

Rearing Media as a Variable in Fruit Fly Fecundity: An Activity to Introduce Scientific Methods of Inquiry to Biology Students

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Abstract: A major challenge in teaching the process of science to students is designing and implementing laboratory activities that emulate what is actually done in a research laboratory. To facilitate this effort, science educators have been encouraged to design exercises that span multiple laboratory periods, encourage independent thinking, promote hypothesis-driven experimentation, and data collection and analysis. We have designed an inquiry-based, semester-long laboratory activity amenable to majors or nonmajors and to introductory or advanced biology students. This activity utilizes *Drosophila melanogaster*, the fruit fly, as a model organism that allows students to investigate how different rearing media additives affect female fecundity measured as numbers of eggs laid. To explore the feasibility of our activity aimed in helping students learn the processes of science, we assigned the activity independently to three different student populations. These included 1) students in an undergraduate biology laboratory; 2) an independent undergraduate research project; 3) a Distance Education Biology Master's graduate student summer research project. The goal of this laboratory activity is to allow students the opportunity to design a controlled experiment, formulate testable hypotheses, identify variables, make quantitative and qualitative observations, and analyze data using a simple computer spreadsheet program.

Keywords: Inquiry-based, fruit fly, fecundity, rearing media

Introduction

Drosophila melanogaster, the fruit fly, was an early model for genetics research, which prompted scientists to develop media that produced consistently high numbers of offspring. Early studies proposed that the different varieties of yeast within the various media were influencing factors in fruit fly nutrition and subsequent development (Baumberger, 1917; Tatum, 1939; Robertson and Sang, 1944). Media containing a variety of additives including pears, raisins, rice, molasses, and oat hulls have been reported (Bridges and Darby, 1933). More recent work showed that developmental and fecundity (egg-laying) rates varied when fruit flies were reared on media of sugar, tomatoes, and grapes (Jaenike, 1986). Currently, commercially available dry mixtures aim to standardize the media used for rearing fruit flies (Flagg, 2005). These media are intentionally produced unsupplemented and uncolored, allowing researchers to personalize it with their own additives.

The effects of different food media \pm yeast on fly fecundity can be easily observed and quantified, which lends itself to use as an activity for biology students.

Fruit flies represent an exemplary investigatory tool for studying genetics both in the research and classroom laboratories. They are inexpensive, easy to rear, have a short life span (15-20 days), are easy to sex, and require very little space or special equipment (Ashburner and Roote, 2000). In addition, fruit flies are commonly used at all levels of biology education, from middle and high schools, to college level biology courses as a tool for teaching introductory Mendelian genetics. For these reasons, fruit flies make an ideal model organism for use in this laboratory activity.

Evidence, models, and explanation are part of the unifying concept and process themes in the National Science Education Standards (NSES). Teaching biology students how scientists use

controlled experiments to answer questions and test hypotheses is essential to their understanding of the scientific process. The NSES (1996) states, "Science as inquiry is basic to science education and a controlling principle in the ultimate organization and selection of students' activities." In this paper, the use of fruit flies as a classroom model for scientific inquiry is discussed.

The investigation of fecundity rates in *D. melanogaster* was performed at three levels of biology education. These included 1) an undergraduate biology laboratory exercise, 2) a two-semester long independent undergraduate senior research project, and 3) a Distance Education Biology Master's graduate student summer research project. In testing this activity, students were allowed to design and perform hypothesis-driven controlled experiments in order to measure the fecundity rates of female fruit flies reared on plain media \pm yeast and media supplemented with a variety of additives including, artificial colors, fruits, cooking additives, grapes used exclusively in wine making, and a commercial energy drink. This simple, but engaging activity was amenable to all levels of science students. This laboratory activity uses inquiry-based learning to help students develop scientific process skills by comparing short-term fecundity rates of female flies reared on different media in a controlled environment over the entire semester. Guidelines are given for setting up culture vials and data collection, but the students choose the independent variable they wish to test in their experiment. At the beginning of the investigation, students are asked to formulate null and alternative hypotheses about the results they expect to obtain and to identify the experimental variables. A discussion with students concerning hypothesis acceptance or rejection using statistical analyses is undertaken. It takes only a few minutes to analyze a set of data for significant differences using appropriate software. By graphing their data, either through the use of a spreadsheet program or by hand, students are better able to visualize the potential differences in their data, aiding them in formulating and writing their conclusions. Lastly, the students were required to present their findings in the form of a written manuscript following the guidelines of a peer-reviewed journal, which included an extensive review of the literature, and a formal discussion of their results. In their discussion, students were encouraged to include observations and perceptions of the process of science after completing the activity. This paper allowed assessment of student understanding of the activity and the process of science in general.

