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A concise *Drosophila* laboratory module to introduce the central concepts of genetics.

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Overview

The quick generation time, ease of handling, and wide availability of striking phenotypic mutants makes *Drosophila melanogaster* a highly attractive system to expose undergraduate and advanced high school students to concepts in genetics. However, it is nevertheless very labor intensive to set up *Drosophila* for a large scale laboratory exercise in a short period of time. The previously developed P{his-hid}Y heat shock system enables a greatly optimized procedure for the collection of virgin females (Venema, 2006; Venema, 2008). Using this system allowed us to develop a short laboratory module that can be completed in only three 2-3 hour long laboratory sessions spaced two weeks apart. This format also permits the *Drosophila* genetics module to be interspersed with other modules in a single semester laboratory course.

This introductory *Drosophila* genetics laboratory module emphasizes hypothesis-driven scientific inquiry by encouraging students to form their own open-ended questions about the nature of mutations and the pattern in which they are inherited from one generation to the next. The two crosses in the module introduce several important genetics concepts including: Mendelian autosomal inheritance, sex-linked inheritance, recombination, genetic mapping, and non-disjunction. Students are also introduced to the method of using statistical tests to validate or reject biological hypotheses.

Methods

The P{hs-hid}Y stocks (Venema, 2006) greatly facilitate setting up and collecting virgin female *Drosophila* for genetic crosses. The P{hs-hid}Y line has a *P* element insertion on the Y chromosome containing a proapoptotic lethality gene, *head involution defect* (*hid*), driven by the Hsp70 heat shock promoter (Grether *et al*., 1995; Starz-Gaiano *et al*., 2001). Heat shocking flies at mid-larval stages for 2 hours activates expression of *hid*, causing the death of all male (Y chromosome-carrying) larvae (Figure 1A and B). Consequently, only the females survive to become adult flies. The P{his-hid}Y system can be applied to any mutant line by crossing P{his-hid}Y males with virgin females homozygous for the mutation of choice and then backcrossing the F1 males with the mutant phenotype to the original stock of homozygous mutant virgin females to create a stable P{hs-hid}Y line with the mutant phenotype.

It is critically important to maintain several parallel cultures of a given P{hs-hid}Y stock that are not intended for heat shock. Heat shocking all cultures eliminates all males and prevents further propagation of the P{hs-hid}Y stock.

