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Fruit fly as a model for alcoholism: Integration of laboratory pedagogy and student-directed research.

Reynolds, Elaine R.[1], David Sison, Taisha Jerez, Gabriel Eusebio, and John Drummond. Biology Department and Neuroscience Program, Lafayette College, 111 Kunkel Hall, Easton, PA 18042; [1]corresponding author: reynolde@lafayette.edu, 610-330-5654.

Overview

Drosophila melanogaster is a good model system for examining genetic predisposition to alcoholism. Flies exhibit a characteristic behavior upon alcohol exposure that includes hyperactivity and then sedation (Wolf *et al.*, 2002; Heberlein, 2000). Flies also become tolerant to drug exposure, with single or multiple exposure (Berger *et al.*, 2004). The alcohol-based behaviors are robust and easy to illicit and analyze, ideal for introducing behavioral analysis to undergraduates. The fly model system also has many tools amenable to genetic analysis that are easily manipulated by students. Pedagogies using the model and tools can be used to engage students in active learning in order to achieve student outcomes related to model systems, genomic analysis, use of computer-based tools, and data analysis.

We developed a laboratory module for our General Biology in which insertion mutations available from the Bloomington Stock Center have been screened for alterations in ethanol sedation behavior and tolerance (http://flystocks.bio.indiana.edu/). These inserts are available through the

gene disruption project (Spradling *et al*., 1995). The students map the insert to the first, second, or third chromosomes using standard crosses and their analysis, and perform the behavioral tests over 4 weeks of labs. Inserts that show altered behavior are confirmed and examined in more detail as part of a student-directed research program. Over two years the project has screened over 200 insert lines and involved about 400 laboratory students and three research students. Once an insert has been identified that alters the behavior, the gene disrupted by the insert can be quickly contextualized by the students using FlyBase http://flybase.bio.indiana.edu/.

The screen thus far has yielded a range of defects and mutants. Mutants that are sensitive to alcohol appear at a higher rate in the insertion population than resistant mutants and altered sedation kinetics are more common than inserts that affect tolerance behavior. We have also demonstrated specific student outcomes related to the general biology course and biology program.

Methods and Procedures

Stocks

All stocks were obtained from Bloomington Stock Center from their EP stock collection. Stocks were raised on standard cornmeal molasses food and maintained with 12/12 light dark conditions at 25^{o}C.

General Biology Laboratories

The lab was run for three or four weeks over the semester and provided two main tasks for the students. First, students were asked to map their insert to a particular chromosome. Second, they tested their insert for altered alcohol sedation kinetics as compared to Canton S wild type flies. Students have worked in pairs in the lab, but we have considered having individual students conduct the screening now that we have the procedures for the lab worked out. The tasks were divided up into the three to four laboratory periods with each lab running for approximately three hours. During week one, students set up crosses and learned to identify the markers that would be used in the crosses. During week two, they conducted the sedation testing and analyzed their data using a t-test. During week three, students used FlyBase to learn a bit more about their insert and began to sort and count flies from their cross. During week four, if they needed to, students finished collecting and counting the progeny of their cross and used a Chi-squared analysis to determine if their results fit with either of the possible expected outcomes.

The first task, mapping of the insert to a chromosome, allows students to gain experience with basic genetics. The cross they performed is diagramed in Figure 1. Logistically, we build up large numbers of *w cv f* bottles to allow virgin collection to occur over a three to four day period prior to the onset of the lab module. One of us (Drummond) serves as a full time lab coordinator for our general biology laboratories, and we have a teaching assistant program (TA) at Lafayette. The research students involved with the project built up and maintain stocks, and all of us collect virgins for the crosses in vials of 10-15 females. The student assistants also do multiple transfers of the insert lines. In the first week of lab, students collect ten males from their assigned insert line using carbon dioxide and cross those males to the virgin *w cv f* females provided. During this week, students also look at markers on the stocks in comparison to wild type (Canton S) to make sure they understand the *w, cv, f* markers and the effect on eye color produced by the insert carrying a wild type allele of the *white* gene. Genetically, these markers provide an introduction to the classic *Drosophila* mutants, but also introduce the idea of an engineered moveable genetic component and the consequence of this engineering in terms of dominance/recessive relationships. For the analysis of the cross, the students generate the two alternative genotypic/phenotypic outcomes for the cross

(depending whether the insert is on the first or second/third chromosome), and they create a data sheet that lists all the possible phenotypic outcomes based on those crosses which they use as they sort the flies. This takes the student through a theoretical exercise about genotype/phenotype and independent assortment such as they may get in a classroom setting, then reinforces that concept with the actual results of the cross and how they fit with the possible outcomes. The students analyze their results by comparing their data to the expected outcomes using a Chi square analysis.

