

Course-Based Research with Bean Beetles, *Callosobruchus maculatus*, and a Y-maze Olfactometer

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Course-based authentic research is an effective and appropriate substitute for mentored research with undergraduates to both engage students in the process of science and to foster the skills and knowledge development required to successfully pursue advanced studies. Here we describe a very flexible olfactometry system that can be used for a wide range of open-ended experimental studies using the bean beetle, *Callosobruchus maculatus*, model organism. The essence of this olfactometer is a Y-maze in which individual beetles choose between two sources of chemical signals. We will use this system to address questions about mate choice by males in this species. This system may be used with a guided-inquiry pedagogy to start a process that leads to students posing their own questions and pursuing authentic research projects.

Keywords: y-maze olfactometer, bean beetles, *Callosobruchus maculatus*, mate choice, course-based research

Link to Supplemental Materials: <http://www.ableweb.org/volumes/vol-38/smith/supplement.htm>

Introduction

One need not look very deep in the literature on the challenges faced by biologist-educators of undergraduates to conclude that the best laboratory learning environments are those in which our students function as genuine scientists (AAAS 2011, PCAST 2012). This means that students design their own experiments, conduct studies of their own design, make original findings, and pose questions that they develop and wish to explore (Hanauer et al. 2006). Yet, authentic course-based research is not common in undergraduate education (Spell et al. 2014). One potential barrier to the adoption of course-based research pedagogy is the scarcity of easily implemented authentic research activities that are inexpensive, adaptable and flexible

enough to be used to address a variety of questions. The Y-maze olfactometer (Mbata et al. 1999) used in this study meets these criteria and uses the commercially available bean beetle, *Callosobruchus maculatus*, model organism (Beck et al. 2011). The protocol described here may be used initially in a guided-inquiry format, in which the instructor presents the question, to provide a scaffold for students to begin asking their own questions and conducting original research on chemical communication in this insect. “A Handbook on Bean Beetles, *Callosobruchus maculatus*” (Beck and Blumer, 2014) describes all the basic methodologies for handling and maintaining bean beetles and is available at www.beanbeetles.org.

Student Outline

Objectives

Design and perform a set of experiments to evaluate whether male bean beetles (*Callosobruchus maculatus*) discriminate between different females of different sizes based on chemical stimuli.

Introduction

Bean beetles (cowpea seed beetles), *Callosobruchus maculatus*, are insects that complete their lifecycle in the dry seeds of beans (Family Fabaceae). The ability of male and female beetles to use visual cues to find the opposite sex may be severely limited in the natural settings of stored supplies of dried beans, so chemical communication (pheromones) may be important modes of communication in this species. Past studies have clearly demonstrated that adult females of this (Lextrait et al. 1994, Shu et al. 1996) and a closely related species (*C. subinnotatus*) produce chemical cues that males use to seek mates (Mbata et al. 1999). Yet, studies on mate choice characterize this species as having no mate choice or a scramble form of mating in which males mate with any female they encounter (Savalli and Fox 1999). In this study, you are to address the question: Can male bean beetles discriminate between different females by means of chemical stimuli? You will design a series of experiments using an olfactometer apparatus to isolate chemical stimuli from all other modes of communication.

Materials

In class, you will be provided with live cultures of bean beetles containing adults that have been raised on black-eye peas, *Vigna unguiculata*. Female beetles are easily identified in the live cultures because they have two dark stripes on the posterior of the abdomen, whereas the posterior abdomen of males is uniformly light in color. Olfactometers consist of a pair of precision air flow meters connected to a small air pump that will permit you to control air flow rates in a simple Y-maze made of clear plastic tubing. Sources of chemical stimuli may be placed in each arm of the Y-maze and a choosing individual may then be placed in the leg of the Y-maze so it may choose which arm of the Y to enter (see Figure 1).

