

LABORATORY 5. *Drosophila* EYE COLOR: A COMBINATION OF PROTEIN PIGMENTS

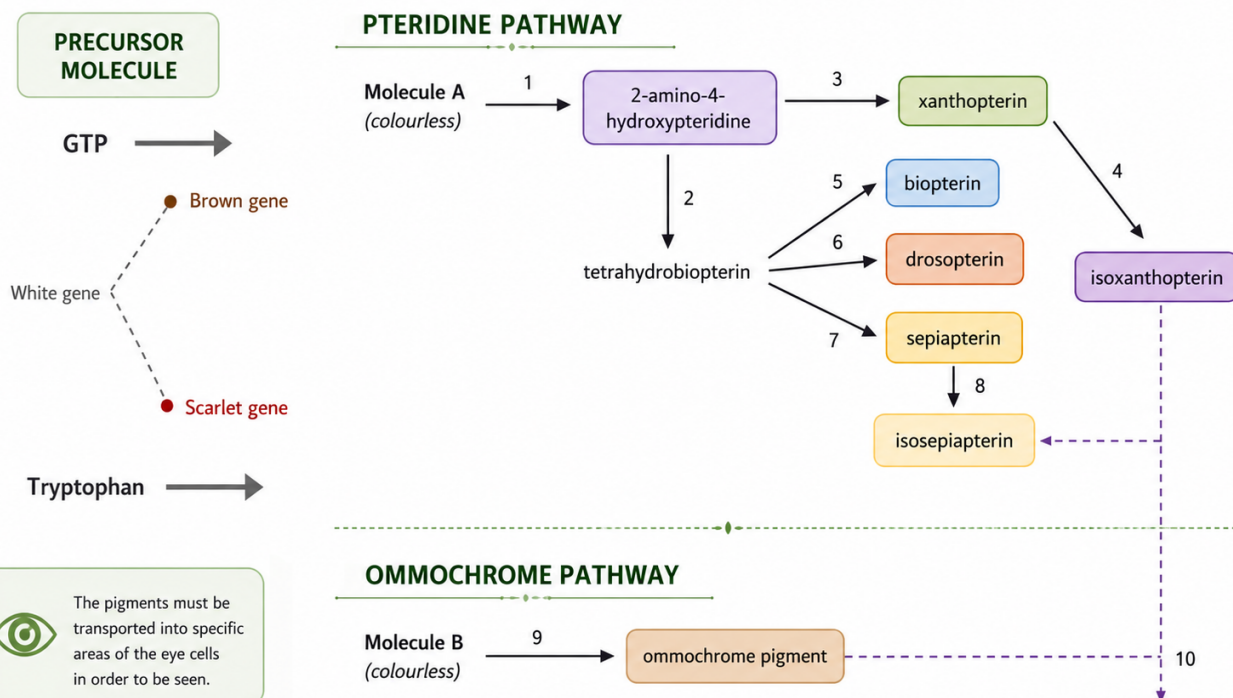
LEARNING OUTCOMES

When students have completed this laboratory practice, they should be able to:

- Describe how the eye color variation in *Drosophila* is based on **protein-pigment** differences.
- Identify some of the **genes** (enzymes) that are involved in the biosynthesis of protein pigments.
- Recognize the **epistatic interactions** between some of the genes responsible for the eye pigment coloration.
- Implement an experiment to illustrate how protein pigments can be separated and analyzed by chromatography.

In *Drosophila melanogaster*, the **brick-red eye** color (wild type) is due to the presence of **two** distinct classes of protein pigments: **pteridines** and **ommochrome**. These two types of pigments are synthesized by **two** separate biochemical pathways and **wild-type flies contain all of them**. Their precursor molecules are **transported** to the compound eyes by **transporter proteins** (ABC proteins) coded by the **scarlet (st)** gene (tryptophan importing), the **brown (bw)** gene (GTP importing) and the **white (w)** gene (common subunit required for importing **both** precursors). The enzymes that are specific to each pathway (# 1 to 9 in the figure) are coded by nuclear genes and the two pathways are under genetic control.

INTRODUCTION





Mutations in one or both pigment pathways will produce mutant flies with eye colors different to the **wild-type brick red**. If a mutation in a gene coding for an enzyme in the **ommochrome pathway**, the **brown** pigment will not be synthesized, and the resulting mutant fly will have **bright red color (scarlet red, st)**.

If a mutation occurs in **one of the genes** coding for any of the **intermediate compounds** in the **pteridine pathway**, the resulting fly may have **brown, dark brown or even black eyes**. For example, the absence of **drosopterin** pigment (#6) will produce flies with **sepia** eye color. Hence, in this case a mutation at the sepia gene (**se**) will inhibit the synthesis of the **drosopterin** pigment. If **none** of the pteridine pigments is produced and **only the ommochrome is present**, the fly will show **brown** eyes (**bw**).

