Visual analysis of eye pigmentation in *Drosophila melanogaster* through paper chromatography
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Biology 2330 – Introduction to Genetics
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Introduction

In *Drosophila melanogaster*, pure-breeding strains share the same biochemical pathways for eye pigmentation. However, each strain exhibits differing levels of enzymatic activity in the biochemical pathways due to genetic mutations and this results in different eye pigment for each strain (this includes Wild-type Red, Scarlet, Sepia, Brown and White). Eye pigmentation is derived from two biochemical pathways that are driven by enzyme activity, namely, the Ommochrome pathway yields brown pigment (in particular, Xanthommatin is produced) and the Pterin pathway yields a range of red pigments (including Biopterin, Drosopterin, Sepiapterin, Isosepiapterin, Isoxanthopterin, and Xanthopterin). Both biochemical pathways are not linked to the other; the phenotypic expression of pigment is controlled by genes expression. Consequently, any mutation that prevents the gene expression directly affects pigment production and the colours are not noticed (Grant et al., 2016). According to the provided lab manual, all pigments should be present in the wild-type and the mutants have varying levels of pigmentation (Langara College, 2021). In this experiment, paper chromatography is used to separate the eye pigments, the chromatogram is then visually inspected to ascertain the levels of pigment produced by each strain, and the resulting data is used to identify the enzyme activity involved with producing the eye pigments as seen in each *D. melanogaster* strain.

Materials and Methods

For this experiment, relevant materials were obtained from the Biology 2330 Lab room at Langara College and the Methods were obtained from the Langara College Biology 2330 Lab manual (Fall 2021 edition) under section titled 'Lab 2 – Genetic Control Of Eye Pigments I Drosophila' (Langara College, 2021).

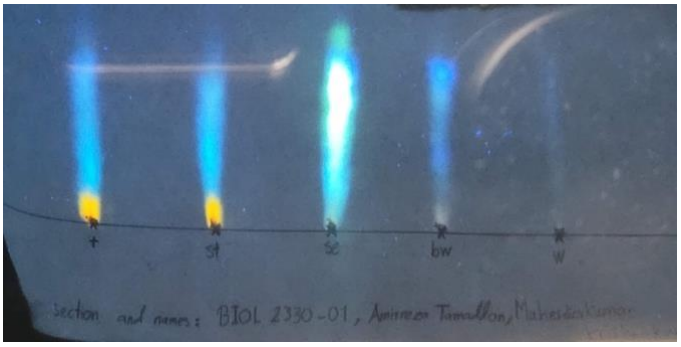
Results

Table 1: *Drosophila melanogaster* chromatogram results and observed amounts of eye pigment (relative to expected wild type)

Pigment	Colour	Wild type (wt)	Scarlet (st)	Sepia (se)	Brown (bw)	White (w)
Isosepiapterin	Yellow	(?)[#1]	(?)	(?)	(-)	(-)
Biopterin	Blue	(?)[#1]	(?)	(?)	(+)[#4]	(?)[#5]
2-amino-4-hydroxypteridine	Blue	(++)	(++)	(?)[#3]	(+)[#4]	(?)[#5]
Sepiapterin	Yellow	(++)	(++)	(?)[#3]	(-)[#4]	(-)
Xanthopterin	Green blue	(-)[#1]	(+)[#2]	(++++)[#3]	(-)[#4]	(-)
Isoxanthopterin	Violet blue	(-)[#1]	(-)	(-)	(++++)[#4]	(+)[#5]
Drosopterin	Orange	(++)	(++)	(?)[#3]	(?)	(-)

Table Legend:

(-) indicates no pigment
(?) indicates inconclusive result, pigment may be present but needs separate testing.
(+) indicates small amount of pigment present (relative to expected wild type)
(++) indicates same amount of pigment present as seen in expected wild type
(++++) indicates large amount of pigment present (relative to expected wild type)
(wt) indicates short form notation of eye color strain type
(++++)[#3] indicates that comment #3 explains this result.



- Figure 1. Eye pigment chromatogram as seen inside the viewport of a UV viewing cabinet. *D. melanogaster* strains pictured (from left to right) are Wild type (*wt*), Scarlet (*st*), Sepia (*se*), Brown (*bw*), and White (*w*).

