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EXPERIMENTS

Exploring the Lotka-Volterra Competition Model using Two Species of Parasitoid Wasps

Christopher W. Beck [1], Judy A. Guinan [2],
Lawrence S. Blumer [3], and Robert W. Matthews [4]

1 - Emory University, Department of Biology, 1510 Clifton Rd., Atlanta, GA 30322, 404-712-9012
cbeck@emory.edu

2 - Radford University, Department of Biology, P.O. Box 6931, Radford, VA 24142, 540-831-5222
jaguinan@radford.edu

3 - Morehouse College, Department of Biology, 830 Westview Dr., Atlanta, GA 30314, 404-658-1142,
blumer@morehouse.edu

4 - University of Georgia, Department of Entomology, Athens, GA 30602, 706-542-2311,
rmatthew@uga.edu

parasitic wasps *Melittobia digitata* (above) and *Nasonia vitripennis* (below) on their host *Neobellierria* pupa
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Table of Contents:

CITATION:

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ABSTRACT:

In this investigation, students first design experiments to examine intraspecific and interspecific competition using two species of parasitoid wasps. Second, students are guided 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. Six weeks later, students gather data on the number of offspring produced by females under each of the initial densities of founding females. The resulting data are used to estimate the parameters of the Lotka-Volterra competition model. The predictions of the model are then compared to the outcome of interspecific competition treatments. Information is also included on using the appropriate statistical analyses to compare the relative importance of interspecific and intraspecific competition on offspring production.

KEYWORD DESCRIPTORS:

Principal Ecological Question Addressed: Are What is the relative importance of intraspecific and interspecific competition in two species sharing resources? Does the Lotka-Volterra competition model accurately predict the outcome of competition between two species of parasitoids?

Ecological Topic Keywords: intraspecific competition, interspecific competition, resource partitioning, Lotka-Volterra competition model, principle of competitive exclusion, parasitism, parasitoid

Science Methodological Skills Developed: hypothesis generation and testing, statistics, graphics, data analysis, quantitative analysis, scientific writing

Pedagogical Methods Used: guided inquiry, cooperative learning

CLASS TIME: This study requires two 3-hour lab periods and weekly, short observation periods in between. In an initial, 3-hour lab period, students work in groups to design experimental treatments and then are guided to establish experimental cultures. Then, students examine cultures weekly for first 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. In a second, 3-hour lab period, students count the offspring produced in each experimental culture and then discuss the analysis of the resulting data.

OUTSIDE OF CLASS TIME: Students may spend several hours analyzing their data and writing papers based on their results.

STUDENT PRODUCTS: Students prepare written scientific papers based on the pooled data from the entire class.

SETTING: The experiment is carried out entirely in the lab. A field component could be added to have students look for parasitoid wasps in their natural habitats (i.e., mud dauber nests).

COURSE CONTEXT: The experiment as described is used in upper-level ecology courses with a maximum of 24 students per lab section.

INSTITUTION: The experiment has been implemented successfully at a small private college, a mid-size private university, and a mid-size public university.

TRANSFERABILITY: A version of this experiment has been implemented successfully in an introductory biology course for non-majors at a large public university by emphasizing qualitative comparisons of the effects of intraspecific and interspecific competition. The version for introductory biology was presented as a major workshop at the annual meeting of the Association for Biology Laboratory Education (ABLE) and will be published in the proceedings of the conference in June 2005. This version of the exercise does not include examination of the Lotka-Volterra competition model, but involves more qualitative analysis of the results. Prior to publication in the proceedings, the version for introductory biology is available from the authors. The study organisms used in this experiment are used for other activities at the pre-college level.

SYNOPSIS OF THE LAB ACTIVITY

WHAT HAPPENS:

In an initial 3-hour lab, students first design experiments to examine intraspecific and interspecific competition using two species of parasitoid wasps. Second, students are guided 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 competitors. Working individually, students set up replicate cultures of the experimental treatments. In subsequent labs, students check cultures for emergence of new adults and record date at first emergence. Cultures are frozen after full emergence, approximately 21 days for *Nasonia* cultures and 40 days for *Melittobia* and mixed species cultures. In a final, 3-hour lab, six weeks later, students gather data on the number of offspring produced by females under each condition. The resulting data are used to estimate the parameters of the Lotka-Volterra competition model. The predictions of the model are then compared to the outcome of interspecific competition treatments.

LAB OBJECTIVES:

At the conclusion of this lab, students will be able to...

1. Describe the life cycle of *Nasonia vitripennis* and *Melittobia digitata*,

2. Explain the possible interactions between two parasite species competing for the same host resource,

3. Design an experiment to determine the nature of the interaction between these two species when competing for a common host,

4. Conduct a consensus experiment to determine the effects of intraspecific and interspecific competition on reproductive output in *Nasonia vitripennis* and *Melittobia digitata*,

5. Use the resulting data to estimate the parameters of the Lotka-Volterra competition model,

6. Relate class research outcomes to the principle of competition exclusion.

EQUIPMENT/ LOGISTICS REQUIRED:

Equipment/ Logistics Required:

Materials 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, $12.85 for 50-100 wasp late stage pupae),
- 2 cultures of *Nasonia vitripennis* (Jewel wasp) newly emerged adults (Carolina Biological Supply, ER-14-4560, $10.75 for at least 50 wasps (you'll need 2 cultures to be assured of a sufficient number of females),
- 72 Young *Neobellierria* (=*Sarcophaga*) pupae (Carolina Biological Supply, ER-17-3480, $11.85 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, $22.80 for 200-250 hosts),
- 72 Glass shell vials, 1 dram, pack of 144 (Carolina Biological Supply, ER-71-5051, $20.00),
- 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.

Pre-lab preparations:

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 refrigerator dairy compartment until day of class. Note: if you need a large number of parasites, you may wish to rear your own. See "Maintaining parasitoid wasp cultures," below, for details.

