

## Article

# Enhancing Undergraduate Teaching and Research with a *Drosophila* Virginizing System

Dennis R. Venema

Faculty of Natural and Applied Sciences, Department of Biology, Trinity Western University, Langley, British Columbia V2Y 1Y1, Canada

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Laboratory exercises using *Drosophila* crosses are an effective pedagogical method to complement traditional lecture and textbook presentations of genetics. Undergraduate thesis research is another common setting for using *Drosophila*. A significant barrier to using *Drosophila* for undergraduate teaching or research is the time and skill required to accurately collect virgins for use in controlled crosses. Erroneously collecting males or nonvirgin females contaminates crosses with unintended genotypes and confounds the results. Collecting adequate numbers of virgins requires large amounts of time, even for those skilled in virgin collection. I have adapted an effective method for virgin collection that eliminates these concerns and is straightforward to use in undergraduate settings. Using a heat-shock-induced, conditional lethal transgene specifically in males, male larvae can be eliminated from a culture before adults eclose. Females thus eclose in the absence of males and remain virgin, eliminating the need to laboriously score and segregate freshly eclosed females. This method is reliable, easily adaptable to any desired phenotypic marker, and readily scaleable to provide sufficient virgins for large laboratory classes or undergraduate research projects. In addition, it allows instructors lacking *Drosophila* expertise to use this organism as a pedagogical tool.

## INTRODUCTION

*Drosophila melanogaster* is commonly used as an example in introductory biology and genetics courses. For example, a survey of introductory biology texts indicates that *Drosophila* is commonly used as a historical example validating the chromosomal theory of inheritance: Campbell and Reece (2005) and Raven *et al.* (2005) use Morgan's historic experiments with the original *white* mutant to illustrate this concept and introduce sex linkage. *Drosophila* is also a common choice for discussing recombination of linked loci: examples include mapping *black* to *vestigial* (Campbell and Reece, 2005; Solomon *et al.*, 2005; Campbell *et al.*, 2006), *white* to *yellow* (Freeman, 2005), and *carnation* to *Bar* (Raven *et al.*, 2005). Introductory genetics texts also rely heavily on *Drosophila* for examples of these and many other genetic concepts. For example, Griffiths *et al.*, (2005), Klug *et al.* (2006), Russell (2006), and Brooker (2005) unanimously use Mor-

gan's experiments to introduce and illustrate recombination mapping (*purple* to *vestigial*, *white* to *miniature*, *yellow* to *white*, and a three-point testcross of *yellow*, *white*, and *miniature*, respectively). Additionally, all of these texts use Morgan's work with *white* to illustrate sex linkage.

I teach Introductory Biology to both majors (Biology 114) and nonmajors (Biology 104) at Trinity Western University (Langley, British Columbia, Canada). The laboratory portions of these courses are combined (such that a given laboratory section will have a mix of both majors and nonmajors students and cover identical material). Both courses contain a unit on Mendelian inheritance, for which I have selected several desired learning outcomes, including 1) to understand basic genetics vocabulary and concepts and 2) to understand the mode of inheritance for a visible phenotype. This requires students to understand the difference between dominant or recessive mutations (relative to wild type) and to distinguish when such mutations reside at autosomal or sex-linked loci. To augment my lecture material (which is identical for both the majors and nonmajors courses), I have introduced a *Drosophila* exercise into the laboratory curriculum. This was also intended to reinforce the genetics mate-

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Address correspondence to: Dennis R. Venema (dennis.venema@twu.ca).

rial covered in the textbooks for both the majors and non-majors courses, which both use *Drosophila* crosses as examples (Campbell and Reece, 2005 and Campbell *et al.*, 2006, respectively). The introduction of this laboratory exercise was intended to accomplish two goals: 1) to increase interest in genetics among students and 2) to improve learning outcomes for the genetics material presented in lectures and in the textbook.

Although using *Drosophila* for undergraduate teaching is desirable, it presents a challenge for large classes. A major limiting factor for using *Drosophila* pedagogically is collecting adequate numbers of virgins to perform controlled crosses. The training and large amounts of time required for collecting virgins present serious challenges for all but the smallest of class sizes. Even if students are trained to collect virgins manually, errors made during collection are common and cause discouraging errors in the results. The most common errors are scoring freshly eclosed males as females and selecting nonvirgin females; both of these errors disrupt the expected phenotypes and proportions of the F<sub>1</sub> generation.

In addition to its use as a teaching example, work with *Drosophila* is a common directed studies or undergraduate thesis topic. Many *Drosophila* researchers supervise undergraduate research, which shares the potential problems of using *Drosophila* in teaching situations described above: New undergraduate researchers must learn to collect virgin flies efficiently and without error, despite a full class schedule. The tendency for virgin flies to eclose in the early morning is not generally helpful at this point. The lack of available time and the problem of nonvirgin flies are prominent limitations to the research productivity of undergraduates and may cause a principal investigator to question the validity of a student's results.

