BIO FAX!

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The Genetics of *Drosophila* Eye Color

An Advanced Inquiry Lab

AP* Biology Big Idea 1

Introduction

Drosophila melanogaster traditionally have brick-red colored eyes. However, some mutations cause their eyes to be different colors—even white! Determine in this activity which pigments are present in the eyes of wild-type and mutant flies.

Concepts

- Dominant vs. recessive
- Genotype vs. phenotype
- Enzymatic control
- Biosynthetic pathways
- Mutations

Background

Wild-type *Drosophila melanogaster* have characteristic brick-red eyes. This organism, also called a fruit fly, is commonly used in the study of genetics and now of gene tracking during embryogenesis. Eye color variation is one of many mutations that have been extensively studied for more than one hundred years. The study of the metabolic pathways and underlying genes that create eye color continues to be of great interest in terms of evolutionary mechanisms. Early on, the study of eye color mutation was chosen because of the easy visibility of the changing phenotypes. Alterations in the enzymatic pathways leading to eye color produced weird eye colors. Now scientists are studying which genes must be expressed to produce the pigments responsible for the various eye colors and the mutant eye colors. The expression of eye color is too involved to simply be the product of a single gene with only two possible alleles. There are actually several genes that must all function properly to produce the red-brick eyes of *Drosophila*.

It is rare that a single trait is controlled by a single gene. More commonly a trait is "built" from a starting material known as a *precursor molecule,* and is changed in a step-by-step fashion called a *pathway*. Each step in the pathway is controlled by a different enzyme. Enzymes are proteins that are coded by DNA and are under genetic control. If the gene for any enzyme undergoes mutation, the expression of that eye color pigment will also be altered. The color may "disappear" from the phenotype or an intermediate product in the pathway can accumulate. The result of these changes to the pathway is a different colored eye.

The red-brick eye color of a wild-type *Drosophila* is actually the product of two biochemical pathways—the *ommochrome* pathway leading to the synthesis of brown pigments and the *pteridine* pathway that contributes to the red pigments. Flies that are missing the red pigments will have brown eyes and any that are missing the brown pigment will have red eyes. Each pathway consists of many steps. The pterdine pigments are soluble in water and can be separated using paper chromatography with an aqueous alcohol solvent system containing a strong base. The ommochrome pigments do not dissolve in water and will not be identified in this lab.

The biochemical pathway that produces the various pteridine eye pigments in wild-type flies is shown below.

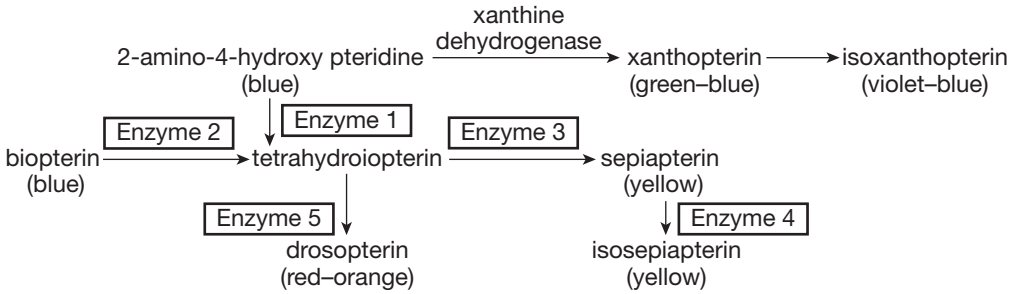

Figure 1. Biochemical pathway of pteridine eye pigments.

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All of the above pigments, plus those of the ommochrome pathway, are necessary to produce brick-red eyes. Enzymes 1–5 catalyze different steps in the biochemical pathway. Notice that the precursor molecule gives rise directly to two pigments and that an intermediate compound is the starting substance for three other pigments and the pigment which is formed depends on which enzyme catalyzes the reaction. For example, if the gene expressing the intermediate pigment is mutated, then the remaining pigments will not be produced. Any change in the pathway produces a failure of development or a change in result.