The *Neobellierria* (=*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) need to sort through the *Nasonia* culture removing all males, so that the wasps provided to the students are entirely female. This is necessary because the sexes are 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. Because the *Melittobia* digitata culture is always about 95% female and the tendency of males 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.

See Notes to Faculty: Preparing Vials with Wasps of Each Sex to help students differentiate the sexes. See Notes to Faculty: Instructions on Maintaining Parasitoid Wasp Cultures.

SUMMARY OF WHAT IS DUE:

During the first lab period, students will produce an experimental design to examine the effects of intraspecific and interspecific competition on offspring production in two species of parasitoids. After collecting and analyzing the data, the students will write scientific papers based on their results.

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DESCRIPTION OF THE EXPERIMENT

INTRODUCTION:

In this investigation, we will examine the effects of competition for resources on reproductive output within and between two species of parasitoid wasps.

Lab Objectives:

At the conclusion of this lab, students will be able to...

1. Describe the life cycle of *Nasonia vitripennis* and *Melittobia digitata*,
2. Explain the possible interactions between two parasite species competing for the same host resource,
3. Design an experiment to determine the nature of the interaction between these two species when competing for a common host,
4. Conduct a consensus experiment to determine the effects of intraspecific and interspecific competition on reproductive output in *Nasonia vitripennis* and *Melittobia digitata*,
5. Use the resulting data to estimate the parameters of the Lotka-Volterra competition model,
6. Relate class research outcomes to the principle of competition exclusion.

Ecological communities are composed of populations of all species in a habitat. The structure of a community will be determined in part by the dynamics of the interactions between the species in the community. Interactions between two species can be direct or indirect (i.e., mediated through other species).

In even a simple natural community, 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). The categories are defined by the direction of the effects on the interacting species.

In addition to interactions among species, interactions among individuals within a species can also be important in structuring a community. Within-species interactions can affect the population dynamics of the species, which in turn will influence interactions among species. *Intraspecific* competition occurs when different individuals of the same species or population 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.

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.
Mutualism	Benefits flow both ways between the interacting species.	+	+	Better viewed as two-way exploitation than as cozy cooperation.
Predation True predators Grazers	Predator attacks and feeds upon a series of prey but does not take up residence in or on them.	+	–	Prey generally dies. With grazers, plant might or might not die.
Parasitism Parasites Parasitoids	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 may flow both ways between species, or the superior competitor may be largely unaffected	–	–	Generally less intense than competition among members of the same species.

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

Consider, however, the theoretical case of two species that occupy the identical niche. 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 utilize identical resources cannot coexist indefinitely or "complete competitors cannot coexist" (Hardin 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 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.

The Lotka-Volterra model was developed to allow ecologists to predict the potential outcome when two species are in competition for the same resources. Basically, the model attempts to account for the effect that the presence of one species will have on the population growth of the other species, relative to the competitive effect that two members of the same species would have on each other.

The equation for the population growth of species 1 is:

$$\frac{dN_1}{dt} = r_1 N_1 \left[\frac{K_1 - N_1 - \alpha_{12} N_2}{K_1} \right]$$

And for species 2, it is:

$$\frac{dN_2}{dt} = r_2 N_2 \left[\frac{K_2 - N_2 - \alpha_{21} N_1}{K_2} \right]$$

where:

N_1 and N_2 are the population sizes of species 1 and 2,
r_1 and r_2 are the intrinsic rates of increase for these species,
K_1 and K_2 are the carrying capacities of the habitat for each species,
$alpha_{12}$ and $alpha_{21}$ are the effects of one species on the population growth of the other. Specifically, $alpha_{12}$ is the effect of species 2 on the growth of species 1, and $alpha_{21}$ is the effect of species 1 on the growth of species 2.

If the values for each equation are known (or can be estimated empirically from the results of an experiment), then the equation can be used to predict the potential outcome of a competition (i.e., whether they can co-exist or if one will eventually exclude the other). The values for K_1, K_2, $alpha_{12}$, and $alpha_{21}$ are used to plot the isoclines of zero growth (i.e., where dN_1/dt or dN_2/dt equal zero) for both species on the same graph, and the resulting sums of population growth vectors (trajectories) are used to determine the outcome of the competition (Figure 1).

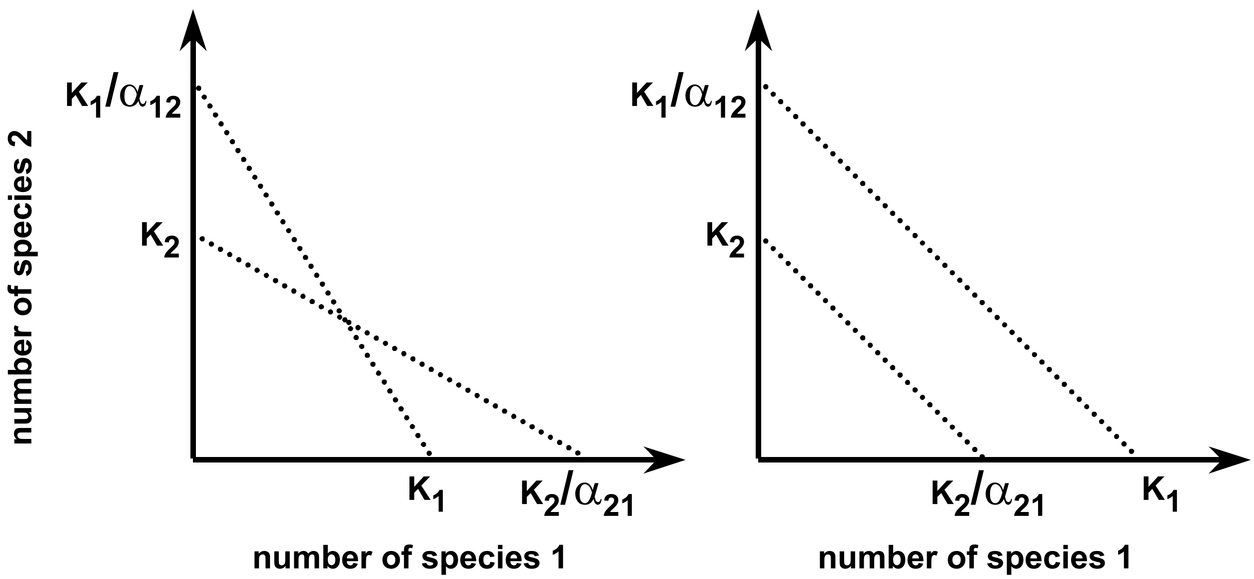

Figure 1. Example graphs of isoclines of zero growth for which species 1 and species 2 coexist (at left), and species 1 competitively excludes species 2 (at right).

