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# Chapter 12

# Competition Within and Between Species of Parasitoid Wasps

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## Contents



## Introduction

This laboratory exercise is meant to acquaint students with the concepts of intraspecific and interspecific competition while observing firsthand the potential effects of each on the reproductive success of individuals. It is meant to act as a "guided inquiry," so that the students are led through the process of experimental design and the analysis of the data. In this investigation, students first design experiments to examine intraspecific and interspecific competition using two species of parasitoid wasps. Second, the instructor guides the students to a consensus experiment that examines the effect of both types of competition on reproductive output in the parasitoids. Third, the students conduct the consensus experiment in which one or two females are placed on a single host, alone, with conspecific competitors, or with interspecific competitiors. In subsequent labs, students check cultures for emergence of new adults. Cultures are frozen after full emergence, approximately 21 days for *Nasonia* cultures and 40 days for *Melittobia* and mixed species cultures. Six weeks after the first lab, students gather data on the number of offspring produced by females under each of the initial densities of founding females. Students use the resulting data to draw conclusions about the intensity of intraspecific and interspecific competition between the two species. This study requires two 2-3 hour lab periods and weekly, short observation periods in between. This lab is suitable for introductory level courses, and, with the additional extension material given at the end of the Notes to Instructor section, for upper-division courses in ecology.

# Student Outline

## Objectives

1. Describe the life cycle of *Nasonia* and *Melittobia*.
2. Explain the possible interactions between two parasitoid species competing for the same host resource.
3. Design and conduct an experiment to determine the nature of the interaction between these two species when competing for a common host.
4. Relate class research outcomes to the principle of competition exclusion.
5. Discuss the concept of resource partitioning as it relates to the natural history and behavior of these two species.

## Introduction

Think of the sparrows darting about in the trees on campus, the robins on the lawn, or the house finches coming to a bird feeder in your yard. The type of place where you will normally find a given bird species is its *habitat* – an inclusive term that includes both the physical and chemical features of the place and the array of other species living in it. Within this habitat, each species of organism is distinct in terms of its "profession," i.e., the sum of activities and relationships in which it engages to secure and use the resources necessary for its survival and reproduction. This is its *niche*. (If there were no constraints at all on its acquisition and use of resources, each species could expand into its *fundamental niche*. In the many cases, however, constraining factors limit a species to its *realized niche*.)

Directly or indirectly, the populations of all species in a habitat associate with one another as a *community*. The structure of this assemblage, in turn, is shaped by many different factors, such as interactions between climate and topography, and the kinds and amounts of food available. A major influence to be considered is the interaction of the species in that habitat. In even a simple natural community, dozens to hundreds of different species of plants and animals interact with one another. In spite of this diversity, however, we can identify categories of interactions that have different effects on population growth (Table 1).

Most species in a habitat have a *neutral* relationship with one another. For example, a robin that feeds on worms is not affected by a hummingbird that feeds on nectar from flowers, even if the robin and the hummingbird live in the same habitat. In other cases, for at least part of the life cycle, individuals of two or more species interact to affect one another's fate directly. Generally, one participant clearly benefits, but the effect on the other can be neutral, positive or negative. For example, flocks of insectivorous birds follow large grazing animals in an African savannah. As the animals move through the grass, they disturb insects that fly up out of the grass. The birds forage on these insects, taking advantage of the disturbance. This type of interaction is called commensalism, where one species benefits and the effect on the other is neutral. In the previous example, the birds benefit by having an easier time finding food, but the large grazers are not impacted by the interaction. In a great many other cases – such as most flowering plants and the insects, birds, bats, and other animals that pollinate them – both parties benefit. This *mutualism* is not only widespread, but often obligatory. One (or sometimes both) species cannot survive without it.

*Predation* and *parasitism* are two more interactions where one participant benefits, though in these cases the other party clearly suffers. Defining the line between these categories can be a fuzzy affair. In general, predators feed on other living organisms that they kill outright. Parasites feed on

tissues of living organisms that they also live on or in, at least for part of their life cycle. Entomologists also recognize *parasitoids,* insects whose larvae live upon and kill what they eat (usually the larvae or pupae of other insect species) (Godfray, 1994).

**Table 1.** Categories of direct interactions between two species in the same community.

| Name of interaction | Type of contact | Direct effect on species #1 | Direct effect on species #2 | Other aspects of the relationship |
|---|---|---|---|---|
| Neutral relationship | Two species are linked only indirectly through interactions with other species. | 0 | 0 | Each species has a neutral relationship with most species in its habitat |
| Commensalism | A relationship that directly helps one species but does not affect the other much, if at all. | + | 0 | Commensalism, mutualism, and parasitism are all cases of symbiosis ('living together"). |
| Mutualism | Benefits flow both ways between the interacting species. | + | + | Better viewed as two-way exploitation than as cozy cooperation. |
| Predation | Predator attacks and feeds upon a series of prey but does not take up residence in or on them. | + | – | Prey generally dies. |
| Parasitism | Parasite feeds on tissues of one or more hosts, residing in or on them for at least part of their life cycle. | + | – | A host might or might not die as a result of the interaction. |
| Interspecific competition | Disadvantages flow both ways between species | – | – | Generally less intense than competition between members of the same species. |

0 means no direct effect on population growth.
+ means positive effect; – means negative effect.

