Drosophila & Beer

An Experimental Laboratory Exercise

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Students appear to like working with living organisms and *Drosophila melanogaster* has historically been a popular organism for studying genetics and development. One exercise that is versatile and applicable to many student levels involves maintaining *Drosophila* on medium prepared with varying concentrations of beer and evaluating the effects on reproduction, life cycle stages, and sex ratios compared to control flies cultured on water-reconstituted medium.

Experimental Design

The basic design of the experiment involves manipulation of the medium in a way that replaces water with varying types and concentrations of beer. The control is the container (tube) that has water as the substance that reconstitutes the medium. I generally use Wards medium, but any basic *Drosophila* medium should suffice. For example, assume that the following experiment is set up:

Drosophila Tube Preparation

All tubes have 3 grams of medium, l grain of yeast, and l0 ml of the designated reconstituting solution (sterile water is used to dilute the beer to the proper concentration).

- Tube 1: water
- Tube 2: 25% beer
- Tube 3: 50% beer
- Tube 4: 75% beer
- Tube 5: l00% beer

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Generally, I have each group (two to four students) prepare at least two replicas of each concentration; certainly more replicas could be set up if supplies and analysis time are available. Once the tubes are prepared, they should be capped with plugs or equivalent tops. Finally, tubes need to be labeled with the date, type of reconstituting solution, and experimenters' names.

Drosophila Sexing

Students should be shown how to sex male and female flies. I have found it helpful to initially use flies that have been recently killed (by ether or temperature) for this purpose. It helps to have a large, overhead illustration to point out the male sex comb (main indicator of sex), abdominal shape, and size.

Placing *Drosophila* in Tubes

This can be difficult for beginning students (especially for nonmajors or for younger students). Students may need to practice the anesthetic procedure (note: ether, fly nap, or any other method can be used). Students tend to overtreat the flies and are frustrated when the flies do not wake up after being placed in the tubes. On the other hand, if the flies are not adequately anesthetized, they will wake up and fly away while students are sexing them. Briefly placing gauze saturated with ether over the inverted petri dishes (containing the awakening flies) can help to minimize this problem. I usually indicate to students that it is good to see some twitching of the flies when they are selected because that is an indicator they will eventually wake up. Flies can be transferred from the binocular stage to the side of a tipped tube. It is important to keep the flies away from the damp and sticky medium until they wake up. It is not

unusual for students to have to redo some of the tubes due to fly mortality. As the alcohol evaporates, it is necessary to add small amounts (0.5 ml) of the various alcohol concentrations twice a week or so during the culturing period. Use a Pasteur pipette and let the solution slide down the side of the tube. It is particularly important to have appropriate alcohol concentrations during the larval stages of development. Note that the flies used for this exercise do **not** have to be virgins.

Observations & Recording of Data

The tubes should be observed and data recorded as frequently as possible. Students need to be made aware of the life cycle of fruit flies and directed to observe the appearance and relative numbers of larvae, pupal cases, and emerged flies in each tube. The observational log should be neat and organized. Also, any unusual events such as fungal or bacterial contamination should be noted and the consequences assessed.

Graphing & Analysis of Data

This can be done in a number of ways. Usually I have the students analyze their data and share the results during entire class analysis. Students frequently elect to do computerized graphs of the data.

Discussion & Conclusions

Discussion of results can be done in a number of ways depending on the time and intent of the instructor. At this point, it is helpful to remind students to consider the normal environment of *Drosophila* and their environmental exposure to alcohol in ripe and fermented fruit and vegetables. *Drosophila* may be more resistant to alcohol than some other organisms would be. In addition, the constituents of beer could be discussed, especially the typical alcohol concentrations, nutrient components, and processing procedures (students are generally interested in this). For appropriate student levels, a trip to a brewery could be considered to observe how beer is brewed.

Experimental Variation & Optional Add-ons

1. Different types of beer or other alcoholic beverages can be used for the reconstituting solution. Remember, a water control needs to be included in all experimental designs.
    - Instead of designing the experiment for combining the data from all the student groups, each group could use a different type of beer and make comparisons of results.
    - Another possible design is to compare alcoholic and nonalcoholic beer of the same brand.
    - Other alcoholic beverages could be used, however, they generally have to be diluted to lower levels to reduce toxicity. An experimental beverage concentration test series will need to be run for each alcoholic beverage. I should caution in this regard that wine generally results in an array of fungus contaminated tubes.
2. Add an associated alcoholic gradient series to the experiment. Consider introducing students to a graded (l% to l0%) ethyl alcohol (ETOH) series. Beer usually contains 6% ethanol. The gradient series serves as a reference standard for assessment of the alcohol component of beer (for example: 25% beer: ~1.5% ETOH, 50% beer: ~3.0% ETOH, 75% beer: ~4.5% ETOH, l00% beer: ~6% ETOH).
3. Gradient series of other beer constituents, such as malt, could also be evaluated. This may require determination of an acceptable concentration range of the ingredient and some experimentation on a feasible way of preparing the medium. Performing a Web search or consulting a brewery could provide help in this regard. Some ingredients in beer are claimed to be nutritious and may contribute positively to fly reproduction, development, and survival.
4. Consider persistent (generational) exposure to beer as part of the experiment. If time allows, interested students may want to study the generational influences of beer exposure. A few flies could be transferred to fresh medium containing the same (or increased) concentration of beer, and assessed for possible development of alcohol tolerance and/or other developmental modifications.
5. Note fly behavior. It is not the intent of this experiment to assess the behavior of intoxicated flies; however, students are frequently interested in this topic and record observations on fly behavior in their reports.
6. Use other chemical substances to replace the water. In this *Drosophila* model, any liquid substance could replace the water in the reconstituted medium. I have had students test a number of substances (some solid, but dissolved in water—such as aspirin or other medication) with interesting results. The model is an effective way to assess the effect of environmental modification on *Drosophila*'s reproduction and development. In one experiment, it was determined that flies are extremely sensitive to even very low concentrations of nicotine. (Note: Nicotine is potentially dangerous to handle and even weak dilutions should be prepared by the instructor.)