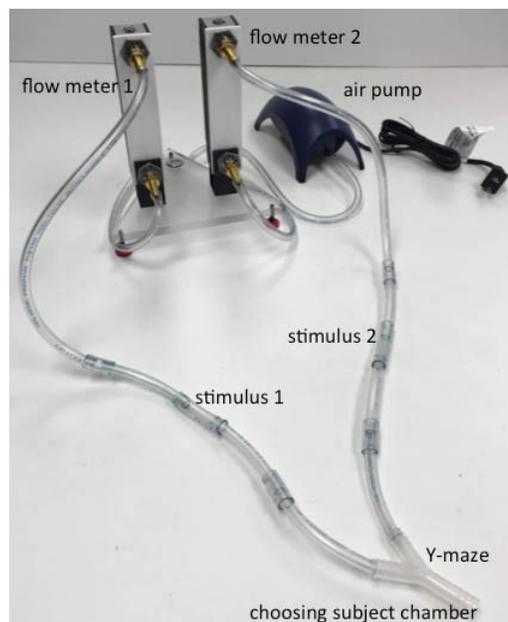


Figure 1. Y-Maze Olfactometer Apparatus. The arms of the Y-maze receive the outflow of two flow meters. The sources of chemical stimuli are held in each arm of the Y-maze and the choosing animal is introduced at the base of the Y (bottom of the photograph).

Experimental Design

Your task is to design an experiment to address the following question: Can male bean beetles discriminate between different females by means of chemical stimuli?

After you have read the background information and before the laboratory class meeting:

- Describe an experimental design for evaluating whether male bean beetles discriminate between different females by responding to pheromone communication.
- Predict the possible outcomes for your experiment.
- Identify and list the variables you would manipulate in the experiment.
- Identify and list the variables you would keep constant in the experiment.
- List the data you would collect to determine if your predictions were true.
- Describe the statistical analyses that you would carry out to test your predictions.

Come to class prepared to present your experimental design. How many replications would you need to conduct to ensure that your findings did not occur by chance alone?

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Materials

Equipment and Supplies

For a class of 20 students working in pairs:

- 20 magnifiers 2.5x, 4" diameter self-standing with folding base ([Fisher #14-648-19](#) or [VWR #62379-535](#), approximately \$50.00 US per unit) or dissection microscopes
- 10 bean beetle cultures with newly emerged adults (Carolina Biological [http://www.carolina.com/bean-beetle/bean-beetle-culture-living/144180.pr?question=Ward's Science](http://www.carolina.com/bean-beetle/bean-beetle-culture-living/144180.pr?question=Ward's+Science) https://www.wardsci.com/store/catalog/product.jsp?product_id=16936668) Canadian faculty should post a message on the ABLE listserv (or email Blumer) to locate sources of bean beetles in Canada.
- 40 Plastic 35mm Petri dishes for isolating adults (Fisher #08-757-100A)
- 20 Plastic 150x25mm Petri dishes to distribute beetle cultures to students (Fisher #08-772-6)
- 32 ounces each of dried black eye peas
- 20 small paint brushes
- 20 soft forceps, Bioquip™ featherweight forceps ([Catalog No. 4748 or 4750](#))
- 20 vernier calipers for measuring beetle size or image analysis software to measure beetles from video microscope images
- permanent markers for labeling Petri plates
- 50 small pieces (1cm²) of fine nylon or polyester mesh to confine beetle movement in Y-maze (mosquito netting mesh available from fabric stores)
- 20 1.5ml plastic microfuge tubes, to transfer beetles to the Y-maze entrance
- small rubber bands (Proclaim Professional Braiding rubber bands #632000, beauty supply store)
- small pieces of Parafilm
- 10 olfactometer units each consisting of:
 - 2 Cole-Parmer 65mm correlated flow meter (#T-32045-00), high-res valve, brass, 5.8ml/min air (\$190.80 each) (Figure 2)
 - 1 #EW-03226-30 Cole-Parmer flow meter tripod base (\$55.80)
 - 4 Threaded adapter, brass, 1/8" NPTM x 1/4" tubing ID, (#T-30904-01) 5 pack (\$8.33) need two for each flowmeter
 - 1 small aquarium air pump, Tetra 77847 Whisper (\$8.99) or similar with a T-

connector to split the airflow (Jarden plastic air valve connectors, 40 pieces, \$4.51)