The Lotka-Volterra competition model describes the outcome of competition between two species over ecological time. Because one species can competitively exclude another species (Figure 1) in ecological time, the competitively-inferior species may increase the range of food types that it eats in order to survive. However, the response of species to interspecific competition in evolutionary time is often the opposite of what occurs in ecological time. Competitors generally will specialize on particular resource types. This resource partitioning that occurs over evolutionary time actually results in decreased or the absence of competition between the two species.

Although they are not particularly closely related to one another, the life histories of two parasitoid wasp species, *Melittobia digitata* and *Nasonia vitripennis*, are quite similar. Both species are capable of using the same host, although in nature they used different hosts. *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 also quite similar (Figure 2). Females lay numerous eggs through the host covering. The eggs hatch to become larvae that consume the host, then change to pupae, and finally metamorphose to an adult stage. In *Melitobbia digitata*, the adult females may have either normal or stunted wings. The normal winged adults disperse from the host to search for new food resources. The flightless females will lay their eggs on the same host from which they emerged, or disperse to a new host within the same nest (Freeman and Ittyeipe 1976, Côsoli and Vinson 2002).

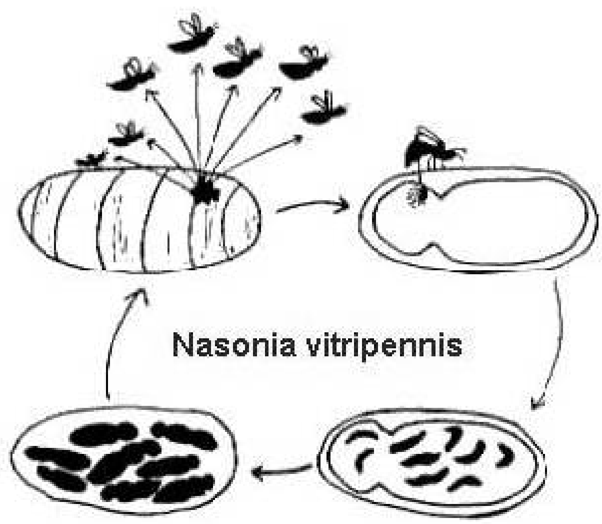

Figure 2. The life cycle of *Nasonia vitripennis* on a *Neobellieria bullata* host pupa (drawing by Bethia King). The life cycle of *Melittobia digitata* is the same, although individuals at all stages are smaller.

There are about 70,000 known species of parasitoids worldwide (9% of all insects), but estimates of their number run as high as 800,000 (Strand 2002). Of the described species, about 80% are members of the order Hymenoptera, as are the two species you are using in this lab. In nature, hosts can be parasitized by more than one species of parasitoid, all competing for the same resource (Stand 2002). Since the host species may itself be a parasite on another species, the ecological effects of that competition on community structure can be very complex. For example, Swaine jack pine sawflies (*Neodiprion swainei* Midd.), which can attack, defoliate, and kill large stands of jack pines in North America, play host to 11 different species of parasitoid wasps. It appears that most of these species coexist because they partition the host resource among them by parasitizing different stages of the host's life cycle, or different segments of the host population (Price 1972). One member of the parasitoid guild that attacks sawfly cocoons is an introduced species (*Pleolophus basizonus*). It is a superior competitor and its presence determines the abundance of some of its competitor species (Price 1970). Although competition can affect parasitoid community structure, spatial and temporal variation in host resources may be even more important in determining parasitoid species richness, even in the presence of interspecific competition among parasitoids (Hawkins 2000).

Parasitoids whose hosts are important pests on crops or forest trees are sometimes intentionally released as biological controls on pest populations. Where more than one species of parasitoid attacks the same host, it is important to understand the nature of that competition before making releases. If the different parasitoid species are capable of co-existing by means of resource partitioning, control of the pest host may be best achieved by introducing some or all of the parasitoid species. On the other hand, if the competitors limit each other's populations because of their competitive interactions, then maximum control of the pest species might be achieved by releasing the most efficient of the parasitoid species by itself (Amarasekare 2000).

MATERIALS AND METHODS:

Overview of Data Collection and Analysis Methods:

Lab 1

The categories of interactions discussed in Table 1 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 similar niches. We are interested in the interactions between the two species. In addition, we want to be able to quantify the effect of one species on the other.

Each group should:

- discuss and list all the possible experimental combinations that could be set up involving two parasitic wasps, *Melittobia digitata* and *Nasonia vitripennis*, and a single host, *Neobellierria*,
- 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,
- identify and list dependent variables you would want to measure to quantify the effect of each species on the other.

Each group will share their experimental design with the rest of the class. Together as a class, we will develop a consensus experimental design. Based on the consensus experimental design, each person should set up one replicate culture for each treatment. See "Handling Parasitoids" below. We will pool the data from the entire class for analysis.

Weekly checks:

Each week, each person should inspect their cultures to see if any adult wasps have emerged. You should record the date that you first see emerged adults for each culture. *Nasonia* cultures should be frozen 21 days after they were established. *Melittobia* and mixed species cultures should be frozen after 42 days.

Lab 2 (6 weeks after Lab 1):

Each person should count the number of offspring produced in each replicate culture. Enter your data into a spreadsheet so that the data for the class can be pooled. Use the pooled data for estimating the parameters for the Lotka-Volterra competition model and for statistical analysis of the effects of competition.

