BIOCHEMICAL SEPARATION OF EYE PIGMENTS OF
Drosophila melanogaster

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Key words: Drosophila melanogaster, pigments, eye, mutant.

Abstract. The accessory pigments in the compound Eye of Drosophila melanogaster will be isolated, and
separated using Paper chromatography. Identify the pteridine pigments present in each of the strains, and
calculated an Rf value for each pigment. The procedure we are using separates many of the intermediate
substances in the pteridin pathway based upon each substance’s relative interactions with the liquid moving
(solvent) phase (paper). Examine the effects of time on Drosophila eye pigments. Since the eye pigments
chanced regularly with period of nine days, we hypothesised that as flies develop their eye pigments darken.
We focused on determining each mutation type of pigment and the changes in genetic make up, as evident by
the change of amount of pigment and by the pattern of how our ten mutation darkened each day.

INTRODUCTION.
Chromatography is a method for the investigation of genetic pleiotropy of Drosophila eye pigments and involves the use of paper chromatography for the separation of various biochemical pigments located in wild-type and mutant eye tissue.

The wild-type eye pigments of Drosophila consist of two major components: ommochromes (brown pigments), which are tryptophan derivates and related pteridines, when present in wild-type eye alone with
omnichromes, result in red eyes.

The ommochromes and pteridines are groups of naturally occurring compounds because they are
gene-dependent for synthesis, and complement each other physiologically in the production of the eye colour of
Drosophila.

Because the pteridine pigments are soluble in mixtures of ammonium hydroxide and n-propyl alcohol, they have been extensively studied by chromatography. The different pigments can be separated out on paper chromatograms by their size and chemical properties.

Each individual pigment can be seen under fluorescent light, and a ratio-to-front (Rf) value can be
calculated for each molecule. 1/

MATERIAL AND METHODS

Materials: chromatographic chamber, propanol, distilled water, ammonium hydroxide, chromatographic filter paper, glass rod with rounded ends, razor blade, dissecting needle, etherizer, UV-black light.

Organism: specimens of Drosophila eyes: wild-type, apricot, brown, cinnabar, eosin, sepia, scarlet, nosy, vermilion, white.

Procedure: Before preparing the samples mix propanol, water, and ammonium hydroxide in a
60:24:6 ratio and pour it into the chromatographic chamber until it reaches approx. 2.0 to 3.0 cm in height.
Place the chromatography paper on top of a clean sheet of notebook paper. Cut the paper with the razor blade
into stripes 30 cm long and 4 cm in width, draw a parallel line, 1 cm from the lower edge with your pencil (indicates the start position).

Etherise the flies (from one sex only - either male or female), select three to four flies using a clean razor blade or dissecting needle, cut off the head of each of the samples. Place one head at a time and thoroughly crash one head with the glass rod. Allow the spot to dry before adding another head to the appropriate spot of the paper. Repeat the step with the other mutants.

Touching only the edges of the paper, fold the paper on the opposite side of the printed samples in a way that the paper with the squeezed heads dips into the chromatographic solution. Seal the top of the chamber and place it at room temperature (away from direct heat or sunlight) at a safe location. After three to five hours, remove the chromatogram from the chamber and allow it to air-dry.

RESULTS AND DISCUSSIONS
The procedure we are using separates many of the intermediate substances in the pteridin pathway based upon each substance relative interaction with the liquid moving (solvent) phase as it moves up and with the solid, stationary phase (paper).

There did be seven types pteridine pigments visible in the chromatogram for a wild type: drosopterins (orange-red), isoxantopterin (deep blue), xanthopterin (green-blue), sepiapterin (yellow), 2-amino-4-hydroxypteridine (light blue), biopterin (light blue), and isosepiapterin (yellow).

Mutant strains of flies produce a different pattern of pigment spots characteristic for each strain, depending on which step in the pathway is blocked in that mutant strain.

Rf value for each of pigments on chromatogram (Rf is calculated by diving the distance from the baseline to the solvent front): isosepiapterin - 0,125, biopterin - 0,7127, 2-amino-4-hydroxypteridine - 0,5045, sepiapterin - 0,3504, xanthopterin - 0,2290, isoxantopterin - 0,1532, drosopterin - 0,0483

The biosynthetic pathways that produce pteridine and ommochromes pigments are not completely understood.

Each step in the biosynthetic pathway for drosopterin, the red pigment, and ommochrome, the brown pigment, is catalyzed by a specific enzyme (as is the rule for all biosynthetic pathways). Thus, a pathway requiring 18 chemical reactions to give a final product needed by the organism will also require that the organism produce 18 separate enzymes to catalyse these reactions. Each enzyme will have to be encoded by DNA and will thus have its own gene.

If a mutation disrupts a gene so that the enzyme encoded by that gene is no longer functional, then a block in the pathway will occur. If the block is severe, then the substrate for the blocked enzyme accumulates. The block also prevents any further biosynthesis in the pathway after the block. Sometimes the block is not severe (called a "leaky" mutant), accumulation is less noticeable, and biosynthesis after the block occurs at a low level, but not at a level high enough to give a wild-type phenotype.

CONCLUSIONS
Paper chromatography of the wild-type Drosophila results in the separation of seven pteridines. Flies with mutant eye colors have pteridine patterns that differ distinctly
from the wild-type flies. Heterozygosity is easily distinguished since even trough they possess a wild phenotype, chromatogram indicate an altered pteridine profile.

Investigation of eye pigments in *Drosophila*, indicates that as a fly develops their eye pigment darkens. All of our mutations darken gradually over the period of nine days. Each mutation darkens at a different rate; however, we are able to distinguish a distinct pattern. The pteridines contain the pattern of changing the most between day 1 and day 3 along with between day 6 and 9. It is demonstrate that each primary colour changed over nine days for each mutant.

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