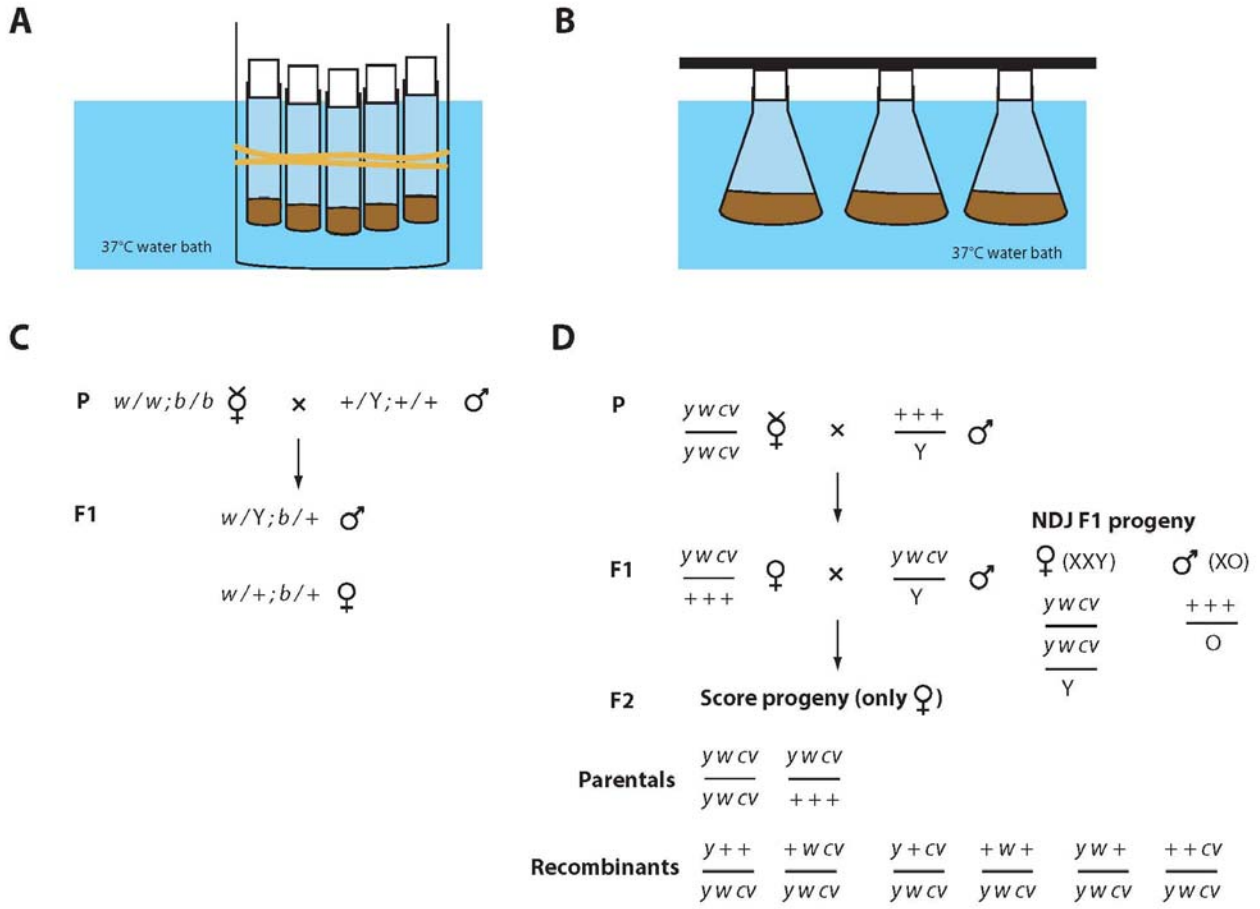

Figure 1. Experimental heat shock setup for P{hs-hid}Y larval culture in vials or bottles. A, Vials with larvae are strapped to the outside of a beaker using rubber bands. The beaker is filled with water and acts as a ballast to ensure that vials stay in place. The vials are positioned such that the water level is up to the bottom of the plug (white box). B, Bottles with larvae are placed in the water bath and held down with a weight (black line). The water bath is filled until the water level is up to the bottom of the plug (white box). It is important to immerse the culture vial or bottle until the water level meets the bottom of the plug. The larvae will attempt to escape from the heat and cluster together below the plug. It is important that there is no area in the vial or bottle where they can escape the 37°C environment. However, it is equally important to keep the inside of the vial or bottle free from water. C, Cross to demonstrate autosomal dominant and sex-linked recessive inheritance. D, Three-point testcross on X chromosome.

Heat shock protocol for P{hs-hid}Y flies

Day 1: Transfer breeding stock into a fresh culture container (vial or bottle) at 25°C.

Day 3 or 4: Remove all adults from the culture. Keep culture at 25°C.

Day 5 or 6: Two days after removing the adults, heat shock the culture by immersing in a 37°C water bath for 2 hours (Figure 1A and B), before returning to 25°C.

Allow the larvae to eclose and quickly scan the adult progeny to confirm the absence of males. While a rare meiotic non-disjunction event may produce XO progeny that are phenotypically male but infertile, it is safest to discard any vials where any males are observed, in case the heat shock procedure was not 100% effective. To date we have never seen any males after the heat shock protocol.

Laboratory module structure

In the first class meeting students working in pairs are initially taught proper *Drosophila* handling technique: how to anaesthetize flies in vials (in our case using carbon dioxide, but alternatively with ether), how to move them onto the stage and handle them using a paintbrush, and how to examine the flies under a dissecting microscope.

Cross to demonstrate autosomal recessive and sex-linked inheritance

Using the microscope, students learn to distinguish between male and female adult flies by recognizing the genitalia. They are then introduced to unlabeled *white* and *black* single mutants and wild-type flies, so that they may learn to recognize the phenotypes. The different mutant flies are given to the students without instruction about the specific phenotypes other than guiding questions asking them to observe the body color and eye color under the dissecting microscope. Students are then given vials containing true-breeding *white, black* virgin females (collected from heat shocking true-breeding *white, black* P{hs-hid}Y larvae) and wild-type males and they set up crosses with 3 females and 3 males in new vials (5 vials per pair of students) (Figure 1C). In a written-exercise, students are then asked to predict potential genotypes and phenotypes of progeny based on hypotheses they make of whether the genes responsible for the phenotypes are autosomal or sex-linked and/or dominant or recessive. Students also learn the genetic nomenclature to write out the cross and expected results.

At least two weeks after setting up the cross the F1 adult progeny are analyzed (Figure 1C). Based on the ratios of the phenotypes observed in the progeny, students are asked to revisit their hypotheses and determine the genetic pattern of inheritance for the *white* (sex-linked, recessive) and *black* (autosomal, dominant) genes.

Three-point test cross on the X chromosome

In the initial class meeting students learn to identify the unlabeled *yellow*, *white* and *crossveinless* single mutant phenotypes. Students are then given vials of true-breeding *yellow, white, crossveinless* virgin females (collected from heat shocking true-breeding *yellow, white, crossveinless* P{hs-hid}Y larvae) and wild-type males, and they set up crosses in fresh vials, each with 3 females and 3 males (5 vials per pair of students) (Figure 1D). In a written-exercise, students are then asked

to predict potential genotypes and phenotypes of progeny based on hypotheses they make of whether the genes responsible for the phenotypes are autosomal or sex-linked and/or dominant or recessive.

Two weeks after setting up the cross, the F1 adult progeny are analyzed (Figure 1D). Based on the phenotypes observed in the progeny, students are asked to determine the pattern of inheritance for the *yellow*, *white* and *crossveinless* genes (all sex-linked, recessive). They then set up crosses between three F1 males and three F1 females in fresh vials (10-15 vials per pair of students). The segregation of phenotypes according to the sex of the F1 progeny in this cross allows for the possibility of observing rare meiotic non-disjunction events that produce XXY females and XO males that exhibit the phenotype normally associated with the opposite sex (Figure 1D, NDJ F1 progeny).

