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A complete *Drosophila* lab for introductory genetics courses.

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Introduction

Drosophila melanogaster is an obvious choice for demonstrating basic genetic principles in a teaching environment. Yet even a simple and limited “fly lab” can be very labor intensive for the instructor. Proper preparation and maintenance of the lab can be a daunting task, especially for newer faculty members or those without teaching assistants or preparatory support. In addition, some of the more sophisticated (and interesting) aspects of fly genetics are intellectually beyond the reach of some (or many) of the students in an introductory course. I find that most students are best served by relatively simple crosses that demonstrate basic genetic principles. Therefore, I present here an overview of the lab I use in my introductory genetics course, offered to first semester sophomores. I also provide the handouts and supporting materials through the following link:

<http://www.iona.edu/faculty/csackerson/genetics/flylab/handouts.htm>

Format of the lab

The entire lab occupies seven weeks; lab sessions are three hours long. The first week is used to introduce *Drosophila* and get the students oriented to the basic techniques and the phenotypes they are using. Students will collect virgin females and set up crosses during the second and third weeks. The fourth week is devoted primarily to the oral presentations of “Predictions”. During the fifth week, most of the time will be spent scoring F_1 flies and setting up the $F_1 \times F_1$ crosses. The sixth week is the earliest that F_2 flies can be collected. The seventh week is when most will be counting the F_2 generation; the lab is usually left open so that the more ambitious students can count flies outside of the regular lab period. The final “Write-Up” is collected during the eighth week.

The instructor should be prepared for labs in which students are finished with their work before the lab period is complete. Such time is easily filled with review of chi-square analysis, other related exercises such as polytene chromosome squashes, computer-based genetics simulations such as Virtual FlyLab, or videos such as [Drosophila as a Model Organism](#) (Cogito).

Fly stocks and crosses

Fly stocks are obtained from Carolina Biological (www.carolina.com). The order is placed 4 – 6 weeks before the exercises will begin, which allows sufficient time for the stocks to be “blown up” for the class. The cost is sufficiently low that the stocks can be ordered new each year, and no attempt is made to maintain the stocks from year to year. Stocks are chosen that have easily scored phenotypes with complete penetrance and high expressivity, even if stocks are maintained at room temperature without an incubator. Ordering information is available through the link above.

Crosses are set up using virgin females recognized by the dark mass of larval material found in the abdomen of newly emerged flies. Although it may be more efficient to clear vials and have students collect females within eight hours, which are thus virgins, I feel that the careful (and frustrating) process of observation required to collect virgins by appearance is a better experience for the students.

Each cross is carried out in two formats. For example, in the cross “dumpy sepia”, the student or group will first cross flies from the *dumpy* stock to flies from the *sepia* stock. Second, the student will cross the double mutant, *dumpy sepia*, to wild-type. In addition, the students set up reciprocals of each cross, if possible. The crosses, and the genetic principles they demonstrate, are presented in Table 1.

Techniques and recipes

Details of the techniques, and recipes for cooking small batches of fly food, are available through the url above. A few points are noted here. I find that cooked food is superior to instant flaked media, especially when some students will be setting up crosses with a small number of flies. I use apple juice for the food, instead of molasses, because apple juice is often less expensive. I have tried to set up CO₂ for anesthetization, but find this to be difficult to accomplish when several stations are being used. The major problems are freezing of the regulators, uneven delivery of gas, and the difficulty of making or buying several platforms. Instead, I use Fly Nap, despite its many draw-backs. Fly Nap has the one advantage of knocking out the flies for long enough for even the slowest students to get their work done.

Keys to success

Sufficient material must be available to the students for each class. This can amount to half a dozen or more vials of each stock, actively producing adults during the early critical weeks when the students are searching for virgin females. When the stocks arrive, begin passaging them to fresh vials every couple of days, and continue through the first week of the lab. Care must be taken to avoid confusion or cross-contamination of the stocks. It is a worth-while investment to set up a database or Word file that will print labels, especially if student help is being used to expand the stocks at this stage.

Students receive their cross by lottery. I ask each group of two students to pick a number between 1 and 8, and give them whatever cross corresponds to their number. In this way, students avoid feeling that they were “given” a tough cross, or that someone else was “given” an easy cross. As each cross is assigned, all the genetics is explained to the entire class. Most students are not prepared to absorb so much material at one time, but their notes will serve as a resource that they can refer to later.

When describing the crosses to the student, specify which stock should be used for the virgin female. In some cases, such as *sepia*, several crosses will require this stock, so students should plan to use the *sepia* stock for the males and get virgin females from the other stock, such as *apterous* or *dumpy*.

When using Fly Nap for anesthetization, it is critical that the vials be left on their side until the flies wake up, or they may get stuck in the food. This can take several hours, or even overnight, despite the claims of the manufacturer.