The final category of contact between species is the only one in which both participants are clearly worse off because of the interaction. *Competition* for required resources is common among living organisms, and may become especially intense when shared resources become limited. *Intraspecific* competition occurs when different individuals of the same species compete for a resource. These interactions can be fierce because the individuals require the same limited resources to survive and reproduce. When different species are vying for the same food, habitat, or some other environmental resource it is called *interspecific* competition. These interactions are typically somewhat less intense. This is because while the requirements of two species might be similar, they can never be as close as they are for individuals of the same species.

Consider, however, the theoretical case of two species that occupy the identical niche. Can such a thing even happen? G. Gause (1934) studied two protist species that both fed on the same bacterial cells. When he combined them in a single culture, one always drove the other to extinction. Many other experiments have since supported "Gause's Law," now called *the principle of competitive*

*exclusion.* It states that any two species that use identical resources cannot coexist indefinitely (Harden, 1960).

Many experiments have demonstrated that the more two species in a habitat differ in their resource use, the more likely it is that they can, in fact, coexist (Krebs, 1994). Even two species with a great deal of overlap may live together for some time, although competitive interactions often suppress the population growth rate of one or both of them. Over time, an interesting phenomenon called *resource partitioning* may occur. Members of each species may come to specialize in a subdivision of some category of similar resources. For example, if both feed upon apples, one may feed upon small green fruits and the other upon larger, riper ones.

Although they are not particularly closely related to one another, the lives of two parasitoid wasp species, *Melittobia digitata* and *Nasonia vitripennis,* are quite similar. Both species lay their eggs on the pupal stages of host insects. In nature, *Nasonia* use *Neobellieria (=Sarcophaga) bullata,* as well as other related species of flies as their hosts, while *Melittobia* lay their eggs on the prepupae or pupal stages of solitary wasps and bees. However, in the lab, *Melittobia* will readily accept *Neobellieria bullata* – the same host as *Nasonia*, While *Nasonia* are more choosey about their host, *Melittobia* might be considered more of a generalist, because they exhibit some flexibility in their host choice. *Melittobia* are about half as large as *Nasonia*, but both are quite small and completely harmless to humans.

Their complete life cycles are relatively short (2-4 weeks at 25$^{o}$C) and quite similar (Figure 1). Females lay numerous eggs through the host covering. The eggs hatch to become larvae that consume the host, then change to pupae, and finally molt to a winged adult stage. Adult females disperse from the host covering to search for new food resources.

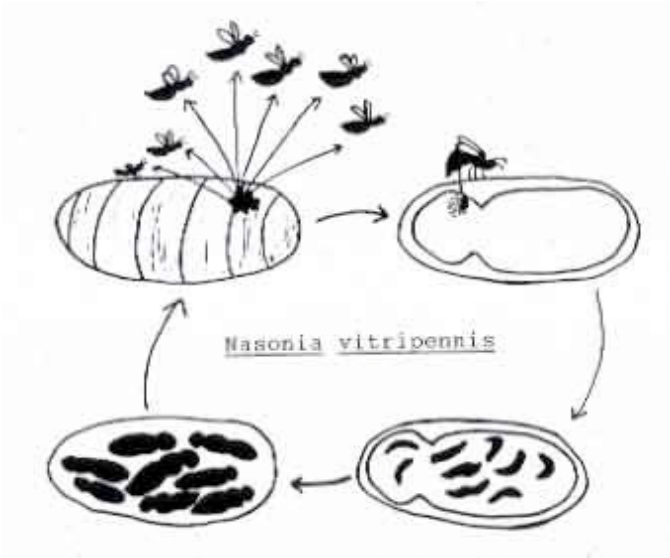



**Figure 1**. The life cycle of *Nasonia vitripennis;* (drawing by Bethia King). The life cycle of *Melittobia* is the same, though individuals at all stages are smaller.

Adults of both parasitoids are very "user friendly." Although females possess normal wings and can fly, they do not do so readily. However, they are negatively geotactic (i.e., they move up, away from gravity). When a few females from a culture are shaken out onto a horizontal surface and covered with a glass vial, they will readily climb into the vial and up the sides. One can readily add a host pupa and then plug the vial tightly with cotton. Large numbers of individuals can be efficiently handled in this way.

**Challenge #1:**
**Design a way to test the interaction between two very similar species in the same habitat**

The categories of interactions discussed above can seem quite straightforward when one is simply reading about them. But if you were to observe two unfamiliar animals interacting, how would you decide what "label" to apply? Could you predict the outcome of the interaction? How could you test your prediction?

The two parasitoid wasps presented in this laboratory investigation seem to occupy very similar niches. What would happen if a female of each species found the same host at the same time? How would it compare to the situation when two females of either species found a host simultaneously? How do either of these situations compare to the outcome when only one female of either species encounters a host? While the situation in nature has not been studied, we can use laboratory trials to make some fairly good predictions. We would need experiments designed to show:

- the reproductive potential for each female in the absence of competition
- whether one species is able to outcompete the other (interspecific competition)
- whether some form of interspecific sharing occurs
- whether two females of a species on a single host (intraspecific competition) produce more or fewer offspring as compared to when they have sole possession of a host
- whether some sort of intraspecific mutualism occurs

Each of these could be approached through a different experimental set-up, and the task of making these observations could be divided among class members. Discuss as a class how you would like to do this.

After you have read the background information and whatever text pages or other material your instructor may indicate, meet with your partner to:

- discuss and list the possible experimental combinations that could be set up involving two parasitic wasps, *Melittobia* and *Nasonia*, and a host, *Neobellieria*
- predict what you think might be the outcome for each possible interaction
- identify and list variables that you would manipulate in your experiment
- identify and list variables you would keep constant in your experiment
- what would you measure to find out whether your prediction was true?

