Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Volume 39, Article 70, 2018

Investigating Critical Periods in Bean Beetle Development: Incorporating Guided Inquiry Research into a Developmental Biology Laboratory Course

Kathryn L. Lipson

Western New England University, Department of Physical and Biological Sciences, Springfield MA 01119 USA
(Klipson@wne.edu)

A guided inquiry of the teratogenic effects of ethanol on developing organisms was incorporated into an upper level developmental biology laboratory course containing 20 students. During this semester-long research project, students work in pairs to design and perform experiments to answer questions about developmental critical periods. As a class, students discuss data collection, and identify the different potential variables. Each student pair typically designs a different experiment so that as a class, we can address as many variables as possible. Students submit detailed research plans to be edited by the instructor before beginning the experiments. The projects require time outside of class meetings, and reinforce the importance of proper experimental planning and routine. This format is useful for instructors wishing to incorporate multiple semi-independent research projects into undergraduate laboratory curricula.

Keywords: developmental biology, bean beetles, teratogens, inquiry-based learning

Link to Original Poster: http://www.ableweb.org/volumes/vol-39/poster/?art=70

Introduction

Integrating research into undergraduate courses allows students to fully experience the scientific process. However, instructors tasked with supervising multiple undergraduate research projects can be challenging for many reasons. To this end, a guided inquiry of the teratogenic effects of ethanol on developing organisms was incorporated into the Developmental Biology Laboratory course at WNEU. Introductory lessons about the bean beetle model system and culturing, principles of teratology, and effects of ethanol on early development are presented. Students are then posed the question. "Is ethanol teratogenic to bean beetles? If so, is there a critical period during bean beetle development when ethanol exposure is particularly detrimental?" During this semester-long research project, students work in pairs to design and perform experiments to answer these questions.

As a class, students discuss data collection, and identify the different potential variables, including concentration of ethanol, volume of ethanol, and duration of exposure. Each student pair typically designs a different experiment so that as a class, we can address as many variables as possible. Students submit detailed research plans to be edited by the instructor before beginning the experiments. The projects require time outside of class meetings, and reinforce the importance of proper experimental planning and routine. At the end of the semester, students write formal research reports detailing their findings, and give an oral presentation to the class. Because much of the work can be done independently by the students on their own schedules, this format may be attractive to instructors looking to incorporate research with larger numbers of students.

What Are Developmental Critical Periods?

In humans, the embryonic period from 18-60 days after conception is the period when the basic steps in organogenesis occur. Exposure to teratogenic agents (such as ethanol) during this critical period has the greatest likelihood of causing a structural anomaly. (Gilbert and Epel, 2008). Since teratogens are capable of affecting many organ systems, the pattern of anomalies produced depends upon which systems are differentiating at the time of teratogenic exposure.

Teratogenic specificity applies to individual species. Thus, even though ethanol has been shown to be teratogenic to humans, it may not be teratogenic to other species. As a relatively new model organism, very little is known about teratogenic susceptibility and critical developmental periods, including susceptibility to ethanol, in bean beetles, *Callosobruchus maculatus*.

Experimental Design

Students typically start each experimental treatment with 30 beans that each have one egg that was laid the previous day. Matings are set up on Day 1, and beans with eggs are collected on Day 2. All eggs are collected out of a single large mating dish and then randomly allocated to each of the experimental group dishes.

Students brainstorm ways to administer ethanol, but most decide to soak a Kimwipe sheet and place the beans with eggs on top of the soaked Kimwipe sheet. Students administer 1-2ml of ethanol (or water) to the wipe once a day for the specified time period. Eggs are cultured in 6cm petri dishes on top of the Kimwipe sheet. Plates are kept in a 30 °C incubator to control for temperature.

At the beginning of the third week after eggs have been laid, students must check their dishes every day to look for emerging adults. Students do 2-3 replicates of each experiment. Over the course of the semester, they can repeat the experiments 2 or 3 three times.