The second task, alcohol sedation testing for the insert mutant, introduces the idea to the student that mutations can have nonvisible phenotypes that can alter a behavior. It also illustrates the concept of a genetic screen, a common practice in fly research but something that undergraduates often do not experience. Students dump, without anesthesia, 15 or so flies from a vial of their insert line into a large glass test tube or a culture flask and cap it with a cotton ball. They repeat this procedure with flies from a wild type stock vial. For the behavioral experiments, we do have them create a behavioral inventory to organize their observations. The behaviors we include are movement, jumping or flight behavior, and sedation. We have the students do observations of the flies' behavior in the vial (at 30 s intervals for 2 min) without alcohol in order for the students to see what normal unexposed behavior looks like. Students make their observations with their test tube on the side to minimize geotactic effects. Next the students saturate a cotton ball with 100% alcohol and carefully place it on one end of one of the test tubes. Students make observations at 30 sec intervals about fly movement and indicate the number of flies sedated at each interval until all the flies are sedated. They perform the same analysis with the other tube. From these data they can make some general observations about the effects of alcohol and compute and an average sedation time with the standard deviation for the flies in the container. We have the student compare the average sedation times for the insert and wild type lines using a t-test with Microsoft Excel.

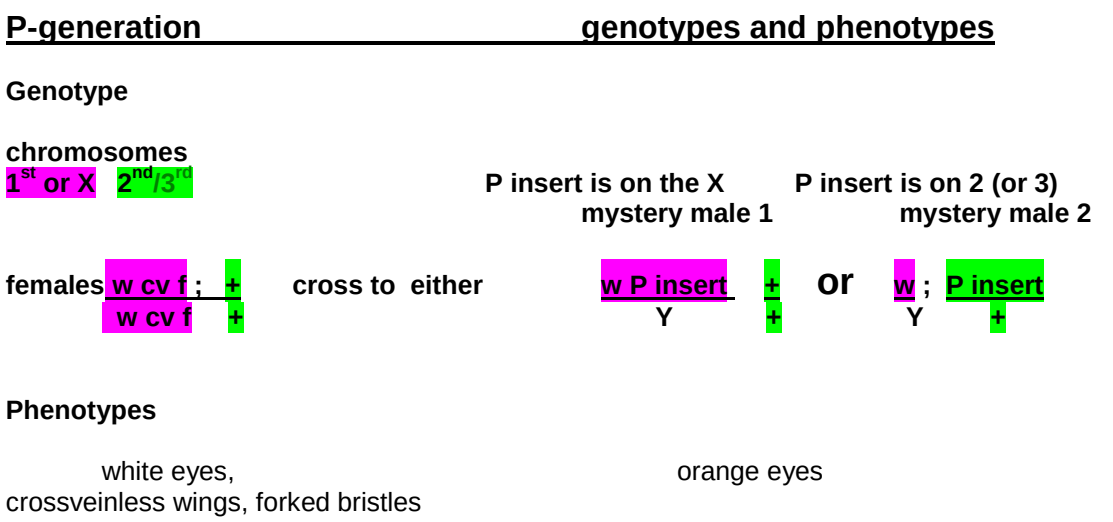

Figure 1. Crosses for mapping insertion mutations.

Also with this lab students explore FlyBase to find out more about the fly genome, their insertion mutation, and the gene disrupted by the insertion. They can compare the chromosomal location of their insert with the entry in FlyBase. We also have them look up the genome map and report specific features of their inserts location. We also ask them to identify the gene that is disrupted by the insert and its putative protein product. We ask them to make inferences from this information about the possible role for this gene in alcohol metabolism or alcohol's behavioral effects.