Once you have completed this step, share your information with the class. Then obtain your instructor's approval and proceed to set up your experiment(s).

**Table 2.** Experimental design worksheet.

| Nature of the question | Experimental conditions (Treatments) | Specific predictions |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

**Challenge #2: Carry out an experiment to test the interaction between two very similar species in the same habitat**

To test validity of the class predictions, each student or pair of students will need to set up a culture for each treatment, observe them periodically over the month of the life cycle, then count the total number of adult parasitoids that are produced in a given treatment. Class results should be pooled for each of the treatments, allowing firmer conclusions about the nature of the interactions observed. You may wish to use a table like the sample below.

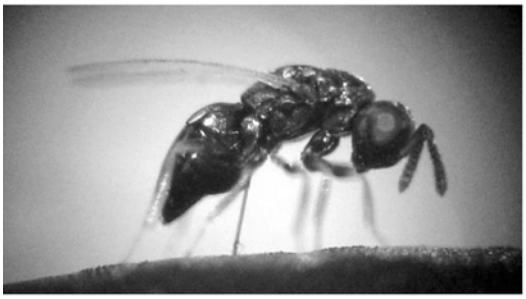
**Table 3.** Sample table for summarizing class data on competition between two species of parasitoid wasps.

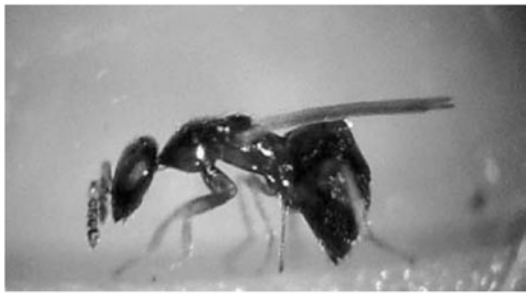
| **Number of Mothers** | **Progeny/ female replicate 1** | **Progeny/ female replicate 2** | **Progeny/ female replicate 3** | **Progeny/ female replicate 4** | **Progeny/ female replicate 5** | **Progeny/ female replicate 6** | **Average progeny/ female** |
|---|---|---|---|---|---|---|---|
| *Nasonia vitripennis* | | | | | | | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| *Melittobia digitata* | | | | | | | |
| 1 | | | | | | | |
| 2 | | | | | | | |
| Both *Nasonia vitripennis* and *Melittobia digitata* | | | | | | | |
| *1 N. vitripennis* | | | | | | | |
| *1 M. digitata* | | | | | | | |
| *2 N. vitripennis* | | | | | | | |
| *2 M. digitata* | | | | | | | |

**Counting offspring in your treatments**

In order to collect the data on the number of offspring produced by females in each treatment, you'll need to open the hosts and count the number of wasps in each treatment. Your instructor will describe how to do this. Be gentle with the hosts. Be careful not to grasp them too tightly, and not to blow the wasps off your bench by coughing or sneezing on them. When you are counting the wasps in the mixed cultures, you'll need to be able to distinguish between *Melittobia* and *Nasonia*. Although *Nasonia* is usually larger than *Melittobia*, that's not always the case, and so size **cannot** be used as a reliable species identifier. Instead you'll need to examine the wasps in those cultures carefully under a dissecting scope and look for the features described below.

The most reliable characters are head shape and body shape. *Nasonia* have a distinctly round head and *Melittobia* a flattened and elongated head when viewed from the side (Fig. 2). The thorax and abdomen are about the same thickness in *Nasonia*. In contrast, in *Melittobia*, the thorax is thinner than the abdomen when viewed from the side (Fig. 2).





*Nasonia* *Melittobia*

**Figure 2.** Side view of both species.

**Data analysis**

Enter your data in an Excel or StatView file to create a class data file. Determine the average offspring production for single females, and the average offspring production per female when two females of the same species share the host. Then, determine the effect of the presence of larvae of the other parasitoid species on the production of adults by each species.

**Discussion and reflection questions**

1. Both of these species are sold commercially. (*Nasonia* are called "jewel wasps" and *Melittobia* are called "WOWBugs.") Imagine you are the laboratory technician facing an ambitious CEO who wants to cut costs and maximize profits. How would you respond to these ideas? What experimental evidence would you present to back up your answers?
    a. Given that they can develop on the same species of host, why can't we just raise them together on the same host?
    b. Setting up cultures costs time and money. If our company's normal rearing protocol is to place one parasitoid female on a single host to establish a culture, wouldn't we do better by using more than one female per culture?
    c. Wouldn't putting two females on a single host result in twice as many offspring produced?
2. Imagine you work for a company that sells these two parasitoid wasps in large numbers to poultry farmers to use to control nuisance flies that breed in chicken manure. You are responsible for rearing cultures of these two parasitoid wasps. Write a memo to your boss with specific recommendations for the optimal rearing conditions that would produce the most parasitoid adults of each species. Justify your recommendations by citing specific results from the classes' pooled experiment data.
3. "Gause's Law" says that complete competitors cannot coexist. This means that the species that most efficiently uses the contested resource will eventually eliminate the other at that location. Does Gause's Law seem to apply to the interaction between *Nasonia* and *Melittobia*? Why or why not?
4. If these two species were to use the same host in nature, how might resource partitioning allow them to coexist? To find out more about their natural history and habitats, visit

<www.wowbugs.com> for *Melittobia,* and <www.bios.niu/bking/nasonia.htm> or <www.rochester.edu/College/BIO/labs/WerrenLab/Nasonia/> for *Nasonia.*