Data Collection

Students collect a variety of data, including:

- Percent emergence: out of the 30 eggs in each dish, how many emerged?
- Time to emergence: students keep track of how many days have passed since the eggs were laid (day one) until they emerge.
- Sex ratios: of all the beetles that emerge, how many are male, how many are female?
- Physical malformations or functional defects: students must closely observe the beetles compared to controls

Experiments typically fall into three categories:

1) testing the effects of different ethanol concentrations
2) testing how the point during development and duration of exposure to ethanol affects emergence rates
3) testing how ethanol exposure affects the time elapsed until emergence

Conclusions

Preliminary data from one semester of work (not shown) suggests that exposure to ethanol effects developing bean beetles in a dose-dependent manner, and that the most critical period is during the second week of the four-week developmental period. Beetles exposed at this stage had the lowest rates of emergence, and also took the longest amount of time to emerge. Our initial work suggests that bean beetles may be a good model system with which to study effects of ethanol and other teratogens in an undergraduate lab with large numbers of students.

Student Outline

Objectives

- Apply the principles of teratology to the study and understanding of teratogenic agents and their effects on developing organisms.
- Design and perform an experiment to determine if critical periods exist during early stages of bean beetle development.

Introduction

A teratogen is any agent that can cause a structural anomaly when acting on a developing embryo or fetus. Disruptions induced by teratogens are important because they are potentially preventable! There are many external factors that negatively impact the developing organism, some of which cause permanent abnormalities (Gilbert and Epel, 2008). These broad range of substances, known as teratogens, are readily found in almost any environment. Teratogens can be airborne contaminants, such as harmful gases, vapors, and pesticides, drugs and medication, such as cocaine, anticonvulsants, anticoagulants, and Thalidomide, congenital infections, such as human immunodeficiency virus and syphilis, consumption of alcohol, cigarette smoking, radiation, and stress.

In 1956, James Wilson put forth six principles of teratology that are still applied to almost all discussions of teratogenesis (Wilson and Warkany, 1965):

1. Susceptibility to teratogenic agent depends on genotype of the embryo, genotype of the mother, and the ways in which their genotypes allow mother and fetus to interact with the adverse environmental factors
2. There are **critical periods** of development when embryos are susceptible to being disrupted by teratogenic agents. These windows of susceptibility are when different organs are forming.
3. Teratogenic agents act in specific ways on genes, cells, and tissues in the developing organism to disrupt normal sequences of developmental events.
4. Several conditions affect the ability of a teratogen to disrupt normal development:
    - Route and degree of maternal exposure
    - Ability of mother to block or detoxify the agent
    - Rate of transfer through placenta
    - Rate of fetal absorption
    - Composition of the maternal and embryonic genotypes
5. There are four manifestations of disrupted development: death, malformation, growth retardation, and functional defects
6. Manifestations of deviant development increase in frequency and degree as the dosage of teratogen increases.

In humans, the embryonic period from 18 to around 54-60 days after conception is the period when the basic steps in organogenesis occur. This is the period of maximum sensitivity to teratogens since not only are tissues differentiating rapidly but damage to them becomes irreparable. Exposure to teratogenic agents during this **critical period** has the greatest likelihood of causing a structural anomaly. Since teratogens are capable of affecting many organ systems, the pattern of anomalies produced depends upon which systems are differentiating at the time of teratogenic exposure (Gilbert and Epel, 2008).

Alcohol (ethanol) has been shown to be a potent teratogen in humans. Embryonic exposure to this teratogen can cause fetal alcohol syndrome (FAS). FAS is a clinical pattern of anomalies characterized by intrauterine growth retardation, which commonly continues postnatally. Postnatal anomalies include: microcephaly, developmental delay, and dysmorphic faces consisting of low nasal bridge, midface hypoplasia, long featureless philtrum, small palpebral fissures and thin upper lip (Thomas and Warren, 2011). Cleft palate and cardiac anomalies also may occur. In addition to these physical characteristics, children with FAS often display learning disabilities, attention or memory deficits, and inability to manage anger, poor judgment, or difficulties solving problems. FAS occurs when a mother consumes excessive alcohol during pregnancy. When alcohol (a teratogen) crosses the placenta, it cannot be removed by the liver since the liver of an embryo is not fully developed until the last trimester; consequently, it depresses the function of the fetal nervous system.

