Dispersal Cues Used by Parasitic Wasps: Phototaxis, Geotaxis and Mated *Melittobia*

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Background

Melittobia digitata is a tiny parasitic wasp with a fascinating life history. Females of this species lay their eggs on the immature forms of other insects, primarily mud-dauber wasps (Matthews, 1999). To do so, they enter the mud nests of their hosts and climb upward until they locate a cell containing a mud-dauber larva. Using their tiny stinger (which is harmless to humans), they oviposit directly on the host (Matthews, 1997). Soon after, the eggs hatch into dozens to hundreds of small *Melittobia* larvae, which slowly devour their host. Eventually the larvae metamorphose into pupae, and within about three weeks from the time the eggs were laid, adult *Melittobia* wasps appear.

First to emerge are the sightless and flightless males. Only about five of every hundred new adults will be males, born to mate and die within the dark cocoon of their hosts. They locate each other and then fight, often to the death, for the chance to court their sisters as the new females emerge (Guinan et al., 2000). Within a week or two, they die, most having completed hundreds of successful matings.

After mating, females leave the host's nest and locate another mud-dauber nest to complete the reproductive cycle. The females have fully functional eyes and wings, and respond to appropriate cues to find their way out of their natal nest and locate a new host (Matthews et al., 1996). Little is known about the way in which females accomplish this dispersal. In these investigations, you will test two possible cues that females may use in this effort, namely, light and gravity. Once you've gained some experience working with *Melittobia*, you'll use the information presented here, in addition to the new knowledge you've gained from your experiments, to develop and test your own hypotheses about *Melittobia* dispersal.

Objectives

The purpose of this laboratory exercise is to help you:

- Understand how light and gravity may affect dispersal of mated female *Melittobia.*
- Develop and test hypotheses about other factors that might affect *Melittobia* dispersal.

Materials Per Pair or Team

- 2 large glass culture tubes (16 x 100-mm disposable tubes without lips), one containing 10 mated *Melittobia* females, one empty
- 2 small glass vials with cotton stoppers, one containing 10 mated *Melittobia* females, one empty
- Poster putty (chewing gum sized wad)
- 2 cotton balls
- 1 piece of black construction paper, approximately 14 x 18 cm
- 1 small piece of aluminum foil, approximately 6 cm square
- 1 roll of transparent tape
- 1 standard sheet of white photocopy paper
- 1 pipe cleaner
- 1 15–cm ruler
- 1 black permanent marking pen

- 1 pair of scissors
- 1 stopwatch, or wristwatch with a second hand
- 1 "cool" light source such as a fiber optic light

Exercise A. Response of *Melittobia* to Gravity

1. Your instructor has provided you with two glass culture tubes, one of which is stoppered with a cotton ball and contains *Melittobia* females. Count the number of females in the tube and record this number on your data sheet.
2. Use a cotton ball to stopper the open end of the *empty* glass culture tube. Make sure to leave enough of the cotton ball outside the tube so that you can remove it again by grasping the end.
3. With the felt marker, draw a short line part way around both tubes at the point where the cotton ball stops inside. Use a ruler to measure the distance between the line you've drawn and the rounded bottom of the tube. Then draw another short line partway around the tubes, halfway between the first line and the rounded end. Put the empty tube aside for use in the next exercise.
4. Cut a piece of black construction paper big enough to encircle the tube containing the females completely and to cover its entire length plus an additional 2.5 cm. Wrap the black paper around the tube, forming a sleeve that is tight enough to exclude light, but loose enough to slide freely up and down. Tape the paper so that it stays rolled (but don't tape the sleeve to the tube!). With the sleeve flush with the open end of the tube, carefully crimp and twist the sleeve closed over the rounded end, being careful not to tear the paper. Remove the sleeve and set it aside.
5. Roll the piece of poster putty into a narrow rope. Invert your tube containing the wasps so that the cotton stopper rests on the lab bench, and wrap the poster putty around the bottom end, pushing the putty against the sides of the tube and the top of the lab bench. The tube should stand upright by itself. Replace the black sleeve over the tube. Make sure that it is secure against the poster putty holder, so that no light enters the tube, but the sleeve can still move up and down freely. See Figure A.