A significant improvement for using *Drosophila* for undergraduate teaching or research would be a system for virgin collection that overcomes these drawbacks. Such a system ideally would have the following features. It would 1) significantly reduce the time required for virgin collection; 2) reduce or eliminate collection errors; 3) be adaptable to a wide range of phenotypic markers; and 4) be simple to use, whether by instructors or students. I have adapted a previously described heat-shock virginizing system for teaching and undergraduate research that readily meets these objectives.

## A VIRGINIZING SYSTEM TO FACILITATE DROSOPHILA USE

A *Drosophila* virginizing system using the proapoptotic gene *hid* has been previously described (Starz-Gaiano *et al.*, 2001). This system uses a heat shock-*hid* transgene within a P-element inserted into the Y chromosome (abbreviated as P{hs-hid}Y; FlyBase Consortium, 2003). This construct acts as a conditional, dominant lethal selectively in males. A 2-h heat shock during larval stages induces expression of the *hid* transgene in males, killing them before they eclose. Female larvae in the culture are unaffected, eclose several days later, and, in the absence of males, remain virgins. These females are genetically identical to virgins collected manually from a non-P{hs-hid}Y stock, because the transgene is carried only on the Y chromosome. This system greatly reduces the time

required to collect large numbers of virgins, because manual collection is no longer necessary. It also eliminates collection errors because males are selected against genetically, without need for a scoring step. This system can be used for any desired phenotypic marker, making it extremely flexible. Finally, this system is simple to use: It requires no special equipment or expertise beyond the ability to culture *Drosophila*.

I obtained a P{hs-hid}Y line as a gift from Ruth Lehmann (Howard Hughes Medical Institute, Skirball Institute, New York University Medical School, NY; Starz-Gaiano *et al.*, 2001). The P-element is marked with a *miniwhite*<sup>+</sup> marker that produces a pale orange eye in genetically *white* males. As a starting point, I made *white*<sup>1118</sup>/P{hs-hid}Y males and crossed them to *white*<sup>1118</sup> virgin females. I have subsequently made P{hs-hid}Y versions of a representative set of common teaching stocks (Table 1). I have constructed stocks that can be used to demonstrate autosomal or sex linkage, independent assortment, recombination mapping, epistasis, and mutagenesis. Although the set I have developed is based on my own teaching needs, this system is easily adapted to other desired markers. An example of how I used this system to facilitate a genetics exercise in the Introductory Biology 114/104 laboratory in Spring 2006 semester is presented here. This example was used to assess both the value of the P{hs-hid}Y virginizing system and the value of the specific exercise with respect to my desired learning outcomes for this laboratory module.

## LABORATORY MATERIALS AND INTENDED LEARNING OUTCOMES

The laboratory exercise consisted of three parts: a set of prelab questions assigned 1 wk before the *Drosophila* laboratory (Supplemental Material 1), scoring the parents and F<sub>1</sub> progeny of a cross during a laboratory period, and interpret-

**Table 1.** P{hs-hid}Y stocks for teaching genetics

Stock genotype <sup>a</sup>	Notes and pedagogical uses
Wild type	Constructed in an Oregon-R wild-type background
<i>white</i>	Used to demonstrate sex linkage, or epistasis in conjunction with <i>sepia</i>
<i>white; black</i>	Used to demonstrate a 9:3:3:1 ratio skewed by gender, also as parental strains for mapping <i>black</i> to <i>vestigial</i>
<i>white; vestigial</i>	Used as a parental strain and tester for mapping <i>black</i> to <i>vestigial</i>
<i>white; black, vestigial</i>	Used to demonstrate a standard 9:3:3:1 ratio
<i>white</i> <sup>+</sup> ; <i>black; sepia</i>	Used with <i>white</i> to illustrate epistasis
<i>white; black, purple, cinnabar</i>	Used to score mutation events at multiple loci with a single cross

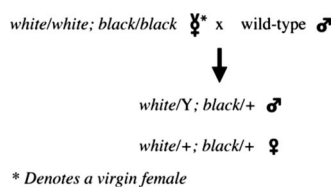
<sup>a</sup> Mutant phenotypes are as follows: *white* mutants, white eyes (wild-type = red); *black*, dark bodies (wild type = tan); *vestigial*, reduced wings; *sepia*, sepia (dark) eyes; *purple*, reddish purple eyes; and *cinnabar*, orange eyes (note that *white* is epistatic to *sepia*, *purple*, and *cinnabar*).

ing the cross results as an in-lab exercise (see Supplemental Material 2). The cross used was that of pure-breeding (*white*, *black*) virgin females (obtained by heat shocking a P{hs-hid}Y, *white*, *black* stock) to pure-breeding wild-type males. This cross exhibits two modes of inheritance simultaneously: the *white* phenotype is inherited as a sex-linked recessive, whereas the *black* phenotype is inherited as an autosomal recessive (Figure 1). Combining both into a single cross heightens the contrast between these two inheritance modes. The prelab questions introduce the two phenotypes and ask students to describe what the outcome of the above-mentioned cross would be assuming different inheritance modes for *white* and *black*. The intended learning outcomes for the laboratory were to increase understanding of sex-linked versus autosomal inheritance and dominant versus recessive alleles (which combine to give four possible modes of inheritance for any given phenotype). The exercise also was intended to implicitly reinforce understanding of the basic genetics concepts and vocabulary presented in the lecture and assigned textbook readings.

