Classroom

In this section of *Resonance,* we invite readers to pose questions likely to be raised in a classroom situation. We may suggest strategies for dealing with them, or invite responses, or both. "Classroom" is equally a forum for raising broader issues and sharing personal experiences and viewpoints on matters related to teaching and learning science.

Rediscovering Genetics with *Drosophila*

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Genetics is taught with minimum experimentation. The alternatives preferred over the years have been the use of colored beads/seeds and a problem-solving approach. To change classroom teaching from dry lab to wet lab exercises, we started working with *Drosophila melanogaster* for teaching genetics to undergraduate students. In a single semester course, they were able to demonstrate both Mendelian and non-Mendelian patterns (such as sex-linked and linkage) of inheritance. In addition, we designed crosses to show phenomena that are either unique to *Drosophila* or achieved differently in these flies such as dosage compensation, absence of crossing over in males and deposition defect in white-eyed flies. In this article, we elaborate two of our experiments.

In a single semester course, undergraduate students were able to demonstrate both Mendelian and non-Mendelian patterns (such as sex-linked and linkage) of inheritance.

1. Dosage Compensation

Male flies have a single X-chromosome and a Y-chromosome that bears very few genes, whereas, female flies have two X-chromosomes. As a result, there is a difference in the number of X-linked genes between males and females. This numerical difference is equalized by an epigenetic mechanism termed as 'Dosage Compensation'. Dosage compensation can operate by

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three different mechanisms which are known to occur in Nature: hypoactivation of both X-chromosomes in females (*C. elegans*); inactivation of one of the two X-chromosomes in females (mammals); and hyperactivation of the single X-chromosome in males (*Drosophila*) [1].

In most classroom lab exercises, mammalian female cheek epithelial cells have been the material of choice for demonstrating dosage compensation. This may be due to the ease of obtaining and staining these large cells to observe the darkly stained Barr body[1]. The presence of a single Barr body in the interphase nuclei of a normal female is generally taken as evidence of equalization of the expression of X-linked genes between the sexes.

[1]A Barr body is a heterochromatin generated by the random inactivation of one of the two X-chromosomes.

Drosophila uses a different strategy to achieve dosage compensation. The males hyperactivate the X-linked genes. Therefore, we devised an alternative approach to functionally demonstrate dosage compensation.

Box 1 highlights the handling of flies for mating.

Experiment

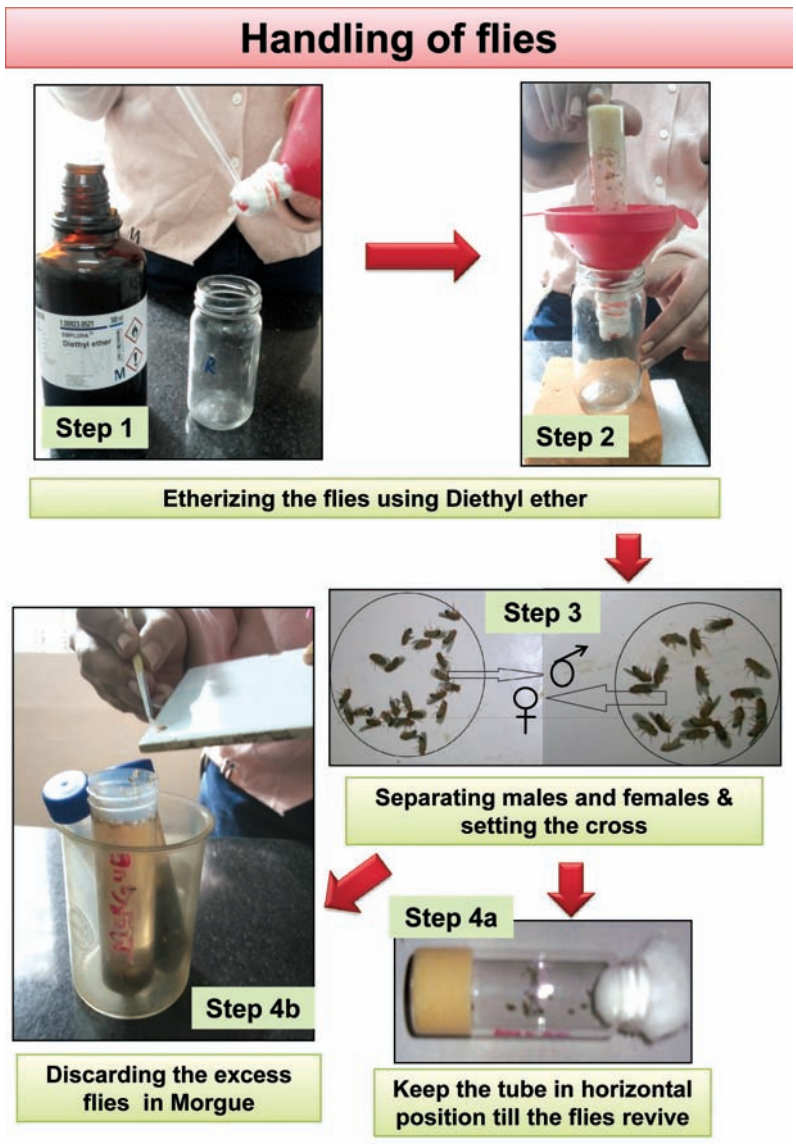
This experiment was originally performed by Edward Lewis [2] and involves the two X-linked mutations *white* and *apricot*. Upon crossing apricot-eyed females and white-eyed males, he found that the F_1 females[2] had a fainter orange eye color. This made him conclude that *white* and *apricot* are non-complementing allelic mutations. We crossed apricot-eyed virgin females (X^aX^a) and white-eyed males (X^wY) and collected the F_1 progeny (*Figure* 1).