5. Based on the results of your experiment, why might the two species not use the same host in nature?

## Materials

Materials required for a class of 24 students working in pairs:

- 1 - 2 cultures of *Melittobia digitata* (WOWBugs) newly emerged adults (Carolina Biological Supply, ER-14-4570, $12.35 for 50-100 wasp late stage pupae).
- 2 cultures of *Nasonia vitripennis* (Jewel wasp) newly emerged adults (Carolina Biological Supply, ER-14-4560, $10.35 for at least 50 wasps). You'll need 2 cultures to be assured of a sufficient number of females.
- 72 Young *Neobellieria* (=*Sarcophaga*) pupae (Carolina Biological Supply, ER-17-3480, $11.40 for 100 – 150 hosts). (Although "flesh fly" is now the preferred common name, these are listed in the catalog as "blow fly" pupae). **Note:** if you are planning to use only hosts of a designated size, you will need to order a sufficient number of hosts to ensure that you have large enough supply of the size you are planning to use. In that case, you might consider ordering more hosts (Carolina Biological Supply, RG-17-3482, $21.90 for 200-250 hosts).
- 72 Glass shell vials, 1 dram, pack of 144 (Carolina Biological Supply, ER-71-5051, $19.45)
- Package of jumbo size cotton balls (purchase locally)
- Package of 24 pipe cleaners (purchase locally)
- Pack of fine tip permanent black marking pens (purchase locally)
- Aluminum foil (for making weigh boats – purchase locally)
- Electronic balance capable of weighing to nearest milligram
- 25 sheets of plain white paper (purchase locally)
- Computer with statistical software, such as Excel
- Dissecting scopes (one for each pair of students)

## Notes for the Instructor

**Pre-lab preparations**

The necessary materials for this exercise are very inexpensive, and set-up is relatively simple. Living *Melittobia digitat*a and *Nasonia vitripennis* cultures should be ordered to arrive within one week (no sooner) of the first lab period. The *Neobellieria* hosts can also be obtained within the same time frame. Within 24 hours of the first lab period, the wasps in *Nasonia* cultures must sorted, and the males removed. The instructor also may wish to partition the wasps from the main cultures into smaller groups in separate vials to facilitate their distribution to the students. Since the students will spend the second lab period counting the offspring from their cultures, no special set-up is required for that lab.

Order the living wasp cultures and fly pupae to arrive at most one week before class. Wasps are shipped as late pupal stages and should be beginning to emerge upon arrival. If emergence appears

complete upon arrival (i.e., numerous adult wasps crawling in culture container), cultures can be maintained fresh for short periods of time by storing them in a refrigerator dairy compartment until the day of class.

Note: if you need a large number of parasites, you may wish to rear your own. See "Maintaining parasitoid wasp cultures" for details.

The *Neobellieria* (=*Sarcophaga*) pupae must be placed in the refrigerator immediately upon arrival and kept there until just before class use. Otherwise, they will begin to develop into flies and if this happens they are unsuitable as hosts for the wasps.

The day before class, you (or the lab prep person) needs to sort through the *Nasonia* culture removing all males, so that all the wasps provided to students are female. This is necessary because the sexes are very similar in appearance, and if the students are asked to distinguish between the sexes, they are not always reliable. However, with a little practice males can be readily distinguished (see "Distinguishing between females and males"). Because the *M. digitata* culture is always about 95% female and the tendency of males is to remain inside the host pupal skin, there is no need to remove the males. There is little chance that a male would end up in an experimental vial. (Male *Melittobia* are also extremely different from females, so in the unlikely event that one is found and chosen by a student, it would be readily apparent.)

If you are planning to have students tally male and female offspring separately, it is also helpful to prepare separate labeled vials containing a single male and female (a few vials for *Melittobia*, some for *Nasonia*). One vial for each species can be handed out to each pair. Students can examine these specimens under a dissecting scope while you explain how to differentiate between the species and the sexes within each species. This can be done in the first lab session, or you can wait until the second lab session, when students will be tallying the results. In the latter case, place the vials in the freezer until they are needed.

**Maintaining parasitoid wasp cultures**

Maintaining your own stock cultures of wasps is an easy and inexpensive way of producing large quantities of wasps when you need them. To maintain a culture, simply place 3-4 hosts in a clean, 1-dram vial, along with 5-6 mated females (almost all should be mated within 24 hours of emerging as adults), and close the vial tightly with a cotton ball plug. The wasps will mature more quickly in an incubator set at about 25-26$^{o}$C, but can be raised at room temperatures as well. *Melittobia* should emerge in 18-28 days, and *Nasonia* in about 14 days. The easiest way to ensure that you have enough mated females available when you need them is to stagger the setup of your cultures. For *Melittobia*, begin by establishing 2 cultures (in case one fails for some reason) about 32 days before you'll need them, and establish more cultures every 3 days or so for about 10 days. Each culture will produce at least 300 females, so you'll have far more females than you need, but as the cultures are so inexpensive to set up, you'll be sure to have enough young females to use for the lab. For *Nasonia*, start about 20 days in advance and establish cultures every 2-3 days for a week. Each *Nasonia* culture should yield about 50 wasps per host.

**Introducing the experimental rationale**

Because this is a guided inquiry, after each lab pair has developed their list of possible interaction experiments, the instructor's role should be to moderate the sharing session during which each pair will present their ideas for experiments. Make suggestions or ask leading questions as dictated by the class dynamics to lead the class to develop a set of logical investigations. Attempt to involve members of every pair in the discussion and avoid letting one student or pair dominate.