In your groups, discuss how the data can be used to quantify the parameters of the Lotka-Volterra competition model (see “Quantifying the Lokta-Volterra competition model” below). Also, discuss what particular treatment comparisons can tell us about the relative importance of intraspecific and interspecific competition in these two species of parasitoids (see “Guidelines for Data Analysis” below).

Handling Parasitoids:

Adults of both parasitoids, *Melittobia digitata* and *Nasonia vitripennis*, are very "user friendly." Although females possess normal wings and can fly, they do not do so readily. However, they are negatively geotaxic (i.e., they move up, away from gravity). When a few females from a culture are shaken out onto a horizontal surface, then covered with an inverted glass vial, they will readily climb into the vial and up the sides. Once you have wasps in a vial, you can easily add a host pupa, then plug the vial tightly with cotton. Large numbers of individuals can be efficiently handled in this way. The adult wasps can also be manipulated with short pipe cleaners, to which the wasps will temporarily adhere.

Quantifying the Lotka-Volterra Competition Model:

The Lotka-Volterra competition model was described and defined with equations in the Introduction. As noted there, if the values for each equation can be estimated empirically from the results of an experiment, then the equation can be used to predict the potential outcome of a competition (i.e., whether the two species will co-exist or if one will eventually exclude the other). The values for K_1, K_2, $alpha_{12}$, and $alpha_{21}$ are used to plot the isoclines of zero growth (i.e., where dN_1/dt or dN_2/dt equal zero) for both species on the same graph, and the resulting sums of population growth vectors (trajectories) are used to determine the outcome of the competition.

Based on our experimental design, we need to determine the values of these parameters. Recall that the carrying capacity for a population is the maximum number of individuals that can survive in a habitat. For simplicity in this experiment, we have defined the habitat of the parasitoids as a single host. In reality, of course, a habitat would likely contain more than one mud-dauber nest or blowfly puparium, and so there would be many potentially exploitable hosts. To determine the carrying capacities of the two species, we need to know the maximum number of offspring of a given species that can be produced on a single host when only that species is present. With this in mind, data from which treatment would be used to estimate the carrying capacities of *Melittobia* and *Nasonia*? (Remember that at carrying capacity all host resources will be used.)

Estimating the competition coefficients ($alpha_{12}$, and $alpha_{21}$) is a little more complicated. Recall that the equation for the population growth of species 1 is:

$$\frac{dN_1}{dt} = r_1 N_1 \left[\frac{K_1 - N_1 - \alpha_{12} N_2}{K_1} \right]$$

When all of the host resource is used by the parasitoids, then a population can no longer grow. In other words, $dN_1 /dt = 0$. This condition will occur when $K_1 - N_1 - alpha_{12}*N_2 = 0$. To find $alpha_{12}$, we need to solve for it (i.e., do a little algebra) and then substitute values for K_1, N_1, and N_2. Above, we described how to find the carrying capacities. Assuming that *Melittobia* is "species 1," use its carrying capacity for K_1. The number of *Melittobia* and *Nasonia* offspring produced in interspecific competition are N_1 and N_2, respectively. With this in mind, data from which treatment would be used to estimate N_1 and N_2?

The same approach that you used to calculate $alpha_{12}$, can be used to calculate $alpha_{21}$.

Now that you have calculated all of the parameter values, you can use these values to plot the zero growth isoclines and predict the outcome of competition between *Melittobia* and *Nasonia*.

Guidelines for data analysis:

We can use comparisons between different treatments to explore the relative importance of intraspecific and interspecific competition. First, identify what type of competition, intraspecific or interspecific, if any, is occurring in each treatment. After you have done this, think about all of the comparisons between pairs of treatments. What does each of the comparisons tell us? It might be helpful to produce a chart that lists the comparisons and what they mean. Since all of the comparisons involve two treatments, they can be analyzed statistically using t-tests.

Questions for Further Thought and Discussion:

1. Based on the parameter values that you calculated for the Lotka-Volterra competition model, what is the predicted outcome of competition between the two species? Was the predicted outcome achieved in every replicate of interspecific competition? If not, why not?

2. “Gause’s Law” states that competitors that share exactly the same resources in the same way 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 *Melittobia* and *Nasonia*? Why or why not?

3. If these two species were to use the same host in nature, how might resource partitioning allow them to coexist?

4. Based on the results of your experiment, why don’t the two species use the same host in nature?

5. Given the estimated values for carrying capacities and competition coefficients, predict the outcome of competition between *Melittobia* and *Nasonia* using the Lotka-Volterra competition model in *Populus* (see References and Links). Is the predicted outcome of competition affected by initial population sizes or population growth rates? If so, how? How is the time to reach equilibrium affected by these values?

6. The carrying capacities and competition coefficients are just estimates. What factors might affect the carrying capacities and competition coefficients for these two species?

7. If interspecific competition occurs in these species, how might we determine what mechanism of competition (interference or exploitative) is occurring?

*** Note: Answers to many of these questions and numerous other comments by the contributing author can be found in the "NOTES TO FACULTY: Comments On Questions for Further Thought" page.

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For information on natural history and habitats, visit:

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

Populus can be downloaded from www.cbs.umn.edu/populus

Tools for Assessment of Student Learning Outcomes:

Assessment has been carried out in a variety of ways at the different institutions that have used this exercise. In all cases, students are evaluated based on a scientific paper written by each student individually, or by students in a group. In some cases, students are evaluated on both first and second drafts of a paper.

The scoring rubric for the papers varies with instructor. Below is an example scoring rubric used at Morehouse College for a “results summary,” which has all of the components of a scientific paper except the methods. In this evaluation rubric, “audience” concerns the choice of appropriate audience by the student. Students are expected to write their report as if it were a scientific paper. So, the appropriate audience is one of peers who have not conducted the experiment but who are scientifically literate. Reports written to the instructor or to other students in the class do not have the appropriate audience. “Format” is the overall organization of the report in sections that have parallel organization and build on each other. For example, the Discussion should evaluate the findings reported in the Results and put those results a larger context. The Discussion should also address the hypothesis stated in the Introduction.