## ASSESSMENT

### Efficiency Gains by Using the P{hs-hid}Y System

First-year biology enrollment at Trinity Western University averages 100 students per year, requiring approximately 250 virgins for the exercise I use. Before introducing the P{hs-hid}Y system, I collected these virgins manually, requiring approximately 8 h at the bench. An additional inconvenience was that this time was restricted to morning hours to maximize collection efficiency. Using a P{hs-hid}Y stock for this exercise reduced the virgin collection time to approximately 30 min: All that was required was to turn over timed cultures (as described below), heat shock, scan the resulting flies to ensure the absence of P{hs-hid}Y (i.e., *miniwhite*<sup>+</sup>) males, remove any sterile *white* XO males, and transfer the virgins to vials for student use. An additional benefit is that all the females in a heat-shocked culture are virgin, whereas only a few will be collected from a traditional stock as virgin. Because a single heat-shocked bottle can produce in excess of 500 virgins, this greatly reduced the number of culture bottles required for virgin collection. When collecting manually I collected from approximately 25 bottles; when using the heat-shock method, I required only one bottle (although I used a few bottles to ensure success). This represented a



**Figure 1.** A cross to demonstrate autosomal and sex-linked inheritance. Virgin females homozygous for *white* on the X and *black* on an autosome are crossed to wild-type males. The F<sub>1</sub> progeny are uniformly heterozygous for *black* and phenotypically tan, demonstrating *black* to be an autosomal recessive mutation. Conversely, F<sub>1</sub> males are hemizygous for *white* and thus phenotypically white-eyed in contrast to red-eyed female progeny, indicating that *white* is inherited as a sex-linked recessive mutation.

significant savings of culture space and media consumption in addition to preparation time. Gains in scoring accuracy were not a factor, because I personally collected virgins when using manual collection; however, this system was perfectly accurate and needed no expertise in scoring virgin females. This aspect of the system will especially benefit those instructors who use teaching assistants or the students themselves to collect virgins.

### Learning Outcomes

Student learning for this laboratory exercise was evaluated in two ways: subjectively through a voluntary, anonymous student questionnaire completed at the end of the laboratory period (see Supplemental Material 3); and objectively by student performance on genetics questions on a subsequent laboratory exam (Figure 2).

### Questionnaire Results

The summary response data for the questionnaire are shown in Table 2. The response rate for the questionnaire was 88/108, or 81%. Although nonmajors students reported lower general genetics interest ( $3.6 \pm 0.19$ ) than did majors students ( $3.9 \pm 0.12$ ), both groups reported equal interest in the specific laboratory exercise ( $3.8 \pm 0.14$  and  $3.8 \pm 0.11$ , respectively). Students who had performed *Drosophila* crosses in high school (16/88, or 18% of respondents) reported the highest interest in genetics ( $4.1 \pm 0.27$ ) and an interest in the exercise equal to the other groups despite having done a similar experiment previously. This group found the prelab questions least helpful to their laboratory preparation (statement 3,  $3.5 \pm 0.22$ ), perhaps reflecting their prior understanding and experience. There was general consensus that the laboratory exercise aided understanding of lecture material (statement 4), and the specific goals of understanding the difference between dominant and recessive alleles (statement 5), and autosomal and sex-linked inheritance (statement 6). Nonmajors students reported slightly higher perceived benefit for the specific learning outcomes (statements 5 and 6) than did majors students. Interestingly, students with high school *Drosophila* experience reported slightly higher perceived benefit than did students without prior experience.

Space for written feedback was also provided on the questionnaire. The most common response was that the *black* phenotype was difficult to score (five responses). Certainly, scoring *black* is more challenging than *white*, and other instructors may wish to substitute a more obvious autosomal phenotype (e.g., *vestigial*). My opinion is that the difference forces students to examine the F<sub>1</sub> flies more thoroughly and methodically than they would for an obvious phenotype (for example, by looking at the thorax, abdomen, and wing veins when comparing *black* with wild type). Other comments from this section suggested that the desired outcomes had been achieved for at least some students:

“Interesting to see the detail of the flies under the microscope and also the whole mode of inheritance.”