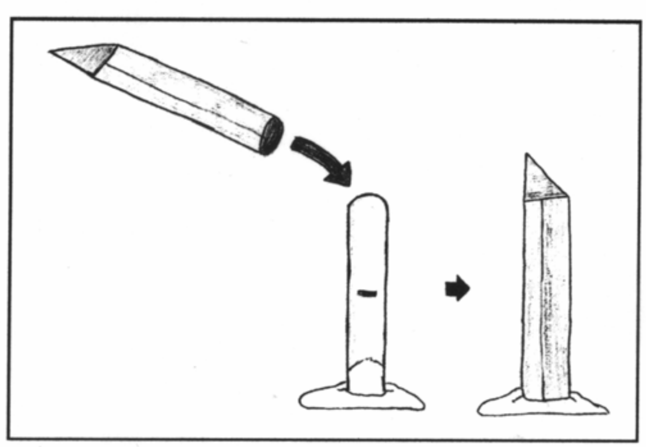

Figure A. Geotaxis chamber (courtesy of Lee Ann Pingel)

6. Let the wasps acclimate inside the tube for 3 – 5 minutes before beginning the exercise, then start your stopwatch. At the end of each minute, remove the black sleeve from the tube, quickly count the number of wasps in the top half, and replace the sleeve. Record the number on your data sheet. Subtract it from the total number of wasps in the sample, and record the result as the number of wasps in the bottom half of the tube. Repeat this procedure for a total of 10 minutes, counting and recording your data at the end of each minute. At the end of 10 minutes, calculate the mean number of wasps in each half of the tube. Add your mean data to the class data on the board or class data sheet.
7. Transfer your wasps to the smaller, empty glass vial by flicking the side of the tube sharply with your finger to dislodge a few at a time onto a plain piece of white paper. Then invert the original vial over them. They should climb back up the sides of the vial on their own. You can use the pipe cleaner to collect any "strays" gently (with a little encouragement, they should crawl up onto it). Replace the cotton stopper in the vial to prevent their escape. Make sure to keep the vial with the "used" females separate from the other vial, so that you can tell them apart.
8. Think about your results. What conclusions, if any, can you reach from the class data about the response of mated female *Melittobia* wasps to gravity? When an organism moves in a direction in response to some external stimulus, scientists call the movement a *taxis*. Movement in response to gravity is called *geotaxis*. If movement toward the stimulus is termed “positive” and away from the stimulus is “negative,” how would you characterize the responses you observed?

Exercise B. Response of *Melittobia* to Light

1. Cut another piece of construction paper, this time just long enough to cover the second tube from the midway line to the open end of the tube. Secure the sleeve with a piece of tape as before.
2. Remove the cotton stopper and, working over a sheet of white paper, transfer the “unused” females from the second container into your experimental tube.
3. Rap your finger against the side of the tube to shake the females toward the closed end, then carefully replace the cotton stopper. Push the cotton stopper in as far as the first line that you drew. Count the number of wasps in the tube and record this number on your data sheet.
4. With a small square of aluminum foil, cover the cotton stopper and part of the bottom of the test tube, crimping the foil tightly against the side of the tube so that no light can enter the tube through the glass at the stoppered end.

Form the poster putty into a ball, and stick this to the piece of white paper on the lab bench. Turn the tube on its side and press it gently into the poster putty ball until it holds securely. Place your head at eye level with the bench top and check to make sure that the test tube side is level with the top of the bench. (Why is this important?)

5. Arrange your light source so that it shines across the rounded end of the test tube, perpendicular to the side of the tube. See Figure B.
6. Allow the *Melittobia* to acclimate for about 5 minutes. Then start your stopwatch. At the end of each minute, record the number of females in the light (uncovered) end of the tube. Subtract this number from the total number of wasps in the tube, and record this result as the number of females in the dark end. Continue counting and recording the information at the

end of every minute until you have 15 minutes of data. At the end of the experiment, calculate the mean number of females at each end of the tube. Add your data to those of the class and transfer your females back to their original vial.

7. Think about your results. What conclusions, if any, can you reach from the class data about the response of mated females to light? Movement in response to light is called *phototaxis*. If movement toward a stimulus is termed "positive" and away from the stimulus is "negative," how would you characterize the light responses you observed? How does the intensity of the phototactic response of these wasps compare with their geotactic response?