Student assessment is in several forms. Students are required to write a laboratory report for this module, which includes a title page, short introduction, methods, results (with a figure laying out the cross, observations, data tables form spreadsheets, and statistical analyses), and discussion. Students are given detailed directions for this report and a rubric by which the report will be graded. Students also have test questions related to this material directly on the lab final and indirectly on the lecture final. We are also teaching and assessing specific outcomes related to departmental program goals with this module. Students are also provided with a feedback sheet on the lab where they can directly assess the module.

Research students

Students enrolled in our neuroscience research course have had a large role in the project and serve as authors on this paper (Sison, Jerez and Eusebio). As noted above, research students are responsible for tasks related to preparation for the screen in general biology labs such as stock maintenance and fly collection. After we gather the results from the fall laboratory, we continue to maintain the stocks that showed promise as affecting alcohol sedation. The insert lines are tested again for alcohol sedation behavior using a larger number of flies and more controlled testing methods. Flies are presorted into groups of 10-20 flies, exposed to 95% alcohol in small glass tubes and the sedation times of individual flies are recorded. A sample of greater than 50 flies is used to determine the sedation times. Insert lines are then statistically compared to wild type flies using t-test with a $P < 0.05$ considered significant. Because so many lines have been showing altered sedation we have further subdivided the lines into three categories, highly significant ($P < 0.0001$), very significant ($P < 0.001$), and significant ($P < 0.05$). Flies that fall into the highly significant category were tested for changes in acute tolerance. Flies were exposed to alcohol until sedation (less than 10 minutes, $N = 50$), sedation times were recorded, and the flies were allowed to recover for 2 hrs. Flies then were re-exposed to alcohol and the sedation time of individuals were again observed. The first and second exposures were compared using a t-test where $P < 0.5$ was considered significant. Student research is not assessed directly at our institution (as of yet we have not defined outcomes for these courses), but the career progress of the students involved in the project will be tracked.

Results and Discussion

Early results of mutant screening:

As of early fall 2010, over 200 insert lines have been screened with 50 insert lines identified as having significant difference from wild type in terms of sedation behavior. This places the number of positive/number screened at about 25%. For each of the classroom exercises, several control lines (3 positive controls and 3 negative controls) were randomly included into the assigned lines. Of those, 83% were accurately assessed by the students. This fall an additional 80 lines were screened, but these have not been examined yet. Of the 50 new lines that determined to be significantly different from wild type, 43 were reconfirmed upon retesting. Of these 37 were found to be more sensitive to alcohol (they sedated more rapidly) while three were found to be resistant (they sedated

more slowly). The sedation data from all 43 lines is shown in Figure 2. Thirteen of the 43 were determined to be highly significant and were tested for acute tolerance behavior. All of these strains showed normal acute tolerance as shown in Figure 3.

