Smith, 1996).

In spring 2016, we integrated student-driven research projects involving parasitoid wasps in three undergraduate courses (Parasitology, Developmental Biology, and Genetics) at Saint Francis College in Brooklyn, NY. The parasitoid wasp *Leptopilina heterotoma* was used to infect *Drosophila melanogaster* fruit flies. All *Leptopilina* spp. are endoparasitoids, with females laying one egg in a *Drosophila larva*. This allows the host to continue growing to pupation (Eijs and Val Alphen, 1999). The growing parasitoid appears dark in

color in the pupae, which makes for easy identification of infected or uninfected hosts. Depending on a variety of factors including host species and temperature, wasps eclose in 25-30 days after infection (Small et al., 2012). *L. heterotoma* specifically parasitize *Drosophila* larva by destroying the lamellocytes, which are blood cells whose normal role is to eliminate foreign bodies and abnormally developing tissues by encapsulation (Rizki et al., 1990). The ability of *L. heterotoma* to evade the *Drosophila* immune system may be why it can parasitize at least 10 different *Drosophila* species (Rizki et al., 1990). Students developed projects focused on infection rate, and the effect of larval stage on parasitism probability. We expand upon the use of the parasitoid wasps for the first time at Saint Francis College in our new Parasitology and Developmental Biology courses. The students in the Parasitology course observed the flies and wasps and learned about their life cycles and phylogenetic relationships. Student groups also cultured new vials of fruit flies, which were reared until larvae appeared (after a couple of days). Adults were cleared by two different methods – either by drowning and pouring off, or by fly nap and clearing. Students then introduced fly-napped wasps to the vials. *L. heterotoma* were not sexed because of time constraints (the females have longer antennae and a more pointed abdomen). After a month, the vials were observed again.

The parasitoid wasps were also used in the Developmental Biology lab curriculum, and the class was separated into four groups. Students separated the *Drosophila* according to early (first or second instar) and late (third instar) larval stages, and then placed the separated larva in different vials with food cultures. Third instar larvae were identified as those that could climb upward out of the food supply and by the presence of dark orange rings at the tips of the posterior spiracles (Tyler, 2000). In order to provide a food source for the wasps, the foam stopper was dipped into honey. Wasps were transferred by tapping the vial on the table, and flipping over the wasp vial on top of the *Drosophila* larvae vials. Group 1 (see Table 1) placed four infected *Drosophila* pupa into the vials with the early and late stage *Drosophila* larva, to see if wasps would emerge from the infected pupa and then infected the *Drosophila* larva. Groups 2-3 placed four adult wasps into each vial, so that infection rate could be measured. Group 4 only tested the wasp infection rate using early instar larvae. The larvae were observed biweekly and observations (number of infected larva) were recorded.

### Student Outline

1. You will be given two vials of flies. One will contain fruit flies alone (*Drosophila melanogaster*) and the other will contain fruit flies that have been parasitized by parasitoid wasps (*Leptopilina heterotoma*). Look for infected pupae which are darker than uninfected ones. After 28 days, many wasps will hatch out of the fruit fly pupae. Your instructor will tell you when the fruit flies were infected in case you will be working with the wasps right away.
2. Take a vial of uninfected fruit flies and make two new cultures from them. Dip the foam stoppers into honey (you may dilute the honey slightly with water if it is too viscous). After 48 hours, you should have some second instar larvae to which you will add wasps. (You will add them to one vial—the other vial will be the control.)
3. Clear the two vials of adult fruit flies according to the method given to you by your instructor.
4. Add fly-nap to your vial of adult wasps (or use CO<sub>2</sub> gas). Do NOT over-nap them!!
5. Add at least 10 adult wasps to your vial of flies with second instar larvae. Try to sex the wasps to include a mix of males and females--although this is a bit difficult because they are so small.
6. Observe the wasps throughout their life cycle as they infect the larvae.
7. After 28 days, count the number of flies in your experimental and in your control. Count the number of wasps that hatched out.