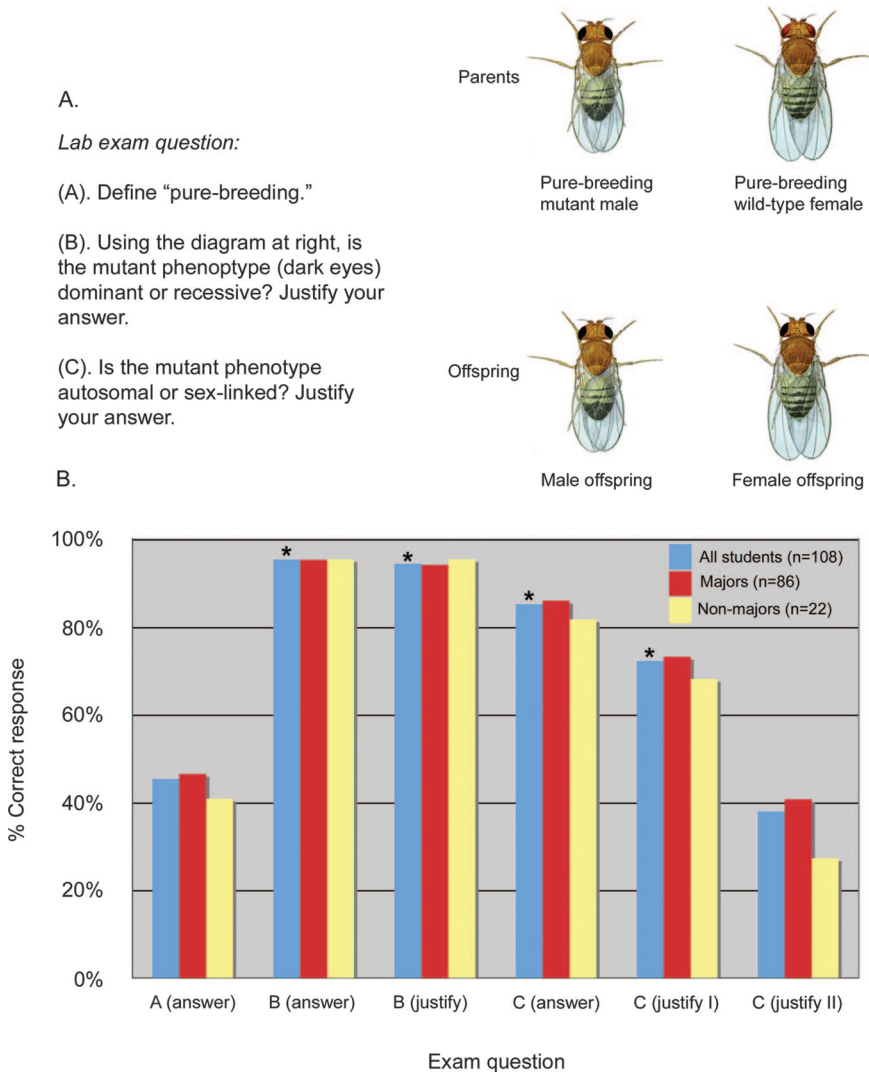
“This is a very good lab to help me see how the genetics I learned in high school really does work.”

“It was a good lab, I enjoyed it, finally understand autosomal and sex-linked! Yah!”

### Exam Performance

Learning outcomes were also assessed objectively with an in-lab exam given 1 wk after the exercise that covered the *Drosophila* exercise as well as other course material. Exams were evaluated for all 108 students (86 students in the majors course and 22 students in the nonmajors course). The relevant exam questions and summary student results are shown in Figure 2. The results were similar for both majors and nonmajors students; all differences between the two groups were not statistically significant (chi-square test,  $p \geq .05$ ). The first question (to define pure breeding) was used as a control question: Although the concept of a pure-breeding (i.e., homozygous) line was presented in lecture, the prelab questions, and the in-lab handout, it was not specifically reinforced during the laboratory exercise. This question was poorly done, with only 45% of students answering correctly (47% of majors and 41% of nonmajors). In contrast, most

students were able to identify that the hypothetical mutation (dark eyes) was inherited as an autosomal dominant mutation and, more importantly, to justify their choice of this inheritance mode. The justification section was a free-form response designed to separate those who merely guessed correctly from those who understood the concept. An autosomal dominant mutation was chosen specifically because this mode was not illustrated in the laboratory exercise. Also, the use of an eye color phenotype was potentially misleading, because of the student’s previous exposure to the *white* mutation as an example of a sex-linked recessive. Still, 95% of students correctly identified that the mutant phenotype acted as a dominant mutation and similarly justified their answer by the observation that the mutant phenotype showed up in the progeny (94% of majors and 96% of nonmajors). This result was significantly improved over the control question (chi-square test,  $p \leq .001$ ). Distinguishing autosomal and sex-linked inheritance was more difficult; yet, most students correctly assigned the mode of inheritance as autosomal (85% overall; 86% of majors and 81% of



**Figure 2.** Exam questions to assess student learning. (A) The exam questions and cross diagram. (B) Student performance for each answer and corresponding justification. Results significantly improved over the first (control) question are denoted with asterisks (chi-square test,  $p \leq .001$ ). For question part C, correct justifications as a whole (“justify I”) and correct “high-level” justifications (“justify II”) are presented separately. All differences between majors and nonmajors students were not statistically significant. See text for details.

