Bean Beetles Make Biology Research Sexy

NABT BioClub RECOMMENDATION

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Abstract

We describe a multiweek laboratory exercise that engages students in class-based research related to sexual reproduction, selection, orientation, and operational sex ratios. Specifically, students discuss contemporary research on sex in the bean beetle, Callosobruchus maculatus, *and then develop and test hypotheses related to bean beetle sex. Working with bean beetles is inexpensive and logistically manageable, allowing instructors to scale up to large-enrollment courses. In addition, live organisms engage students in meaningful dialogue related to evolution, sex, and the process of science itself.*

Key Words: *Sexual selection; bean beetles;* Callosobruchus maculatus; *course-based undergraduate research experience (CURE).*

○ Can Introductory Biology Be Sexy?

The *Vision and Change* report (AAAS, 2011) articulated several priorities for biology education, among them the incorporation of authentic research experiences at all levels and for all students. In doing so, the report echoed the sentiments of numerous investigators – namely, that authentic research experiences contribute to increased student engagement (Hunter et al., 2007), understanding of scientific processes (Seymour et al., 2004), long-term learning (Laursen et al., 2010), and interest in science as a career (Lopatto, 2007; Russell et al., 2007; Eagan et al., 2013; for a review, see Auchincloss et al., 2014).

Course-based undergraduate research experiences (CUREs) allow students to engage in authentic research in the context of an existing course. By incorporating CUREs into the curriculum, a large number of students can participate meaningfully in the process of science, with the palpable expectation of discovery that is often lacking in traditional, inquiry-based laboratory exercises (Auchincloss et al., 2014, and references therein). Fortunately, several excellent models, or CUREs, exist for incorporating research experiences into course settings (see Adams, 2009, and references therein), among them ideas for student-driven research projects in molecular biology (Harrison et al., 2011), cell biology (Hurd, 2008), microbial ecology (Kloser et al., 2011), and experimental evolution (Ratcliff et al., 2014). However, we are unaware of existing models for course-based research related to sex (e.g., sexual selection, reproduction, orientation, or operational sex ratios); this shortcoming is notable, given student interest in sex-related topics (Cotner & Gallup, 2011; Hagay & Baram-Tsabari, 2011).

We have developed a multiweek laboratory exercise that engages students in meaningful research that is sex-related and authentic, while being inexpensive and logistically manageable. Specifically, students discuss contemporary research on sex in the bean beetle (*Callosobruchus maculatus*) and then develop and test hypotheses related to bean beetle sex. The exercises described below are suitable for college and advanced high school students.

> *Bean beetles are agricultural pests that occur throughout the tropics and subtropics.*

○ Who Are the Bean Beetles?

Bean beetles (Figure 1) are agricultural pests that occur throughout the tropics and subtropics. The larvae feed on, and develop within, the seeds of legumes (especially favoring black-eyed peas, mung beans, and adzuki beans). The adults live around two weeks, in which they restrict their activity to reproduction: courtship, copulation, and laying eggs. Several features of their life history make bean beetles ideal for student-driven research projects:

- they are inexpensive (and available to ship internationally; e.g., from Carolina Biological Supply);
- they cost even less to breed, requiring merely a bean and high humidity for egg laying and larval development;
- the adults do not eat, thus minimizing the animal-care requirements;

The American Biology Teacher, Vol. 78, No 3, pages. 233–240, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: 10.1525/abt.2016.78.3.233.

Figure 1. Bean beetles on mung beans. One male (center) is flanked by two darker and larger females.

- they die, naturally, after a couple of weeks of adulthood, eliminating the need for – and possible psychological costs associated with – euthanasia;
- they are large enough to be observed with the naked eye, but small enough to require little classroom "real estate";
- with a little practice, males are easily distinguishable from females; and
- they have stereotypical mating behaviors that are easily interpreted.

