HOW TO DO IT

# Guided-Inquiry Labs Using Bean Beetles for Teaching the Scientific Method & Experimental Design

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**Abstract**

*Guided-inquiry lab activities with bean beetles (*Callosobruchus maculatus*) teach students how to develop hypotheses, design experiments, identify experimental variables, collect and interpret data, and formulate conclusions. These activities provide students with real hands-on experiences and skills that reinforce their understanding of the scientific method and experimental design. This teaching method can easily be adapted to other test organisms or alternative themes.*

**Key Words:** *Guided inquiry; bean beetles; scientific method; experimental design; experimental variables.*

Although students in college-level introductory biology courses know the steps of the scientific method, many have not had the chance to apply them in a laboratory setting. Students lack the skills to design an experiment and do not understand the importance of experimental variables. To address this problem, we chose to conduct guided-inquiry labs using bean beetles (Prince & Felder, 2007). A guided-inquiry approach uses inductive teaching to challenge the students while still providing explicit and scaffolded instruction.

*Bean beetles are significant agricultural pests of stored food in Africa and Asia.*

## ○ Life History & Life Cycle

*Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) is a small, 3- to 4-mm beetle. Bean beetles are significant agricultural pests of stored food in Africa and Asia. The larvae of this species grow inside and feed exclusively on the seeds of legumes (Fabaceae) (Mitchell, 1975; Messina, 1991). Males and females are sexually dimorphic. Females are larger and have a black abdomen with a white stripe down the center. By contrast, males have a brown abdomen (Figure 1).

Males and females mate shortly (10–15 minutes) after contact. Once inseminated, an adult female will lay a single fertilized egg on the surface of a bean (e.g., mung, blackeyed-pea, etc.). The white egg is 0.5 to 1 mm in length. After a few days, the larva hatches and burrows through the seed coat into the bean's endosperm. The larva feeds on the bean endosperm and embryo. Over the next month, the larva undergoes a series of molts. After the fourth molt, the larva burrows to a position just underneath the seed coat and enters the pupa stage. After a few days, an adult emerges. The newly hatched adult chews through the seed coat and emerges from the bean. Adults are fully mature 24–36 hours after emergence. Males seek females to inseminate, and females continually lay eggs during their short life span of 10–14 days. During this period, adults are believed not to feed or drink water. The complete life cycle from egg to adult takes about 25–30 days at 30°C (Mitchell, 1975; Messina, 1991; Blumer & Beck, 2011).

Bean beetles are an excellent model system for use in undergraduate laboratory courses (Blumer & Beck, 2011). They are cheap, available at Carolina Biological Supply (http://www.carolina.com), grow on dry beans (mung, adzuki, blackeyed peas, etc.), are easily handled and sexed by students, and take up little space in the lab.

## ○ Procedure

A five-step scaffolded approach was taken to successfully train students in the use of the scientific method and experimental design. This approach required two laboratory periods.

### 1. Introduce the experimental organism: the bean beetle

A brief (10–15 minutes) lecture introduces students to the bean beetle. Using a series of images, the instructor trains the students to recognize this species and identify the sexes. The bean beetle's life cycle (egg → larva → pupa → adult) is then discussed. Next, students are given Petri dishes containing beetles growing on mung beans and are trained how to observe and handle the beetles.

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**Figure 1.** A female (left) and a male (right) adult bean beetle. Females are typically larger than males and have a black abdomen with a light-colored stripe down the center (arrow). By contrast, the male body is all brown.

### 2. Engage the students in experimental design and data analysis using a previously set-up experiment

Immediately following the introduction, a brainstorming session is facilitated by the instructor. The whole class participates in the design of a controlled experiment to answer the question "If given a choice of various bean types, do beetles prefer to lay their eggs on the bean type from which they hatched (natal bean)?"