If **both** precursor molecules are not **transported** to the pigment granules by **transporter proteins** (for example, a mutation in the **white** gene), the absence of pigmentation will cause the mutant fly to have **white** eyes. This mutation in the white gene (**w**) is the famous sex(x)-linked mutation discovered in 1910 by Thomas Hunt Morgan. If you would like to improve your knowledge about the biology and chemistry of pigmentation, please visit the following website:

Metabolic pathways: investigating the biology and chemistry of pigmentation

<https://droso4schools.wordpress.com/14-enzymes/>

In today's laboratory experiment, students will be extracting the eye pigments from **wild-type, sepia, white, scarlet, and brown flies**. The extracted pigments will then be used for a Thin Layer Chromatography (TLC) experiment to separate seven **pteridine** compounds. **Only** these compounds **fluoresce under UV light**, not the ommochrome ones.

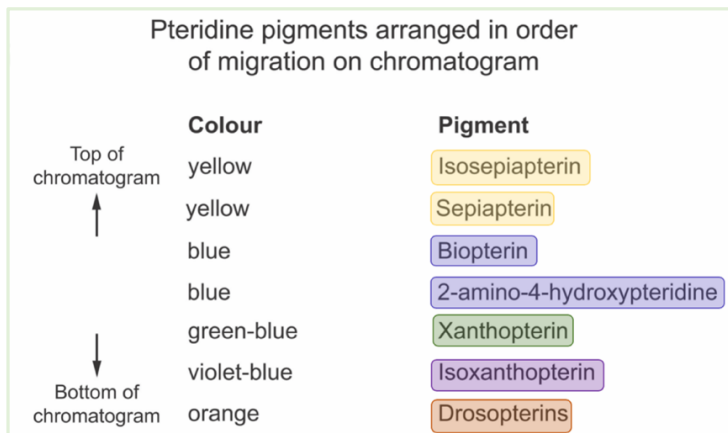
Before setting up the experiment, observe the eye colors of the flies provided in separate vials. Based on the mutant phenotypes and the **pteridine pathway**, think about a suitable hypothesis and try predicting the expected results for the experiment.

Please **watch a video** describing the practical component of the TLC experiment in the following link:

TLC *Drosophila* eye-pigments

<https://youtu.be/x8bXqC-Au04>

A diagram representing the different **patterns of migration** of the pigments in the TLC plate is presented in the following figure:



**Wild type (+)**

(Pteridine
AND
ommochrome
pigments)

Brown (bw)

(Only
ommochrome
pigment)

White (w)

No pigments
are present

Scarlet (st)

(Only
pteridine
pigments are
present)

Sepia (se)

(Only
Drosopterin
pigment is
absent)

MAIN MATERIALS

- Your vial containing F₂ flies
- *Drosophila* Flies: Wild, Sepia (se), White (w), Scarlet (st), and Brown (bw) (5 phenotypes)
- Fluorescent UV light
- Dissection microscope
- Plastic pestles
- Microtubes (1.5 ml)
- A TLC plate (glass-backed silica gel)
- Paintbrush
- Solvent (1:1 mixture of n-propyl alcohol and 28% ammonium hydroxide)
- Marking pens, pencil, ruler

PROCEDURE FOR LABORATORY WORK

1. Work in **pairs** of students. Collect your vial from the previous lab practice. They are in the cart at the front of the lab room.

Remember the cross in this vial:

F₁ females (WT) × F₁ males (yellow, white, miniature)

2. Check your tube for the presence of F₂ flies.
3. Transfer the flies to a clean vial and use ice to anesthetize them. Observe them using the dissecting scope.
4. Using a brush, move the flies around and observe the phenotypes. Identify male and female flies.
5. Score the flies. Remember that in this generation **you are expecting a wide range of phenotypic variation** (see page # 46). Keep this information safe, as you need this data to complete your assignment (question # 1). Discard the flies in the morgue.
6. Discard the vial in the bin located in the front of the lab. No further phenotyping is required.



THIN LAYER CHROMATOGRAPHY (TLC)

EXPERIMENT

1. Work in groups of **5 students**. Each student should collect 2 flies **per each** of the **5 phenotypes**. Deposit the flies in the 1.5 ml tubes.

2. Collect one TLC plate. Draw a light pencil line **1-2 centimeters** from one end of the plate. This would be the bottom of the plate. Place **5 tick marks** at even intervals on the line. Label the tick marks with the mutant symbols: **+, se, w, st, bw**.

3. Each student in the group will extract the eye pigments as follows:

- Add 2-3 drops of solvent. Crush/grind the flies with a plastic pestle.
- Allow the vial to sit for a 1 minute.
- Use the pipette tip to spot the extracted pigment onto your TLC plate.
- Let the spot dry before applying the next drop (apply 5-10 drops in total).

- Dispose the tube and the tip into the appropriate container.
- Allow the TLC plates to dry (3 minutes)
- Use forceps to hold the top end of the TLC - plate, and gently lower the bottom of the TLC plate into a beaker (do this step in a fume hood or ask a TA to do it for you).
- Run the experiment for 35 to 50 minutes in the dark as pteridines are light sensitive.
- Check the experiment periodically to make sure that solvent does not go beyond the end of the plate.
- Allow the TLC plate to air dry.
- Wrap your TLC plate in aluminum foil, label it with your name, and leave it for **analysis until next week**.

Please **watch a video** describing the practical component of the TLC experiment in the following link:

TLC *Drosophila* eye-pigments

<https://youtu.be/x8bXqC-Au04>

If you need further assistance, please contact your assigned TA or the lab coordinator. Their contact information is available in Canvas