Comments on results:

#1: Isosepiapterin and Biopterin were hardly noticeable, presence is inconclusive. Wild type (wt) did not show Xanthopterin and Isoxanthopterin.

#2: Brief streaks of green blue were present. This was indicative of Xanthopterin presence in Scarlet (st) sample.

#3: 2-amino-4-hydroxypteridine and Sepiapterin presence was inconclusive due to over production of Xanthopterin. Drosopterin was almost nonexistent, but it could still be present in the Sepia (se) sample, so result is inconclusive.

#4: Brown (bw) sample indicated the presence of dark blue pigments, and no yellow pigments were seen.

#5: Small bright streaks of violet colours were seen in the White (w) sample. There were also bluish streaks (possibly indicative of Biopterin and 2-amino-4-hydroxypteridine of the same size but hardly distinguishable, these results were inconclusive.

Discussion

The results from the chromatogram does not agree with the expected result of noticing all 7 eye pigments in wt strain of *d. melanogaster*.

This disagreement could be because of low amounts of Isosepiapterin and Biopterin produced by *wt* and mutants and these pigments could mask brighter pigments like Drosopterin or Xanthopterin. The chromatogram could have been slightly contaminated with oils and small debris from the experimenters' hands, and this could introduce slight changes in the solubility of the pigments which could have skewed the results or masked the pigments altogether. The pigment variations seen in the results are caused by enzyme or protein deficiencies somewhere in the two biochemical pathways associated with *d. melanogaster* eye pigment production. According to literature, each mutant has a distinct deficiency which results in a noticeable eye color difference.

Considering the *st* mutant, the sample of flies did not show any dark colours arising from pigments such as Isoxanthopterin in the chromatogram. This is potentially owed in part to the mutation seen in scarlet flies that affects the transport of a tryptophan-derived precursor (3-hydroxykynurenine) needed for xanthommatin production by the ommochrome pathway, resulting in phenotypic expression of pteridine pigments (Howells & Ryall, 1975). Note that the non-expression of xanthommatin was out of the scope of this experiment, however the over expression of pteridine pigments may have masked the Isoxanthopterin.

Considering the *se* mutant, the sample of flies showed bright green blue colors arising from the Xanthopterin but was not able to show any yellow or orange colour from pigments such as Sepiapterin or Drosopterin in the same chromatogram. This is in part due to a mutation affecting a key intermediate enzyme known as, PDA synthase. This enzyme is responsible advancing the process towards producing the yellow and orange-coloured pigments in the Drosopterin biochemical pathway. Sepia mutants were found with no PDA synthase activity because of this mutation, resulting in the darker eye colour being visible (Kim et al., 2013). In this case, Xanthopterin presence in the chromatogram is probably due to being produced earlier in the Drosopterin pathway before Sepiapterin and Drosopterin were inhibited by the mutation.

Considering the *bw* mutant, the sample of flies showed presence of some bluish colours potentially arising from Biopterin, 2-amino-4-hydroxypteridine, and Isoxanthopterin. However, literature tells us that the mutant's ability to produce Drosopterin pigments is stunted due to a mutation that affects the ATP-binding cassettes (ABC) transporter from delivering the pigments to the granule site in the eyes. When this mutant's ABC transporter was unable to transport the pterin pigments, only the ommochrome pigments were present in the eye (Grubbs et al., 2015). Biopterin, 2-amino-4-hydroxypteridine, and Isoxanthopterin were potentially seen in the chromatogram because of trace amounts of pteridine pigments still produced by the mutant.

Considering the *w* mutant, the sample of flies showed presence of trace amounts of blue colour arising from Biopterin, 2-amino-4-hydroxypteridine, and Isoxanthopterin pigments. From literature, we know that this mutant also affects the ABC transporter in helping pigments reach the granule site in the eye. In these mutants, the mutation results in the disruption in transport of pigments arising from both the ommochrome and pterin pathways and this leads to a whitish colour seen in these flies (Ismail et al., 2018). The presence of other pigments in the *w* chromatogram result could also be of the same reason why pigments were seen in the *bw* chromatogram result, some trace amounts of pigments may still be produced in these flies.

If trace amounts were not produced, the streaks found in the *w* chromatogram result could be due to contamination of the glass rod which may have introduced some trace amounts into the chromatography paper.

References

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