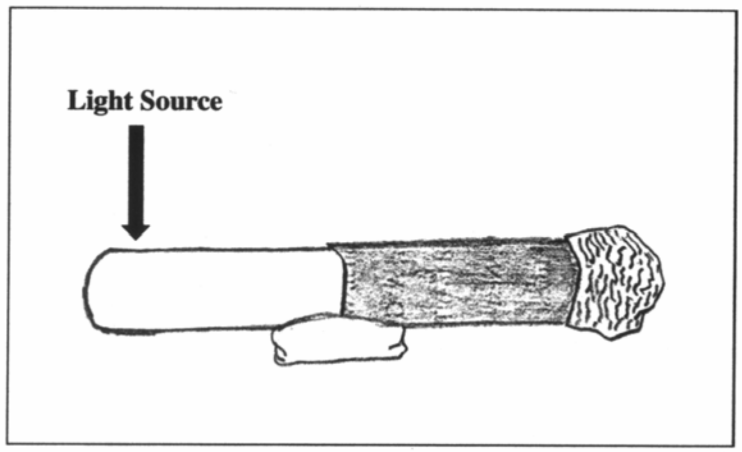

Figure B. Phototaxis Chamber (courtesy of Lee Ann Pingel)

Exercise C. Develop your own hypotheses

1. Use the information in the background section, as well as your results from the experiments you've just performed, to develop a new hypothesis about another cue or condition which might influence the dispersal of mated female *Melittobia* wasps. It might help to imagine what life is like for newly mated females inside the dark host nest. What kinds of cues might guide them to finding their way free? In what ways could you vary the quality or quantity of a particular potential cue to test how that affects the females' response? What about the females themselves? What characteristics of females might affect how they respond to cues? Write down at least one idea that you could test about female dispersal. Your hypothesis should be biologically relevant. In other words, it should involve a condition that these animals might actually face in their natural environment.
2. To test your hypothesis, plan an experiment that uses the supplies available on the lab bench. (Some additional supplies are available beyond those you've already used — check them out!)
3. Conduct your experiment, recording your data as you did for the previous experiments.
4. When everyone has completed their tests, discuss with your classmates the new knowledge you've gained about *Melittobia* dispersal. To which kind of cues do they respond? From the information you now have, is it possible to tell which cues tend to exert the most influence? Why or why not? Are there particular characteristics about the females themselves that affect their response? What influences, cues, or characteristics appeared to have no effect?

Does their behavior in the laboratory seem consistent with their life history? In what ways might it be advantageous in a natural situation? What other ideas for testing can you think of to explore aspects of *Melittobia* dispersal further?

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Literature Cited

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Notes for the Instructor

Background and Rationale

Here, we introduce a three-part laboratory investigation that we hope will stimulate students to consider factors that may influence dispersal behavior. A goal of these activities is to lead students away from the idea that laboratory experiments always have predictable outcomes, or in other words, "right answers." To accomplish this, we begin with an activity (A) that produces quite clear results in the form of negative geotaxis, but then move on to a second exercise (B) for which the results may be somewhat ambiguous. Together, these serve as a springboard from which students start to generate their own questions and experiments (C) about the response of these wasps to environmental or internal cues that might affect dispersal behavior.

Besides being very easy to raise and to work with, *Melittobia* have the added advantage of being relatively unstudied. This characteristic makes them ideal subjects for independent student experimentation. We hope the fact that the answers to many of these questions are unknown will free students from the constraints they may feel to "get it right." Enterprising students may find *Melittobia* to be useful subjects for advanced experimentation, such as senior theses or independent directed study.

Advance Preparations

The *Melittobia* needed for this exercise can be purchased from Carolina Biological Supply Company or can be easily raised from your own cultures; for details, see Guinan et al. (2000). At least 20 mated female *Melittobia* per student will be needed for the first two exercises. We find it convenient to use cotton-stoppered 1-dram glass vials as holding containers for the wasps. We suggest that you load the 10 Melittobia females into the culture tubes for the first experiment prior to class. The easiest way to load that many females into a tube is to invert the tube over one or more of them at a time, thereby taking advantage of their natural tendency to climb upward. Because this lab exercise investigates that very behavior, we don't want to tell the students this trick beforehand.