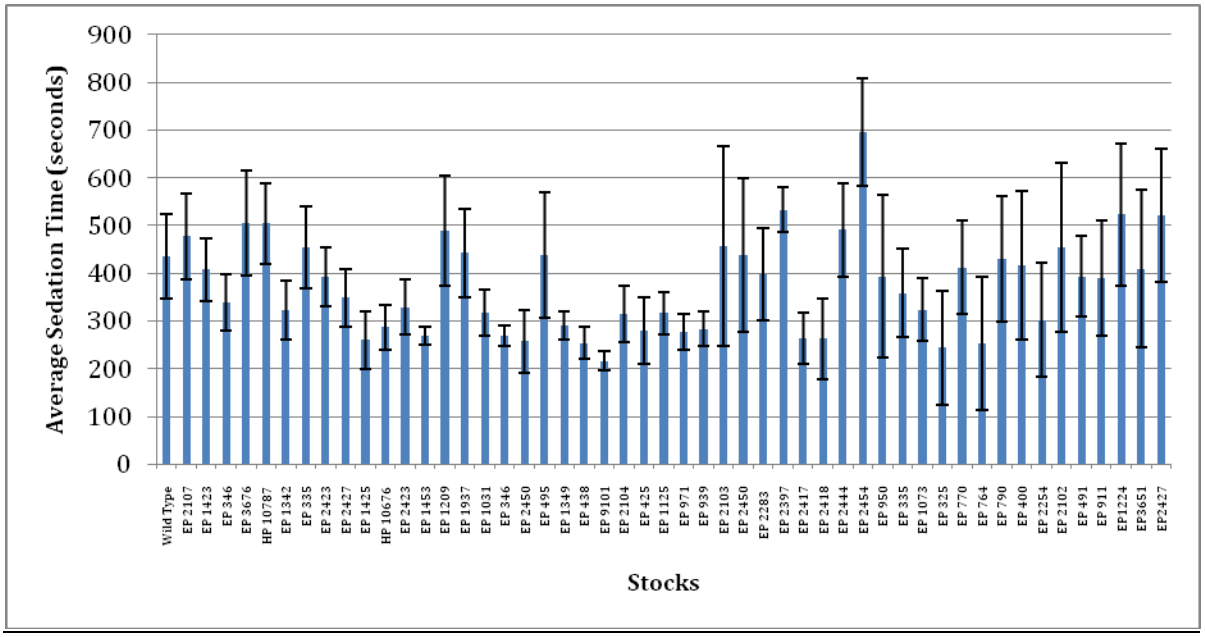

Figure 2. Average sedation time of insertion lines that are significantly different from wild type.

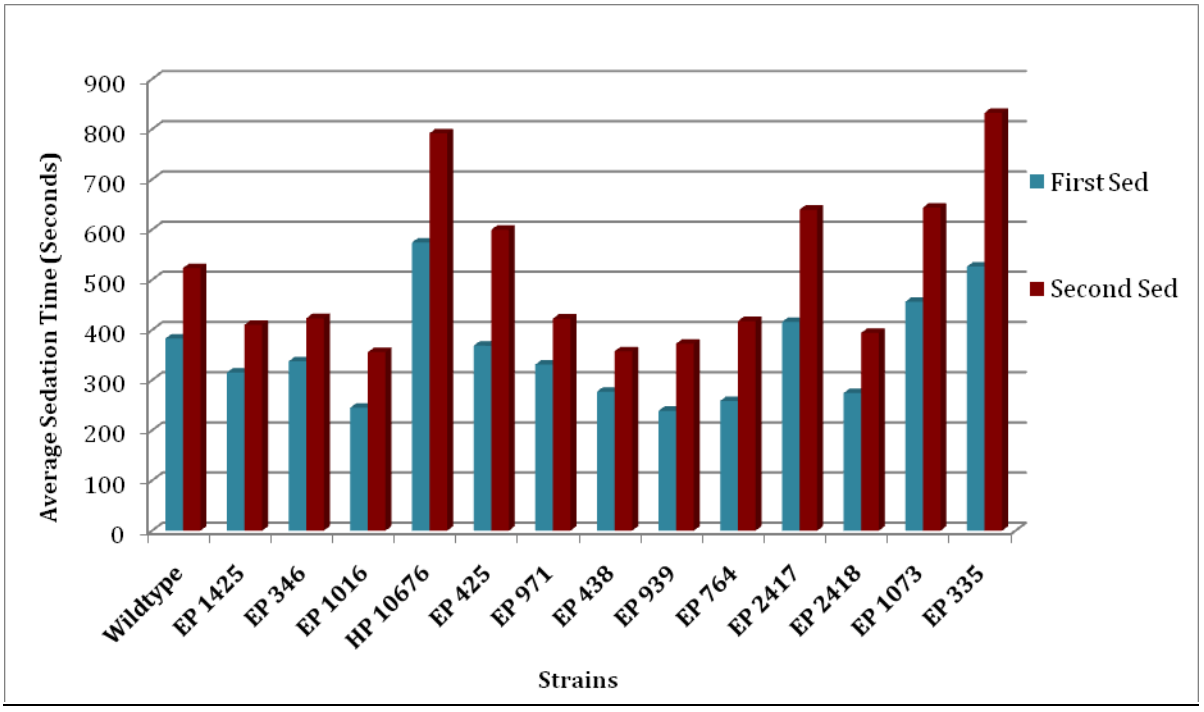

Figure 3. Tolerance data from insert lines that are significantly different from wild type.