[2] F_1 refers to the first filial generation or the first generation from a mating.

Results and Discussion

In the experiment, the eye color of both, F_1 males (X^aY) and F_1 females (X^aX^w), was compared with each other and with that of the mother (X^aX^a). The F_1 females had a fainter orange eye color as compared to the males (*Figure* 2A). Both of them have one *apricot* allele, although females have two X-chromosomes. The counting mechanism signals the dosage compensation machinery

Box 1. Handling of Flies



to hyperactivate the X-linked genes in males. This is evident from the difference in the intensity of eye color. Incidentally, the F_1 males have the same intensity of apricot eye color as the homozygous mother with two *apricot* alleles (*Figure* 2A). This demonstrates dosage compensation in the F_1 males by

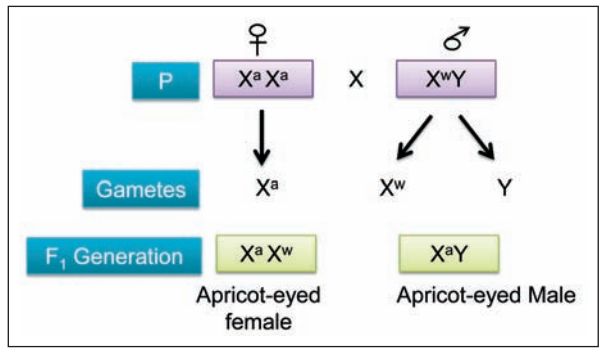


Figure 1. A simple cross performed to functionally study dosage compensation.

hyperactivation. *Drosophila* males hyperactivate X-linked genes to twice their potential to match levels of gene expression in females.

To further confirm our findings, we went ahead to separate pterin from the F_1 progeny by thin layer chromatography. It required four fly heads of females versus two of the males to obtain the same intensity of resolved pterins (*Figure* 2B). This again supports the results interpreted by visual observation.