Bean beetles are already employed as a model system for research (Zuk et al., 2014) and educational purposes (Beck et al., 2013; Schlueter & D'Costa, 2013; also see examples referenced at http://beanbeetles.org), and several reports convey their role in recent developments in sexual selection (e.g., Maklakov & Arnqvist, 2009), sperm competition (Hotzy & Arnqvist, 2009), sexual orientation (Maklakov & Bonduriansky, 2009), and operational sex ratios (Miller & Inouye, 2013). Thus, there is no scarcity of information on bean beetles, nor do we lack resources for breeding them for use in a laboratory setting (http://beanbeetles.org).

○ Bean Beetles by the Week

This research project was developed at the University of Minnesota for a nonmajors introductory biology course focused on the evolution and biology of sex. The course has no prerequisites, enrolls students from disparate backgrounds, and includes 600–1000 students per year (in ten to twenty 24-person lab sections per semester). The work we describe is appropriate for nonmajors or majors-level biology students in introductory courses, as well as courses in evolution, animal behavior, sexual selection, or research methods. Our goals are for students to (1) apply science-processing skills in a weeks-long research project that they design, execute, present, and evaluate; and (2) learn lab techniques that apply to bean beetles (sexing beetles, identifying stages of the bean beetle's life cycle, etc.).

In our laboratory curriculum, students begin the bean beetle work during the fifth week of class. At this point in the term, they've practiced reading scientific literature, identifying hypotheses, and conducting small, time-constrained experiments that can be completed in a single, two-hour lab session. Students have been assigned several readings from "Understanding Science 101" (http://undsci.berkeley.edu/article/scienceflowchart), including a section on the empirical nature of science, "The Core of Science" (http://undsci.berkeley.edu/article/0_0_0/coreofscience_01). They've been introduced to concepts such as experiment, control, replication, standardization, data collection, probability, and statistical significance, concepts that are succinctly described at http://www.visionlearning.com/en/library/Process-of-Science/49/Statistics-in-Science/155 (Carpi & Egger, 2008). At this point, they are ready to conduct their own multiweek experimental research project. We allow eight weeks for this minimally structured lab exercise (Figure 2).

Week 1

Students are introduced to bean beetles via a handout, several live organisms, and a short video on sexing bean beetles (available at http://youtu.be/ZVm89AvElGY). During this introduction, students are encouraged to test their abilities to (1) manipulate the beetles and (2) determine the sex of the beetles. Then students are assigned one of four articles on bean beetle sex, such as the following (see References):

- Gay et al., 2009: This short article, using accessible language and simple figures, describes how male harassment results in females laying fewer eggs and thus leads to a reduction in female fitness.
- Maklakov & Bonduriansky, 2009: After a short discussion of homosexual interactions in nature, the authors describe how the costs of homosexual and heterosexual interactions are similar for males, but dissimilar for females: specifically, the costs of female homosexual interactions are small compared to the costs of heterosexual interactions.
- Eady et al., 2000: The authors report on the results of two experiments designed to evaluate the possible evolutionary benefits of polyandry in bean beetles.
- Edvardsson & Canal, 2006: After a discussion of sexual conflict in bean beetles (male intromittent organs have spines that puncture the female reproductive tract, and females kick their mates to lessen copulation duration), the authors describe the complex positive relationship between prolonged copulation and fitness.

Although there are myriad other examples of suitable papers, we've found the ones listed above to be clear, succinct, and excellent fodder for student questions and projects. Students read their assigned article and complete a short questionnaire about the paper before the next lab session.

Week 2

Using a jigsaw format, students discuss the four assigned papers. Specifically, students initially form groups consisting of peers that have read the *same* paper; these groups discuss the assigned papers, using the prompts included in the accompanying questionnaire (e.g., "State the rationale for this work," "Explain what the researchers did," and "In your own words, explain the significance of this work"). Then, students disperse into groups in which *all* papers are represented; individual students present the findings from their assigned paper and learn about other work on bean beetles. Ultimately, students are familiar with several research projects on bean beetles. This exercise is intended to introduce them to the type of investigation that can be pursued; the selected papers describe work that is at least partially feasible within the time and space constraints of the laboratory schedule.