### Sample Results

In the Parasitology course, the vials that had more than seven wasps introduced produced new wasps, and the fly-napped and cleared adults yielded more results than the vials in which the adults were drowned. In the Developmental Biology course, vials were checked for infected *Drosophila* pupae 2-weeks and 4-weeks post-wasp introduction. Results for each group are shown in Table 1. Groups 1 and 4 had successful wasp infections, but only in vials containing *Drosophila* with early instar larvae. No group had successful wasp infection using late (third) instar larvae. Group 1 was the only group that used *Drosophila* infected pupae to try to infect *Drosophila* larvae, and they had the highest success rate, with at least 50 wasps observed.

**Table 1.** Results for Developmental Biology lab groups by week and larval stage at time of infection. Early instar = first or second instar larvae; late instar = third instar larvae.

Weeks after wasp introduction	Larval stage at time of wasp introduction	Group 1	Group 2	Group 3	Group 4
2	<u>Early instar</u>	10 infected pupae observed; no new wasps observed	No infected pupae or new wasps observed	No infected pupae or new wasps observed	Several dark colored pupae present (possible infection)
	<u>Late instar</u>	No new wasps observed	No infected pupae or new wasps observed present	No infected pupae or new wasps observed present	N/A
4	<u>Early instar</u>	Many wasps observed, at least 50	No infected pupae or new wasps observed	No infected pupae or new wasps observed	15 wasps observed, 30 <i>Drosophila</i>
	<u>Late instar</u>	No new wasps observed	No infected pupae or new wasps observed present	No infected pupae or new wasps observed present	N/A

## Discussion

The students in the Parasitology and Developmental Biology courses at St. Francis College were introduced to the system of parasitoid wasp—fruit fly infection successfully. The animals are easy to grow and manipulate and tangible results were obtained after two distinct experiments. For the Developmental Biology students, only early-stage *Drosophila* infections yielded successful results (see Table 1). An example in the literature in which *Leptopilina heterotoma* successfully infected fruit fly larvae at the second instar stage (after 48 hours) is in Stokl and Herzner (2016). The students' results were consistent with this. The third instar stage is probably too developed for the wasp to infect. Interestingly, Group 1, which transferred infected *Drosophila* pupae to vials containing uninfected early-stage larvae, had the highest infection rate, with almost 50 wasps produced after a month. It would be interesting to test this again, to see if high infection rate can again be obtained with infected pupae. Two groups did not have any successful infections. This could be for various reasons, including the possibility that the four wasps placed in the vials were not females or had not mated before introduction to the *Drosophila* larvae, which is necessary for infection (Small et al., 2012).

## Materials

Various stocks of *Drosophila melanogaster* (We get ours from Carolina Biological)

Standard materials for rearing flies:

vials  
fly food  
foam stoppers  
paint brushes  
fly-nap or CO<sub>2</sub>  
honey

There are several sources available for obtaining parasitoid wasps. For example, Todd Schlenke ([schlenke@reed.edu](mailto:schlenke@reed.edu)) at Reed College maintains a variety of parasitoid wasps, and, according to his web site (<http://people.reed.edu/~schlenkt/index.html>), “We maintain a large number of live strains of parasitoid wasps that infect *Drosophila*. Our lab policy is to make these strains available to everyone once we have published on them.”

Several species are also available online for purchase for use in biological control, including: *Trichogramma pretiosum*, *Trichogramma brassicae* and *Trichogramma minutum* from Planet Natural (<https://www.planetnatural.com>). *Trichogramma platneri* is also available for purchase from Biological Control Systems (<http://www.buglogical.com/trichogramma/>).

## Notes for the Instructor

It is evident from our initial wasp trial at Saint Francis College that these projects lend themselves well to hypothesis-driven, student research projects. In each course, students worked together to develop a hypothesis, and the classroom was partitioned so that replicate trials of that hypothesis could be conducted. We suggest further experiments such as infecting different *Drosophila* species and mutants, with other *Leptopilina* spp. Additionally, infection rates could be measured under various conditions such as temperature, food availability, and light exposure. Students can also examine *Drosophila* spp. physiological responses including ability to kill wasp eggs and diversify offspring (Singh et al., 2015), as well as behavioral defenses such as decreased egg-laying when exposed to male or female wasps (Lefèvre et al., 2012). Other class projects can investigate immune responses to parasitoid wasps, which have been extensively studied in model organisms such as *Drosophila* (Keebaugh and Schlenke, 2014).

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