Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 37, Article 10, 2016

Bean Beetle Nutrition and Development Lab: An Iterative Approach to Teaching Experimental Design

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In this lab, students work first independently and then collaboratively to formulate a novel hypothesis and design an experiment to test it. Bean beetle (Callosobruchus maculatus) larvae can develop inside multiple types of host beans, but they develop more quickly and grow larger in more nutritious beans. Students test the effects of a nutrient of interest on larval development by adding it to flour made from nutrient deficient beans. A female beetle will lay eggs on a gelatin capsule full of the modified bean flour, as she would on a natural bean. The larvae developing inside the experimental capsules are compared with control capsules.

Keywords: inquiry-based learning, hypothesis formulation, experimental design, bean beetles

Introduction

When covering the nature of science, biology educators often emphasize that science is a process of inquiry, not simply a collection of facts. Students rarely experience this full process in lab courses because original research is time consuming, resource-intensive, and seldom successful the first time around. The step-by-step procedures commonly used in lab courses shield students from much of the failure and frustration of authentic research, but they also generate misconceptions about the nature of science.

Inquiry-based labs allow students to actively participate in observation, hypothesis formulation, prediction, and protocol development. The openness of inquiry can range from guided lab activities to original research of publishable quality. For example, Hawkins and Ferzli (2014) developed a successful model for incorporation of authentic, hypothesis-driven research into an undergraduate biology curriculum. One challenge they noted is that novice students are particularly reluctant to formulate hypotheses before they fully understand the science behind the experimental system. In a review of the recent literature on inquiry-based teaching in undergraduate biology lab courses, Beck et al. (2014) found that the majority of inquiry-based labs are intended for upper level majors students. Thus inquiry-based labs that are accessible to novice students are needed.

Bean beetle labs are particularly attractive for introductory and non-majors courses because students face fewer technical and conceptual hurdles than they would in microbiology or molecular biology labs. Less time is required for students to understand the system, and more time can be spent designing, refining and implementing experiments. Bean beetles are also a great low-cost, low-tech option for educators with limited resources or large class sizes (Beck and Blumer, 2006).

This module serves as an inquiry-based introduction to experimental design for first year and non-majors biology labs. Students learn about bean beetle development while becoming familiar with hypothesis testing, independent and dependent variables, controls, and other experimental concepts. Students develop and refine their own designs in an iterative process that begins with independent thinking, then continues with group work, and then is finalized in a guided class discussion. Thus, students are presented with the challenge of designing an experiment from scratch, but they reap the benefits of collaboration with their peers and guidance from the instructor before actually conducting the experiment.

In this module, students are provided materials and background information, and are guided through the process of designing an experiment from scratch. The materials include flour from two bean types, gelatin capsules, and a variety of vitamins, minerals, amino acids,

and sugars. Students search the USDA National Nutrient Database to compare the composition of two different beans. Based on this information, they chose a nutrient to test and add it to a bean flour of low nutritional quality. The powder is packed into gelatin capsules, female beetles oviposit on the capsules, and larvae develop inside. After a few weeks of development, students evaluate the impact of their nutrient of interest by comparing developmental rates in their experimental capsules to positive and negative controls. The entire module is designed to take place over four lab periods, with the second and fourth lab separated by approximately three weeks.

Student Outline

Bean Beetle Nutrition and Development
Handout 1

Objective

In this lab module, you will formulate a novel hypothesis about bean beetle nutrition and development, and design an experiment to test it. You will set up your experiment during the next period, and data will be collected later in the course.

Background

While female beetles typically prefer to lay their eggs on the same type of bean they emerged from, they will readily lay eggs on other bean types if the natal bean is not available. In fact, they will even lay their eggs on a marble if they are not given another choice! This characteristic of bean beetles makes them very amenable to experimentation. When placed in a petri dish with gelatin capsules full of bean powder, a female will lay her eggs on the capsule, and the larva will chew through the gelatin shell and burrow down into the powder where it grows and develops.

At 30°C, it takes 3-4 weeks from the time an egg is laid for a beetle to emerge as an adult from a mung bean. This generation time is about twice as long for beetles reared on adzuki beans, and fewer larvae emerge successfully. The nutritional quality of a bean will influence the rate of survival and development of beetle larvae. Your task is to design an experiment that evaluates the importance of a specific nutrient.