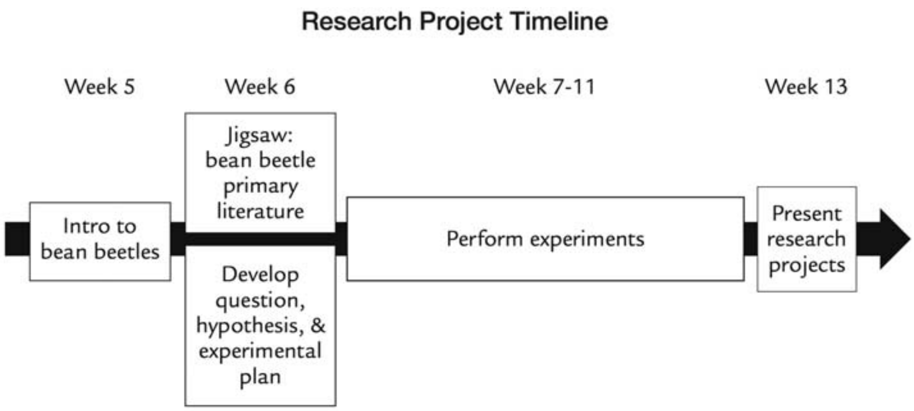


Figure 2. A working schedule for incorporating bean beetles into a laboratory curriculum.

After the literature jigsaw, students have time, in small groups of four or five, to discuss their own interests, identify potential research questions, and discuss experimental plans and feasibility. Instructors assist by asking guiding questions (e.g., "Can you really analyze three generations of beetles in five weeks?"), highlighting the available equipment (in our case, this is essentially limited to a light table, Petri dishes, stock dishes of bean beetles, dissecting microscopes, and different types of legumes), and encouraging students to think carefully about whether the data they'll collect (e.g., number of eggs per bean, female fecundity and life span) actually align with their hypotheses and predictions. Students submit project ideas to their classmates and instructors via our electronic course-management system and select a project just before, or at the beginning of, week 3.

Weeks 3 to 7

Students perform experiments, conduct observations, collect data, and interpret results. In some cases, they can accomplish this during the short periods of available time in our standard laboratory curriculum. Some groups are required to visit the lab outside of normal lab hours. In either situation, communication among group members is key. Also, instructors work with groups ahead of time to ensure that the work can be done with minimal assistance, once the initial setup is complete.

Week 8

Students present the results of their work. Using a rubric for guidance (Appendix), groups develop a short presentation (~15 minutes) describing the rationale for their study (the "background"), methods, results, and significance. Afterward, students complete confidential evaluations of the contributions of each of their group members. Graded elements of the project include the initial jigsaw-paper questionnaire (week 2), their experimental plan (week 2), the results of the group-member evaluation (week 8), and the final presentation (week 8).

○ Results

Student Products

Sample hypotheses, methods, and results are depicted in Figure 3. As of this writing, >1600 students have conducted research into bean beetle sex, and several themes have emerged. In our experience, the students' choices of research projects are influenced by the information given to them about bean beetles and the papers that are used for the literature jigsaw. In general, student projects investigated various aspects of the evolution and biology of sex, including how sex ratios, sexual orientation, sexual selection, and environmental factors influence mating, reproductive success, and life span. Some groups chose to alter a normal aspect of bean beetle life history to determine how that alteration may influence reproductive success. For example, students investigated whether access to water – a resource not required by adult bean beetles – would alter their reproductive success (Figure 3). Other groups used the results of one paper as a jumping-off point for their project, in which they investigated a next-step question. For example, Maklakov and Bonduriansky (2009) reported that the life span of female bean beetles is reduced when females are housed with males, in comparison to when females are housed only with other females. This result led some groups to investigate potential causes for the reduction in the female bean beetle's life span in the presence of increasing numbers of males – including a potential increase in an energetically demanding activity like egg laying (Figure 3).

Among the most tangible benefits of authentic research experiences is the reward for sound experimental design. Students who adhere to best practices – clearly defined controls, use of replicates, vigilant note-taking, and so on – are rewarded with reliable data that can be interpreted in light of their initial hypotheses. When students are unable to interpret their data meaningfully, their final presentations become a litany of "should haves": they *should have* monitored activity during the same time each day; they *should have* used the same number of beetles in each dish; they *should have* used more replicates; and so on. These problems can be mitigated by careful instructor feedback during the design phase but, at a minimum, are parts of the process and educationally valuable (see student comments below, e.g., "I learned that you need many repetitions to get accurate data").