Typical Experimental Observations & Results

1. Alcohol at low concentrations (1-2%) appears to act as a sterilizing agent. Elimination of bacterial growth enhances the general state of the culture. When student experiments indicated this result, I immediately applied the information, with positive results, to culturing flies for genetic crosses in my genetics classes. Bacterial contamination is frequently a problem in routine *Drosophila* genetic crosses. This problem can be reduced by merely adding a small amount (usually1-2%) of ethyl alcohol to the water used to reconstitute the medium.
2. Beer concentrations of 25% have more pupae and flies than the controls. Higher concentrations tend to reduce the number of pupal cases and fly numbers, and to slow the developmental periods (larval stages, pupal period, and time for emergence of the flies). The l00% beer concentration is particularly detrimental to fruit fly reproduction and survival.
3. Sex ratios (female to male) of emerging flies can also be determined; however, this process requires time and is a tedious process. I usually offer this as an optional activity for interested students. Female flies tend to increasingly outnumber the males as the beer concentrations increase. Whether this is an actual sex difference in reproduction or an alcohol-induced feminizing effect was not determined. The emerging male flies could be more sensitive to the beer (alcohol).
4. A few cultures (both control and experimental) will have fungal contamination. Any fungal growth dramatically reduces the numbers and growth rate of *Drosophila*. The larvae appear to be very sensitive to fungal toxins. Students like to speculate as to possible nutritional benefits of beer. At this point, I should mention a rather strange but inconsistent observation. Once in a while, giant larvae appear in the beer cultures. I'm not sure as to the cause; however, I mention it as a matter of intrigue since I have never seen larvae this large in routine *Drosophila* cultures.

Conclusion

I have had fun developing and implementing this exercise. The format is flexible and the hands-on experience valuable for all levels of students. The younger students and nonmajors demonstrate obvious excitement and interest in setting up the experiment. The genetics students gain hands-on experience in dealing with flies, which helps them in more difficult crosses. The obligations of data accumulation and assessment can become a bit tedious; however, at the end of the exercise students generally like to discuss the data and draw conclusions. They also like to speculate about potential human applications of the data.

The basic model of using *Drosophila* cultures, in which the medium contains various unique components (chemicals, drugs, or other substances), and of determining their effects on fly reproduction, growth, and development, demonstrates to the students how a basic protocol can be used to study and draw conclusions on the exposure of living organisms to a number of environmental substances. Students learn how to design an experiment that contains both test (experimental) organisms and controls. They learn how to carefully record data and do statistical analysis. The sophistication of the statistical analysis depends on the level of the students and their experience using computer software, as well as the type and amount of data collected.

In terms of biological information, students learn about the normal life cycle of *Drosophila* and the factors that can modify it, as well as about fly reproductive processes. Although it is frustrating, students quickly learn that living organisms do not always respond as anticipated and sometimes basic elements of the experiment need to be modified or redone. For example, when I first tried this experiment using hard liquors instead of beer, most of the flies died and the liquor concentrations had to be dramatically reduced. Notably striking is the negative effect of fungal toxins on *Drosophila*, especially during the larvae stages. Some contaminating fungal spores are normally present in the classroom environment; others may be in the beer. Students are always upset when they realize one or more of their cultures is contaminated; however, this experience is an opportunity to learn about the critical environmentally-limiting factors in *Drosophila* survival. The possible influences of beer on sexual development or sex-related survival of the flies is intriguing and deserves further study. Beer has been implicated in estrogenic influences on human males; perhaps similar effects are occurring in the flies.

The topic of beer and its influences on *Drosophila*'s reproduction and development may extend in a number of ways to the human situation. I usually use this exercise as a lead-in to a discussion on the effects of alcohol on human embryonic development, especially in regard to the occurrence of Fetal Alcohol Syndrome.

This laboratory exercise can be done in class or independently. The protocol is flexible and can be creatively expanded by the instructor and/or students. One test of the interest and enthusiasm of students is whether they discuss course work with their friends and/or parents. A great deal of discussion took place outside of the classroom with this exercise.