<u>Results Summary Evaluation (50 points possible)</u>

Introduction and Title Page (10 points)	______
Results (10 points)	______
Discussion and Conclusions (10 points)	______
Literature Use and Citations (10 points)	______
Format, Audience (10 points)	______

Comments:

At the introductory level, students turn in all class-generated tables, the responses to the Discussion Questions, and weekly notes on the progress of the investigation. In addition to individual scientific papers, students could present the results of the experiment in the form of group scientific papers, group oral presentations, or group poster presentations. However, since all of the students are carrying out the same experiment and therefore presenting the same results, individual or group scientific papers would be the most effective.

Tools for Formative Evaluation of this Experiment:

In the ecology course at Morehouse College, each student is asked to evaluate each laboratory exercise on a 10-point scale in terms of the perceived efficacy of each study in reinforcing their knowledge and understanding of the subjects covered in the ecology lecture course. In addition, the students are asked which exercises were the least and most enjoyable and which increased their understanding of the scientific method the most. This information is used to choose and modify exercises used in subsequent semesters.

In the ecology courses at Emory University and Radford University, each student is asked which exercises they liked the best and which they liked the least. Students sometimes provide comments as to why they rated the exercises in the way that they did. Again, this information is used to modify exercises used in subsequent semesters.

An extensive discussion on Evaluation appears in the Teaching section of the TIEE website: http://tiee.ecoed.net/teach/teach.html.

NOTES TO FACULTY

Comments by Contributing Authors - Christopher W. Beck, Judy A. Guinan, Lawrence S. Blumer, and Robert W. Matthews

CHALLENGES TO ANTICIPATE AND SOLVE.

1. Distinguishing between species: At times in the mixed cultures, students have difficulty distinguishing between *Melittobia* and unusually small *Nasonia*. Preparing labeled samples of each species will help the students be able to distinguish between the species. Oyster-eyed mutants (Carolina Biological RG 17-3425, $10.20) of *Nasonia* also can be used in place of the wild type to help students distinguish between the two wasp species.

2. Quantitative literacy: We have found that students have difficulty determining the values of the parameters of Lotka-Volterra competition model. After allowing the students to discuss it in groups, the instructor may want to review the proposed calculations. We have this discussion after the data are collected during the second lab period. However, it could take place after the consensus experimental design is determined during the first lab period. See “Quantifying the Lotka-Volterra competition model” below for detailed description of the calculations.

3. Statistical comparisons: Students also have difficulty determining the appropriate statistical comparisons and then interpreting the results. After allowing the students to discuss the comparisons in groups, the instructor may want to review the possible comparisons and their interpretation. We have this discussion after the data are collected during the second lab period. However, it could take place after the consensus experimental design is determined during the first lab period. Note: pairwise comparisons should be made on offspring per foundress. Therefore, in treatments with two females of the same species, average number of offspring should be divided by two prior to analysis. See “Statistical analysis of competition” below for a detailed description of the comparisons that can be made and their interpretation.

4. Culture problems: Laboratory conditions, especially during winter heating season, can be excessively dry and this may cause cultures to desiccate resulting in low levels of emergence or high rates of culture failure, which can be frustrating to students. It is best if cultures can be maintained in an incubator (about 26^0 40-60%RH). Under poor culture conditions, as high as 50% of cultures may fail. The highest failure rate is with cultures with single foundresses. As a result, we recommend establishing a minimum of 20 replicates. Those cultures that do not produce any offspring should be removed from analyses.

COMMENTS ON THE LAB DESCRIPTION.

The "Detailed Description of the Experiment" above is intended as a student handout. Although all of the students ultimately will perform the same experiment, the student handout is designed to lead students through the process of experimental design and analysis of data. As a result, we have intentionally left out details on the exact experimental design and analysis of data. Those details are presented here for instructors. They could be inserted into a student handout; however, in our experience, giving students the details up front leads students to think that there is only one correct approach to the question. In addition, we use this laboratory exercise after we have discussed competition, including the Lotka-Volterra competition model, in lecture. As a result, we do not present a detailed discussion of the model and all possible outcomes in the student handout. The details that are presented are intended as a reminder for students of what was covered in lecture already. However, if the exercise is used independent of a lecture course or before discussion of competition in lecture, a more detailed discussion of the Lotka-Volterra competition model may need to be included in the student handout.

Introducing the Lab to Your Students:

Because this is a guided inquiry, after each student group has developed their list of possible interaction experiments, the instructor's role should be to moderate the sharing session during which each group 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 group in the discussion and avoid letting one student or group dominate.

On the board or overhead projector, set up a table with four columns. In the first, help students think through the important experimental questions. In column two, develop a running list of various possible treatments that address the experimental questions in the first column. Help students see that this should be various combinations of the two wasps. In column three, for each possible experimental condition list specific predictions of anticipated outcomes proposed by your students. 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.

Samples of Student Thinking About the 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."

Ultimately guide students to appreciate that the most complete way to investigate and understand the possible interactions between two wasp species 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 between foundresses. Treatment 2 will show if two 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 (see Statistical analysis of competition, below). This is also a good opportunity to discuss the need for developing testable predictions. For example, although the student's third and fifth predictions in the table above might be possible outcomes, given the structure of this experiment, they are impossible to evaluate.

COMMENTS ON THE ACTIVITIES IN THE LAB.

Control of variables:

To control for possible host effects, there are at least two considerations that should be discussed and agreed upon prior to starting the experiment. First, 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 "Other Extensions," below).

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 group 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. It 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, $4.50 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 initiate the experiment. Because the materials are relatively inexpensive, each student can be responsible for conducting one replicate (a total of five vials). Alternatively, replicates can be divided up so that each group is responsible for one replicate. The former is recommended, however, as having more replicates increases the confidence in the results and also helps mitigate against the occasional experiment failure or unforeseen disaster.