Materials

Fruit flies were reared in seventy-five mL plastic culture vials or 250 mL plastic culture bottles with foam plugs. Boiled baby food jars with cheesecloth rubber-banded around the mouth could be used as an inexpensive alternative. An insect culture chamber maintained the fruit flies at a constant temperature ranging between 24–26°C during all experiments. If you do not have access to an incubator, a room in which the temperature can be maintained at ~25°C during the entire experiment can be used. It is important to note that the warmer the temperature, the faster the fruit flies will complete their life cycle. The plain medium for control vials or plain medium used in mixtures with different food additives was purchased from Carolina Biological (Catalog # 17-3200). The yeast used was Fleischman's brand baker's yeast and can be obtained from almost any medium-sized grocery store. For immobilizing the fruit flies, a homemade etherizer consisting of a 250 mL glass bottle with cotton balls in the bottom, a 50 mL plastic conical tube with holes punched in the side, and a powder funnel was used with ether as the anesthetic. In order to dispose of fruit flies, they were "morgued" or dumped into a fly morgue that consisted of a flask containing water with a pinch of salt, a drop of liquid detergent, and a powder funnel inserted into the mouth of the flask. Other supplies that were needed included a soft-bristled paintbrush to manipulate the fruit flies, a white note card for contrast when viewing fruit flies, a dissecting microscope for analyzing fruit flies, and the food additive of choice to be tested (Ashburner and Roote, 2000; Flagg, 2005).

Procedures and Results From An Undergraduate Biology Laboratory

For the student investigation performed in the undergraduate biology laboratory, the guidelines were explained and the students separated into groups of four. The groups brainstormed to choose their experimental food additive. They were restricted to an additive that was legal to have on campus and that a fruit fly may encounter in the "wild". The students outlined in their laboratory notebooks the procedure they were going to follow in making their experimental food and the process they would follow for the duration of their experiment (approximately 3 months). During this initial class period, the students were trained in fly handling and sorting, especially on how to properly etherize the fruit flies. Briefly, approximately 1 mL of ether was added to the cotton balls in the etherizer. The fruit flies were placed into the conical tube, and watched until the fruit flies stopped moving. The fruit flies were dumped on a white note card, placed on the stage of a dissecting microscope, and sexed. For sexing of fruit flies, the students are instructed on the differences in the size of the females and male fruit flies, as well as other

distinguishing characteristics. Males are slightly smaller than females and have a dark-colored anal ring at the posterior end of their abdomen. The sex combs on the male, which are black hairy tufts on their forelegs, are especially helpful in sexing newly emerged fruit flies. In contrast, females have somewhat pointed posterior ends and no sex combs (Flagg, 2005).

During the second laboratory period, students prepared their experimental media. One group prepared the control media (plain \pm yeast) and the other groups prepared media \pm yeast with additives including apples, a commercial energy drink, and red or purple food coloring. For the initial set-up, students were provided with two stock bottles of the media \pm yeast for a total of 4 bottles per group. Each bottle had approximately 5 grams of plain, uncolored, commercial medium. Apples were pureed in a blender with nanopure distilled water prior to adding to the commercial food mix. Liquid was added to the commercial medium until it reached the consistency of mashed potatoes. For the yeast containing bottles, approximately 1 milligram of yeast was sprinkled on the top. To each of the bottles, 50 female and 50 male wild-type fruit flies were added and allowed to mate for 96 hours, after which time the fruit flies were morgued. For the remainder of the experiment, the students were instructed that they would have to come into the lab outside of their regularly scheduled class period to set-up the experiment and to sex and count fruit flies. This step helps the students to realize that science experiments are not done in a scheduled block of time, one day a week. For the next part of the experiment, the students prepared 3 bottles each of their media \pm yeast for a total of 6 bottles per group. They collected virgin females from their stock bottles and mated them to males. To collect virgins, the bottles were "cleared" (i.e., all the fruit flies morgued) early in the morning and females collected within the next 8 hours. For each bottle, 10 virgin females and 10 males were added from the stock bottle, allowed to breed for 72 hours, then morgued. The larvae were left to feed on the media, develop, and eclose (emerge). Once the fruit flies started to eclose, the students had to come in every day to sex and count fruit flies. This had to be done at 8 hour intervals so that the newly eclosed fruit flies would not mate in the bottles. The fruit flies were counted until the bottles did not produce any more fruit flies,