**Table 2.** Summary data from the student questionnaire<sup>a</sup>

Group	n	Questionnaire statement <sup>b</sup>					
		1	2	3	4	5	6
All responses	88	3.8 ± 0.10	3.8 ± 0.09	3.8 ± 0.09	3.9 ± 0.08	3.9 ± 0.09	3.9 ± 0.09
Majors	69	3.9 ± 0.12	3.8 ± 0.11	3.9 ± 0.10	3.9 ± 0.09	3.9 ± 0.11	3.8 ± 0.11
Nonmajors	19	3.6 ± 0.19	3.8 ± 0.14	3.7 ± 0.17	3.8 ± 0.18	4.1 ± 0.14	4.1 ± 0.16
Previous experience	16	4.1 ± 0.27	3.8 ± 0.25	3.5 ± 0.22	4.1 ± 0.17	4.1 ± 0.21	3.9 ± 0.26
No experience	72	3.8 ± 0.11	3.8 ± 0.10	3.9 ± 0.09	3.8 ± 0.09	3.9 ± 0.10	3.8 ± 0.10

<sup>a</sup> Students were asked to rate their agreement with six questionnaire statements (1, strongly disagree; 2, disagree; 3, neutral; 4, agree; and 5, strongly agree). The average response value for each statement is presented ± s.e.m. Values are also given for various student subgroups: those in the majors class versus the nonmajors class, as well as those students indicating high school *Drosophila* experience (Previous experience) versus those who had no previous exposure to *Drosophila* crosses (No experience). The number of respondents in each category is given (n).

<sup>b</sup> The questionnaire statements queried general genetics interest (1); specific interest in the exercise (2); the perceived value of the prelab questions (3); and the perceived value of the exercise with respect to student understanding of general genetics concepts (4), recessive and dominant alleles (5), and autosomal and sex-linked inheritance (6). The full questionnaire is provided in Supplemental Material.

nonmajors). This result was also significant (chi-square test,  $p \leq .001$ ). Fewer students were able to correctly justify their choice (72% overall; 73% of majors and 68% of nonmajors); yet, this improvement was significant (chi-square test,  $p \leq .001$ ). Correct justifications for this section were further divided into two categories: a “low-level” justification that stated that the mode was autosomal because there were no differences in phenotype between offspring based on gender and a “high-level” justification that specified that sex-linked inheritance would have produced male offspring with wild-type (red) eye color. Overall, 38% of students provided a high-level justification for sex-linked inheritance, with more majors students providing this level of detail than nonmajors students (41% of majors and 27% of nonmajors). Together, the results suggest that the laboratory exercise improved the desired learning outcomes for both majors and nonmajors students.

## ADDITIONAL TEACHING POSSIBILITIES

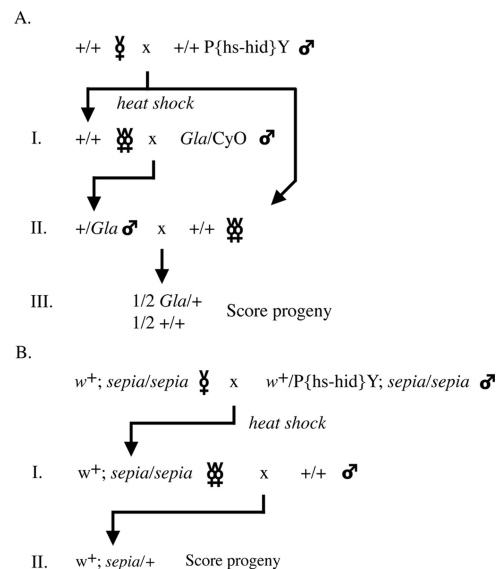
One benefit of the P{hs-hid}Y system is that it is easily adaptable to numerous teaching situations. Some additional, more complex examples of how I have used these stocks in the laboratory portion of an introductory Mendelian genetics course (at the junior level) are outlined next.

### Examining Autosomal Monohybrids

In the first laboratory of the semester, I present students with wild-type and several mutant *Drosophila* lines. Students examine and describe the wild-type line and then examine the mutants to find the altered characteristic. Once they have discovered the mutant phenotype in each line, they are given the F<sub>1</sub> progeny of that mutant crossed to a pure-breeding wild-type line and asked to determine the following by examining the F<sub>1</sub> individuals:

1. Is the mutant characteristic recessive or dominant to its wild-type allele?
2. Was the original mutant used in the cross homozygous or heterozygous for the mutant allele?