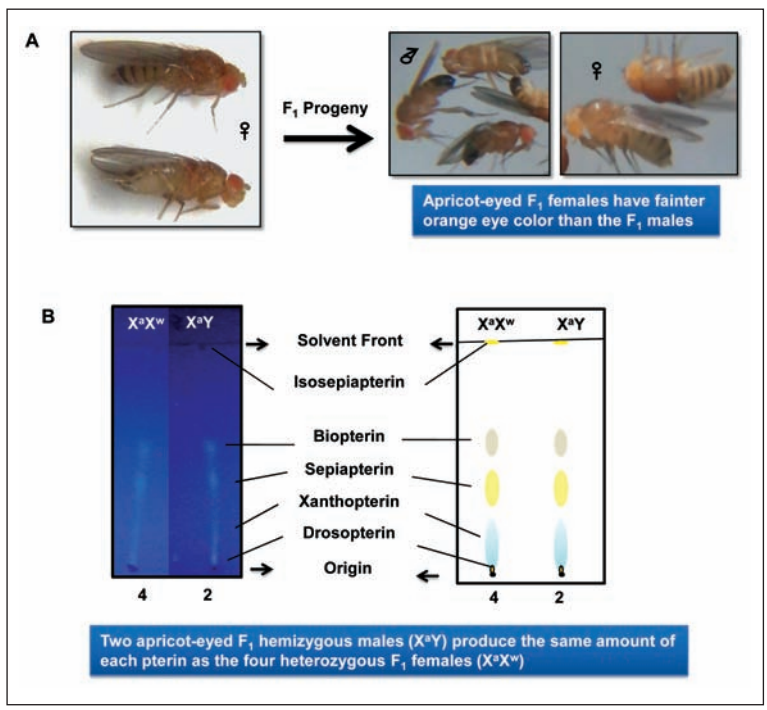


Figure 2. Dosage compensation in *Drosophila melanogaster*.
(A) Comparison of F_1 progeny males and females with P_1 female. The F_1 males have the same intensity of apricot eye color as that of the mother. **(B)** Separation of pterins of two apricot-eyed F_1 males and four F_1 females.

The beauty of this experiment is the simplicity and a visual demonstration of the hyperactivation of an X-linked gene in males under a dissecting microscope (10X). What can go against this experiment is the time taken to complete a generation (10-12 days). It is worth the effort as we are visually observing the effect of dosage compensation. It is unlike the exercise of looking for a Barr body in smears of epithelial cells. We strongly believe that such experiments generate more excitement and leave a lasting impact on students.

The beauty of the experiment is the simplicity and visual demonstration of hyperactivation of an X-linked gene in *Drosophila* males.

2. Deposition Defects

The wild-type eye color (brick-red) of *Drosophila* results from the deposition of pterins (red pigments) and ommochromes (brown pigments) in the eye. They are metabolites of tryptophan and GTP respectively (*Figure* 3). White-eyed flies, generally used in genetic crosses, result from a mutation in an X-encoded gene. It is a loss of the function allele (*w*) that results in a white eye color. The wild-type gene (w^+) encodes a protein located in the cell membrane that transports pigment precursor molecules into the ommatidium (individual units) of the compound eye. Most white-eyed flies can synthesize the pigments but have a transportation or deposition defect [3]. The other mutant used is *vermilion*. The *vermilion* gene is involved in the ommochrome pathway. It encodes for tryptophan oxygenase. Loss of function mutations in *vermilion* results in a bright red eye color phenotype due to the absence of brown pigments.

Complementation between eye color mutants was used to demonstrate that they can synthesize eye pigments.

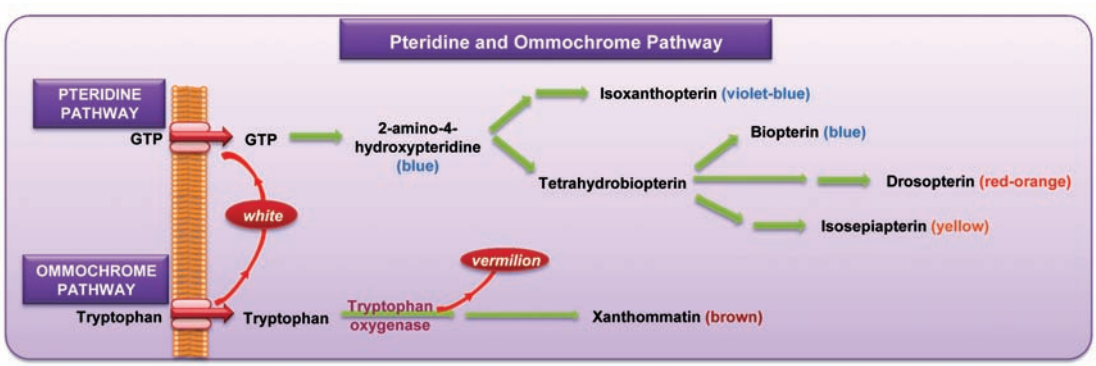


Figure 3. The pteridine and ommochrome pathway.

Experiment

There is more than one way to demonstrate this:

(i) The ommatidia of white-, apricot- and red-eyed flies were carefully observed at higher magnification (40X).
(ii) We also crossed vermilion-eyed females with white-eyed males and observed the F_1 progeny for their eye color.