- 60cm or more clear plastic tubing, Tygon 3/16" ID to connect to aquarium pump to flow meters and to connect flow meters to Y-connector
- 15cm clear plastic tubes, Tygon 5/16" ID to join pieces of 3/16" tubing and create holding chambers in y-maze
- 1 polypropylene 3/8" Y connector

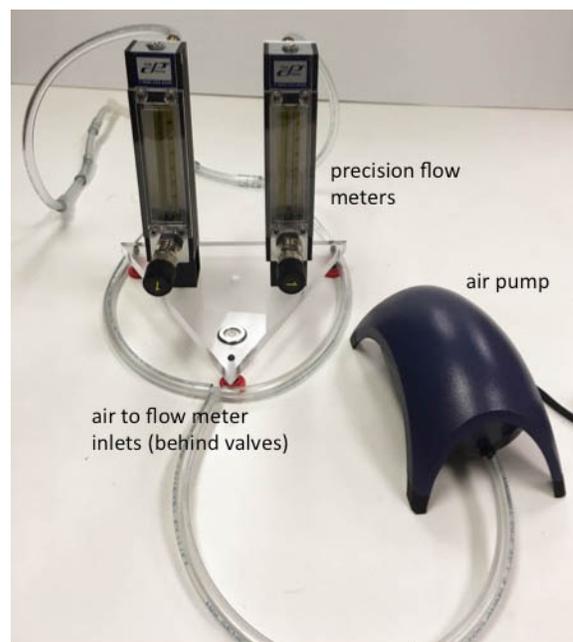


Figure 2. Y-Maze Olfactometer Components. Each olfactometer consists of two flow meters that receive air from a single aquarium pump. The air flow in each arm of the Y-maze can be adjusted to ensure the flow rates are the same. An aquarium pump is a better choice than connecting to laboratory compressed air, unless the compressed air is filtered to remove grease and the pressure does not exceed 200psi. The float ball in these meters is glass and may stick inside the meter tube if the air is contaminated with grease.

Notes for the Instructor

Olfactometer Assembly

The olfactometer consists of two 65mm flow meters mounted as a pair on a tripod base. Each flow meter must also be supplied with two threaded hose adapters that permit the attachment of 3/16" ID tubing. The lower hose adapter on the flow meter is the inflow from the air pump and the upper hose adapter is the outflow to the Y-maze. Pieces of 5/16" ID hose will fit snugly over the 3/16" tubing so small holding chambers can be made in the two arm branches of the Y to contain odor sources. If the odor sources are a live animal, fine mesh screening should cover both pieces of 3/16" tubing that connect in a 5/16" piece. Individual beetles may be easily introduced to the Y-maze entrance by first placing them in a 1.5ml microfuge tube from which the conical end is removed and covered with fine mesh. A confined beetle is introduced to the Y-maze by opening the cap and placing the microfuge tube at the Y-maze entrance. A 3.0cm square of fine mesh can be held on the end of the microfuge tube with a small rubberband (Figure 3A). This arrangement permits air flow through the tube when the cap end is open and applied to the opening of the Y-maze. A small piece of 3/16" tubing connected to the entrance of the Y-maze fitted with a gasket of Parafilm to form a tight fit with the cap end of the microfuge tube will facilitate the introduction of a choosing beetle to the Y-maze (Figure 3B, 3C).

