CHROMATOGRAPHY OF *DROSOPHILA* EYE PIGMENTS

Introduction:

Drosophila mutants with various eye colors occupy a significant place in the history of genetics. T. H. Morgan was the first to characterize the "white eye" (w) mutation, and subsequently Beadle and Tatum extended his work to include many other eye mutant types. This early genetic work demonstrated that a change in a gene (mutation) may affect the structure, function, or regulation of a protein, in this case, an enzyme. Eye color mutants have a defect in one or more enzymes required for the biochemical pathways of pigment synthesis. As a consequence, a pigment may be missing, and/or a different pigment may accumulate because of a defect in a pigment biosynthesis pathway. The work of Beadle and Tatum resulted in the "one gene: one enzyme" hypothesis, which was one of the early foundations for the development of molecular biology during the second half of the 20th century. The present experiment allows a visualization of this hypothesis.

There are two classes of pigments in the eye of *Drosophila*, brown pigments called **ommochromes**, which are the products of tryptophane metabolism, and red pigments called **pteridines**, which are the products of purine metabolism. The wild type eye color is due to a mixture of several different pigments. The wild type eye of *Drosophila melanogaster* contains, for example, seven pteridines.

It is important to realize that if there is a mutation in the ommochrome (brown pigment) pathway, the semi-dull effect of the brown pigments will be missing and the eye color will be brighter red. On the other hand, a mutation in the pteridine pathway will result in a duller, darker color. The second critical point is that mutants were named based on their actual eye color, not the corresponding biochemical defect. For example, the mutant "brown" has a brown eye color, therefore, it is missing the pteridines, which, in turn, means that the mutation affects one of the enzymes in the pteridine biosynthetic pathway.

Experimental Procedure:

Eye pigments from a variety of mutant *Drosophila* will be separated via the technique of thin layer chromatography (TLC). Comparison with wild type eye pigments will allow a visualization of the differences in the various mutants.

Each group of students should have available the following: two plastic-backed silica gel chromatography sheets (TLC plates), a microspatula, razor, glass rod, forceps, small test tube, small vial, pencil, and ruler. Several different vials of flies will be provided. The TA will sacrifice the flies, using ether. Because there are sex differences in the pteridine pathway, ALL OF THE FLIES USED SHOULD BE OF THE SAME GENDER. Use either male or female, but be consistent for the entire experiment.

1. While wearing gloves, take the TLC plates provided and lightly draw a pencil line one centimeter from the bottom. Make the line parallel to the short edge. Place 2 tick marks at even intervals along the line. Lightly label each mark below the line with the pigment extract to be applied. Record, on a data sheet, which fly-type is located at each spot.

2. Each group will use four types of flies: wild type, white eye, one brown eye mutant and one red eye mutant. Each student in the group will prepare an extract of one fly type as follows. Using the razor, decapitate fifteen flies of one gender. Put the heads in the small test tube containing 0.25 ml of solvent. Crush the heads using the glass rod. Transfer the extract to a clean vial, leaving debris behind. Apply 15 microliters of each extract, one microliter at a time, to a spot on the TLC plate. Wait until the spot has dried before making the next application. Wait five minutes after all of the samples have been applied to allow the spots to dry. Each group should now have two TLC plates, each with two extract samples.

3. Using forceps, place the two TLC plates, samples down, in a Coplin jar containing 3 mls of solvent. Be careful that the edges of the plate do not touch the edges of the jar. The solvent is composed of equal volumes of n-propyl alcohol and 28% ammonium hydroxide. Screw the lid on tightly without splashing the solvent. Close the sash of the fume hood. The pigments are light sensitive so the Coplin jar is kept in the dark.

4. Allow the solvent front to approach the top edge of the TLC plate. Check periodically so the solvent does not run off the end of the plate. This should take 25 - 40 minutes. Remove the plates. Immediately mark the TLC plate with a pencil along the solvent front, *i.e.*, the wet/dry interface near the top. Allow the chromatogram to air dry. Examine the chromatograms using a hand-held UV light source in the darkroom. USE UV RESISTANT GLASSES AND GLOVES, AND AVOID EXPOSING YOURSELF TO THE UV LIGHT!! Using a pencil, gently outline each spot in each lane. Note the color and intensity of each spot.

Analysis:

Using the table on the following page, identify the pteridines for each fly type and record them. Note any which appear reduced or increased in quantity in comparison to wild type. Using the equation below, calculate the Rf values for each pigment. Use the distance from the baseline to the center of each spot for the calculation.

The lab report should include conclusions about pigment patterns. Why are wild type and white eye mutants included? What conclusions can be made regarding genotype and phenotypic expression?

The pigments are listed in the order in which they will be separated in the chromatogram, with the pigment listed at the bottom of the table being found at the bottom of the chromatogram, nearest the sample application spot.

Pigment	Color	+ +	W				
Isosepiaterin	yellow						
Biopterin	blue						
2-amino-4-hydroxy pteridine	blue						
Sepiapterin	yellow						
Xanthopterin	green-blue						
Isoxanthopterin	violet-blue						
Drosopterins	orange						

Calculation of R_f Values:

The distance that a particular compound moves is related to the chemical nature of the compound itself, its relative solubility in the solvent, and to the overall distance traveled by the solvent front. The distance is usually reported in terms of a ratio-to-front value (R_f) and is a characteristic of the particular compound in the particular solvent.

$$R_f = \frac{\text{Distance from the baseline to the center of the spot}}{\text{Distance from the baseline to the solvent front}}$$

R_f values can be calculated for the various pteridines only if 1) the location of the solvent front is marked at the time the chromatogram is removed from the chamber and 2) if the various pigment spots are labeled when examined under UV light.