Student Impressions

The bean beetle project consistently meets its goals, which are for students to (1) apply science-processing skills in a weeks-long research project that they design, execute, and evaluate; and (2) learn lab techniques that apply to bean beetles. Measurable objectives include explaining the bean beetle life cycle; describing the research, from rationale to hypothesis, methods, results, and conclusions; and presenting the results of the research in an oral presentation.

Furthermore, students realize the value of the activity, especially as it pertains to giving them an authentic experience with scientific methodology. In a postterm evaluation, when asked to rate how much the bean beetle exercise had engaged them in the process of science (using a Likert scale, in which 1 = "not at all engaged" and 4 = "extremely engaged"), students averaged a 3.30 response, with 95 of 188 (50%) recalling that they were "fairly engaged" and 75 of 188 (40%) saying they were "extremely engaged." When asked

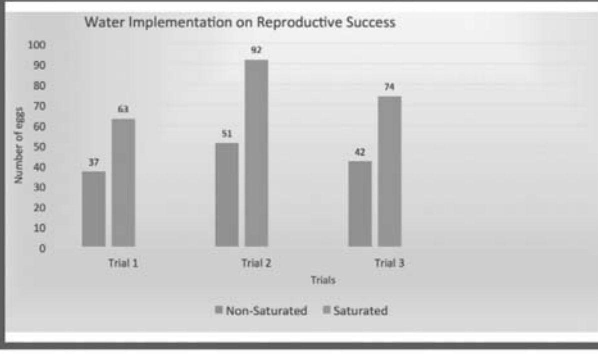
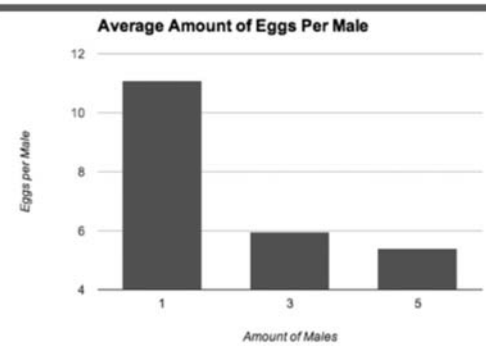
Hypothesis	Water implementation positively affects reproductive success.	Bean beetle fecundity increases with more potential mates.
Methods	1. Put 5 female and 5 male bean beetles in each of 2 medium petri dishes 2. Add 5 dry crystals in one and 5 water-saturated crystals in the other 3. Count the eggs	1. Fill six petri dishes with 25 new beans (without any eggs) each 2. Put 1 female in each dish 3. Put males in dish (two dishes of each 1, 3 or 5 males) 4. Place the labeled petri dishes under lamps for 48 hours 5. Count the number of eggs laid in each dish 6. Repeat process
Results		

Figure 3. An overview of two sample student projects.

to rate how much they had learned in the lab (on a scale in which 1 = "less than I expected" and 4 = "much more than I expected"), students averaged a 3.29, with 77 of 183 (42%) recalling that they had learned "about what I expected" and 52 of 183 (28%) and 21 of 183 (11%), respectively, claiming to have learned "more" and "much more" than expected.

As part of an in-class assessment in fall 2014, students were asked (via a classroom response system, or "clicker") to evaluate various aspects of the laboratory experience. Specifically, students were asked to compare several inquiry-based laboratory exercises to several authentic, discovery-based projects (of which the bean beetle project was the most time- and energy-intensive). Figure 4 summarizes their perceptions of how authentic research projects engaged them in the process of science. Namely, 67% (or 102/153) of the students rated the discovery-based labs more engaging than the traditional lab activities.

Auchincloss et al. (2014) described features that contribute to qualifying course-based research experiences, specifically (1) use of scientific practices, (2) discovery, (3) broadly relevant or important work, (4) collaboration, and (5) iteration. The students' open-ended comments, sampled below, indicate why the bean beetle lab is an effective model for authentic research, using our interpretations of the criteria established above.