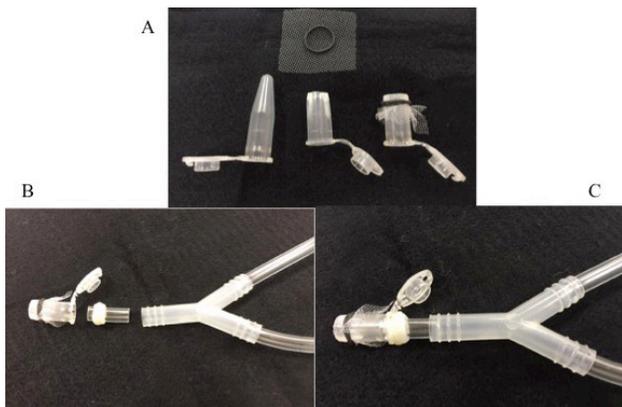


Figure 3. Microfuge Beetle Chambers. We modified 1.5ml microfuge tubes to confine individual beetles prior to introducing them to the entrance of the Y-maze (A). The microfuge chamber makes it relatively easy to introduce an individual to the Y-maze in a standardized manner. A small piece of 3/16" tubing connected to the entrance of the Y-maze fitted with a gasket of Parafilm to form a tight fit with the cap end of the microfuge tube (B) will facilitate the introduction of a choosing beetle to the Y-maze (C).

Given that the most expensive components of this apparatus is the flow meters, faculty facing budgetary constraints may wish to consider alternative designs for the olfactometers. We have not tested these alternatives but suggest that you try them, even with your students, as an authentic investigation of experimental methods. For example, you may be able to conduct experiments without precise airflow control if care is taken to ensure that both sides of the Y-maze consist of the same lengths and diameters of tubing coming from a single air pump. Similarly, a single flow meter may be sufficient to control air flow in both sides of an olfactometer while permitting you to ensure that different set-ups have the same air flow rates.

Handling Bean Beetles

Consult "A Handbook on Bean Beetles, *Callosobruchus maculatus*" (Beck and Blumer, 2014) for detailed information on growing cultures, handling techniques, and methods of safe disposal (available for downloading at: <http://www.beanbeetles.org/handbook> and in the supplemental materials for this major workshop). In addition, tips on identifying the sexes including pictures of a male and female are available at: <http://www.beanbeetles.org/handbook/#IS>.

The student handout is written for students to design their own experiments, rather than instructors giving students explicit directions on how to conduct their experiments. No matter the exact experiment that students design, the experiments will require having dense cultures of bean beetles from which females and males can be isolated. If new cultures are initiated approximately 2 months before the lab period, there will be sufficient time for two generations of beetles, which will result in dense cultures. When possible, we supply one culture to each group of students. However, cultures should have sufficient beetles for multiple groups. Newly emerged cultures work better for this experiment than older cultures. Some student-designed experiments may require virgin males and females. Virgins are easily staged by isolating single eggs beans in the wells of tissue culture plates until adult emerge as described in Beck and Blumer (2014).

Instructors should caution students to prevent the accidental release of bean beetles from the laboratory environment. *Callosobruchus maculatus* is a potential agricultural pest insect that is not distributed throughout the United States and Canada. It is essential that you keep your cultures secured in a laboratory environment to ensure that they are not released to the natural environment. Disposal of cultures (and beans [seeds] exposed to live beetles of any life cycle stage) requires freezing (0°C) for a minimum of 72 hours prior to disposal as food waste. If you have any questions about

the handling or disposal of bean beetles, please contact Larry Blumer at lblumer@morehouse.edu or 470 639-0283 (voice or FAX). Information also is available at: www.beanbeetles.org in the Handbook section.

Experimental Design

We use a guided-inquiry method to begin this study, by posing a starting question for students to use as the basis for their first experiments using this Y-maze olfactometer. After the first experiments are conducted and the findings analyzed, students should begin asking additional testable questions that they may explore.