Two weeks after setting up the cross between the F1 adults, the female adult progeny in the F2 generation are analyzed for recombination events on the X chromosome. Specifically, the number of female flies with each possible phenotypic combination is counted (Figure 1D). There are eight possible phenotypes, representing the parental and recombinant genotypes (including single and double crossover events). Data collected from the whole laboratory section are pooled to determine the observed numbers in each phenotypic class. Using these data, students are asked to examine whether the observed ratio of phenotypes corresponds to the expected values for unlinked genes. Students then calculate the map distance between the linked *yellow*, *white* and *crossveinless* genes on the X chromosome, as well as the double cross-over Interference value. Students are also introduced to the chi-square statistical test and use it to analyze the expected and observed frequency of the phenotypes to determine the validity of the different predicted inheritance patterns.

Results

When this module was taught in spring 2009 with 6 pairs of students in each of two laboratory sections, a total number of 2495 F2 generation flies from the three-point test cross were characterized and scored by the students (Figure 2A). All possible recombination events were observed in the combined data set (including the double cross-over) with alignment to the published map positions (FlyBase Consortium, 2003). From combined class data, the calculated map distance between *yellow* and *white* was 1.2 cM (published distance = 1.5 cM) and between *white* and *crossveinless* was 5.8 cM (published distance = 12.2 cM) (Figure 2A). No evidence of Interference (i) of the double crossover was detected in this data set, possibly reflecting the over-representation of the double crossover in a small sample size (Figure 2A).

In this module, students are exposed to a very concise introduction to a number of genetic concepts and are encouraged to develop and test their own hypotheses about different modes of genetic inheritance. Students wrote a final lab report describing their observations, genetic hypotheses and results for both crosses in the laboratory module. A post-course survey revealed that student evaluation of the learning outcomes from the module were generally very positive (Figure 2B).

This module could readily be scaled up for larger and more numerous laboratory sections. The laboratory manual, additional instructional materials and the P{hs-hid}Y stocks described are available upon request.

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A

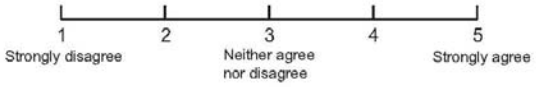
Group # / Phenotype	1	2	3	4	5	6	7	8	9	10	11	12	Total
w y cv	70	58	78	67	101	71	138	112	75	123	75	76	1044
+ + +	94	89	70	106	117	118	182	105	84	105	134	79	1204
w y +	2	2	4	5	12	10	12	4	4	6	10	6	77
+ + cv	8	5	3	3	6	3	7	6	4	6	6	4	61
w + +	0	0	0	0	2	0	0	0	0	0	0	0	2
+ y cv	0	0	0	0	0	0	0	2	0	2	0	0	4
+ y +	0	1	0	0	1	0	1	0	0	0	0	0	3
w + cv	2	3	0	2	1	0	0	3	0	3	1	6	21
Total	176	158	155	183	240	202	340	232	167	245	226	171	2495

Genes	Distance
y to *w*	1.20
w to *cv*	5.77

$$i = 1 - C \text{ of } C = 1 - \left[\frac{\text{Observed \# of double recombinants}}{\text{Expected \# of double recombinants}} \right]$$

Expected	1.73
Observed	6
C of C	3.47
Interference (i)	-2.47

B



Q1 Enhanced my ability to frame scientific questions and form testable hypotheses
Q2 Enhanced my ability to design a good experiment
Q3 Enhanced my ability to analyze, present and verbalize scientific results
Q4 Exposed me to an area of biology I had not experienced previously
Q5 Had a positive impact on my interest in biology

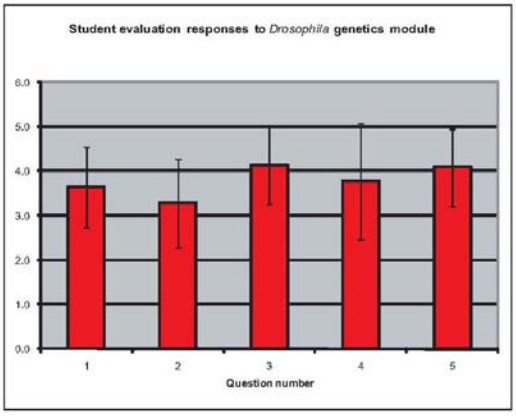


Figure 2. Laboratory results and learning outcomes. A, Results from three-point testcross. B, Student evaluation responses to *Drosophila* genetics module.

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Submissions to Drosophila Information Service are welcome at any time. The annual issue now contains articles submitted during the calendar year of issue. Typically, we would like to have submissions by mid-December to insure their inclusion in the regular annual issue. but articles can be accepted for this volume until 31 December. Details are given in the Guide to Authors or on the DIS web site: www.ou.edu/journals/dis.