Teratogenic specificity applies to individual species. For example, aspirin and corticosteroids have been found to be teratogenic in mice and rats but appear to be safe in humans. Thalidomide, on the other hand, was not shown to be teratogenic in rats, a tragic fact that resulted in significant human morbidity. Thus, even though ethanol has been shown to be teratogenic to humans, it may not be teratogenic to other species. Bean beetles, *Callosobruchus maculatus* are agricultural

pest insects of Africa and Asia that presently range throughout the tropical and subtropical world. The larvae of this species feed and develop exclusively on the seed of legumes. As a relatively new model organism, very little is known about teratogenic susceptibility and critical developmental periods, including susceptibility to ethanol, in this species. In this lab, you will design and carry out experiments that expose bean beetles to ethanol at different periods during development to determine the effects of ethanol on bean beetles and to determine if critical periods in bean beetle development exist.

Materials

In class, you will be provided with live bean beetle cultures, supplies of dried mung beans (*Vigna radiata*), 100% ethanol, petri dishes, Kimwipes™ sheets, paper towels, stereoscopes, and any other materials you decide you need.

Experimental Design

In this study, you will be designing experiments to address the following questions:

1. Is ethanol teratogenic to bean beetles?
2. If so, is there a critical period during bean beetle development when ethanol exposure is particularly detrimental?

Prior to our next lab, each pair of students should design a set of experiments using ethanol as a potential teratogen to determine and define critical periods of bean beetle development. You will turn this in at our next lab meeting and I will look it over and provide comments and additional suggestions. Be as detailed as you can in regards to number of embryos, method(s) of exposure to the teratogen, time of exposure, duration of exposure, controls, etc. In your research plans, you should include the following information:

- Describe an experimental design for evaluating the effects of ethanol on early bean beetle development.
- Predict the outcomes for the experiment
- Identify and list the variables you will manipulate in the experiments
- Identify and list the variables you will keep constant in the experiments
- Describe what you will measure, observe, or tabulate
- Describe any statistical analyses you will carry out to test your predictions

This is a semester-long project that will require some time outside of our normal class periods. You will write a research report documenting your findings; this will be due the last day of classes. Be sure to keep good notes and good records of data, observations, and/or measurements in your laboratory notebook. At the end of the semester, you and your laboratory partner will be asked to orally present your results via a 10-15 minute Power Point presentation.

Cited References

Gilbert, S.F., and Epel, D. 2008 *Teratogenesis: Environmental Assaults on Development*. In: Ecological Developmental Biology. Sinauer Associates, Inc. Pp 167-175

Wilson, J.G and Warkany, J. 1965. Teratology: Principles and techniques. The University of Chicago Press.

Warren, K.R., Hewitt, B.G., and Thomas, J.D. 2011. *Fetal Alcohol Spectrum Disorders: Research Challenges and Opportunities.* Alcohol Research and Health. 34: 4-14

Materials

We supply each student pair with live bean beetle cultures, supplies of dried mung beans (Vigna radiata), 100% ethanol (non-denatured), 10cm and 6cm petri dishes, Kimwipes™, paper towels, pipettes, and stereoscopes.

Notes for the Instructor

Experimental Design

There are several questions that students may address in this laboratory activity, including 1) Does the developmental period (week1, week 2, etc.) during which bean beetles are exposed to ethanol affects their survival (i.e., Is there a critical period in development?) 2) Does the length of time the beetles are exposed to ethanol affect developmental timing or survival? 3) Does the ethanol concentration affect the survival time or physical characteristics of the bean beetles that were exposed?

Instructors should remind students to consider:

- How did your experimental animals compare to the controls?
- What were the major effects of the teratogen?
- Were you able to observe a dose-dependent response to the teratogen?
- Were you able to observe differences in effects based on time of exposure and/or duration of exposure?
- Was there any correlation between the way your experimental animals responded to the teratogen and the way humans or other animals have responded? (they will need to do some research here)
- Were you able to repeat your results?