At least once a week over the next four weeks, have students briefly examine their cultures, noting any changes that are evident. 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 5 weeks after the students have established their cultures. Four or five weeks after initiating the experiment, the new generation of *Melittobia* adults should be emerged. For cultures only containing *Nasonia foundresses*, 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. Consider also having students maintain records of the sex and body size of the offspring (see “Other Extensions,” below.)

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 larval and in the early pupal stage, so if your students are keeping track of sex ratios, they would not be able to classify these offspring.

When the students' experiments have been concluded, class results can be pooled onto a spreadsheet, with copies made available for each student. Results from multiple class sections may also be compiled to provide larger numbers of replicates.

Quantifying the Lotka-Volterra Competition Model:

First, it is important to note that traditionally the Lotka-Volterra competition model has been applied to systems in which the resource for which species are competing is renewable such that multiple generations can use the resource. However, in this experiment, only a single generation of wasps can be produced because the host is not a renewable resource. As a result, this experiment allows us to examine whether the Lotka-Volterra model can accurately predict the outcome of competition for a non-renewable resource.

To quantify the Lotka-Volterra competition model, students must determine the carrying capacities and competition coefficients for both species. We allow students to work in groups to determine which treatments should be used to estimate these values and then discuss their ideas as a class. Remind the students that estimates should be based on treatment averages for the pooled data and not on just their individual replicates. Also, if students are dividing offspring by sex, parameter estimates should be based on the total number of offspring, because of female-biased sex ratios, especially in *Melittobia*.

To estimate the carrying capacities for each species, it is critical that the entire host is consumed by the parasitoid larvae. In some cases, single foundresses may not produce sufficient offspring to consume an entire host. On their natural hosts, the first *Melittobia* females that hatch are non-dispersing, which suggests that the host can be used by multiple generations (Freeman and Ittyeipe 1976, Côsoli and Vinson 2002). Therefore, carrying capacities are best estimated by the total number of offspring produced with two foundresses (i.e., 2M or 2N treatments). It is important to emphasize that in determining the carrying capacities, we are interested in the total number of adult offspring that can be produced on a given host and not the number of offspring per foundress.

When estimating the competition coefficients, we are interested in the effect of interspecific competition alone. As a result, the 1+1 treatment should be used to determine the values of N1 and N2 needed to estimate the competition coefficients. Carrying capacities also are needed to estimate the competition coefficients. Therefore, we discuss estimating carrying capacities first. In the 2+2 treatment, both intraspecific and interspecific competition are occurring, and intraspecific competition can be strong enough to limit the effects of interspecific competition. Therefore, it is not appropriate to use the 2+2 treatment for estimating the competition coefficients.

Below is an example based on data from an Emory University ecology class that is present below.

$K_N = 31.8$
$K_M = 132.9$
$alpha_{NM} = (K_N - N_N)/ N_M = (31.8 - 18.1)/6.7 = 2.04$
$alpha_{MN} = (K_M - N_M)/ N_N = (132.9 - 6.7)/18.1 = 6.97$

In all of the trials that we have conducted, the estimates of the parameters of the Lotka-Volterra competition model suggest an unstable coexistence between the two species. As a result, the outcome of competition will depend on the initial densities of the two species. In interpreting the results of the Lotka-Volterra competition model, students often think that the number of foundresses of each species represents the initial densities of the two species. It is important to emphasize that it is the larvae that are competing for the resource.

Because an unstable coexistence between the two species is typically predicted based on the data, a possible extension of the experiment is to vary the number of foundresses of each species independently (e. g., 1M+2N, 2M+1N). However, it is important to keep in mind that an increase in the number of foundresses may not necessarily lead to a proportional increase in the number of competing larvae, as parasitoid are known to adjust their clutch size based on the presence of conspecific and heterospecific offspring (e. g., Werren 1984, Mackauer et al. 1992).

Statistical analysis of competition:

The experiment is designed to permit students to 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.

Treatment:	**Type of Competition:**
1 foundress (Trt 1)	No competition between offspring of different foundresses
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

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 between two treatments, t-tests can be used for all of the analyses. The analysis can be done using data on offspring production or offspring production per gram host mass (see “Variation in host mass” under “Other Extensions,” below). 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. It is also important to note that we are assuming no (or limited) competition when there is only a single foundress. Currently, it is unknown whether competition is occurring among offspring of a single foundress, as the number of eggs laid has not been determined. How host size and number of foundresses affects number of eggs laid also is unknown. However, in *Melittobia* on their natural hosts, the first females that hatch are non-dispersing, which suggests that the host can be used by multiple generations (Freeman and Ittyeipe 1976, Côsoli and Vinson 2002) and that host resources are not limited for the first generation.

Sample of expected results:

Interspecific Competition - At the University of Georgia, we have run nearly 600 trials in sets of 100 replicates 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: 24-33%
* Neither produce any offspring: 7-15%

Intraspecific Competition - The table that follows 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.

Sample Outcomes of Studies of Competition Between *N. vitripennis* and *M. digitata* on the Same *Neobellierria* 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	

Conclusions:

Interspecific competition

A single individual either species alone with a host produces significantly more progeny than it does even if it wins in an interspecific competition situation. When both produce some progeny in the competition, the total production per species decreases even 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 might 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*. Also, females of both species feed on host fluids. Thus, the greater the number of females, the greater an effect they have on depletion of the host resource. Finally, there is also the possibility that venom or other chemicals injected into the hosts at the time of initial parasitoid attack alter the host's physiology in ways that affect the outcome of the competition. This aspect is so far totally uninvestigated, but could be a topic for speculation.

