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Wild Type Drosophila melanogaster Eye Pigments: Examining Absorbance Spectra and Light Sensitivity

Erin Brown
Parkland College

Taylor M. Delaney
Parkland College

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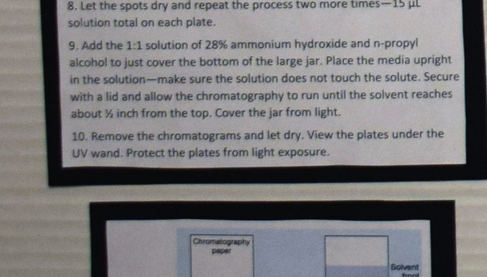
Erin Brown and Taylor Delaney
Bio-141, Section 002
Professor David Wilson

1. Abstract

- The eyes of wild type fruit flies, Drosophila melanogaster, contain various pigments that contribute to a brick red color.[1]
- Chromatography techniques are used to separate and view these pigments.
- In our project, we extract and gather absorbance spectra for these pigments using various methods.
- We compare how efficient these techniques are at separating and extracting the pigments and providing reliable results when examining absorbance spectra.
- To explore the photosensitivity of the pigments, we compare absorbance spectra changes to a pigment solution.

2. D. melanogaster Eye Pigments

- Compound eyes of fruit flies have pigment cells, which prevents too much light passing through the structure.[1]
  - These pigments are suspected to degrade in light.[4]
- The eyes contain two biochemical pathways to create pigments.[1]
  - Pteridine and ommochrome pathways.
  - Mutations in enzymes can create different eye colors.[2]
- We are interested in wild type fruit flies.
  - They have no mutations and produce every pigment.[3]



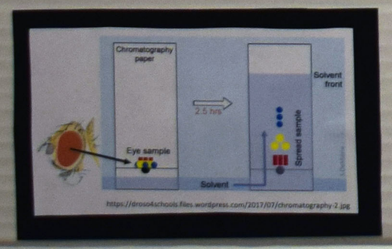
3. Procedure: Eye Pigment Chromatography

Materials

- Alumina TLC plate
- Silica TLC plate
- Strip of filter paper
- 10 wild type flies
- 1:1 mixture, 28% ammonium hydroxide and n-propyl alcohol
- DI water
- Microfuge tubes (2)
- Paint brush
- Centrifuge
- 100-1000 µL and 2-20 µL micropipette
- Large jar with a lid
- Mineralight UV lamp 254/366 nm

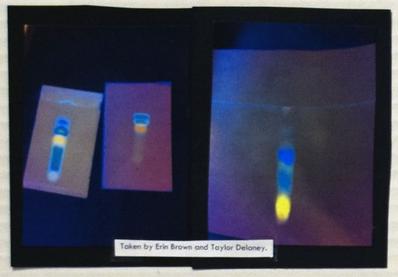
Methods

1. Anesthetize and decapitate the flies.
2. Transfer the head to the microfuge tube and crush with the end of the paint brush.
3. Use the large micropipette to add 100 µL of 1:1 solution of 28% ammonium hydroxide and n-propyl alcohol to the microfuge tube. Crush and mix the heads. Close the tube.
4. Use the large micropipette to add 100 µL of DI water to the other microfuge tube. Place both tubes in centrifuge exactly opposite of each other. Centrifuge for 5 seconds.
5. Crush and mix the tissue into the solution again with the paint brush. Centrifuge again for 5 seconds. Set the pigment tube aside without disturbing the pellet at the bottom.
6. Gather your chromatography media. Lay the alumina and silica plates- plastic side down.
7. Use the small micropipette to add 5 µL of solution from the microfuge tube to each plate 1 cm from the edge. Do not disturb the pellet.
8. Let the spots dry and repeat the process two more times—15 µL solution total on each plate.
9. Add the 1:1 solution of 28% ammonium hydroxide and n-propyl alcohol to just cover the bottom of the large jar. Place the media upright in the solution—make sure the solution does not touch the solute. Secure with a lid and allow the chromatography to run until the solvent reaches about ½ inch from the top. Cover the jar from light.
10. Remove the chromatograms and let dry. View the plates under the UV wand. Protect the plates from light exposure.



