FLY EYE PIGMENTS LAB

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THIN LAYER CHROMATOGRAPHY OF EYE PIGMENTS OF
Drosophila melanogaster
STUDENT HANDOUT

Introduction

The purpose of this lab is to examine the biochemical factors influencing the eye color of Drosophila melanogaster. Eye color is controlled by genes. Changes in eye color can occur either by defects in the genes for the synthesis of pigments, or by defects in the genes for the transport of pigments.

This activity will demonstrate the relationship between phenotype (the appearance of the trait) and the genotype (the genes present in the organism.)

Equipment

Ultraviolet illuminator, Dissection microscope or magnifying glass.

Supplies

Safety goggles, aluminum foil, TLC plates, filter paper, 600mL beaker, solvent (1:1 mixture of n-propyl alcohol and 29% aqueous ammonia), flies, tweezers, paintbrushes, kimwipes, marking pens, ruler, glass rods, pencils.

Procedure

1. Obtain a TLC plate and lightly draw a line 1.5 cm from the bottom of the plate with a pencil. See Figure 1. Warning: Be careful not to gouge the coating. Space a series of x's on the line, 1 cm apart from each other and 1 cm from the sides of the plate. At the top of the plate, with a pencil, lightly label which eye types you will use, i.e., wild type (+), scarlet (st), brown (bw), white (w).

2. Obtain a labeled vial with at least 3-4 flies of one type.
3. With tweezers or paintbrush, place a fly of the appropriate type on the x mark and gently crush the head with a glass rod, following your teacher's instructions and demonstration. Do the same with a second, third and fourth fly of the same type, trying to place each fly on the exact same spot as the one before. Gently brush fly parts off the TLC plate.

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+ bw st w
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x x x x x
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Figure 1 (TLC plate 10 cm x 5 cm)

4. Repeat with a different fly eye type on the appropriate spot. Do this until you have all four types of flies on your TLC plate.

5. Next, carefully place your TLC plate into the chromatography chamber as instructed by your teacher. (See Figure 2). Place a card with your name below the beaker for identification.

6. Allow about 30-40 minutes for the solvent to move up the TLC plate. **SOLVENT MUST NOT REACH THE TOP OF THE PLATE!** Remove the TLC plate from the solvent and draw a line on the TLC plate marking the final level of the solvent. If time is a problem, steps 4 and 5 may be done by your teacher.
7. Set the plate aside in the hood to dry. When it is dry, wrap it in aluminum foil and place it in a plastic bag with the identification card for later interpretation.

STOP HERE AND CONTINUE IN YOUR NEXT LAB PERIOD.

8. Carefully remove the TLC plate from the aluminum foil. Place it on the UV illuminator with the smooth surface up. Be sure to wear UV goggles while working with the illuminator. Note the presence and location of the eye pigments. Carefully outline and label the pigments found. Compare the pigments found in the wild type (+) with the pigments found in the other eyes.

Data Collection

Make a drawing of the pigment outlines observed on your plate.

Interpretation

Interpreting the results: Carefully observe all spots that are visible on the wild type fly lane(s) on your TLC plate. Then, compare these with each mutant lane in turn. See whether or not all the wild type pteridine pigments you can see are also present in each type of mutant. Record if there is less or more of any pigment than in the wild type (the more there is, the more intense and bright the fluorescence you see).
Questions

1. Which pigments are found in the wild type flies?

2. Are there any other strains of flies on your plate which have the same pigments? Which strain(s)?

3. Are there one or more strains on your plate in which one or more of the pigments are missing? Which pigments are missing from which strain or strains?

4. Do all the pteridine pigments appear in all the mutants?

5. What pigment(s) are absent whenever the eyes are white in color?

6. Which of the mutants has more severely affected the pteridine pigments, brown or scarlet?

7. Reread the introduction to this laboratory, and answer this question: how can these mutations affect the presence of eye pigments?

Compare your results with other students' results.