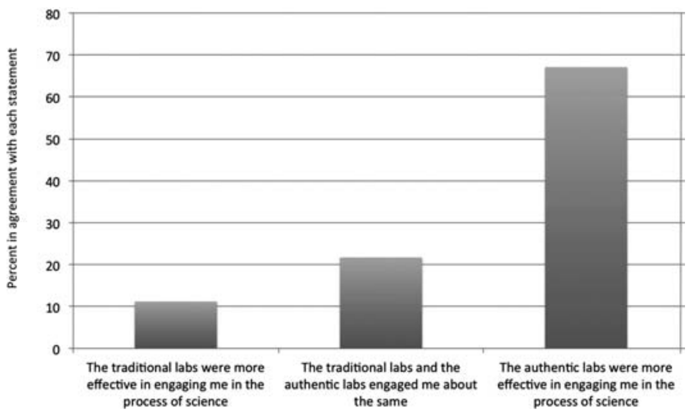


Figure 4. Student impressions of traditional versus authentic laboratory exercises (n = 153).

Use of Scientific Practices:

- Designing our own experiments from start to finish really helped me understand the scientific process better.
- This project really highlighted that research is sloppy and hard to do 100% correctly. After gathering all the data and trying to put it all together you can really see the areas where you should've gotten some more data or where there were a lot of confounding variables that messed with the results.
- This was a project where we were more in charge of what happened. So while the other labs still engaged us in the process of science, this project made us take that into our own hands and figure out how to do it for ourselves.
- Turns out, it takes much more thought to plan and perform experiments.
- Going every week waiting for the beetles to hatch and trying to decide how our experiment would affect the outcome was pretty interesting and engaging.

- It engaged me because I haven't really ever done my own experiment where I collected data for weeks so that was very interesting for me.
- I learned how much work it is and how long it takes to collect all of the data in an experiment.

Discovery:

- In the project I learned that even beetles maybe can see colors!
- It was also great to have the chance to work on an experiment that no one knew the answer to, so we weren't just doing pointless work to prove that we were capable of following directions.
- I learned that I really don't like bean beetles, especially when one flew into my face, but it was still cool to come into lab and do our own thing, although inconvenient that it had to be on our own time, it was nice not to follow our lab notebook.
- Even though ours was not as successful my group ended up learning from the other group and noticed that more eggs were laid in the darker environment like the other group.
- I could feel myself getting excited about the eggs hatching.

Broadly Relevant or Important Work:

- In terms of a career and what I generally care about, sexuality plays a large role. So these labs related back to what I care about and what interests me, which is why I was personally engaged by them. I feel like these labs did a good job of engaging all of us (not just people with specific interests like mine) in a way that was scientific and fun.
- Being able to answer a question that I have is a much more valuable learning experience, to me, than answering one I know has been answered a hundred times already in the week. Knowing that I was doing something different from my neighbor, that we were contributing in different ways, to better understand a particular function of something, gave it more of a sense of purpose than the lab otherwise would have.
- It was nice to have labs that connect science to real life situations.
- We're not just retaining information, we're exploring the topics and getting into them headfirst, which I think is very cool for a lower-level science course. I think that we could have done a little more with talking about how to use the data we found in our own lives.

Collaboration:

- This allowed me to work with a team and the freedom to design and complete an experiment.
- I liked the bean beetle project and thought it was a good way to work in groups.
- I learned that a lot of things can go wrong and good group members are crucial for success.
- Some challenges we faced is that a lot of our group members didn't show up to count eggs so it was frequently just me and one other lab member counting a ridiculous amount of eggs.

Iteration:

- We also did replicate experiments to get better validity.
- I learned that you need many repetitions to get accurate data.

○ Advice to Adopters

Bean beetles are an excellent model for course-based authentic research, and this case has been made with numerous previous examples (see http://beanbeetle.org for a complete, and growing, list). We contribute to this list by making the case for bean beetles as a model for course-based investigations related to sex – sexual selection, sexual orientation, and the effects of operational sex ratios. Given how interested college students (and humans in general) are in sex-related topics, sex is an excellent "wedge" through which to engage students meaningfully in the process of science. Along the way, students will be exposed to experimental design, the empirical nature of science, the concept of science as a group endeavor, bruchid beetle life-history traits, and alternative reproductive strategies – in addition to critical topics such as evolution, adaptation, fitness, and sexual selection. In addition, students develop key science-communication skills via in-class presentations and short written summaries of their experimental designs, results, and conclusions.

