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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 hypnotised that as files 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 pleyotrophy 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: omochromes (brown pigments), which are triptophan derivates and related pteridines, when present in wild-type eye alone whith omochromes, result in red eyes.

The ommochromes and pepteridines 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 (R_f) value can be calculated for each molecule /1/.

MATERIAL AND METHODS

Materials: chromatografic 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, rosy, 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 4cm in width, draw a parallel line, 1cm 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 nteraction 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 filles produce a different pattern of pigment spots characteristic for each strain, depending on which step in the pathway is blocked in that mutant strans.

Rf value for each of pigments on chromatogram (R f 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 ommochrome 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 often 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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