Thus far the discussion has focused on the pigment pathways but they are just one of many pathways that are being studied. A gene known as Pax6 was identified by geneticists and found to be the universal regulator of eye formation. This is important because when a gene in the beginning of a regulatory network is switched on, it triggers all the genes downstream into a series of action that creates a complex structure. One example is a fruit fly that was found to have eye pigment in its wing. This mutation was mapped back and attributed to the inappropriate expression of the Pax6 gene. Discovering unusual gene expression with a simple switch is an example of the nature of developmental genetics. These simple switches also exemplify evolution, since small changes can lead to dramatic variations. If the variation leads to an advantage in the survival of the fruit fly or in an increase in the survival or number of its offspring, the change in the expression of that gene could be visible in generations to come. In the case of the Pax6 gene's expression on the wing, one hypothesis is the expression of eyes on the wings was a developmental advantage because the fruit flies looked larger to predators. These complex developmental networks are not a barrier to evolution but instead provide opportunity for evolution.

Experiment Overview

In this laboratory students will determine which pigments are present or missing in wild-type and mutant *Drosophila*. The pigments will be separated and identified using paper chromatography.

Materials

Drosophila melanogaster, 2 flies of each phenotype
- Wild-type
- Sepia
- White-eyed mutant
- Scarlet-eyed mutant

Isopropyl alcohol, $(CH_3)_2CHOH$, 70%, 5 mL

Chromatography solvent, 20 mL

Aluminum foil, 12" × 12" sheet,s 2

Beaker, 600-mL

Chromatography paper, 20 x 10 cm

Cotton balls, 5

Dissection pin

Forceps

Paper, white

Pencil

Staples

UV Lamp

Safety Precautions

Isopropyl alcohol and n-propyl alcohol are flammable liquids and a fire risk. They are also harmful to the eyes and respiratory tract. Ammonium hydroxide vapors and liquid are extremely irritating—especially to eyes. Dispense in a hood and make sure an eye wash is accessible. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

Baseline Activity: Day One

1. Wrap aluminum foil around the outside of a 600-mL beaker to protect the chromatography chamber from light. The pigments are light sensitive.
2. Obtain a piece of chromatography paper. *Note:* Only handle it by the edges since oils from skin can affect the movement of the pigments on the paper.

3. Using a pencil, draw a line 1.5 cm from the longer edge. This will be the bottom edge of the paper. Make four marks along the line at approximately 2-cm intervals. Label the intervals on the paper as shown in Figure 2.

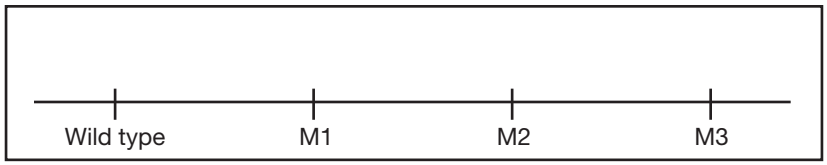


Figure 2.

4. Obtain two anesthetized flies from each of the four phenotypes.
5. Obtain a large dissecting pin and clean the head of the pin with a cotton ball and isopropyl alcohol.
6. Using forceps hold the wild-type fly so that its head is positioned on the line at the wild-type marker. Crush the head of the fly onto the paper using the head of the dissection pin. Discard the body of the fly.
7. Wait for the spot to dry. Lightly blow on the paper to decrease drying time.
8. Repeat steps 5 and 6 with the remaining wild-type fly—crush the head at the same location as the first.
9. Repeat steps 5–8 with the three mutant strains, placing each in the proper location of the chromatography paper. *Note:* It is important to clean the head of the dissection pin between each different strain of *Drosophila* to ensure no leftover pigment will contaminate each successive area.
10. After the head samples have been dry for about five minutes, roll the chromatography paper into a cylinder with the blank side inward. Remember to handle the paper by the edges only. Carefully staple the top and bottom edges of the chromatography paper avoiding the sample spots (see Figure 3).
11. Pour 20 mL of chromatography solvent into the 600-mL beaker, to a depth of approximately 10 mm.
12. Place the stapled chromatography paper in the beaker as shown in Figure 4. Check the solvent level in the beaker— the liquid should just cover the bottom of the paper but the pigment samples should not be immersed in the solvent.
13. Cover the top of the container with aluminum foil to prevent evaporation of the solvent and protect the light-sensitive pigments.