which indicated the first generation was finished. The students were instructed to make qualitative and quantitative observations during the experiment, including changes in the media, describing the developing larvae, numbers of larvae, and phenotype of adults. Their data was compiled using a Microsoft[®] Excel spreadsheet, and their experimental data was compared to the control bottles and included differences observed between males and females. They performed statistical analyses of their data in the spreadsheet using the student's *t*-test function as provided in the software's statistical analysis package (Microsoft[®] Corporation, Redmond, WA). Lastly, they were instructed to write a complete discussion including published literature to support their ideas.

For all treatments, statistically significant differences in the numbers of offspring from fruit flies reared on all experimental treatments compared to those reared on plain media \pm yeast was found (**Figure 1**), suggesting that the additives had a detrimental effect on fly numbers. There were no significant differences in any treatments between the numbers of males and females. In this experiment, the energy drink caused the internal organs of the fruit flies to turn black, which the students found a bit disturbing and concluded that the drink was "rotting" the fruit flies. Other interesting observations were that adding yeast had no effect on the fruit flies reared on purple or apple containing food. The apple containing food was explained by the high amount of fructose in the fruit. The students found that the red food coloring killed the fruit flies. The more likely cause was not found until after some deep probing revealed that the students had used the cooper laden tap water from the building to make their media.

Procedures and Results From a Two-Semester Long Undergraduate Senior Research Project

For the two-semester long independent undergraduate senior research project, the protocol outlined above was followed except that the addition or removal of additives was based upon the preference of the student and yeast was only included for the control. The additives not repeated in this experiment were the red and purple food coloring and the apples. The new additives tested were 10% honey, 25% vinegar, and Traminette and DeChaunac