Apparently there are many genes in the genome that can alter sedation rate. Of the strains that showed the most difference in sedation rate, none showed a difference in tolerance behavior. Although it is early in its implementation, it is not clear whether sedation behavior is a good initial screen for genes that could be a predisposing factor for alcoholism. We may need a secondary screen that can eliminate confounding factors such as altered general metabolism. When compared to the wild type, the majority of the insertion strains displayed increased sensitivity to alcohol, which might indicate that some of them have impaired alcohol metabolism. The secondary screen for acute tolerance is most likely a good secondary screen since both acute and chronic tolerance do not result from alterations in alcohol pharmokinetics, although strains selected for tolerance can alter ADH levels (Berger *et al.,* 2004; Malherbe *et al.,* 2005). We also plan to look at chronic tolerance. In addition, we also will look for alterations in learning and memory in these mutants, since any of the mutants that affect alcohol sedation also affect learning and memory (Berger *et al*., 2008). These initial screens done by the general biology labs have, however, provided a useful foundation for determining which mutants should be chosen for further analysis.

Student Outcomes and Assessment

Implementation of this module in its current form occurred two years ago as part of a revamped Gen Bio lab curriculum. Assessment of the module is ongoing. For these laboratories we defined a set of student outcomes. Since most of the outcomes are for a 100 level course, the expectations are modest. We looked for students to explain:

1) The role of model organisms such as *Drosophila* in understanding basic cellular biology and human disease
2) Some basic genetic concepts such as dominant, recessive, genotype and phenotype, sex chromsomes and autosomes, independent assortment and sex linkage
3) An understanding of genome projects and how the information from those projects might be stored and accessed through a genome data base
4) How behavior can be observed, quantified, and analyzed.
5) How to use simple statistical tests to analyze and assess data.

Student mastery of these outcomes was assessed through a lab report, performance on a lab exam, and performance on a lecture exam on genetic concepts. We also provided the students with feedback sheets, although only a handful of these sheets have been returned and, therefore, they have not been very useful.

The biology department has not defined a clear set of outcomes for its research program. We have, therefore, not assessed the outcomes for the research students. We will track their career progress. One student is currently applying for graduate school and the other two are current students.

The general biology laboratory that was in place before this two-year period was very different than the current module, and so we cannot compare lab reports and lab exams directly from the years before this module was implemented. However, one of us (Reynolds) taught the lecture portion of the course to students who had the previous laboratory experience and the new genetic module described above. On the lecture exam related to this material, the mean on that exam rose from 79 to 81 (N = 64 and 128, respectively).

We have done assessment to see how students are performing relative to the outcomes listed above. The table below summarizes the data from the last year's class (N = 144 students).

Table 1. Assessment tools for general biology module.

Description	Outcome assessed	Mean (%)	Range (%)
Lab report-summarize data and demonstrate mastery of concepts	1-5	85.7 ± 8.4	42-99
Punnet Square Homework showing genetic concepts	2	91.9 ± 17.2	0-100
Lab Exam Questions			
Module concepts (cross)	2	48 ± 50	0-100
Basic genetic concepts	2	87 ± 10	3-100
Module concepts (fly model, genome)	1, 3, 4	56 ± 40	0-100
Experimental design	1, 4	77 ± 20	20-100
Genome/FlyBase	3	79 ± 25	0-100
Statistical test-Chi square	5	45 ± 50	0-100
Statistical test-t-test		50 ± 50	0-100

We intend to compile data over multiple years to assess whether we are meeting our outcomes and also to make changes in areas where we are not successful. Based on these limited assessment findings, we are successfully meeting some outcomes but not others. For example, we seem to be successfully getting across basic genetic concepts and concepts about genomics. This group of students, however, did not master concepts about the role of genetic screens and the use of model systems. Some of the student outcomes, such as using statistical tests and being able to interpret and analyze data, are also larger student outcomes we have developed for our biology majors. After one semester of biology, very few students are able to use statistical tools well, but our goal is teach this skill over the four years of the program to our majors.

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Confirmation of the Calvin B. Bridges study: Based on nondisjunction, the white gene is located on the X chromosome of *Drosophila melanogaster*.

Marvin, Rachel K., Christopher C. Wagner, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

In 1910, Thomas Hunt Morgan reported on the recovery of a *Drosophila melanogaster* male with white eyes, instead of the usual red eyes (Morgan, 1910; see the figures below; RCW; http://www.cas.vanderbilt.edu/bsci111b/drosophila/supplemental).