Intraspecific competition –

In *Melittobia digitata*, the total number of progeny is higher with two foundresses, but the number of progeny per foundress when two foundresses are placed with a host decreases (Cooperband et al. 2003, Silva-Torres and Matthews 2003). However, one big unknown, and a serious limitation of the data, is that we are unable to know the relative contributions of the two individual wasps in a competitive context. We assume that both are equivalent and infer that the reduced per capita output is a result of competition. Students should be led to think critically about assumptions being made in any experiment. In addition to offspring number, competition apparently affects offspring size and viability. Forewing length and hind tibia length of female progeny from single-female cultures are larger than those from cultures where two or more females are together on a single host. Also, progeny from single female experiments live significantly longer (Silva-Torres and Matthews 2003).

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 also changes dramatically, with the proportion of males being much greater when two females share hosts (Werren 1983, King 2000). However, having more than one female *Melittobia* in the initial set-up does not change the sex ratio of the offspring from that found with a single female (Abe et al. 2003, Cooperband et al. 2003, Silva-Torres and Matthews 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 the species:

Because adult *Nasonia* can be small, especially when there is competition for host resources, the two species cannot always be distinguished by body size. Therefore, it is important to point out to students the characters that can be used to distinguish between the species. 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 (see figure below). 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.

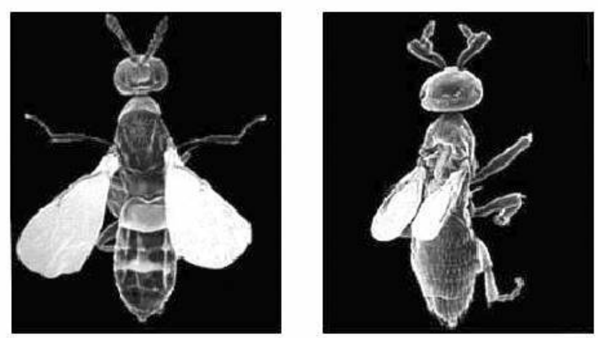
Nasonia vitripennis

Melittobia digitata

Nasonia vitripennis (left) and *Melittobia digitata* to show typical size differences (photos © Jorge M. González)

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 (see figure below).



Sexes of *Melittobia digitata*, with a female at left and a male at right. (photos © Robert W. Matthews)

Nasonia sex identification 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.

It's important to stress to students that size is not a reliable indicator of sex, as some of them might assume otherwise. 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 group. 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 part two of the lab, 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 tightly with a cotton ball plug. The wasps will mature more quickly in an incubator set at about 25-26oC, but can be raised at room temperatures as well. *Melittobia* should emerge in about 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 two cultures (in case one fails for some reason) about 32 days before you'll need them, and establish additional 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.

Other Extensions:

Although the experiment is intended for students to investigate the Lotka-Volterra competition model, the experiment can be extended or adapted to examine other related questions.

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. 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,” above). 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.

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. For the species used in this study, wing length, head width, or hind tibia length are often used as a measure of body size. These 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 or ignore the males. 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. 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.

Effect of Female Number on Sex Ratio. \

Published data for sex ratio adjustment in *Nasonia* and *Melittobia* differ strikingly. For *Nasonia*, presence of multiple females on a host results in an increased proportion of males (Werren 1983, King 2000), whereas for *Melittobia* the sex ratio remains constant with increasing numbers of females (Abe et al. 2003, Cooperband et al. 2003, Silva-Torres 2003). Careful counts of the sexes produced in each treatment would allow students to confirm whether these trends hold under different treatments. It is noteworthy that *Melittobia* males also engage in lethal combat, and the extent of this could be partly assessed by simply tallying the numbers of intact vs. dismembered males in treatments where *Melittobia* emerged. Male fighting is postulated to be related to the apparent failure of *Melittobia* to conform to predictions from local mate competition theory (Abe et al. 2003).

Comments On Questions for Further Thought:

Although the questions for further thought are included in the student handout, most of us do not have the students answer the questions explicitly, as the students are required to write a scientific paper based on the results of the experiment. The questions would be most appropriate if students are not required to submit a written report. Below are comments on expected answers.

Comment on Question 1:

- Based on the parameter values that you calculated for the Lotka-Volterra competition model, what is the predicted outcome of competition between the two species? Was the predicted outcome achieved in every replicate of interspecific competition? If not, why not?

In all trials that we have run, the predicted outcome of competition is an unstable coexistence. In some replicates of one *Nasonia* and one *Melittobia*, one species competitively excludes the other, as predicted by the Lotka-Volterra model. However, in many replicates, we see both species coexisting. The difference between the prediction of the model and the actual outcome may be due to the fact that the model is most appropriate for systems in which multiple generations can feed on the same renewable, but limited, resource, which is not possible in this system. In addition, the parameter estimates for the Lotka-Volterra model are point estimates and do not consider the variation across replicates.

Comment on Question 2:

- "Gause's Law" states that competitors that share exactly the same resources in the same way 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?

We have asked this question in introductory biology courses. The responses of students seem to depend on whether students consider trends in the data as a whole or whether they consider individual replicates independently. If they consider the trends in the data as a whole, they find that Gause's law holds in that *Nasonia* tends to exclude *Melittobia* in interspecific competition. However, if they consider each replicate, they state that Gause's law does not hold, because there is coexistence in some replicates. In a very few cases, students consider coexistence as evidence that the two species are not complete competitors.

Comment on Question 3:

- If these two species were to use the same host in nature, how might resource partitioning allow them to coexist?

Since the two species use the same life cycle stage of the host, they could only partition the host resource by using different parts of the host, rather than different life cycle stages.

Comment on Question 4:

- Based on the results of your experiment, why don't the two species use the same host in nature?

The results suggest an unstable coexistence, in which one species is excluded. As a result, both species cannot coexist on the same host in nature. Therefore, if the two species competed for the same host early in their evolutionary history, they have since diverged in what host species they use to avoid competition (niche partitioning or ecological character displacement).

Comment on Question 5:

- Given the estimated values for carrying capacities and competition coefficients, predict the outcome of competition between *Melittobia* and *Nasonia* using the Lotka-Volterra competition model in *Populus* (see References and Links). Is the predicted outcome of competition affected by initial population sizes or population growth rates? If so, how? How is the time to reach equilibrium affected by these values?