On the board or overhead projector, set up a table with four columns (Table 4). In the first, help students think through the important experimental questions. In column two, develop a running list of various possible treatments that illustrate possible variables to be manipulated. Help students see that these should be various combinations of the two wasps. In column three, for each possible treatment, list specific predictions generated by the students as to the anticipated outcome. Accept all predictions students make about the outcomes at this time, but allow student generated discussion concerning them. In column four, elicit their prediction for how the relative numbers of the offspring of each species will change compared to when each is alone on a host, assuming that competition is present.

Set up a second table to develop lists of variables to be kept constant or controlled in each experiment. Encourage student brainstorming on this topic until it seems that all relevant matters have been addressed.

Have students copy these two tables and submit them as part of their laboratory report at the close of the investigation.

Ultimately, guide students to appreciate that the most complete way to investigate and understand the possible interactions between two wasps competing for a single host resource would include the following four treatments.

1. A single female alone on a host (Treatment 1 – one for each species)
2. Two females of the same species on a host (Treatment 2 – one for each species)
3. A female of each species together on a host (Treatment 1+1)
4. Two *Melittobia* and two *Nasonia females* together on a host (Treatment 2+2)

Treatment 1 will show the reproductive potential for each female in the absence of competition. Treatment 2 will show if two conspecific females sharing a single host (intraspecific competition) produce more or fewer offspring as compared to when they have sole possession of a host (Treatment 1). Treatment 1+1 will reveal whether one species is able to outcompete the other for a single limiting resource (interspecific competition) or whether some form of sharing occurs. Treatment 2+2 will demonstrate the interaction between interspecific and intraspecific competition. For example, a comparison of Treatment 2 (intraspecific competition) with Treatment 2+2 (both intraspecific and interspecific competition) will suggest the importance of interspecific competition when intraspecific competition is present. For introductory level courses, you could leave out the 2+2 treatment altogether, to simplify the interpretation of the data.

This is also a good opportunity to discuss the need for developing testable predictions. For example, although the student's third and fifth predictions about interactions in the table above might be possible outcomes, given the structure of this experiment, they are impossible to evaluate.

To prevent students from arriving at the suggested protocol by reading ahead, you may wish to break the student handout into two sections, one containing Challenge 1, and the other beginning with Challenge 2. You can then withhold the section containing Challenge 2 until the class has had the opportunity to work through developing the experimental protocol.

**Table 4.** Sample table to guide student thinking about experimental set-up.

| Nature of the question | Treatments - # of parasitoid(s) on a host | Specific predictions | |
|---|---|---|---|
| | | Types of interactions | Effect on offspring number |
| What is the reproductive potential for a female *Melittobia* without competition? | One *Melittobia* | "This will be the highest number because these wasps are smallest so more of them fit." | N/A |
| What is the reproductive potential for a female *Nasonia* without competition? | One *Nasonia* | "There will be fewer of these because they are larger, but more of them than when they have to share a host with another wasp." | N/A |
| Is the outcome of the interspecific interatction competition, neutral, commensalism, or mutualism? | One of each species | "I think they'll share the host, one taking the head and the other the tail end." | "There will be slightly more *Melittobia* than *Nasonia*, but the total will not be greater than either species alone since the host is a finite amount of food." |
| Is the outcome intraspecific interaction in *Melittobia* competition, cooperation, or neutral sharing of the resource? | Two *Melittobia* | "There will be fewer offspring per female because they will be crowded together." | "The total number of offspring will be the same as with one female by herself since the host is a finite amount of food." |
| Is the outcome intraspecific interaction in *Nasonia* competition, cooperation, or neutral sharing of the resource? | Two *Nasonia* | "They'll fight each other and end up with only one alive to lay eggs." | "The total number of offspring will be the same as with one female by herself since the host is a finite amount of food." |
| Which is more important, intraspecific or interspecific competition? | Two of each species | "Because *Nasonia* are larger they should be better interspecific competitors. But *Melittobia* produce more offspring, so intraspecific competition will be more important." | "The total number of offspring of each species will be the same as with just one of each species, if interspecific competition is most important." |

## Control of variables

To control for possible host effects, there are considerations that should be discussed and agreed upon prior to starting the experiment. Fly host weights vary rather greatly, with the larger (ca. 0.125g) being more than twice the weight of the smaller (ca. 0.055g). Such variation can obviously affect the potential number of parasitoid progeny, with lower yields from smaller hosts compared to larger hosts. Lead students to consider the importance of weighing the hosts and using relatively uniform host sizes for all experiments. Alternatively, they could calculate a conversion or adjustment factor, i.e., average number of progeny per milligram of host and adjust their data accordingly (see "Extending the exercise for a General Ecology course").

Note: an interesting extension would be to run one set of treatments on the largest size hosts and a parallel set on the smallest size hosts to explore whether host weight changes the results in a

consistent or predictable fashion. There is evidence that host size influences the outcome of intraspecific competition in *Melittobia* (C. Randall and J. Guinan, unpublished data).

**Handling techniques**

Prior to having the students set up their individual or pair experiment, demonstrate how to remove a few wasps onto a piece of white copy paper by gently brushing them with the side of a pipe cleaner. Demonstrate how to use an inverted shell vial to readily capture one, which will immediately crawl up into the vial. Finally, and this is *critically important*, make a big deal about tightly plugging the vials with a cotton ball once the wasps and host are inside. Loose cotton plugs will result in escaped wasps and experiment failure. Discuss with students the matter of how to label their experimental vials, and have them write legibly.