Since this course is not long enough to track beetles until they emerge, you will need to think about other possible ways to measure the progress of bean beetles.

Materials

As in the previous lab, you will be given live cultures of beetles that have been raised on mung beans. You will also be provided with ground adzuki and mung bean powder, and empty gelatin capsules. The following substances will be available:

Table 1. List of available nutrients.

Vitamins	Minerals	Sugars	Amino Acids
-Vitamin C (ascorbic acid) **Other nutrients** - Soy protein - Albumin (protein)	- Calcium Chloride - Sodium Chloride - Potassium Chloride - Zinc Chloride	- Glucose - Sucrose - Maltose - Lactose - Raffinose (sugar found in beans)	- Alanine - Aspartic acid - Glutamic acid - Isocitric acid - Isoleucine - Lysine - Tryptophan - Valine

Online Resources

In order to generate an informed hypothesis, you will have to do some research on the nutritional composition of different bean types. Use the USDA National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/) to compare components of mung beans and adzuki beans. Based on your findings, formulate a specific hypothesis about why emergence rates are higher and development is faster for beetles reared on mung beans than for beetle reared on adzuki beans. When you search mung and adzuki, a list of options will come up. Be sure to choose mature seeds, raw. Then click on "Full Report (All Nutrients)" for a complete list of nutrients.

For more information about bean beetles, the following website is a great resource: www.beanbeetles.org. Under the Laboratory methods header, you will find detailed information on bean beetle development and generation time.

Reference your textbook or online resources to check that your design includes all of the key components of a good scientific experiment.

Your Assignment: Design an Experiment

After you have read the background information:

- Formulate a specific hypothesis about bean beetle development and nutrition.
- Design an experiment to test it.
- Predict the outcome of your experiment. What will you learn from the data you collect?

Handout 2

Table 2. Bean composition comparison chart, adapted from the USDA National Nutrient Database for Standard Reference.

Nutrient	**Unit/100g**	**Mung Bean**	**Adzuki bean**
Proximates			
Water	g	9.05	13.44
Energy	kcal	347	329
Protein	g	23.86	19.87
Total lipid (fat)	g	1.15	0.53
Ash	g	3.32	3.26
Carbohydrate, by difference	g	62.62	62.9
Fiber, total dietary	g	16.3	12.7
Sugars, total	g	6.6	
Minerals			
Calcium, Ca	mg	132	66
Iron, Fe	mg	6.74	4.98
Magnesium, Mg	mg	189	127
Phosphorus, P	mg	367	381
Potassium, K	mg	1246	1254
Sodium, Na	mg	15	5
Zinc, Zn	mg	2.68	5.04
Copper, Cu	mg	0.941	1.094
Manganese, Mn	mg	1.035	1.73
Vitamins			
Vitamin C, ascorbic acid	mg	4.8	0
Thiamin	mg	0.621	0.455
Riboflavin	mg	0.233	0.22
Niacin	mg	2.251	2.63
Pantothenic acid	mg	1.91	1.471
Vitamin B-6	mg	0.382	0.351
Amino Acids			
Tryptophan	g	0.26	0.191
Threonine	g	0.782	0.674
Isoleucine	g	1.008	0.791
Leucine	g	1.847	1.668
Lysine	g	1.664	1.497
Methionine	g	0.286	0.21
Cystine	g	0.21	0.184
Phenylalanine	g	1.443	1.052
Tyrosine	g	0.714	0.591
Valine	g	1.237	1.023
Arginine	g	1.672	1.284
Histidine	g	0.695	0.524
Alanine	g	1.05	1.16
Aspartic acid	g	2.756	2.355
Glutamic acid	g	4.264	3.099
Glycine	g	0.954	0.756
Proline	g	1.095	0.874
Serine	g	1.176	0.976