Two examples are illustrated in Figure 3. The F<sub>1</sub> of a *Glazed* × wild-type cross is 1:1 *Glazed*:wild type, indicating that *Glazed* is dominant to its wild-type allele and that the original *Glazed* mutant used in the cross was heterozygous (Figure 3A). The F<sub>1</sub> of a *sepia* × wild-type cross is uniformly wild type, indicating that *sepia* is recessive to wild type and that the original *sepia* mutant was homozygous (Figure 3B). I find this analysis successfully reinforces the concepts of phenotype versus genotype, dominant versus recessive alleles, and hetero- versus homozygosity presented in lecture. It also propels students into a problem-solving mentality early in the course. For this exercise, I have also used other



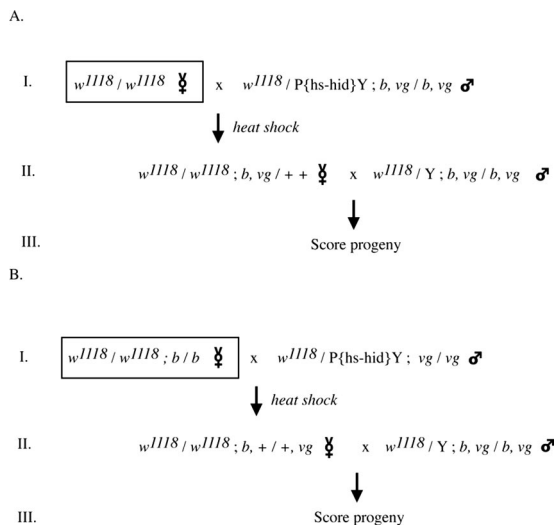
**Figure 3.** Autosomal monohybrid crosses by using the P{hs-hid}Y system. (A) A cross of a *Glazed*/CyO stock to wild-type virgins can be used to demonstrate a 1:1 ratio in the third generation, proving *Glazed* is dominant to wild type. (B) Demonstrating that the *sepia* mutant is recessive to wild type can be accomplished in two generations.

standard balanced stocks with dominant markers available from the Bloomington *Drosophila* Stock Center (Department of Biology, Indiana University, Bloomington, IN) such as *Stubble*, *Curly*, and *Lyra* in the same manner as *Glazed*. Because only males were used, it was not necessary to make P{hs-hid}Y versions of these stocks.

### Illustrating Recombination Mapping

As noted, *Drosophila* is commonly used to illustrate recombination mapping in textbooks. Because recombination mapping requires several generations and the collection of F<sub>1</sub> virgin females, the P{hs-hid}Y system is especially suited for this type of experiment. A series of crosses used to map *vestigial* to *black* are outlined in Figure 4. In keeping with the problem-solving approach I try to emphasize, I have students design the crosses from scratch in teams before setting them up. I find that using both a *black* to *vestigial* cross and a *black, vestigial* to wild-type cross provides an excellent way to reinforce the concept that nonrecombinant (i.e., parental) and recombinant allele combinations depend on the parental strains used.

The above examples are not exhaustive, and this system can be tailored to many desired learning outcomes. For example, I have also used a *black, sepia* to wild-type cross followed by an F<sub>1</sub> intercross cross to illustrate the 9:3:3:1 ratio; similarly, a cross of *white, black, purple, cinnabar* females to mutagenized wild-type males can be used to compare mutation frequencies between these loci with various mutagens.



**Figure 4.** Using the P{hs-hid}Y system to map *black* to *vestigial*. A box indicates virgin females obtained from a P{hs-hid}Y stock. Crosses may be set up to map the recessive alleles in *cis*-configuration (A) or in *trans*-configuration (B). For both approaches, the P{hs-hid}Y chromosome is used to ensure dihybrid virgin females in the second generation for testcrossing. Note that the P{hs-hid}Y chromosome is not necessary for the (*black, vestigial*) tester males and should be avoided to prevent skewing the male:female ratio.

## APPLICATIONS FOR UNDERGRADUATE RESEARCH

### Using the P{hs-hid}Y System for F<sub>2</sub> Screens

As a Ph.D. student, I performed an F<sub>2</sub> screen for novel alleles of *Glilotactin* (*Gli*). Over 4 mo, I was able to screen approximately 5000 chromosomes, resulting in six new *Gli* alleles (Venema *et al.*, 2004). In my first year as a principal investigator, I had an undergraduate repeat this exact screen by using the P{hs-hid}Y system to provide the required virgins. This student was able to screen 7900 chromosomes in approximately 3 mo, successfully identifying numerous additional alleles of *Gli*. The bulk of the time and effort for the first screen was collecting virgins manually and setting up the single-pair matings; for the second screen, the bulk of the time was spent doing scaled-up mutagenesis and single-pair matings. Virgin collection consisted merely of heat-shocking bottles of the appropriate cultures and transferring the eclosed females into single-pair matings. That the second screen was approximately 60% larger and accomplished in less time by an undergraduate with only a few months of experience with *Drosophila* demonstrates the power and efficiency of this system and its suitability to short-term undergraduate research projects.