The treatments should be stored in an upright position. An excellent way to organize and store the vials is to use the box in which they were sent, which contains dividers that will hold the vials in an upright position. If the box is placed in a convenient drawer, students can have easy access to check the progress of their experiment. Another option is to purchase heavyweight cardboard vial trays that will store up to 112 cultures upright (Carolina Biological Supply, ER-71-4906, $4.35 each).

**Conducting the investigation**

*Part One.* Once everyone has agreed on the treatments to be used and the appropriate protocols, students can be directed to the materials table to set up the experiment. Because the materials are relatively inexpensive, each student can be responsible for conducting one replicate. Alternatively, replicates can be divided up so that each pair is responsible for one replicate. The former is recommended, however, as having more replicates increases the confidence in the results, and helps mitigate against the occasional experiment failure or unforeseen disaster. If you decide to have each student responsible for a replicate, you'll need to adjust the required materials upward accordingly (i.e., you will need twice the number of wasps, hosts and vials).

At least once a week over the next four weeks, have students briefly examine their cultures, noting any evident changes. This should take only a few moments, and should not interfere with other laboratory activities you have scheduled.

*Part Two.* It is best not to schedule the lab for the second half of this experiment for at least 6 weeks after students have established their cultures. After 4-5 weeks from setup, the new generation of *Melittobia* adults should have emerged. For *Nasonia*, emergence will take about half as long. Several days after the adults have emerged, you should collect the vials into a resealable plastic bag and place the bag in the freezer compartment of a refrigerator until class. This will serve to euthanize remaining live wasps and keep all of them relatively soft and pliable so they can be counted more easily.

To test validity of their predictions, students will need to count the total number of adult wasps produced in each treatment. (For more advanced classes, consider also having students maintain records of the sex and body size of the offspring. See "Extending the exercise for a General Ecology course".) Comparing the pooled class results for each of the treatments will lead to conclusions about the nature of the interaction.

When it comes time to examine the offspring, suggest that students empty the contents of their experimental vial onto a piece of white copy paper. They can then use a pipe cleaner to move the

dead wasps into small groups for tallying totals. Caution them to exercise care during counting; wasps are easily lost if the student sneezes or breathes heavily on them. Also remind them that because some wasps will die inside the host pupa skin, it will be necessary to break open the host remains and brush out any wasps remaining inside. In some cases, students may find larvae or pupae as well as adults. It is probably best not to include them in the counts. Some of these may not be viable and would never have emerged. In addition, sex is impossible to determine in the larval and early pupal stage, so if your students are keeping track of sex ratios, they would not be able to classify these offspring.

When the experiments are concluded, class results can be pooled in a spreadsheet (such as EXCEL, see "Using Microsoft Excel for statistical analyses"), with copies made available for each student. Results from multiple class sections also may be compiled to provide larger numbers of replicates.

Communicate your expectations to students regarding grading, laboratory reports (form and timing of submission), any statistical treatment expected for the data, and other mechanics. We suggest that class-generated tables, the responses to the Discussion Questions, and weekly notes on the progress of the investigation all be accounted for in the report and grading process.

## Sample of expected results

*Intraspecific Competition.* Table 5 lists outcomes of research on different numbers of *Melittobia* and *Nasonia* alone on a single host fly pupa. Although the activity, as written, does not include sex ratio data, we've included it here in case you wish to make this an optional addition for more advanced classes or extra credit.

**Table 5.** Sample outcomes of studies of competition between *N. vitripennis* and *M. digitata* on the same *Neobellieria* host.

| Number of Mothers | Sons | Daughters | Total Progeny | Sex Ratio (% males) | Sample Size | Source |
|---|---|---|---|---|---|---|
| *Nasonia vitripennis* | | | | | | |
| 1 | 10.0 | 54.6 | 64.6 | 16% | 10 | B. King, 2000 |
| 2 | 24.6 | 34.1 | 58.7 | 43% | 9 | |
| *Melittobia digitata* | | | | | | |
| 1 | 3.1 | 93.7 | 96.8 | 3.2% | 11 | Silva-Torres and Matthews, 2003 |
| 2 | 4.2 | 128.4 | 132.6 | 3.2% | 16 | |
| Both *Nasonia vitripennis* and *Melittobia digitata* | | | | | | |
| *1 N. vitripennis* | 6.8 | 9.3 | 16.1 | 42.2 | 146 | Matthews, unpublished |
| *1 M. digitata* | 0.7 | 7.1 | 7.8 | 8.97 | 146 | |
| *2 N. vitripennis* | 16.9 | 18.6 | 35.5 | 44.4 | 44 | West, unpublished |
| *2 M. digitata* | 0.02 | 1.34 | 1.36 | 1.0 | 44 | |

*Interspecific Competition.* At the University of Georgia, we have run nearly 600 trials placing one female of each species with a single host pupa at 26°C with the following general outcomes:

| | |
|---|---|
| Only *Nasonia vitripennis* results: | 30-36% |
| Only *Melittobia digitata* results: | 22-27% |
| Each produce some offspring: | 26-33% |
| Neither produce any offspring: | 7-15% |

**Conclusions**

*Interspecific competition.* Either species alone with a host produces significantly more progeny than it does even if it wins in a competition situation. When both produce some progeny in the competition, the total production per species falls still further. Thus, the presence of a competitor seriously impacts reproductive success (fitness). One can also speculate about whether the relative sizes of the two competing species should be a factor in the outcome, given that *Nasonia* require about twice as much host resource per offspring as do *Melittobia*. Other possible topics for discussion include the effect of differing generation times between *Melittobia* and *Nasonia*, and the fact that blowflies are not the natural host of *Melittobia*, but are for *Nasonia*.