Handout 3

Group Experimental Design Sheet Group#_____

Names: _____

1. Hypothesis:
2. Independent variable:
3. Dependent variable, how will you quantify it?
4. Experimental group:
5. Negative control group:
6. Positive control group:
7. What concentration of nutrient will you use? _____(unit/100g)
8. How many capsules per dish will you use? _____
9. How many replicate dishes will you make for each group? _____
10. Total number of capsules: _____
11. Each capsule holds about one gram of powder. Estimate the total mass of bean powder you will need for:
    a. Mung: _____(g)
    b. Adzuki: _____(g)
12. Estimate the total mass of nutrient you will need: _____(g) of _____
13. Draw the layout of your data table below:

14. What kind of data would (a) support and (b) refute your hypothesis?

Handout 4: Bean Beetle Nutrition and Development Data Collection

Name_____ Group #_____

Instructions for Data Collection:

1. Examine the capsule for "emergence windows." After going through several developmental stages, larvae will burrow into a position right next to the outer shell of the bean, or capsule in this case, and form 1-2mm windows before they chew through the capsule and emerge. That means the larva is almost fully developed. Make note in the table below of how many of these windows you find on your capsules.
2. Count the number of eggs on each capsule.
   Optional step: to ensure that eggs were viable, use the point on a dissection probe to feel for an indentation inside of the capsule where see eggs. If you feel a groove, then the larva has successfully chewed through the capsule. You can take down the total number of eggs and/or the number of viable for each capsule in your data table.
3. Open up one capsule at a time and empty the contents into a petri dish. Sift through the powder with a dissection probe and gently break open clumps to search for larvae. When in doubt: if it's not squishy, it's not a larva. Record the number of larvae you find for each capsule in your emergence data table. Do NOT waste time searching for larvae if you don't see any eggs on the capsule!
4. Record the mass of 3 weight boat (1 for each treatment) and use forceps to gently transfer any larvae you find. Do your best not to squish them, and be sure to keep the larvae from different treatment groups separate, as you will weigh them and get an average larval mass when you are done. Also count how many larvae appear to be molting, or shedding their skin. This means that the larvae are far along in development.
5. After you have thoroughly searched the powder of one capsule for larvae, dump the powder and capsule into a waste collection dish.
6. Fill out the two data tables provided and note any additional observations in your lab notebook.

Calculations

Emergence rate = (number of adult beetles emerged) / (# of eggs laid)

Expected emergence rate = (number of larvae found) / (# of eggs laid)

Table 3. Larval Development Raw Data

Mung (Positive Control Group)				
Capsule	# of emergence windows	# of eggs	# of larvae	Expected emergence rate
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
Average:				
Total number of larvae:			Total mass of larvae:	

Experimental Group:_____ (Describe treatment)

Capsule	# of emergence windows	# of eggs	# of larvae	Expected emergence rate
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
Averages:				
Total number of larvae:			Total mass of larvae:	

Adzuki (Negative Control Group)

Capsule	# of emergence windows	# of eggs	# of larvae	Expected emergence rate
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
Average:				
Total number of larvae:			Total mass of larvae:	

Table 4. Larval Development Averages

	Mung (Positive Control)	**Experimental Group**	**Adzuki (Negative Control)**
Average expected emergence rate:			
Average larval mass:			

Handout 5

Your Assignment: Describe your Results

When you are done collecting data, you will need to find out if your results are statistically significant. A t-test is a way of seeing if the averages of two groups are significantly different. It is not sufficient to just enter the averages; the test requires that you enter all of the data points. In this case, the data you enter will be the emergence rate for each capsule. **If you had any capsules without eggs on them, do not enter an emergence rate of zero for that capsule: simply omit it from your data set.** However, for capsules with eggs but no larvae, you should enter zero as the emergence rate. To test if emergence rates between your treatment groups are significantly different, enter the emergence rate from each capsule into the following website:

http://www.graphpad.com/quickcalcs/ttest1.cfm

You can only compare two treatment groups at a time, so you will have to run two separate tests to see if your experimental group is different from the positive control, and if it is different from the negative control. You may want to compare the difference between your positive and negative control groups as well.

Written Assignment

Write a short paper summarizing your experiment, and create a figure that depicts your results. Are your results statistically significant? Was your hypothesis supported, refuted, or was your experiment inconclusive? Do any of your measures (larval mass, expected emergence rate, etc.) suggest conflicting results? Discuss potential sources of error, and explain how those errors might be remedied in future studies.