Facilitated by the instructor, the students formulate a hypothesis and design an experiment to test it. The class defines the control group, experimental group, independent variable, dependent variable, and controlled variables and decides the number of replicates that are appropriate. The instructor then provides the class with the results of the previously set-up experiment. Students tally the data and make conclusions on whether the data support or reject the hypothesis that was developed. The lab is concluded with a discussion of the strengths and weaknesses of the experiment.

*Experimental setup:* The instructor sets up the experiment as follows, a week before the laboratory. This will provide "real data" for students to tally while in the lab. The instructor places three virgin male beetles and three virgin female beetles hatched from mung beans (natal bean) into Petri dishes containing an equal number of mung, adzuki, and black-eyed peas (10 beans each) for about 1 week. At this point the plates are ready for the students to tally the number of eggs on each bean type to determine whether the beetles had a preference for their natal bean or not (Blumer & Beck, 2011).

### 3. Student groups choose a question and design their own experiment with appropriate sample sizes and controls

Students are divided into groups of four and are told that they will perform their own group experiment during the next lab period. They are provided a list of bean beetle observations and questions, as well as a list of materials that will be available for their use (Figure 2). Students meet together outside of class before the next laboratory period and use an experimental design worksheet for guidance (Figure 3) to pick a question, develop a hypothesis, identify the experimental variables, and design a protocol with appropriate controls and replicates to test their hypothesis.

*An example of a typical student experiment:*

**Observation** (from Figure 2): It is claimed that adult bean beetles do not need to eat or drink.

**Question:** Would adding suitable food or water increase the life span of the adult bean beetle?

**Hypothesis:** If food or water was provided, adult bean beetles would live longer.

**Control group:** 2 males and 2 females placed in empty Petri dish, no beans

**Experimental group A:** 2 males and 2 females placed in Petri dish containing a few drops of water

**Experimental group B:** 2 males and 2 females placed in Petri dish containing some fruit fly media

**Experimental group C:** 2 males and 2 females placed in Petri dish containing some baker's yeast

**Experimental group D:** 2 males and 2 females placed in Petri dish containing whole mung beans

**Independent variable:** The food item or water given to the beetle

**Dependent variable:** Life span of the beetle measured in days

**Controlled variables:** Equal number of newly hatched males and females, all placed at the same temperature in a lab drawer.

*Experimental Design:*

- There will be 5 groups (1 control and 4 experimental).
- Each group will have 3 replicates, or 3 Petri dishes.
- Petri dishes will be stored in a lab drawer at room temperature.
- Each group will be checked every day around the same time and the number of dead beetles will be recorded.
- The experiment ends when all the beetles are dead.

### 4. Perform the experiment in class

At the beginning of the lab, the instructor reviews each group's experimental design, and the students present it to the class. The students set up their experiment and, in the next few weeks, gather data and make conclusions on whether their data support or reject their hypothesis.

### 5. Reflection step

Students consider what worked well or did not work with their experiment. Was their design flawed? Were there enough replicates? Were there unanticipated factors that flawed the experiment? What would they do different next time? All of the results and conclusions are summarized in a brief laboratory report.

## ○ Conclusions

Students who performed our scaffolded five-step approach understood the scientific method and experimental design much better