Many additional experiments are possible; for ideas, see Matthews et al. (1996). For students to design and carry out their own experiments after conducting parts A and B, allow at least an additional 10 females per student. In addition, have available some unmated (virgin) females and some mated females of various ages (e.g., 1-day-old vs. 5- day-old). Other suggested materials for part C might include cellophane in a variety of colors, resealable plastic bags, ice, and a heating pad. Students will also need at least 40 fresh 16 x 100-mm disposable glass culture tubes (without lips) and more cotton balls.

Conducting the Laboratory Exercises

Prior to beginning the exercises, students should have read and become thoroughly familiar with the background information on *Melittobia* and the laboratory procedures (A brief quiz may encourage compliance.) The construction process should take no more than 10 minutes per exercise, including the time required to introduce the *Melittobia* to the culture tubes. Exercise A will take about 15 minutes to complete, including the incorporation of individual results into the class data set. Exercise B will take about 20 minutes for the same process. After exercise B, the instructor should facilitate a discussion of the results. The time required for exercise C will vary, depending on individual student design. The instructor may wish to allow at least 10–15 minutes for discussion of students' results from the first two exercises, and 20–30 minutes for students to develop their own experimental designs. Alternatively, allow students to develop and discuss their ideas for further experimentation for the remainder of the class period, and to conduct student-designed experiments during the following laboratory session. (One advantage of this approach is that it gives students and the instructor time to gather additional materials that may be needed to carry out their designs.)

Expected Results and Applications

The investigation of geotaxis will likely produce very clear results. *Melittobia* show a strongly negative geotactic response. Most will crawl to the top of the culture tube and will usually stay there throughout the exercise. On the other hand, the results of Exercise B may be highly variable. In some cases, mated females appear to be phototactic and congregate at the "light" half of the tube. Sometimes, however, the opposite occurs, and females aggregate at the opposite end. We suspect that their behavior is influenced by a number of factors, including age, mating condition, and the presence of other females (an “aggregation effect”). As you guide a discussion of the results, it is important that you redirect any students who may feel that since the experiment "didn't work", they did something wrong. Rather, encourage them to look at the results and analyze the possible effects of various factors on the behavior of the wasps.

It may be useful to encourage students to visualize life for the *Melittobia* females inside the dark host cocoon. How would light be likely to enter the host mud-dauber nests? Is it likely that there would be large holes in the nest, or tiny cracks through which light might enter? Can students use these ideas to devise experiments that adjust the quantity of the light to determine how that might affect female behavior? To test this idea they can use the black construction paper and scissors or hole-punches to experiment on the effects of different quantities of light on female response.

Help students realize that different organisms perceive the world differently with their senses. It is likely that the spectrum of light that is visible to *Melittobia* is different from that which we see. Do females respond more strongly to some colors than to others? By wrapping the "light" end of the tube with cellophane of different colors, students could easily change the wavelength of the light that the females perceive.

The students might also want to use the ice in plastic bags and the heating pad to investigate the possible effects of temperature on the dispersal behavior of mated females.

Age and reproductive status also might affect female behavior. Therefore, some students may wish to consider the responses of mated vs. virgin females, and older vs. younger females to stimuli.

Invite several students to tackle each of the major possible factors identified by the class. For example, if an aggregation effect seems possible, one possible design would be to run the phototactic experiment again, but with only one female per tube. Each student interested in this question could run several trials simultaneously, and then compare those results with the class results for exercise B. Help students realize that it would be important to use fresh tubes for each trial, because if there is an aggregation affect, it is possible that females leave behind pheromones to signal one another.

These are just some possibilities with which students can experiment. They are likely to come up with still others. Their results can be shared with others through a dedicated email address, <bugdoc@arches.uga.edu> or through *The WOWBugs Bulletin* (Riverview Press, P.O. Box 5955, Athens, GA 30604). There is also a WOWBugs web site <www.entomology.ent.uga.edu/wowbugs>.

We have encountered no difficulty in using these investigations with students who have no previous knowledge of *Melittobia*. However, if you prefer to begin with a more directed activity so that students can become familiar with the species and handling methods, consider starting with “The Great WOWBug Roundup” (pp. 39-42, Matthews et al. 1996) or the similar activity found on the Animal Behavior Society website, <http://www.animalbehavior.org/ABS/education/ABSwebactivity.html>.