Anyone who has worked with class-based research projects knows that giving students control of the investigation may lead to chaos and confusion in the beginning of the process. This is an excellent opportunity to illustrate that sometimes, science is messy. We've found that it helps to give students time to struggle, to explore experimental procedures that may fail to deliver results, and to learn from their peers. Future work will involve developing a database of student results for in-depth, comparative, or longitudinal investigations. Ultimately, our own experience suggests that the chaos is worth the pain, as students experience real science, tackle meaningful questions, and work with live animals.

○ Acknowledgments

This work was made possible by a Howard Hughes Medical Institute grant awarded to Robin Wright (Chair, Department of Biology Teaching and Learning, University of Minnesota) and by a National Science Foundation IUSE grant (Integrated Science Education for Discovery in Introductory Biology, proposal no. 1432414) awarded to Robin Wright, Sehoya Cotner, and Catherine Kirkpatrick. We thank our colleagues – especially Paul Nelson and Sarah Borgen – and our many intrepid students!

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Appendix. Presentation rubric for bean beetle research project (modified from Cotner & Nelson, 2014).

	Incomplete (0)	Needs Work (0.5)	Satisfactory (0.75)	Exemplary (1)	Score
Introduction (1 point possible)	Does not state question or hypothesis	States question and hypothesis Does not include background information	Clearly states question and hypothesis Provides some background information; only uses sources provided through class materials	Clearly states question and hypothesis Provides detailed background information using multiple sources in addition to the sources provided through class materials Provides a rationale for the hypothesis	

	Incomplete (0)	Needs Work (0.5)	Satisfactory (0.75)	Exemplary (1)	Score
Methods (1 point possible)	Does not include experimental methods	Includes general experimental methods; lacks details OR Does not identify independent and dependent variables or control group (if applicable)	Includes detailed experimental methods Identifies independent and dependent variables and control group (if applicable)	Includes detailed experimental methods Identifies independent and dependent variables and control group (if applicable) Identifies controlled variables	
Results & Data Analysis (2 points possible)	Does not include an appropriate graphical representation of the results	Includes an appropriate graphical representation of the results Describes and explains trends	Includes an appropriate graphical representation of the results Graphs are labeled with independent and dependent variables on the correct axes Data presented includes replicates Describes and explains trends	Includes an appropriate graphical representation of the results Graphs are labeled with independent and dependent variables on the correct axes with units Data presented includes replicates Describes and explains trends Uses basic statistical methods (i.e., calculates and graphs means)	
Conclusions (1 point possible)	Does not discuss what the results mean in terms of the big picture (i.e., applications or real-world connections)	Discusses what the results mean in terms of the big picture (i.e., applications or real-world connections)	Discusses what the results mean in terms of the big picture (i.e., applications or real-world connections) Discusses obstacles experienced during the research project Discusses how the current experiment could be improved if it were done again	Discusses what the results mean in terms of the big picture (i.e., applications or real-world connections) Discusses obstacles experienced during the research project Discusses how the current experiment could be improved if it were done again Discusses potential follow-up experiments	

	Incomplete (0)	Needs Work (0.5)	Satisfactory (0.75)	Exemplary (1)	Score
Overall Presentation (1 point possible)	Presentation is not organized OR Presentation is not legible (does not use appropriate sizes and colors for background, text, and images)	Presentation is organized Presentation is legible (uses appropriate sizes and colors for background, text, and images) Presentation does not include references	Presentation is organized Presentation is legible (uses appropriate sizes and colors for background, text, and images) Presentation includes references	Presentation is organized Presentation is legible (uses appropriate sizes and colors for background, text, and images) Presentation includes references Presenters provide thoughtful evidence-based answers to questions	
Total Score (6 points possible)