Data Collection

There are several variables the students can test, including:

- Concentration of ethanol
- Volume of ethanol
- Duration of exposure (how many days during the developmental period will they receive ethanol) (once a day, twice a day, etc)
- Point during the developmental period (1st week only, 1st and 2nd week, 3rd week only, etc)

There are a lot of possibilities, and each student pair typically designs a different experiment. **We stress the importance of having just one variable per experiment**.

Students typically setup two different experiments: 1) one with varying concentrations of ethanol, and 2) one varying the developmental period at which the embryos are exposed.

For example:

1) Use 0% (control), 10%, 20%, and 40% ethanol, administered once a day for the first week of embryo development. This tests a possible dose-dependent response.

2) Use 0% (control) or 20% ethanol and administer during the first week of development only, some during the second week only, some during the third week only, and some during 4th week only. This tests for a critical period in development.

We have students brainstorm the best ways to administer the ethanol, but most decide to soak a Kimwipes™ sheet and place the beans with eggs on top of the soaked Kimwipe™ sheet. Students administer 1-2ml of ethanol (or water) to the wipe once a day for the specified time period. We have also had students try soaking the beans before or after the eggs have been laid. Note that beans soaked in water will sprout. Eggs are cultured in 6cm petri dishes on top of the Kimwipe ™ sheet. Plates are kept in a 30 degree C incubator to control for temperature.

Students typically start each experimental treatment with 30 beans that each have one egg that was laid the previous day. Matings are set up on Day 1, and beans with eggs are collected on Day 2. For an experiment with four different treatments, students must collect at least 120 day one eggs (each on a single bean). All eggs are collected out of a single large mating dish and then randomly allocated to each of the 4 experimental group dishes.

At the beginning of the third week after eggs have been laid, students must check their dishes every day to look for emerging adults. We encourage them to keep detailed notes about the emergences. Students do 2-3 replicates of each experiment. Over the course of the semester, they can repeat the experiments 2 or 3 three times. (Keep in mind any holidays or breaks and try to plan so that beetles are not emerging that week!)

Data Analysis

Students can collect a variety of data, including

- Percent emergence: out of the 30 eggs in each dish, how many emerged?
- Time to emergence: students keep track of how many days have passed since the eggs were laid (day one) until they emerge. Typically, we have seen that eggs treated with ethanol take longer to emerge!
- Male to female ratios: of all the beetles that emerge, how many are male, how many are female?

- Physical malformations or functional defects: students must closely observe the beetles compared to controls

After emergence, we have had students mate the ethanol treated beetles with each other, and mate the control beetles with each other, and then compare the number of day one eggs. We have preliminary data suggesting that beetles treated with ethanol as embryos produce fewer eggs as adults than control beetles.

Other follow up studies could be done as well: do the offspring of the ethanol treated beetles have any functional defects? How long do the ethanol-treated adults live?

Statistical tests could include a student's t-test and ANOVA, or Mann-Whitney and Kruskal-Wallis tests if the data are not normally distributed. If the students are comparing two groups only (example: percent emergence of control versus 20% ethanol treated), they can use a t-test or Mann-Whitney test. If the students have more than two experimental groups, then an ANOVA or Kruskal-Wallis test will allow them to see if there is a significant different between the groups.

Cited References

Gilbert, S.F., and Epel, D. 2008 *Teratogenesis: Environmental Assaults on Development*. In: Ecological Developmental Biology. Sinauer Associates, Inc. Pp 167-175

Acknowledgments

Thank you very much to all of the students who worked so hard on their semester projects, and took such good care of their beetles. Also a big thank you to Chris Beck and Larry Blumer for introducing us to the bean beetle model system, our students love it.

About the Author

Kathryn Lipson is an Associate Professor of Biology at Western New England University, where she has worked since 2009. In addition to Developmental Biology, she also teaches A & P, Animal Behavior, and General Biology.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/.**

Papers published in *Tested Studies for Laboratory Teaching: Peer-Reviewed Proceedings of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Citing This Article

Lipson K L. 2018. Investigating Critical Periods in Bean Beetle Development: Incorporating Guided Inquiry Research into a Developmental Biology Laboratory Course. Article 70 In: McMahon K, editor. Tested studies for laboratory teaching. Volume 39. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). **http://www.ableweb.org/volumes/vol-39/?art=70**