Results and Discussion

Allelic variants of white, such as apricot, are partially functional and have pigment deposition to some extent. When viewed at 40X magnification, apricot-eyed *Drosophila* showed deposition in some ommatidia while others were empty. Wherever deposited, the pigment was brick red in color like the wild-type (*Figure* 4A). Ommatidia were completely empty in white-eyed flies while wild-type *Drosophila* showed all the ommatidia filled with brick

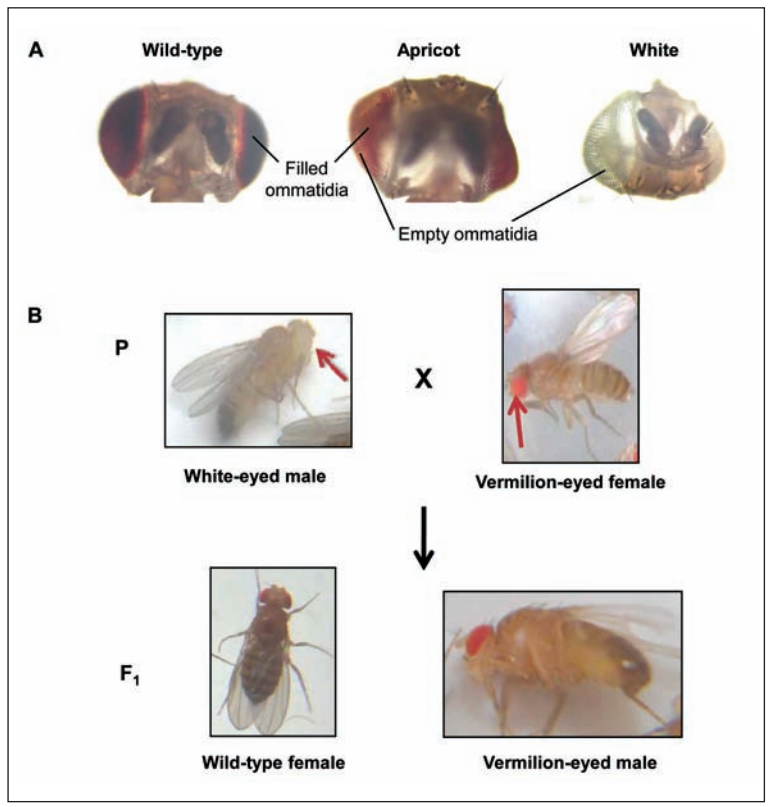


Figure 4. White-eye color mutant can synthesize eye pigments but cannot deposit it in the ommatidia. **(A)** The eyes of wild-type, apricot-eyed and white-eyed *Drosophila* when viewed at 40X magnification. **(B)** Cross between a white-eyed male and vermilion-eyed female.

red color pigments. This partially proves that white eye color mutant has a deposition defect but can synthesize both pterins and ommochromes.

To further confirm our initial results, we performed a cross of vermilion-eyed females with white-eyed males. In the F_1 progeny, all males had vermilion eye color as they are hemizygous and receive their X-chromosome from their mother, while all females were red-eyed (*Figure* 4B). The heterozygous females carrying one X-chromosome with the *vermilion* allele and the other with the *white* allele complemented the defects of either parent and hence had wild-type eye color. This implies that the ability to synthesize ommochromes has come from the white-eyed parent confirming that they are lacking in deposition.

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We welcome suggestions and will readily support any undergraduate department interested in initiating work on *Drosophila*.

Suggested Reading

[1] **T Conrad and A Akhtar, Dosage compensation in *Drosophila melanogaster*: epigenetic fine-tuning of chromosome-wide transcription, *Nat. Rev. Genet.*, Vol.13, No.2, pp.123–134, 2012.**

[2] **E B Lewis, The Pseudoallelism of white and apricot in *Drosophila melanogaster, Proc. Natl. Acad. Sci. USA*, Vol.38, No.11, pp.953–961, 1952.**

[3] **http://www.public.asu.edu/~thoffman/commonfiles/lsc348/lsc348drosophilaeyepigment.pdf**

[4] **H A Ranganath, Teaching and Learning Genetics with *Drosophila*, *Resonance*, Vol.5, No.7, pp.59-70, 2000.**