An experimental design we have conducted consists of a large female and a small female in different arms of the Y-maze (Figure 4). Control treatments may consist of a Y-maze with a female in one arm of the Y and nothing in the other arm, or an empty Y-maze containing no stimuli in the arms of the Y. Conducting a minimum of two trials with each choosing animal but with the location of the stimuli alternating between trials would control for side bias (for example, preferring the right side of the Y-maze) by the choosing animal.

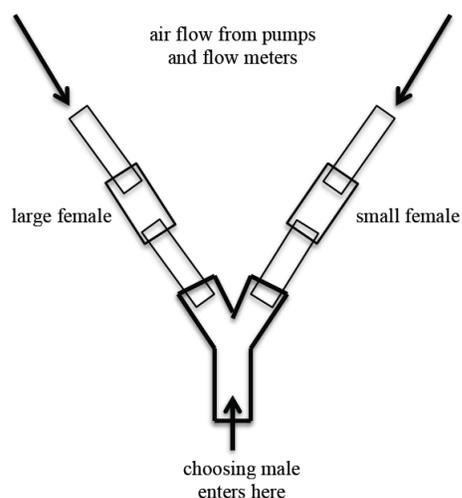


Figure 4. Example Experimental Design. In this example, a large female is the stimulus in the left arm of the Y-maze and a small female is the stimulus in the right arm. A choosing male enters at the base of the Y-maze and choice is indicated by the arm of the Y-maze to which he moves during a 10-minute trial. A second trial with the same male would be conducted after flipping the Y-maze (leaving the females in their original positions) to put the large female on the right and the small female on the left. A male who consistently chose the same female (for example, the large female) in those two trials would be scored as a chooser who chose the large female. Males who did not choose consistently in their two trials were scored as non-choosers.

Data Analysis

Regardless of the type of stimuli presented in the arms of the Y-maze, the data consist of the choice that the choosing male makes, which arm of the Y-maze he enters. These binomial data (like coin flipping data) are most appropriately analyzed with a binomial test. This statistical test is easily performed using the on-line VassarStats website <<http://vassarstats.net>>. On the VassarStats homepage, select "Frequency Data" from the left side menu and then "Binomial Probabilities" from the page that opens. Enter the number of independent trials conducted (n), the number of responses toward a given stimulus (k), and the expected probability for the null hypothesis (p) which should be 0.5 if responses are random. Then click "Calculate" to calculate the probabilities. The probabilities "For hypothesis testing" are the appropriate values to consider. Both one-tailed and two-tails probabilities are calculated.

Previous Results

In 2014, 43 different males were evaluated in the Y-maze olfactometer to choose between a small female (smaller than 4mm total length) and a large female (larger than 4mm total length). Every male was tested twice, with the larger female on the right side and then on the left side of the Y-maze. These 43 males made consistent choices regardless of the location of the larger female. A significantly greater number of males (32) chose the larger female, while 11 chose the smaller female (Binomial test, $p < 0.01$, two tailed). In 2015, 54 different females were evaluated in a similar manner to choose between a small male (smaller than 3mm) and a larger male (larger than or equal to 3mm). Every female was tested twice to control of side bias. Only 32 of the 54 females made consistent choices and there was no significant preference shown by those females. A total of 17 females chose the larger male and 15 chose the smaller male (Binomial test, $p = 0.86$, two tailed).

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Acknowledgments

We thank the John Hopps Scholars Program at Morehouse College for supporting the mentored research that became the basis for this laboratory teaching protocol. We also thank the participants in our ABLE workshops who provided critical and constructive peer reviews that helped us improve this manuscript.

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Citing This Article

Smith RT, Blumer LS. 2017. Course-Based research with bean beetles, *Callosobruchus maculatus*, and a y-maze olfactometer. Article 17 In: McMahon K, editor. *Tested studies for laboratory teaching*. Volume 38. Proceedings of the 38th Conference of the Association for Biology Laboratory Education (ABLE). <http://www.ableweb.org/volumes/vol-38/?art=17>

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