Materials

For a class of 25 students working in groups of five:

- 5 bean beetle cultures with newly emerged adults
- 5 large plastic petri dishes to hold beetle cultures and for picking adults females from cultures
- Organic mung beans, *Vigna radiata*, dried beans, organically grown
- Organic adzuki beans, *Vigna angularis*, dried beans, organically grown
- 1 flour mill
- 500 gelatin capsules (size "0")
- 10 small funnels
- 15 racks from 200 µL pipette tip containers
- 15 dissection probes for packing capsules
- weigh boats for weighing nutrients and larvae
- weigh paper
- 2 balances (for weighing .001- 100g)
- 15 containers for mixing bean powder and loading capsules
- 25 paintbrushes
- 25 soft forceps, Bioquip™ featherweight forceps (Catalog No. 4748 or 4750)
- 50 petri dishes (plastic) for holding capsules (35mm) (Falcon 351008)
- 5 permanent markers
- Optional: 25 magnifiers 2.5x, 4" diameter self-standing with folding base (Fisher #14-648-19 or VWR #62379-535, approximately $50.00 per unit) or dissection microscopes
- All or some of the nutrients from table 1 in powdered form
- A 30 °C incubator

Notes for the Instructor

An Iterative Approach to Teaching Experimental Design

The activities in this lab module are intended to take place over three to four class periods: (1) introduction to bean beetles, (2) discussion and revision of experimental designs, (3) setting up the experiment, and (4) data collection 3-5 weeks later. Periods 1 and 2 can be combined if the experimental design homework assignment is handed out before the first period. The handouts are designed to facilitate an iterative process of experimental design, and thus students should receive them one at a time. A detailed timeline for the module can be found in Appendix A.

Observation and Familiarization with the Subject (Period 1)

At this stage, students are introduced to the natural history and life cycle of bean beetles. Students observe and interact with them, identify eggs on a natural bean, and learn how to sex them. A complete handbook with background, culturing and handling information can be found at http://www.beanbeetles.org.

Independent Hypothesis Generation and Experimental Design (Homework)

After becoming familiar with bean beetles, students are prompted to generate a specific hypothesis and design an experiment to test it. Students are directed to the USDA National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/). Table 1, the list of available nutrients can be modified based on existing lab supplies.

Group Sharing and Consensus (Period 2)

Students share their designs with 2-4 peers, and work together to come up with a common experimental design. Handout 2 (optional) contains a comparison chart of the nutrient compositions of mung beans and adzuki beans to facilitate discussion.

Guided Class Discussion and Consensus (Period 2)

One representative from each group briefly shares their proposed design. Then the class as a whole will discuss the strengths and weaknesses of different approaches. The instructor has the liberty to guide the discussion towards desired learning objectives and clarify misconceptions. Topics include independent and dependent variables, positive and negative controls, nutrient dosage, sources of error, replication, and statistics. The instructor may want to delve deeper into some concepts and gloss over others. The class should come to a consensus on a basic protocol and the number of replicates.

Group Design and Instructor Approval (Period 2)

In this final stage, groups receive handout 3, which calls for an explicit statement of the full design and details of how it will be carried out.

Tips for Guiding Experimental Design

After looking at the USDA database, students will notice that mung beans are nutritionally superior to adzuki beans in most respects. It is important for students to understand that developing organisms require a complex suite of nutrients, and differences in developmental rates are not likely due to one nutrient or one group of nutrients alone. The hypothesis should be something a long the lines of "Bean beetles develop more quickly on mung beans than adzuki because mung beans have a higher concentration of nutrient X."

Vitamin C is often identified as a nutrient to test because it is absent in adzuki beans and present in mung. Calcium and glucose are other common choices. One group of students successfully added a whole suite of

amino acids to adzuki powder to match their respective concentrations in mung beans, so a group of nutrients can be tested if calculations are done carefully. Depending on how many replicates groups will be making, it may be a good idea to encourage multiple groups to test the same nutrient and compare results.