It must be stressed repeatedly that students should

Table 1. The crosses, and the genetic principles they demonstrate.

cross_name	independent assortment	linkage	sex linkage	epistasis	other
dumpy sepia	X				
apterous sepia	X				
white apterous	X		X		
brown scarlet	X				X
cinnabar brown		X			X
white sepia	X		X	X	
sepia ebony		X			
yellow Bar		X	X		X
white plum curly	X		X	X	X

not put unused flies back in the vials they came out of; for this reason, I provide vials for the students to use to set up stock vials, long after such stocks could be useful to them. Students can be very linear and rigid in their thinking, and are best served by clear rules that are followed throughout the lab.

Students must also be cautioned (repeatedly) to not anesthetize flies in the stock vials, or to put Fly Nap contaminated plugs into stock vials. Long exposure to even low levels of Fly Nap will kill the flies.

The students should maintain their own stock vials, once the experiments have begun. By having stock vials of the same age as their crosses, they can make direct comparisons to help them sort out the presence or absence of specific phenotypes. This is especially important with eye and body colors that can darken with age, such as brown and yellow.

All crosses must be duplicated by the instructor a sufficient number of times to allow each student to receive a backup, if their own crosses should fail to be productive. These backup crosses should be begun during the first week, and setting them up should continue through the third week. It is often convenient to examine the vials several hours after they have been emptied by the students and simply take all the females present as virgins. Since the F_2 generation is the interesting one, and in most cases the $F_1 \times F_1$ cross can be set up without requiring virgin females (since most crosses give only those F_1 flies that are desired), this gives the students the opportunity to feel as if they have actually done the important part of the experiment themselves and that the F_2 data is “their own”.

A set of “back-up” data must be available to the student, in case any crosses fail to give a chi-square value that allows them to accept their predictions. My back-up data is available through the url above, but each instructor may wish to save student data from year to year to use in the future as back-up.

Pedagogy

I feel that the major value of doing the fly lab lies not in the genetics, *per se*, but rather in the opportunity to expose the student to a living animal which they rear and manipulate to generate real data. Often, a student will initially find a vial seething with larvae to be disgusting, but later that student will be found to be staring, fascinated, through the microscope at just such a vial. Similarly, many students claim initially that they cannot see the difference between their stock and wild-type, and only after several weeks be reliably scoring their flies. The basic scientific skills of reliability, consistency, and especially careful observation are strongly reinforced by these labs.

Two assignments are associated with the lab. The first is called “Predictions”. Predictions are due in the fourth week of lab. The predictions fulfill several functions: First, they force the student to think through their genetics before any F_2 flies emerge. Second, they provide the instructor with the opportunity to detect and correct any errors or misconceptions the students may have regarding their cross. Third, the oral presentation strengthens the students’ presentation skills (and motivates them to do a good job!). Fourth, all the students are exposed to every cross and the genetics being illustrated. Predictions are presented orally, at the blackboard; each student or group must diagram the two crosses through the F_2 generation, using Punnett squares or branch diagrams to predict the phenotypes and proportions that will be found. Each student must also clearly state and describe the genetic principles being illustrated. If errors are detected by the instructor during the presentation, the class is “encouraged” to “help” the student, by directing them toward the error, rather than pointing it out directly. In this way, the class participates in each presentation, and the student is not “called out” publicly by the instructor.

The second assignment is the “Write-Up”. The write-up is a full research paper, containing all the features associated with a modern research paper. This is most students’ first attempt at data analysis and presentation. To assist them, I precede the fly lab with two other labs in which they have had to present a research-style paper, but with varying degrees of it already done for them. Their first paper is a “model”: it is largely written, leaving out key sentences and paragraphs which they have to fill in. The second paper has the introduction and methods sections done, but requires that they complete the results and discussion sections. This third paper, the fly lab write-up, is supported by extensive discussion and written instruction, but must still be constructed entirely by the student. The data section includes a chi-square analysis of their data, as well as a figure presenting their predictions and a table presenting their data. This paper gives the instructor a comprehensive view of each student’s scientific understanding and language skills.

I find that students consistently have trouble with each of the areas of the paper. First, many students will copy handout material for the Introduction, presenting the life cycle of *Drosophila* in great detail rather than focusing their attention on introducing the crosses they have done and the genetics these crosses illustrate. Second, students frequently present the Materials and Methods section as a diary or timeline of events, rather than taking advantage of the chance to present techniques as “as described ...” in the handouts or other sources. Third, students may have difficulty interpreting a chi-square value with a low probability of being acceptable (say, 15%) as being good enough to accept the predicted genetics. This can be a difficult lesson: that the F₂ offspring will not appear in exactly 9:3:3:1 ratios, yet support a 9:3:3:1 ratio. Fourth, students often fail to use the Discussion section to elaborate on the genetic principles being demonstrated. Instead, they may largely repeat the Results section. It is useful to keep these common errors in mind when discussing the write-up, and helping individual students think through their presentations.

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