As stated above, the predicted outcome is unstable coexistence. In this case of the Lotka-Volterra model, the outcome of competition is affected both by initial population sizes and by population growth rates. In general, the species with the larger initial population size and higher population growth rate will competitively exclude the other species. In all other cases of the model, the outcome of competition is not affected by either initial population size or population growth rates. Larger initial population sizes and higher population growth rates will lead to a decreased time to reach equilibrium.

Comment on Question 6:

- The carrying capacities and competition coefficients are just estimates. What factors might affect the carrying capacities and competition coefficients for these two species?

Carrying capacities and competition coefficients can be affected by a variety of factors, including host quality, initial population densities of competitors, characteristics of the founding populations of competitors, and environmental conditions such as temperature and humidity.

Comment on Question 7:

- If interspecific competition occurs in these species, how might we determine what mechanism of competition (interference or exploitative) is occurring?

In this system, it would be very difficult to prevent interference competition among larvae to examine the effects of exploitative competition among larvae alone. Therefore, direct observations on competing larvae would be necessary to determine whether interference competition is occurring.

However, Hawkins (2000) suggests that all competition among larvae is interference. Exploitative competition among parasitoids occurs when one adult parasitoid attacks or kills a host before another parasitoid, thus limiting the availability of the host to the later parasitoid. Adult female parasitoids also can engage in interference competition while searching for hosts (Hawkins 2000). By examining the effect of invasion sequence, students could determine the effect of competition among adults.

COMMENTS ON THE ASSESSMENT OF STUDENT LEARNING OUTCOMES

Details of the assessment methods are presented in the "Description: Tools for Assessment of Student Learning Outcomes" section above.

Assessment of student learning in this experiment has been evaluated in two different ways. At Morehouse College, students are given a pre-test and post-test over the range of subjects taught in general ecology. At Emory University and Radford University, students are given a pre-test and post-test specifically on interspecific competition (see below). Student performance on the two tests is then compared.

At Morehouse College, there was a significant improvement on the post-test as compared to the pre-test. However, the degree of improvement was not influenced by whether students were enrolled in the laboratory or not. Yet, since the assessment does not address just competition, we cannot draw specific conclusions about this exercise.

At Emory University, in one semester, students exhibited significant increases in overall score and in scores on questions related to the Lotka-Volterra model (Q3 and Q4) on the post-test as compared to the pre-test (paired t-test, one-tailed $P < 0.5$). In another semester, there were no differences between pre-test and post-test scores. Students at Radford University exhibited a similar pattern with significant differences between pre-test and post-test scores in one semester, but not in another.

An example student assessment instrument:

Assessment for *Melittobia - Nasonia* Competition Lab

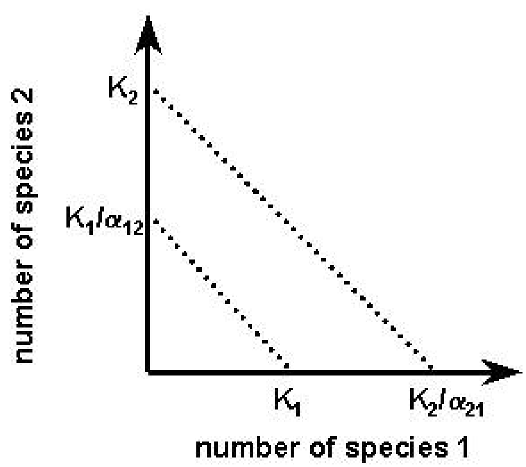
1. Gause's competitive exclusion principle states that:
   a. intraspecific competition is always stronger than interspecific competition,
   b. interspecific competition is always stronger than intraspecific competition,
   c. two species cannot occupy the same ecological niche,
   d. two individuals of the same species cannot occupy the same ecological niche,
   e. both a. and d. are correct.

2. For competition to occur, what must be true about resources in the environment?

3. Two very similar species, ditzy-headed dingbats and nasty-tempered meanbats, which use the same limiting resource, are introduced into the same area. When a dingbat has a habitat all to itself, it can produce 60 offspring a year. When two dingbats share a habitat, they each produce an average of 30 each offspring a year. A solitary meanbat can produce 50 offspring per annum, while two meanbats sharing a habitat produce only 25 offspring each per year. However, when a ditzy-headed dingbat and a mean-tempered meanbat share a habitat, the dingbat produces just 25 offspring and the meanbat produces 40 offspring. Answer the following questions regarding this interaction:

   Which type of competition is stronger for dingbats, intra- or interspecific competition? Explain your answer.
   Which type of competition is stronger for meanbats, intra- or interspecific competition? Explain your answer.
   Using dingbats as species 1 and meanbats as species 2, calculate alpha12 of the Lotka-Volterra equation.

4. If the graph of the Lotka-Volterra equation for this interaction is as shown below, what will be the probable outcome of this interaction?



   a. dingbats will exclude meanbats,
   b. meanbats will exclude dingbats,
   c. either species will eventually exclude the other,
   d. the two species will coexist.

Comments On the Evaluation of the Lab Activity:

Details of the evaluation methods are presented in the “Tools for Formative Evaluation of this Experiment” section.

In addition, extensive notes on how to conduct formative evaluation are in the Teaching Resources sector of TIEE under the keyword "Formative Evaluation."

Comments On Translating the Activity to Other Institutional Scales

A version of this experiment has been implemented successfully in an introductory biology course for non-majors at a large public university by emphasizing qualitative comparisons of the effects of intraspecific and interspecific competition. The version for introductory biology was presented as a major workshop at the annual meeting of the Association for Biology Laboratory Education (ABLE) in 2004 (www.zoo.utoronto.ca/able/conf/able2004/abstracts.htm) and will be published in the proceedings of the conference in June 2005. This version of the exercise does not include examination of the Lotka-Volterra competition model, but involves more qualitative analysis of the results. Prior to publication in the proceedings, the version for introductory biology is available from the authors.

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