2018

Absorbance of 0.15 Seen at 440 nm Double Peak in Sepia Eyed Drosophila melanogaster

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Absorbance of 0.15 seen at 440 nm double peak in sepia eyed *Drosophila melanogaster*

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Bio 141

Objectives:

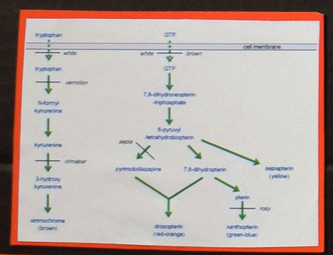
Our objective was to observe the eye pigments and its absorbance in both sepia and white eyed fruit flies.

Abstract:

One way to observe the eye pigments in fruit flies is by performing chromatography. In our experiment three different types of chromatography were used with the sepia and white eyed fruit flies. Regular paper chromatography was performed on both flies allowing one to visibly see the colored pigments found in the eyes of the fruit flies separated. However, since white fruit flies have no pigment in their eyes observance of their pigments was quickly dismissed. The second method required a different type of paper—alumina paper. Thin layer chromatography (TLC) was performed using the alumina paper on wild fruit flies rather than sepia eyed fruit flies. Thinking that the organs of the fruit flies interfered with the absorbance found in the spectrophotometer the heads of both sepia and white eyed fruit flies were left to sit in a chromatography solvent of ammonium hydroxide for a little over an hour. The results from the spectrophotometer had showed a double peak in absorbance in the sepia eyed fruit flies. A second experiment including the microfuge of sepia eyed fruit flies had confirmed the double peak around 420-440 nm. The third method of chromatography was 2D chromatography, in which 2 solvents are used on the same paper to get a better separation of pigments. Acetone and ammonium hydroxide were used for the first 2D chromatography; methanol [4] and ammonium hydroxide were used for the second 2D chromatography. We had found the 2D chromatography gave us the most interesting results.

Eye pigments of fruit flies:
The various pigments produced in fruit flies results from the epistasis of genes. Brown pigments are produced from the ommochrome pathway while scarlet pigments are produced from the drosopterin pathway. Both pathways produce different eye pigments using specific amino acids. According to Laurence A. Morgan, a professor in biochemistry at the University of Toronto, mutations in the transport system lead to mutations like, bright red eyes, and sepia eyes. Morgan goes on to explain that sepia eyed mutations result from the disruption of enzymes in the drosopterin pathway while the white eyed fruit flies result from mutations in both the ommochrome and drosopterin pathways. [4]
Chromatography: The process or technique of separating molecules or components in a mixture according to the differential absorption and elution [1]

We believe no real results will be seen when observing white eyed fruit flies, but we believe regular paper chromatography will show us the best separation of eye pigments.



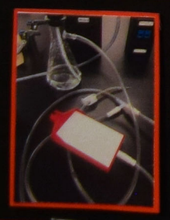
Pathway of the eye pigments

Swift, David W. "Phenotypic Variations Caused by Corrupting Genes." *Evolution under the Microscope*, evolutionunderthemicroscope.com/evolution07.html.

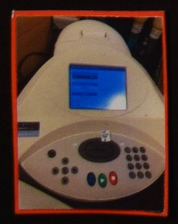
Tools:

CO_2 device
- Crafted by Parkland's 3D printer
- Flask has two tubes inserted into it. One tube is connected to the CO_2, while the other tube connects to two other tubes at a "T" intersection. One tube is connected to a flat fly bedding, which releases CO_2 from its pore like surface while the second tube releases CO_2 from its pointy end.

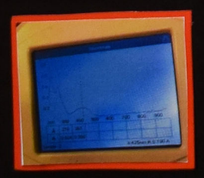
Spectrophotometer
- An optical instrument for measuring the intensity of light relative to wavelength. [3]
- Device that allows one to measure the absorbance of liquids in nm.



CO_2 device used to put the fruit flies to sleep



Spectrophotometer used to find the absorbance level and the peaks

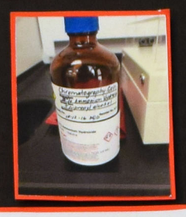


Methods:

Regular paper chromatography of white and sepia eyed fruit flies
Thursday October 11th, 2018
1. 4 sepia eyed and 4 white eyed fruit flies heads were decapitated.
2. Decapitated heads were put into a clear plastic medicine cup
3. A long thin piece of paper was cut, and the white eyed fruit fly heads were placed together on the bottom left side of the strip while the sepia eyed fruit fly heads were placed together on the bottom right of the strip.
4. Both the sepia and white eyed fruit fly heads were smashed with a glass rod. We made sure to label both sides with an s for sepia and a w for white.
5. A small amount of ammonium hydroxide was placed into a beaker, afterwards the strip of paper was placed inside the beaker covering the top with aluminum paper
6. Before the solvent reached the top of the paper, we took out the strip and let it dry.
7. Once dry we observed the strip under fluorescent the light and under a UV light.