3. Results

- The alumina had the best separation.
- The silica and paper showed three pigments: orange, green and blue.
- The brown pigments do not dissolve into the solution.


Taken by Erin Brown and Taylor Delaney.

4. Procedure: Pigment Extraction and Spectrophotometry

Materials

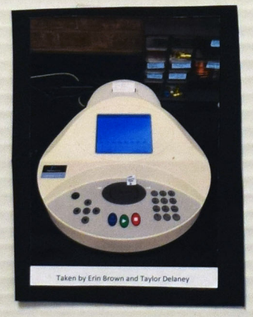
- Strip of filter paper
- 10 wild type flies
- 1:1 mixture of 28% ammonium hydroxide and n-propyl alcohol
- DI water
- Microfuge tubes (2)
- Paint brush
- Centrifuge
- 100-1000 µL and 2-20 µL micropipette
- Large jar with a lid
- Mineralight UV lamp 254/366 nm
- Test tubes (3)
- Scissors
- Glass stir rod
- Wax parafilm
- Pasteur pipette
- Thin glass cuvette
- PerkinElmer Lambda XLS+ UV/Vis spectrophotometer

Methods

1. Proceed to create a paper chromatogram using the previous methods and materials.
2. Using the UV lamp, cut out the three separate pigments. Cut the pigment papers into tiny pieces and transfer to separate test tubes.
3. Add 1000 µL of 1:1 solution of 28% ammonium hydroxide and n-propyl alcohol to each tube. Stir with a stir rod. Cover with parafilm and let dissolve for 15 minutes. Cover from light.
4. Calibrate the UV/Vis spec by selecting absorbance spectrum and a range of 200-950 nm.
5. Prepare a blank by filling the thin cuvette ¾ full of 1:1 solution of 28% ammonium hydroxide and n-propyl alcohol. Wipe with a kim wipe and insert the cuvette into the sample holder with the frosted glass touching the sides. Press the 0.0 button to calibrate the spec.
6. Use the Pasteur pipette to remove and dispose the solution from the cuvette. Transfer the first pigment solution from the test tube to the cuvette with the pipette. Wipe the cuvette and insert into the sample holder with the frosted glass of the cuvette touching the sides. Press the green "play" button to generate an absorbance spectrum.
7. Remove the pigment solution from the cuvette and replace it into its proper test tube.
8. Repeat steps 6 and 7 with the two remaining solutions.

4. Results

- We saw consistent peaks with the orange pigment.
- The blue and green pigments were not easily separated.
  - We did not get consistent results of the individual pigments.
- Two-dimensional chromatography could produce better separation.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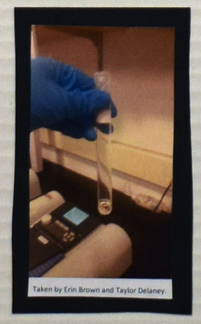
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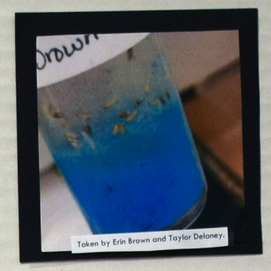

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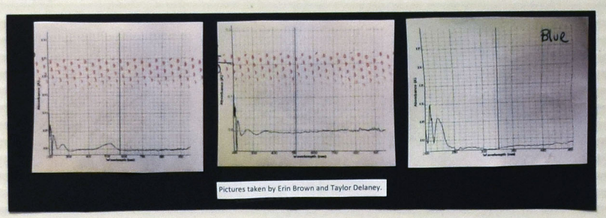


Pictures taken by Erin Brown and Taylor Delaney.