The most biologically relevant experimental setup would be to add enough of a nutrient to adzuki powder to match its concentration in mung beans. Students in the past have done experiments to test the optimum level of a certain nutrient by testing multiple concentrations. Be sure to warn students that increasing the concentration of one nutrient means simultaneously decreasing the relative concentration of all other nutrients that might also be important. Students should use capsules full of plain mung powder as a positive control and capsules full of plain adzuki powder as a negative control. Students might also be interested in comparing the rate of development of bean beetles reared on capsules with the development rate on real beans.

Tips for Setting up the Experiment

Preparing Powder

Stocks of mung and adzuki beans should be very finely ground in a four mill before the lab. Students will calculate the amount of mung and adzuki flour they will need, and the appropriate concentration of their chosen nutrient (handout 3). Each group should mix the nutrient of interest into their experimental bean flour in batch quantity.

Filling Capsules

The instructor should demonstrate how to fill capsules before the start of lab. Most funnels are not small enough to channel powder into the capsules, so a 1mL pipette tip can be attached to decrease the diameter of the funnel tip (Figure 1). The end of the tip can be cut at an angle such that powder only flows out when the funnel is tapped. Capsules can be propped up using the frame of a 200 µL pipet tip rack (Figure 1). Line up open capsules in the rack. Insert the funnel tip into an empty capsule, and use a spatula or spoon to scoop a small amount of powder into the funnel. Gently tap the top or side of the funnel with the spatula until the first capsule is full, and repeat to fill all of the capsules down the line. Then use the flat, round end of the dissection probe or a pen to pack the powder down. Fill capsules a second time and pack. Fill a third time to overflowing and cap. It may be helpful to place some powder in the lid before closing.

It is very important for capsules to be tightly packed, otherwise larvae will have reduced weight or die because they cannot feed properly. A capsule of size '0' should hold about 0.8g of powder. It is a good idea to have students mass their first few capsules to make sure they are filling them consistently.

Setting Up Dishes

Fill the bottom of each dish with capsules (about 3-8 depending on the size of the dish), and place two females in each dish to ensure that eggs are laid. Dishes should be incubated at 30° C until data collection.

Figure 1. Modified pipette tip and rack for filling capsules with bean flour.

Data Collection and Analysis

Data should be collected 3-5 weeks after eggs are laid. Larvae will be small, and thus difficult to find if the capsules are opened earlier. Students can calculate expected emergence rates and average larval mass for different treatment groups. If data are collected after emergence, students can simply calculate the emergence rate by dividing the number of emerged adult beetles over the total number of eggs laid on the capsules. Taking the mass of adult beetles is not recommended, as it will begin to decline after emergence. Data for individual capsules can be analyzed using a student's T-Test in GraphPad: http://www.graphpad.com/quickcalcs/ttest1.cfm.

Assignments and Evaluation

Final assignments and evaluation should be tailored to the learning objectives and needs of a particular class. At minimum, groups can share their results informally in a class discussion following data collection. Possible concepts to cover include graphical representation of data, sources of error, and statistical significance. Students can also formally report on their results in a group presentation or a written scientific paper.

The Experimental Design Ability Test (EDAT), developed by Sirum and Humburg (2011), has been used in a pre-test/post-test format with this module to detect learning gains. A suggested exam question tailored to the Bean Beetle Nutrition and Development Lab can be found in Appendix B, along with a modified EDAT rubric.

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Acknowledgments

The authors would like to thank the Emory University Department of Biology for the opportunity to develop and pilot this lab module, and especially Megan Cole, Kathleen Campbell, and Christopher Gilson for their time and feedback. We also thank the Emory University SURE (Summer Undergraduate Research Experience) program for funding and support.

About the Authors

Julie Laudick is a high-school biology drop out. It wasn't until she read Darwin's *Origin of Species* in a college philosophy class that she developed a passion for biology, and her interest in pursuing a science career was sparked while participating in an inquiry-based lab. She completed a B.S. in Biology and a B.A. in Philosophy at Emory University, where she designed this module with Chris Beck. She is currently working on an M.S. in Environmental Science at The Ohio State University. Her research evaluates the potential role of plant-beneficial microbial inoculants in organic farming systems.