Results:
A yellow and red-orange pigment was seen in both the sepia eyed and white eyed fruit flies. The pigment seen in the white eyed fruit flies was caused from an accidental mix of sepia and white eyed fruit flies head. It is still assumed that no pigment is seen from white eyed fruit flies.

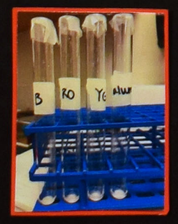
Paper chromatography with white eyed and sepia eyed fruit flies



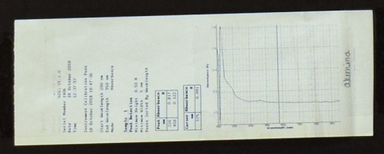
28% Ammonium Hydroxide solvent used for the Chromatography

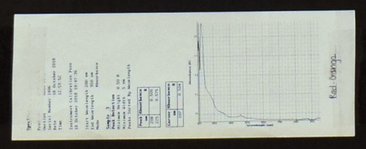
Alumina chromatography of wild type fruit flies
Thursday October 19th, 2018
1. 8 wild type fruit flies heads were decapitated and put into a clear plastic medicine cup.
2. 4 wild type fruit fly heads were laid together on the bottom right of the alumina paper while the remaining 4 wild type fruit fly heads were laid together on the bottom left of the alumina paper.
3. With a glass rod the heads were crushed together.
4. The alumina paper was put inside a beaker containing ammonium hydroxide while the top was covered with parafilm.
5. We took out the alumina paper before the solvent reached the top of paper and left it out to dry in the fume hood.
6. Once dry we had marked off the colors seen on the paper under the UV light and scraped off each color off the alumina paper and scraped off a bit of the alumina paper. Each sample of color was set into their own test tube.
7. With a micropipette we pipetted 250 ml of ammonium hydroxide into four test tubes and covered the test tubes with parafilm. We let the test tubes laid out for an hour.
8. We cleaned the cuvettes with ammonium hydroxide using pasteur pipettes.
9. We transferred the alumina sample into a cuvette using a Pasteur pipette and added ammonium hydroxide until the sample reached halfway of the cuvette. We used the alumina sample to zero the spectrophotometer.
10. We then made paper tunnels for each sample and used a real funnel to filter out the alumina from the pigments into a beaker and then into the cleaned cuvette using new pasteur pipettes with each pigment.
11. When using the spectrophotometer, we had set the mode to absorbance and had a start wavelength of 200 nm and an end wavelength of 950 nm.

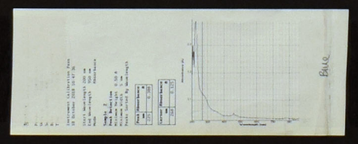
Results:
The observed pigments seen under the UV light were blue, red-orange, and yellow. The pigments all had similar absorbances ranging from 0.1-0.8 at similar peaks of 208-225 nm. No useful results were seen from the alumina chromatography, therefore we decided not to use TLC for the sepia eyed fruit flies.

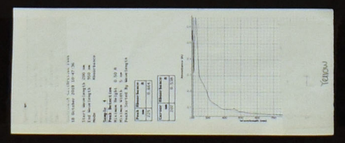


4 test tubes containing extracted pigments from the thin layer chromatography (TLC) in 28% ammonium hydroxide solution





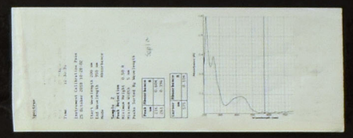


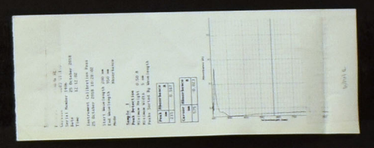


Peaks and absorbances seen with alum (the control variable) and the yellow, blue, and red/orange pigments in wild type fruit flies.

Discovery of possible double peak in sepia eyed fruit flies
Thursday October 25th, 2018
1. Decapitated 6 sepia eyed fruit flies and 6 white eyed fruit flies.
2. We pipetted 1000 microliters of ammonium hydroxide into 2 tubes and placed 6 sepia eyed heads into one test tube and 6 white eyed heads into another test tube. We then wrapped the top of the test tubes with parafilm.
3. We let the test tubes sit in the fume hood for a little over an hour.
4. We then used the spectrophotometer to check the absorbance of both fruit flies.
5. This is where we discovered a double peak in the absorbance of sepia eyed fruit flies.