| BEAN BEETLE EXPERIMENT |
|---|
| **Goal:** To formulate a hypothesis and design an experiment to test your hypothesis. |
| Today, each lab group will choose one of the following observations and questions to pursue further. Next week, your group will perform an experiment, designed by you, to test the question that you have chosen to investigate. Prior to beginning the experiment next lab, each group will meet outside of class and prepare a 5-minute presentation on their question, hypothesis, and the experiment that was designed by the group. |
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| OBSERVATIONS AND QUESTIONS ABOUT BEAN BEETLES |
| **Males are driven to find females and mate with them. Typically, males find females and begin mating within 15 minutes. Male beetles have been observed attempting to mate with other male beetles.** |
| (1) What senses do males use to find their mates? |
| (2) Does mating decrease or increase a beetle's life span? |
| (3) Does the presence of females reduce or increase the number of male-to-male mating attempts? |
| (4) Does the presence of extra male beetles increase or decrease the time it takes for a male to successfully mate with a female beetle? |
| |
| **It is claimed that adult bean beetles do not need to eat or drink.** |
| (5) Does the presence of food increase the life span of a bean beetle? |
| (6) Does the presence of light or darkness affect the life span of a bean beetle? |
| |
| **Females show a preference for laying eggs on their natal bean (the bean they hatched from).** |
| (7) Does the size of a natal bean affect whether a female will lay an egg on it? |
| (8) Does the presence of an egg on a bean affect whether a female will lay a second egg on it? |
| (9) Is a seed coat necessary to lay eggs? |
| (10) What makes the natal bean attractive to the female: its color or shape? |
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| SUPPLIES AVAILABLE |
| Virgin male and female beetles |
| Non-virgin male and female beetles |
| Mung beans with seed coat, without seed coat, and with eggs |
| Other bean types (adzuki beans, black-eyed peas, chick-peas, black beans) |
| Water, yeast, fruit fly media |
| Petri dishes, scissors, microscopes, electronic balances |
| Beetle "storage" areas include (a) cool area, (b) warm area, (c) dark area, and (d) light area |

**Figure 2.** Guided-inquiry experiment guidelines and resource worksheet given to students to assist them in developing a hypothesis and designing their experiment.

than students who did not participate. Data from assessment tests are reported in a separate article (D'Costa & Schlueter, 2013). In post-laboratory surveys, more than 90% of students indicated that they enjoyed performing their own experiments, compared to traditional laboratory exercises. More than 90% felt that this experience improved their understanding of the scientific method and experimental design.

## ○ Suggestions for Classroom Implementation

Fresh cultures of bean beetles can be obtained from Carolina Biological Supply. Stocks are made by growing the beetles to sufficient numbers in mason jars containing whole mung beans (or other bean type) covered with mesh. After 4–6 weeks at room temperature

## EXPERIMENTAL DESIGN WORKSHEET

**You will need to answer each of the following questions in your experimental design paper. This paper is due at the beginning of the lab, before you perform the experiment. Each group will give a 5-minute presentation defining their question and detailing the experiment that they plan to perform.**

1. State your question (or reword the question).

2. State your purpose.

3. State your hypothesis.

4. List your variables.
   a. What is your independent variable?
   b. What is your dependent variable(s)?
   c. What is your controlled variable(s)?

5. Design your experiment.
   a. What materials or organisms will you need? How many?
   b. Write out a step-by-step procedure.

* Consult your "Available Supply List"
* Remember to include a control group
* Remember to include replicates in your experimental design
* Statistics might be useful (e.g., t-test, ANOVA)

**Figure 3.** Experimental design worksheet.

(or 3–4 weeks at 30°C), new beetles will hatch in large numbers, and they are ready to divide further.

Stocks in mason jars should be replenished with fresh beans every 4–6 months. Because bean beetles are potential agricultural pests, the old cultures should be placed in a freezer for 4 days and then thrown in the trash.

To obtain virgin females and males, add fresh mung beans to a Petri dish along with several males and females. Allow females to lay eggs for several days. Beans with a single egg must be isolated (for example, each placed in a well of a 96-well plate), so that when the adult hatches, it remains a virgin.

## Alternative Approaches

The five-step approach outlined above with bean beetles can be easily modified to other organisms such as daphnia, flour beetles, and fruit flies, which are inexpensive and easy to obtain. Different question themes can be used to tailor this method to the class. Teachers can focus on specific physiology themes (e.g., digestion or reproduction) or behavior themes (e.g., mate detection or temperature tolerance). Ecology themes could be explored using organisms such as daphnia (prey) and hydra (predator).

Because it is a relatively new model system, there is still much to learn about the behavior, development, and genetics of the bean beetle. Questions leading toward experimental design can be tailored to these different themes.

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