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

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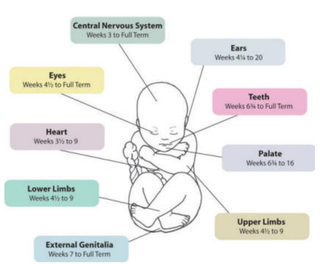
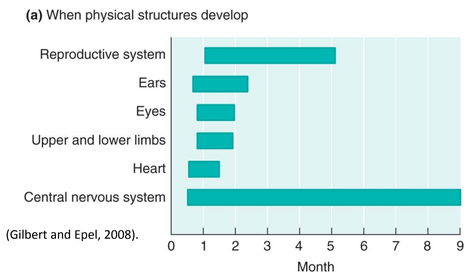
Abstract

Integrating research into undergraduate courses allows students to fully experience the scientific process. 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 a Powerpoint presentation to the class. This format is useful for instructors wishing to incorporate multiple semi-independent research projects into undergraduate laboratory curricula.

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. 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.



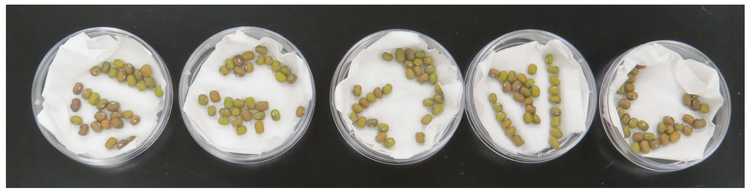


(Gilbert and Epel, 2008).

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 Kim Wipe and place the beans with eggs on top of the soaked Kim Wipe. 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 Kim Wipe. 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?

Students tally the number of beetles emerging from the bean, and look for the presence of emergence holes

- 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?

Students record sex ratios for each treatment group. Here a male is picture on the left, female on the right.

- 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

Sample Student Data

Pairs of students enrolled in Developmental Biology developed experiments to determine the effect of ethanol on bean beetle development with the goal of identifying a critical period where larvae are most sensitive to ethanol exposure. The following is aggregate data collected by a mixed class of junior and senior undergraduate biology majors.

Effect of Ethanol Concentration on Emergence

The number of beetles that emerged was compared to the total number of eggs laid for each ethanol concentration as shown in Figure 1. There is a clear dose dependent relationship between the percent of beetles emerging and ethanol concentration, with 95% ethanol resulting in zero emergences over a 2-week period from the time of expected emergence.

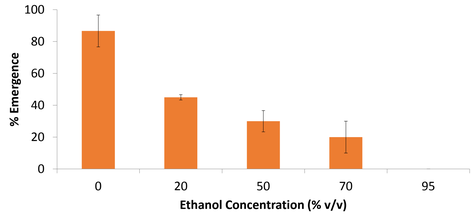


Figure 1. Effect of Ethanol Concentration on Emergence. The number of beetles emerging were tallied for each treatment group of 30 beans, each with a single egg. Error bars reflect standard error, n = 5.

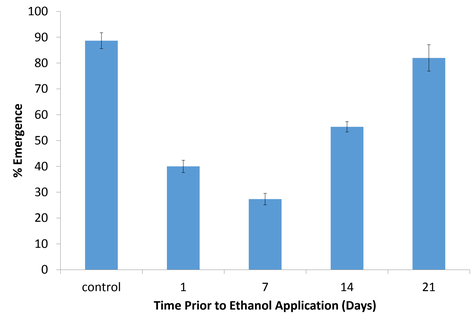


Figure 2. Relationship between the time of ethanol application and been beetle emergence. The number of beetles emerging were tallied for each treatment group of 30 beans, each with a single egg. Error bars reflect standard error, n = 5.

Identification of Critical Periods of Sensitivity Beans harboring a single egg were selected and set aside one day after mating. After 1, 7, 14, and 21 days, beans were placed on Kim wipes soaked in 1 ml of 20% ethanol once a day for 1 week. Emerging beetles were counted over a 2 week period. Figure 2 shows that larvae exposed to ethanol 7 days following egg deposition had a lower rate of emergence than those exposed to ethanol earlier or later in development. These same larvae also began emerging later than control beetles that were not exposed to ethanol and those exposed to ethanol later in development (Figure 3).

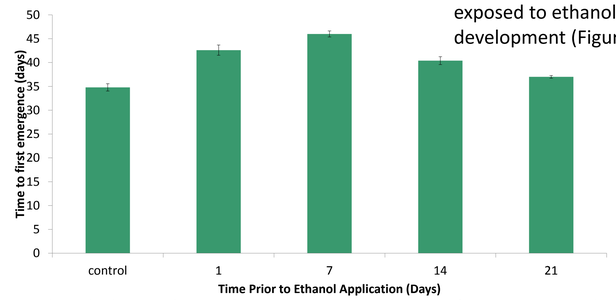


Figure 3. Relationship between the time of ethanol application and the time to first emergence in each treatment group. Error bars reflect standard error, n = 5.

Conclusions

- Exposure to ethanol effects developing bean beetles in a dose-dependent manner, with 20% ethanol causing over a 50% reduction in emergence rates as compared to control.
- The most critical period is during the second week of development, beetles exposed at this stage had the lowest rates of emergence, and also took the longest amount of time to emerge.
- Bean beetles may be a good model system with which to study effects of ethanol and other teratogens in an undergraduate lab.