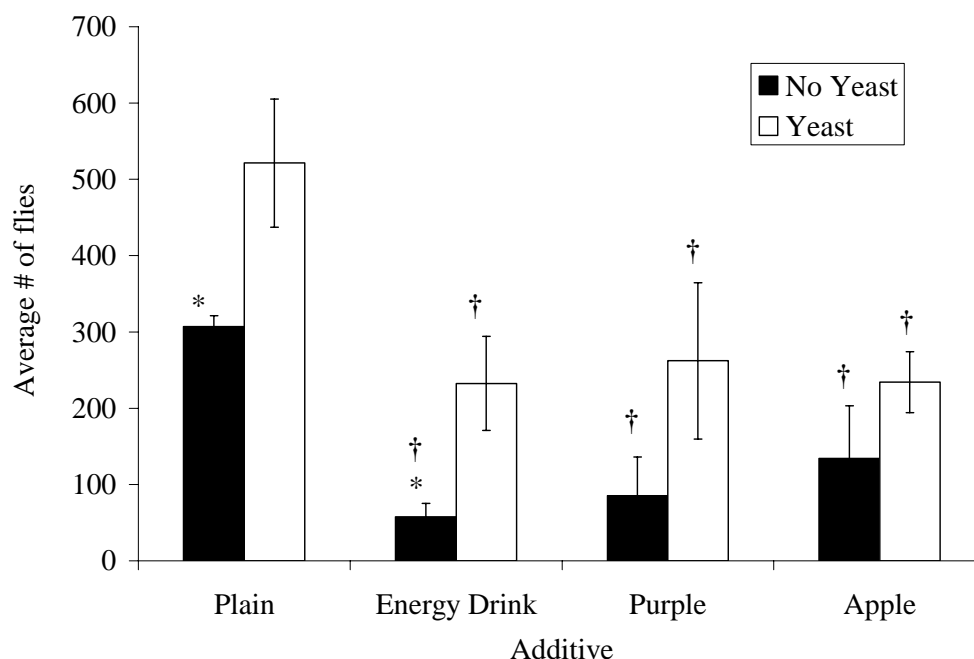


Figure 1. Experiment #1: Comparison of average number of total fruit flies collected for each media ± yeast. * $p < 0.05$ when no yeast medium was compared yeast containing counterpart (i.e., energy drink – yeast compared to energy drink + yeast); † $p < 0.05$ media ± yeast was compared to plain media ± yeast (i.e., energy drink + yeast compared to plain + yeast or energy drink – yeast compared to plain – yeast); error bars are standard error of the mean (SEM).

grape juices obtained from Mac's Creek Vineyards and Winery in Lexington, NE, which is where the student was employed. These were selected because the student noticed a large number of fruit flies associated with these juices compared to the other juices used in the wine making process. The honey and the vinegar treatments arose from the old adage, "You catch more fruit flies with honey than vinegar".

In this study, an interesting observation was that the first emergence of flies from the media containing the energy drink was a full week ahead of all the other treatments, even though the bottles were all started at the same time. The student concluded that this may be due to the high concentration of caffeine found in the energy drink. For all treatments, statistically significant differences in the numbers of offspring from fruit flies reared on all experimental treatments compared to those reared on plain medium with yeast was found (**Figure 2**). The student concluded that there are a couple factors important in considering the effect yeast has on increasing fecundity. One is that the yeast produces an abundance of riboflavin, or vitamin B₂. This vitamin is important in the development of the flies in their larval stages (Bruins, Scharloo, and Thorig, 1997). The other factor is that increased yeast metabolism causes a large amount of yolk protein to be produced. Yolk protein is one of the main

constituents of proteins that a female *D. melanogaster* uses in reproduction (Carlson and Harshman, 1999a). There were no significant differences in the numbers of males versus females between any of the treatments.

Procedures and Results From A Distance Education Biology Master's Graduate Student Summer Research Project

The graduate student summer research project was performed to modify the protocol possibly for high school use. In this study, fruit flies were reared in 75 mL culture vials to determine whether the experiment could be scaled down and still be successful. Also, different additives from the previous examples were tested based upon the preference of the graduate student, including plain ± yeast, bananas, and kiwis. Each vial contained 15 mL of total food media, with control vials containing 100% plain medium and each experimental vial containing 25% of the additive. Once again, whole fruits were pureed in a blender prior to adding to plain medium. The yeast treatment had 0.1 mg of yeast sprinkled on top. Stock vials were maintained on plain medium without yeast or any other additives. For the experiment, six virgin females and six males from the stock bottles were added to six vials of each treatment and each treatment was repeated three times over a period of two months. All males were

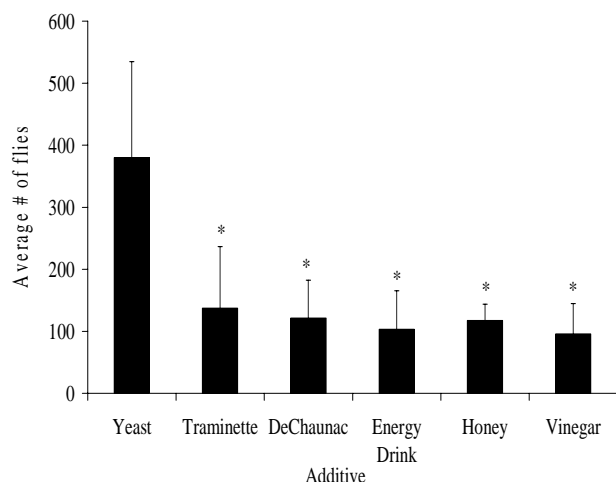


Figure 2. Experiment #2: Comparison of average number of total fruit flies collected for each treatment . * $p < 0.05$ when compared to yeast; error bars are SEM.

removed after forty-eight hours and after another forty-eight hours, the females discarded. The offspring were collected every 12 hours until offspring no longer emerged. The data was compiled using a Microsoft® Excel spreadsheet. Data collected from the control group was compared to the experimental fruit flies reared with or without yeast. Statistical analyses of the data were again done in the spreadsheet using the student's *t*-test function.