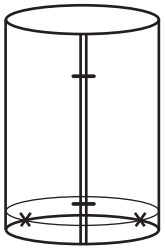


Figure 3.

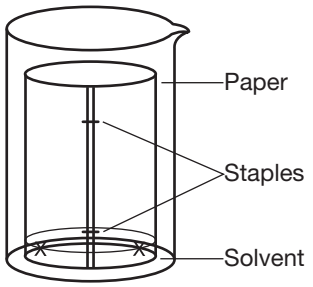


Figure 4.

14. Allow the chromatogram to run until the solvent level is about 1 cm from the top of the paper. If the solvent has not reached this point by the end of class, notify your teacher.
15. Remove the chromatography paper from the beaker and mark the solvent line with a pencil.
16. Carefully remove the staples from the chromatography paper.
17. Give sample to your instructor to refrigerate overnight.

Baseline Activity: Day Two

18. Obtain your chromatogram from your instructor.
19. Turn out the lights in the laboratory. The room should be as dark as possible. *Note:* If you have black lab table tops, place your chromatogram on a white sheet of paper.
20. Observe the results for the wild-type flies. The pigments will have separated on the chromatogram. Using a pencil, circle each pigment spot and label it. *Note:* Adjacent spots will blend together.
21. Repeat step 19 with the three remaining mutant samples on the chromatogram.

Opportunities for Inquiry

1. Consider the following questions while reflecting upon your knowledge of *Drosophila* genetics and evolution.
   a. How might the age of the fruit fly change the results of the pigment chromatography?
   b. What other organisms could be studied in a similar fashion?
   c. Develop a method to study the ommochrome pathway.
   d. Of the factors identified in the above questions, which can be replicated as an experiment in the laboratory?
2. Plan, discuss, execute, evaluate, and justify an experiment to test a question regarding the evolution of insect eye pigments.
   a. Decide upon one question that your group would like to explore.
   b. Develop a testable hypothesis.
   c. Discuss and design a controlled experiment to test the hypothesis.
   d. List any safety concerns and the precautions that will be implemented to keep yourself, your classmates, and your instructor safe during the experimental phase of this laboratory.
   e. Determine what and how you will collect and record the raw data.
   f. How will you analyze the raw data to test your hypothesis?
   g. Review your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning the experiment.
   h. Once the experiment and analysis are complete, evaluate your hypothesis and justify why or why not the hypothesis was supported by your data.
   i. Present and defend your findings to the class.
   j. Make suggestions for a new or revised experiment to modify or retest your hypothesis.

Teacher's Notes

The Genetics of *Drosophila* Eye Color

Materials Included in Kit (for 8 groups of students)

- Aluminum foil, 12″ × 25″
- Ammonium hydroxide solution, NH_4OH, 3 M, 120 mL
- Isopropyl alcohol, 100 mL
- Lull-A-Fly™ solution, 15 mL
- n-Propyl alcohol, 100 mL
- Anesthetizing wands, 8
- Chromatography paper, 10 × 20 cm, 15 sheets
- Cotton balls, 50
- Dissection pins, large, 10

Additional Materials Needed (for each lab group)

- *Drosophila* cultures
  - Wild-type
  - Sepia
  - White-eyes
  - Scarlet-eyes
- Beaker, 600-mL
- Forceps
- UV light source

Additional Material Needed (for *Pre-Lab Preparation*)

- Beaker, 600-mL
- Graduated cylinder, 100-mL
- Stirring rod

Pre-Lab Preparation

Ammonium Hydroxide Dilution

1. To make 100 mL of 2 M ammonium hydroxide, pour 66 mL of 3 M ammonium hydroxide into a 100-mL graduated cylinder.
2. Fill to the 100-mL mark using distilled or deionized water.