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Citing This Article

Laudick J. L. and C. Beck. 2016. Bean beetle nutrition and development lab: An iterative approach to teaching experimental design. Article 10 in *Tested Studies for Laboratory Teaching,* Volume 37 (K. McMahon, Editor). Proceedings of the 37th Conference of the Association for Biology Laboratory Education (ABLE). http://www.ableweb.org/volumes/vol-37/?art=10

APPENDIX A
Timeline for a Full Bean Beetle Module

Period 1: Introduction to Bean Beetles (1 hour)
Agenda
- Take pre-test (optional)
- Present background information on bean beetles (15 minutes)
- Students observe beetles and learn to sex them (15-30 minutes)
- Pass out handout 1 for next day's lab, students will be asked to design an experiment for homework

Period 2: Discussion and Revision of Experimental Designs (1-2 hours)
Agenda
- Discussion in groups of 3-5 (15-30 minutes)
    - Students will share their designs from the homework assignment
    - Groups will collectively deicide on a design. Handout 2: Bean Composition Comparison Chart is optional, and may help students chose a nutrient of interest.
    - Each group will share
- Class Discussion (15-30 minutes)
    - Potential topics to weave into the discussion:
        - Independent & dependent variables
        - Positive &. negative controls
        - Replication
        - Statistics
        - Biologically meaningful doses of nutrients
    - Review math for concentration calculations
- Give handout 3 to groups for completion and approval before leaving lab (15-30 minutes)

Period 3: Setting up the Experiments (2-3 hours)
Agenda
- Show students how to mix bean powder (5 minutes)
    - Make sure they're measuring their bean powder in batch quantities (not massing the amount required per capsule)
- Show how to fill capsules (5 minutes)
    - **Make sure they are packed tightly: e**ach capsule should weigh about 0.8g if it is sufficiently packed.
- Remainder of period is left for filling capsules, setting up dishes (1-2 hours)

Period 4: Nutrition and Development Data Collection (2-3 hours)
Agenda
- Students will open capsules, mass larvae, and calculate expected emergence rates
- Instructions and data tables are provided in Handout 4
- Groups should record and graph data to present and discuss with the class
- Modify Handout 5 according to your learning objectives

APPENDIX B
Post-test and Evaluation

The Experimental Design Ability Test (EDAT) is an open-ended test of experimental design ability with a simple rubric for rapid evaluation (Sirum and Humburg, 2011). Here, we have tailored the EDAT prompt and rubric to the Bean Beetle Nutrition and Development Module. The original EDAT was designed to be content-independent, and students are not explicitly prompted to design an experiment. For the purposes of this module, some content is incorporated into the question, and students are explicitly asked to design an experiment. After participating in the module, students should be able to apply the same design principles to answer a question about the effects of harmful, rather than beneficial chemicals on bean beetle development.

Exam or Post-test Prompt

In addition to being less nutritious than mung beans, adzuki beans also contain chemical compounds that plants produce to protect themselves from herbivores (e.g. bean beetles). Saponins are one type of plant defense compound present in adzuki beans, but absent in mung beans, and they are known to be toxic to insects. Formulate a hypothesis about saponins and bean beetle development, design an experiment to test it, and predict the outcome of your experiment.

Modified EDAT Rubric

1. *Formulates a specific, testable hypothesis*
2. *Identifies what variable is manipulated (independent variable)*
3. *Includes a negative control*
4. *Includes an positive control*
5. *Identifies what variable is measured (dependent variable)*
6. *Describes how the dependent variable is measured*
7. *Consideration of dosage*
8. *Consideration of sample size and/or replication*
9. *Consideration of statistical significance*
10. *Explains how evidence gathered will support a conclusion*

The experimental design homework assignment (Handout 1) can also be evaluated with this modified EDAT rubric. In the first assignment, the independent variable is the concentration of a given nutrient, the positive control would be plain mung powder, and the negative control would be plain adzuki powder. In the post-test question, the structure of inquiry is the same, but a toxic compound is evaluated instead of a nutrient. The independent variable is the concentration of saponins, the positive and negative controls are reversed, and the dependent variable remains the same.