*Intraspecific competition.* In *Melittobia digitata*, the total number of progeny is higher with two females, but the number of progeny per female when two females are placed with a host decreases (Cooperband et al., 2003).

Having more than one female *Melittobia* in the initial setup does not change the sex ratio of the offspring from that found with a single female. However, if the size (head width and tibia length) of the female progeny is measured, those of the single-female experiment are significantly larger than progeny from experiments where two females are together on a single host. Also, progeny from single female experiments live significantly longer (Torres, unpublished).

In *Nasonia vitripennis*, two females on a host produce slightly fewer total progeny, and the number per female (per capita rate) is considerably lower compared to a single female alone. Interestingly, the sex ratio changes dramatically, with the proportion of males being much greater when two females share hosts (King, 2000). The sex ratio adjustment contrasts strikingly with *Melittobia,* where there seems to be no change in sex ratio under the conditions tested (Cooperbrand et al., 2003). Attempting to understand such differences leads into the fascinating area of local mate competition theory and how differences in the life histories and mating behaviors define behavioral expression in the two species.

**Distinguishing between females and males**

*Melittobia* males and females are easy to tell apart. Females have straight dark bodies, straight antennae, and fully developed wings. Males are amber colored, have branched antennae, and stunted wings (Figure 3).

Distinguishing between the sexes in *Nasonia* is a little trickier, but students in advanced classes can learn to do it with practice. The most reliable difference between the sexes is that males have stunted wings, while females' wings are fully developed (Figure 4).

It's important to stress to students that size is not a reliable indicator of sex, as some of them might assume otherwise.

**Figure 3.** Sexes of *M. digitata.* The female is on the left, male on the right.

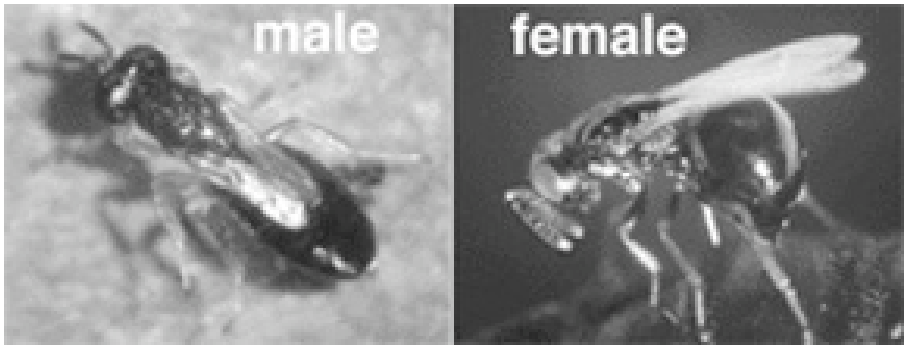



**Figure 4.** Sexes of *N. vitripennis.* Males have noticeably shorter wings than females.

**Extending the exercise for a General Ecology course**

The exercise as outlined above is intended for use in an introductory biology course. However, we have found the exercise to be effective in a general ecology course by making it more quantitative. Below, we outline this more quantitative approach.

*Variation in host mass.* In the general protocol, students are provided with hosts that are greater than 0.1g. However, the hosts still may vary considerably in mass. As a result, students could consider the effect of host mass. To do so, students weigh the hosts prior to the initiation of the experiment. With data on host mass, students can examine the effect of host mass on offspring production (male, female, and total) in each treatment by plotting offspring number versus host mass and carrying out a linear regression analysis (see "Using Microsoft Excel for statistical analyses"). In addition, students can control for host mass in their analysis of the effects of competition by dividing the number of offspring produced per female by host mass for each replicate prior to analysis (see "Statistical analysis of competition").

The importance of host mass could be explored to an even greater extent by using a wider range of host masses, rather than limiting hosts to those greater than 0.1g.

*Statistical analysis of competition.* The experiment is designed such that students can examine the effect of both intraspecific and interspecific competition on offspring production (male, female, total) using planned statistical contrasts. To understand the contrasts, we have the students first identify what type of competition, if any, is occurring in each treatment. Then, we ask students to determine what particular comparisons of pairs of treatments tell us about competition. Below are the treatments and comparisons and how they relate to competition. We would not give these tables to students, but ask them to generate the tables themselves.

**Table 6.** Types of competition illustrated by treatments.

| Treatment | Type of Competition |
|---|---|
| 1 foundress (Trt 1) | No competition |
| 2 foundresses of the same species (Trt 2) | Intraspecific competition |
| 1 foundress of each species (Trt 1+1) | Interspecific competition |
| 2 foundresses of each species (Trt 2+2) | Intraspecific and interspecific competition |

**Table 7.** Interpretation of treatment comparisons.

| Contrast | What it tells us |
|---|---|
| Trt 1 vs Trt 2 | Strength of intraspecific competition |
| Trt 1 vs Trt 1+1 | Strength of interspecific competition |
| Trt 1 vs Trt 2+2 | Strength of combined competition |
| Trt 2 vs Trt 1+1 | Relative strength of intraspecific and interspecific competition |
| Trt 2 vs Trt 2+2 | Relative strength of interspecific competition in the presence of intraspecific competition |
| Trt1+1 vs Trt 2+2 | Relative strength of intraspecific competition in the presence of interspecific competition |

Since all of the contrasts are pairwise, t-tests can be used for all of the analyses. See "Using Microsoft Excel for statistical analyses" for an explanation of how to carry out a t-test. The analysis can be done using data on offspring production or offspring production per g host mass (see "Variation in host mass"). In either case, offspring production should be expressed per foundress before analysis. In treatments with more than one foundress of a particular species, we cannot determine which foundress produced the offspring. Therefore, we assume that offspring production was equal for each foundress and just divide the number of offspring produced by the number of foundresses.