Chromatography Solvent

1. Using a graduated cylinder, measure 100 mL of n-propyl alcohol and transfer to a 600-mL beaker.
2. Using the same graduated cylinder, measure 100 mL of 2 M ammonium hydroxide and transfer it to the same beaker.
3. Stir so the solution is evenly mixed and cover with aluminum foil to prevent evaporation.

Safety Precautions

Isopropyl alcohol and n-propyl alcohol are flammable liquids and a fire risk. They are also harmful to the eyes and respiratory tract. Ammonium hydroxide vapors and liquid are extremely irritating—especially to eyes. Dispense in a hood and make sure an eye wash is accessible. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Ammonium hydroxide may be disposed of according to Flinn Suggested Disposal Method #10. n-Propyl alcohol may be disposed of according to Flinn Suggested Disposal Method #18b. Isopropyl alcohol may be disposed of according to Flinn Suggested Disposal Method #18a.

Alignment with AP Biology Curriculum Framework

Big Idea 1: The process of evolution drives the diversity and unity of life.

Enduring Understandings

1A1: Natural selection is a major mechanism of evolution.

1A2: Natural selection acts on phenotypic variations in populations.

1A3: Evolutionary change is also driven by random processes.

1C3: Populations of organisms continue to evolve.

Big Idea 4: Biological Systems interact and these systems and their interactions process complex properties.

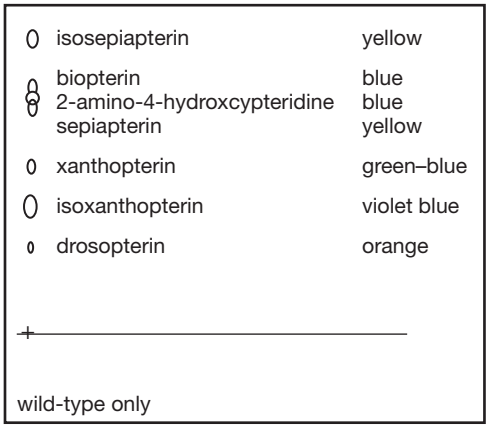
Enduring Understandings

4A2: The structure and function of subcellular components, and their interactions provide essential cellular processes.

Lab Hints

- It is important that the chromatography is run in the dark, which is the reason for covering the beaker with aluminum foil. The pigments are light-sensitive.
- Results are best viewed in a room that is completely dark. If windows are present close the blinds before viewing with the black light.
- As an extension to this activity brown-eyed flies can also be run. However, the brown (ommochrome) pigments seen in the brown-eyed fruit fly are not soluble in the solvent used in this experiment and thus remain on the initial sample locations. Allow students to experiment with different solvents to determine a solvent system that will dissolve and separate these pigments.
- This experiment does not work with flies that have been dead for several hours. The flies may be anesthetized to death but they must be used immediately following. If allowed to sit dead for too long the pigments dry up and do not transfer to the chromatography paper well.

Sample Data and Observations



Above is a sample of what the chromatogram should look like for the wild-type fruit fly. This is an ideally separated model for reference purposes. The colors tend to run together and will not separate to the degree which they have in the diagram. The mutant results are as follows:

Sepia: no drosopterin; large smear of sepiapterin (yellow)

White: no pigment

Scarlet: same as wild-type

Brown (optional): no pigment

Acknowledgment

Special thanks to Kathy Van Hoeck, York High School, Elmhurst, IL for sharing this activity with Flinn Scientific.

References

Van Hoeck, Kathy. *Genetics—Laboratory and Classroom Activities;* Flinn Scientific: Batavia, IL; 2001; pp 98–105.

AP Biology Investigative Labs: An Inquiry-Based Approach, College Entrance Examination Board: New York; 2012.

The Genetics of Drosophila *Eye Color—Advanced Inquiry Laboratory* and supporting supplies are available from Flinn Scientific, Inc.

Catalog No.	Description
FB2046	The Genetics of *Drosophila* Eye Color
LM1115	*Drosophila,* wild-type
LM1117	*Drosophila,* mutant culture, white eyes
LM1125	*Drosophila,* mutant culture, sepia
LM1245	*Drosophila,* mutant culture, scarlet
LM1244	*Drosophila,* mutant culture, brown-eyes

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