### Applications to Other Undergraduate Projects

Although a virginizing system is most commonly used in large screens, I use similar approaches to smaller-scale crosses for two reasons: 1) the lack of time available for undergraduates to collect virgins and 2) the virtual guarantee of virginity by using the P{hs-hid}Y system. As a principal investigator, it is very convenient to construct and verify a P{hs-hid}Y stock, give a portion to the undergraduate researcher to maintain, and allow he or she to heat shock portions of the culture as needed for the required virgins. The heat shock can be scheduled at any time during the day, and the resulting virgins similarly can be collected at any time after eclosing. This feature of scheduling freedom is reason enough for me to use this system with undergraduate researchers, because this benefit alone greatly boosts their productivity. Indeed, the undergraduates I was supervising while developing this system liked nothing better than receiving a heat-shock version of a stock they had previously used for manual collection. There was even a friendly rivalry in the laboratory between those already using the system and those awaiting their own heat-shock stocks, so great was the advantage for those students using this method.

## DISCUSSION

### Optimizing the P{hs-hid}Y System

There are a few ways to maximize the efficacy of the P{hs-hid}Y system, whether for teaching or research. First, cultures intended for heat shock must be free of adults lest a male be present when virgins eclose. Second, cultures should not be heat shocked when pharate (i.e., dark) pupae are present, because a late heat shock may fail to kill all the males. Third, very early embryos will not express the *hs-hid* transgene until the onset of zygotic transcription and thus will survive if heat shocked too early in development. These

issues are easily addressed by setting up timed cultures as follows: allow adults to lay freely for 1 or 2 d, transfer the adults to a new bottle, culture the original bottle for an additional 2 d, heat shock for 2 h at 37°C in a water bath, and allow females to eclose. When carefully prepared in this manner, a culture will produce only females and rare (sterile) XO males that arise from nondisjunction of the sex chromosomes in either males or females. XO males can be scored for the absence of the *miniwhite*<sup>+</sup> marker associated with the Y if they are *white* on the X (for this reason I have made as many of the stocks as possible in a *white*<sup>1118</sup> background). Such XO males are of no concern because they are sterile. When it is not possible to score a possible XO male for the *miniwhite*<sup>+</sup> marker owing to the presence of *white*<sup>+</sup> on the X, storing putative virgins for at least 3 d and examining the vial for larvae is advised. Another issue of note is that rapid transfer of breeding stock not intended for heat shock may inadvertently exclude males from the culture, because males with the P{hs-hid}Y chromosome are delayed in their development and less abundant than in a wild-type Y background. Thus, it is not advisable to have students score male progeny harboring the P{hs-hid}Y chromosome, because the 1:1 male:female ratio will be skewed in such crosses. When designing pedagogical crosses using this system, use P{hs-hid}Y males only when you intend to kill the male larvae that result from the cross, but use an identical stock with a wild-type Y chromosome otherwise to ensure normal male viability.

### Adopting the P{hs-hid}Y System

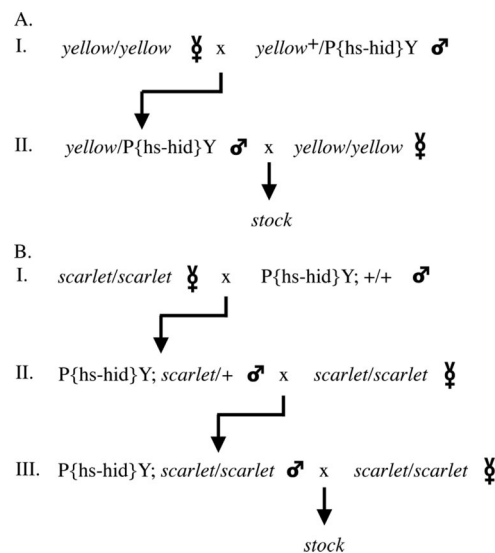
The specific benefits and drawbacks of adopting this system will depend on the circumstances of individual instructors. Perhaps the most likely adopters of this system are instructors who currently use *Drosophila* crosses in their curriculum and collect virgins manually (especially those who happen to use the markers presented here). For such instructors, obtaining a P{hs-hid}Y version of a stock already in use will greatly expedite virgin collection.