This investigation found that the majority of offspring emerged within the first five days of the first emergence. Emergence time was 9-11 days for fruit flies reared on plain medium with yeast, and 13-14 days when reared on banana or kiwi containing media. Statistically significant differences in the numbers of offspring from fruit flies reared on kiwi, banana, or plain media compared to those reared on plain medium with yeast was found (**Figure 3**). No significant differences between kiwi, banana, and plain media or between the numbers of males and females were found. In this experiment, all kiwis were found to not be equal. The first few batches of food killed all of the fruit flies. This was rationalized to be due to the use of pesticides or chemicals during the growing season. Therefore, the fruit was scrubbed before use. This proved to be of no help and the source of death was never identified. Also, mold inhibitor was not used and there were several incidents of mold overtaking vials. In the future, this will be rectified by adding mold inhibitor to the vials. This investigation also found that the experiment could be scaled down and still produce similar results to the larger experiments. The graduate student rationalized that there are many factors affecting

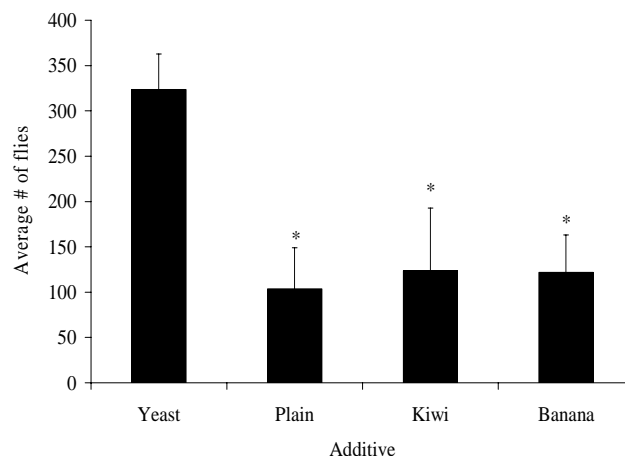


Figure 3. Experiment #3: Comparison of average number of total fruit flies collected for each treatment. * $p < 0.05$ when compared to yeast; error bars are SEM.

fecundity in fruit flies. Female fruit fly body size has been identified as an attributing factor. Large female flies have been shown to lay an average of 80 eggs per day, while medium sized flies lay approximately 30 eggs, and the smallest females lay an average of 15 eggs per day (Chiang and Hodson, 1950). Nutrition can also impact the body size of a female fruit fly by increasing the percentage of ovarioles containing vitellogenic or yolk containing egg stages (Carlson and Harshman, 1999b). Other factors such as age of the female (Shorrocks, 1970) and population density (Sameoto and Miller, 1966) have been identified as factors influencing fecundity.

Discussion

This laboratory activity has now been successfully repeated a number of times at different levels of biology education. Most conventional college laboratory class periods are designed to fit in the allotted time, and are “cookbook” exercises completely laid out for the students to follow with little chance of error. Based upon student comments found in the discussion section of their formal laboratory manuscript, it was found that they learned that science is not performed in the confines of a 1-3 hour lab period and that critical thinking and data analysis are paramount to the scientific process. They appreciated not having the laboratory worked out for them and having some input in what they were studying. This carries over with the fact that many of them stated that they did not mind coming in on their own time to carry out the experiment. The undergraduate student is pursuing a career in teaching

high school biology and the graduate student is a middle school science teacher. Both of these students have stated that they can amend this project for their classrooms and will do so in the future. This process is important because many high school students do not get exposed to this type of hypothesis-driven laboratory activity that more closely resembles an actual research project, unless they do an independent science fair project. Therefore, this activity can be successfully used to expose all students, majors or nonmajors, introductory or advanced biology students, and high school or undergraduate students, to the process and excitement of doing a “real” research project. This investigation gives students an

engaging activity to help them learn about the process of scientific inquiry and the components of a controlled experiment.

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