Results:
The spectrophotometer showed us a peaks in abs around 216 nm, 261 nm, and 575 nm. Additionally a double peak around 420-440 nm in the sepia eyed fruit flies was observed.



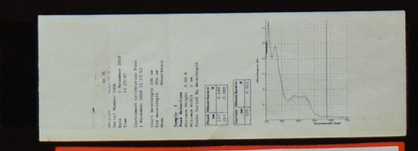


Peaks and absorbances seen in white eyed and sepia eyed fruit flies after letting the heads soak in separate test tubes containing 1000 microliters of 28% ammonium hydroxide.

Confirmation of double peak in sepia eyed fruit flies
Thursday November 1st, 2018
1. 12 sepia eyed fruit fly heads were decapitated and put into a microfuge tube which contained 250 microliter of ammonium hydroxide. The heads were then crushed inside the microfuge tube.
2. The heads were put inside the microfuge for 1-2 seconds and then taken out. We then transferred this solution inside of a cuvette having already zeroed the spectrophotometer with ammonium hydroxide.
3. Here we were able to have a closer look at the double peak and were able to confirm the location of the double peak to be around 420 nm-440 nm.

Results:
The experiment was successfully confirmed the double peak in the sepia fruit flies around 420-440 nm.

Microfuge of sepia eyes head



Double peak seen around 420-440 nm after having put 12 sepia heads in the microfuge.

2D chromatography with ammonium hydroxide and acetone
Thursday November 8th, 2018
1. 8 sepia eyed fruit flies were decapitated
2. The 8 sepia eyed fruit fly heads 1-2 cm from the bottom of a large strip of paper.
3. We stapled the opposite edges together and put the paper inside a jar of ammonium hydroxide letting sit inside the jar until the solvent was close to reaching the top of the paper.
4. We then laid the paper to dry in the fume hood and took a picture of the paper. Once dry we once again stapled the paper and put it inside a jar with acetone and let it sit until the solvent was close to reaching the top.
5. When done we let the paper sit out in the fume hood again.
6. Once dry we looked at the paper under the UV light and saw no significant change in colors.

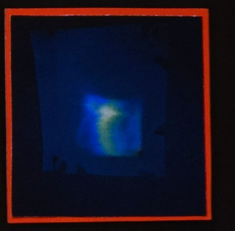
Results:
The 2D chromatography had no effect on the pigments; green and blue pigments were seen from the chromatography.

2D chromatography using acetone under light

2D chromatography with ammonium hydroxide and methanol
Thursday November 15th, 2018
1. Steps 1-6 were repeated from the previous 2D chromatography, only a change in one solvent was done.

Results:
The change of the second solvent (methanol) had allowed us to see better separation of the blue and green pigments. The pigment had also moved upwards.

2D chromatography using methanol under light



2D chromatography using methanol under UV light

Conclusion:

With the comparisons made between paper, TLC, and 2D chromatography we have come to the conclusion that 2D chromatography depicts the best separation of pigments. With a solution of 50 ml of methanol and 50 ml of distilled water (50% v/v), the solvent was able to separate the green and blue pigments seen under the UV light (red-orange and yellow under fluorescent lights) and had moved the visible pigments. The same paper from the previous 2D chromatography with acetone was used, therefore there is a chance the acetone may have contributed to the results of the methanol 2D chromatography. Not only did we see a great separation of pigments, but a double peak in absorbance was seen in the pigments of sepia eyed fruit flies around 440 nm. If more time were available we would have liked to experiment with other solvents and would have liked to further explore the double peaks. We wish we would have kept the number of heads worked with at a constant. We recommend that those working with 2D chromatography use various solvents and for those who work with different mutant colored fruit flies to be careful not to mix the eye colors together.

Work Cited:
[1] "Chromatography." *Biology Online*, www.biology-online.org/dictionary/Chromatography.
[2] "Identification of an Ommochrome in the Eyes and Nervous Systems of Saturniid Moths." *ACS Publications*, pubs.acs.org/doi/pdfplus/10.1021/bi00784a028#.W-2lPpEEyyM.email.
[3] Martin, Paul. *How Does the Ultraviolet Absorption of Proteins Impact Analysis?*, www.microspectra.com/support/learn/what-is-a-spectrophotometer.
[4] Moran, Laurence A. "Eye Color in Fruit Flies." *Sandwalk*, 18 Sept. 2007, sandwalk.blogspot.com/2007/09/eye-color-in-fruit-flies.html.
[5] pharyngula. "Epistasis and Pathways in Fly Eye Pigmentation." *Science Blogs*, 23 Feb. 2012, scienceblogs.com/pharyngula/2012/02/23/epistasis-and-pathways-in-fly.