To assess potential interest in, and transferability of this system to other institutions, I contacted colleagues at neighboring universities and colleges. Three institutions expressed interest, and upon reviewing a preprint of this article, they decided to adopt the P{hs-hid}Y system for pedagogical crosses. One institution (University of British Columbia, Vancouver, British Columbia, Canada), is a medical-doctoral university with a first-year biology laboratory enrollment of approximately 150–180 students per year. University of British Columbia currently uses *Drosophila* crosses in its first-year laboratories, with four recessive eye color stocks: *white*, *scarlet*, *brown*, and *sepia*. The crosses used require approximately 500 virgins to be collected by the instructor with help from work-study students (C. Pollock, personal communication). The instructor related how inconvenient and labor-intensive manual virgin collection was (she estimated it required approximately 20 h to collect the required virgins, not including training time for student assistants). Despite the inconvenience of having to cross the P{hs-hid}Y chromosome into two stocks (*scarlet* and *brown*), she is enthusiastically converting over to this system for the coming year (C. Pollock, personal communication). Two other institutions are similarly adopting this system for use

in both large introductory and smaller upper-level courses (P. MacDonald and B. Moon, personal communication). Together, these responses indicate that this system has broad appeal despite the initial setup phase to adapt existing exercises to use P{hs-hid}Y lines.

Instructors who already use flies (but with other markers) and wish to adopt this system may do so in one of two ways. The first option is to change laboratory exercises currently in use to use the available markers. For example, an exercise based on a sex-linked recessive marker such as *yellow* could be changed to use *white*. Similarly, laboratories based on autosomal recessive markers could be changed over to an available autosomal marker such as *black* or *sepia*. The second option would be to create P{hs-hid}Y stocks of the markers already used. These crosses are straightforward for both autosomal and sex-linked loci. Crossing schemes to generate novel P{hs-hid}Y derivatives from existing marker stocks are outlined in Figure 5.

Instructors who use this system to introduce *Drosophila* crosses into their curriculum may simply use the available P{hs-hid}Y stocks, because there is no prior commitment to a particular set of markers. For example, the exercise I have developed and described here is efficient in that it simultaneously examines sex-linked and autosomal inheritance, forcing students to consider both modes of inheritance within a single cross. Implementing this exercise would not require developing new laboratory material, because it is provided here. Additionally, this exercise requires maintaining only two stocks (*white*, *black* double mutant in the P{hs-hid}Y background for virgins plus a normal Y wild-type stock for males). This exercise thus provides an easily acces-



**Figure 5.** Crossing schemes to generate novel P{hs-hid}Y stocks for sex-linked (A) and autosomal (B) loci using wild-type males from a P{hs-hid}Y stock. The sex-linked recessive mutation *yellow* is used as an example for generating a novel sex-linked stock in two generations (A); the autosomal recessive mutation *scarlet* is used as an example for a three-generation scheme to generate a novel autosomal stock (B). Analogous crosses could be used for other desired markers.

sible way to demonstrate sex-linked and autosomal inheritance in an introductory laboratory.

### ***Pedagogical Value of *Drosophila* Crosses for Majors and Nonmajors Students***

Student feedback indicated general agreement that the specific laboratory exercise evaluated here was interesting and increased student understanding of genetics over a range of student backgrounds and interest levels. Several students indicated in the feedback section that the exercise had improved on their high school experience of learning genetics. The exam results also demonstrate marked improvement in understanding for those outcomes explicitly reinforced in the laboratory exercise (determining inheritance modes and justifying one's choice) compared with one merely presented in lecture and implicit in the laboratory (defining pure breeding). Together, the data suggest that this exercise improved student interest and enhanced learning outcomes over and above the lecture presentation. Additionally, the *Drosophila* exercise benefited majors and nonmajors students equally. The value of "hands-on" genetics exercise in the laboratory in a freshman biology course is also heightened because this is a terminal course in biology for many students. The survey revealed that only 26 of the 88 respondents planned to pursue a degree in biology; the rest are presumably using Intro Biology as a science credit for their degree in a different subject. Thus, freshman biology is a "last chance" for hands-on genetics for many students; however, this requires introducing a genetics exercise into what are often very large classes with multiple laboratory sections. The P{hs-hid}Y system provides an elegant way to introduce the benefits of genuine genetics experiments into a large class with minimal effort.

### ***Applications Before and Beyond the Undergraduate Level***

Although I present this system as a means to enhance teaching and research productivity of undergraduates, this system is equally amenable to other levels of instruction. High school science instructors could easily use this system to introduce basic crosses to their curriculum or to facilitate crosses already used. At the postgraduate level, a student in the first years of graduate school suffers from time pressures similar to undergraduates. Similarly, a student starting out with *Drosophila* at any career stage will need time to gain expertise in virgin collection. Strategic use of this system could thus prove of great benefit to graduate students. Finally, as a principal investigator with a large teaching load at a primarily undergraduate institution, I find this method extremely useful to enhance my own productivity at the bench. During the summer I am able to build the virginizing stocks I require for the teaching months and then use them when my schedule is too full to permit large-scale virgin collection by traditional methods. This allows me to main-

tain a higher level of productivity year-round, despite the increased load when teaching.

### **ACCESSING MATERIALS**

The P{hs-hid}Y teaching stocks I have developed and described here are freely available for a nominal fee to cover shipping and stock maintenance costs. The laboratory exercise handouts are also available as Adobe Portable Document Format (.pdf) or Microsoft Word (.doc) files upon request.

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