5. Procedure: Spectrophotometry and Photosensitivity

Materials

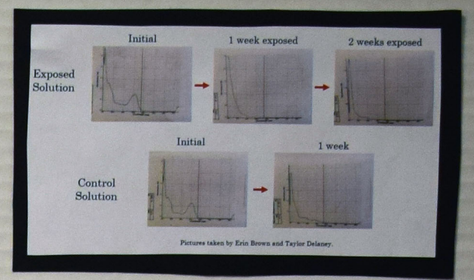
- Strip of filter paper
- 20 wild type flies
- 1:1 mixture of 28% ammonium hydroxide and n-propyl alcohol
- DI water
- Microfuge tubes (2)
- Paint brush
- Centrifuge
- 100-1000 µL and 2-20 µL micropipette
- Large jar with lid
- Test tube
- Wax parafilm
- Pasteur pipette
- Thin glass cuvette
- PerkinElmer Lambda XLS+ UV/Vis spectrophotometer
- Aluminum foil
- LED light bulb (16 W, 120 V, 1600 lumens)

Methods

1. Anesthetize and decapitate 10 flies.
2. Transfer the head to the microfuge tube and crush with the end of the paint brush.
3. Use the large micropipette to add 1500 µL of 1:1 solution of 28% ammonium hydroxide and n-propyl alcohol to the microfuge tube. Crush and mix the heads. Close the tube.
4. Use the large micropipette to add 1500 µL of DI water to the other microfuge tube. Place both tubes in centrifuge exactly opposite of each other. Centrifuge for 5 seconds. Crush and centrifuge as needed.
5. Use the large micropipette to transfer 1000 µL of the pigment solution to a test tube and seal the top with parafilm. Do not disturb pellet. Cover from light exposure.
6. Repeat steps 1-5 with the rest of the flies.
7. Calibrate the UV/Vis spec using the technique from the previous procedure.
8. Transfer the first pigment solution from the test tube to the cuvette with the pipette. Wipe the cuvette and insert into the sample holder. Press the green "play" button to generate an absorbance spectrum.
9. Remove the pigment solution from the cuvette and replace it into its proper test tube; seal the tube with parafilm. Rinse the cuvette with plain 1:1 solution of 28% ammonium hydroxide and n-propyl alcohol.
10. Repeat steps 10-11 with the second test tube.
13. Construct an area with constant light from a lamp with the bulb. Place one test tube under the light with no cover. Cover the second test tube completely with aluminum foil. Keep the tubes next to each other under the light.
14. In one week, repeat steps 9-11 with both the light and control samples.

5. Results

- We measured the control solution after one week and the exposed solution at one week.
- The exposed solution after one week showed a very smooth curve with no peaks.
- The control solution showed a decrease in visual color and in peaks.
- The UV range showed less change than the visible range.

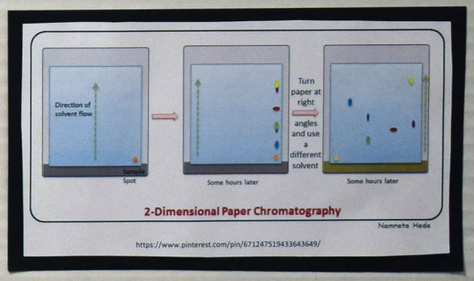


6. Conclusion

- Alumina thin-layer chromatography produced the best separation.
- We discovered characteristic absorbance peaks in the pigments.
  - The orange drosopterin pigment showed a unique peak at 520 nm.
  - The blue and green pteridines shared overlapping peaks.
- Light plays a slight role in pigment decay.
  - The control solution showed decay, but not at the rate of the exposed solution.
  - The visible range showed the most change in absorbance.

7. Limitations and Improvements

- Separating flies between gender and age.
- Performing two-dimensional chromatography to separate the pigments more effectively.
- Using different light bulbs and conditions in the photosensitivity experiment could yield different results.



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