*Effect of competition on offspring quality.* In addition to affecting offspring number, competition can influence offspring quality. Students can determine offspring quality by measuring body size in a subset of offspring from each replicate. In the species used in this exercise, head width is often used as a measure of body size. Head width can be determined by using a dissecting scope equipped with an ocular micrometer. Because *Melittobia* males have significantly larger heads than females (C. Randall and J. Guinan, unpublished data), students should analyze the data for males and females separately. Students can investigate the effects of host size and competition on offspring

quality itself by using the analyses described above. In addition, students may want to determine the relationship between offspring number and offspring quality for each treatment, by using linear regression with offspring number as the independent variable and offspring quality as the dependent variable. If offspring number does significantly affect offspring quality, then students could examine the effects of host size and competition on offspring quality after controlling for the effects of offspring number. Perhaps the easiest way to do this is to save the residuals from the regression of offspring number and offspring quality and then analyzing the residuals as described above. (See "Using Microsoft Excel for statistical analyses" for instructions on saving residuals.) The residuals describe the variation in offspring quality that is not explained by variation in offspring number.

*Effect of invasion sequence.* In interspecific competition treatments (1+1 or 2+2), the experimental protocol calls for students to introduce foundresses of both species into the culture at the same time. However, if the two species were to use the same host in nature (remember that they don't), it is unlikely that both species would find the host at the same time. As a result, students could investigate the effect of invasion sequence by staggering when foundresses are introduced.

***Lotka-Volterra Competition Model.*** An extension to this exercise, which uses the data generated from the competitive treatments to estimate parameters of the Lotka-Volterra model, is available on the Ecological Society of America's TIEE website (http://tiee.ecoed.net/ ) (Beck et al., 2004).

**Other labs using *Melittobia digitata***

As they are so easy to culture and maintain, you may wish to consider using *Melittobia* as study organisms for other laboratory exercises as well. One exercise, suitable for beginning students in biology, examines the responses of mated females to light and gravity. It also contains an investigative component that asks the students to design experiments to explore other variables affecting female dispersal (Guinan and Matthews, 2000). The fact that male *Melittobia* produce a pheromone to attract females provides the basis for a laboratory that examines courtship reaction chains (Guinan and Matthews, 1999). That lab is suitable for students in Introductory Biology, Animal Behavior, or Ecology classes.

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# Appendix A

## Using Microsoft Excel for statistical analyses

The following appendix includes details on the use of Microsoft Excel for carrying out the statistics in this exercise.

Before you can use Excel for statistics, the Analysis ToolPak must be installed. If it is installed already, there should be a "Data Analysis" option in the tools menu. If not, to install the ToolPak, go to "Add Ins" under the tools menu and select "Analysis ToolPak" and "Analysis ToolPak – VBA." Now, you are set to do data analysis with Excel.

*Simple linear regression*

Simple linear regression can be used to examine relationships between host mass and offspring production, host mass and offspring quality, and offspring production and offspring quality. To run a regression, first enter the dependent variable in one column and the independent variable in another column. Next, select Tools → Data Analysis → Regression. Click OK. The dependent variable is the Y Range and the independent variable is the X range. If you want to see a plot of the data, select the "Line Fit Plots" box. Then, click OK. Excel will now create a new worksheet with the results of the regression. The most important parameter is the slope of the regression model. The slope is the coefficient of the X variable in the lower table. The P-value associated with the slope will tell you whether the slope is significantly different from zero. P-values less than 0.05 are generally considered significant.

The residuals from the regression also can be generated. When setting up the regression in the dialog box, select the "Residuals" box. The residuals for each observation will be displayed in the bottom table of the results worksheet. These values can then be copied to the original worksheet and used for additional analysis.

To return to the original data, click on the appropriate worksheet tab at the bottom of the page.

*T-test*

T-tests can be used for the pairwise comparison of treatments. First, enter the data for each treatment in a separate column. Next, select Tools → Data Analysis → t-Test: Two-Sample Assuming Unequal Variances. Click OK. Select the data for the treatments that you want to compare. It doesn't matter which treatment is Variable 1 and which is Variable 2. The "Hypothesized Mean Difference" is zero. Click OK. Excel will now create a new worksheet with the results of the t-test. The important parameters are the t-Stat and the P-values. Whether to use the one-tail or two-tail P value depends on the contrast. If we can make a directional prediction about which treatment will lead to higher offspring production, then we would use the one-tail P value. Otherwise, we would use a two-tail P value. For example, in the Trt 1 vs Trt 2 comparison, we would predict that offspring production per female would be lower in Trt 2 than in Trt 1, because there is the possibility of intraspecific competition in Trt 2 but not in Trt 1. Therefore, we would use a one-tail P-value. (Keep in mind that even if the P-value is significant, i.e., $< 0.05$, the mean values for the treatments also have to differ in the direction predicted for the result to be considered significant.) In contrast, when we compare Trt 2 to Trt 1+1, we would use a two-tail P value because we have no a priori expectation of whether intraspecific or interspecific competition will have a greater effect.

To return to the original data, click on the appropriate worksheet tab at the bottom of the page.

*Options other than Microsoft Excel.* Another option for data analysis is VassarStats. This is a free web-based program available at http://faculty.vassar.edu/lowry/VassarStats.html.