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The Effect of Light Environment on the Development of Eye Pigments in *Drosophila melanogaster*

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ABSTRACT

Drosophila melanogaster have complex, compound eyes that have been extensively studied and continue to be an area of interest for visual system research. Wild-type fruit flies have seven different photopigments that are each receptive to different wavelengths of light. The way that these flies interact and respond to light has led to the question of whether the developmental light environment affects the type and abundance of photopigments in adult flies. To investigate this, we exposed fruit flies to six different wavelengths of light (no light, white light, red light, blue light, green light, or UV light) during the first 14 days of their life cycle. The eyes of the adult fruit flies were then extracted and the type and amount of photopigments were analyzed using paper chromatography and fluorescence spectrophotometry. Two pigment bands were seen in the chromatograms, orange and blue-green, but there was no difference in the retention factors of these main bands. However, the emission intensity of these two bands were found to be significantly different across the light treatments. Specifically, between those flies developing in the green light environment and those developing in the UV light environment. This finding suggests that *D. melanogaster* eye development is not based purely on genetics, but instead is relatively malleable and depend on their developmental environment to some degree.

Keywords: *development, Drosophila melanogaster, eye pigments, photomorphogenesis, UV light.*

INTRODUCTION

An individual's phenotype is a product of their genotype, the environment they develop in, and often an interaction between the two. This suggests that the environment plays a significant role in phenotypic expressions of organisms (Auld, et al 2009). Sensing and responding to light in the environment is an important function of plants and animals. Changes in light can be critical in terms of competition and survival. Organisms have to be able to adapt to changes over time in their environments. One way organisms can do this is through their visual system. The visual systems of *Drosophila melanogaster* have been studied extensively, leading to a wealth of information regarding the organism.

The compound eyes of fruit flies consist of around 750 ommatidia (optical units) which each contain 8 photoreceptor cells (Yamaguchi et al., 2010). Photoreceptors are the cells in the eye tissue that respond to light. There are two categories of photoreceptors in *D. melanogaster*: R1-R6, and R7/R8. Each photoreceptor cell surface contains microvilli structures. The microvilli in the R1-6, R7, and R8 cells provide a large plasma membrane surface to pack in a high concentration of rhodopsin, the light sensitive pigment containing sensory protein that converts light into an electrical signal. This serves as the site for most of the proteins that function in phototransduction (Wang and Montell, 2007). The outer photoreceptors R1-R6 are responsible for motion detection, and the inner receptors R7 and R8 are important for color vision (Yamaguchi et al., 2010). Yamaguchi et al. (2010) found that R1-R6, R7, and R8 all affect phototaxis differentially based on the spectral properties of the light. The visual system of *D. melanogaster* is dependent on light to sense and respond to their environment.

There are many pigments found in the photoreceptors of *D. melanogaster.* The main pigment found in rod photoreceptors is rhodopsin. This particular pigment is very sensitive to light. Rhodopsin absorbs light very efficiently in the middle of the visible spectrum (Berg et al, 2002). In terms of pigments responsible for the eye color of *D.* melanogaster, there are two classes of pigments, brown 'ommochromes' and red 'drosopterins' (Kim, et al 2006, Euphrussi and Herold, 1943). Within these two classes, the wild type eye color is due to the mixture of seven different color compounds (Kim, et al 2006). According to a genetics lab done by many universities, these seven pigments each absorb at a wavelength correlated to a color. Isosepiaterin (yellow at ~ 570nm), biopterin (blue at ~ 475nm), 2-amino-4-hydroxypteridine (blue at~ 475nm), sepiapterin (yellow at ~570nm), xanthopterin (green-blue at ~ 490nm), isoxanthopterin (violet-blue at ~ 445nm), and drosopterins (orange at ~590nm). These seven pigments are produced by the pteridine pathway.

Due to the separation of photopigment sensitivities to different wavelengths, flies living in light environments that only have certain wavelengths available would be limited in their vision by the amount of photopigment produced that absorbs in

that spectrum. Thus, flies may be well served to respond to their developmental environment by adjusting the type of pigment produced. By studying the eyes of *D. melanogaster*, researchers can create a clearer understanding of how organisms sense and react to light availability in different environments.

MATERIALS AND METHODS

A culture (~100 flies) of wild-type *D. melanogaster* was ordered from Carolina Biological Supply. Six test tube vials were needed for this experiment, one vial for each light treatment. The six different light treatments included: no light, white light, red light, green light, blue light, and UVA light. A DC circuit of five LED bulbs (adafruit.com) was made by wiring up bulbs in parallel on a breadboard with one resistor for each bulb. The bulbs were: Bright Red 5mm LED #297, UVA LED 5mm Purple, Clear 5mm Blue LED #301, Clear White LED #754, Clear Green LED #300. An input of 9V was plugged into a wall and used as the power source. For each treatment of light, one vial was prepared with *Drosophila* plain instant medium ordered from Carolina Biological Supply Company. Instant *Drosophila* medium needs neither sterilizing nor cooking. Medium was prepared by putting one scoop of the dry powdered medium into a vial, and mixing with ~18 mL of DI water, and a dash of baker's yeast. Each vial was covered with aluminum foil and a small hole in the foam top of the vial was created for the LED bulb to be inserted. In the case of no light, the tube was completely covered with aluminum foil. This was done to control for the amount of light entering the vials. In order to mate the flies, two males and two females were placed in each test vial and were assigned to a specific light treatment, to ensure complete development of the offspring in the light treatment. It takes approximately two weeks for D. melanogaster to fully develop, which is when the light treatments were finished. The fruit flies were then anesthetized in order to work with them for the next step. Carolina Biological Supply Company's FlyNap solution was used as an anesthetic, as it was a safe alternative to work with compared to ether. A swab was immersed in the FlyNap solution, and carefully inserted into the vial of fruit flies for two to three minutes, or until the last fly was asleep. FlyNap safely knocked the fruit flies out for about an hour. After flies were anesthetized, a dissecting scope was used to extract the eyes from the body. This was done by pinning the abdomen of the fly down with a needle, and pushing the eyes off of the body with the edge of a scalpel. Four replicates of four sets of eyes from each vial were ground up in a plastic mortar and pestle container with a drop of DI water from a pipet to create a liquid solution. This solution was bright red in color. To separate and identify the pigments, paper chromatography was used. The eye pigments that were ground up (as described above) were spotted on Whatman cellulose-based chromatography paper. A solvent of one-part ammonium hydroxide and one part propanol was used as the solvent. The chromatograms were placed in a 100 mL graduated cylinder with ~5 mL of solvent, covered with aluminum foil, and placed in the dark photo room for three hours to develop. The chromatograms were then exposed to UV light to fluoresce. During this fluorescence, the distance that the solvents and pigments travelled were recorded, and the retention factor was taken for each replicate. Following this, each chromatogram was cut into sections with each specific pigment on it and was re-dissolved into a liquid form by using 3 mL of the 1:1 propanol and ammonium hydroxide solvent. The papers containing the pigments were placed in the solvent inside small plastic tubes and placed on the rotary shaker overnight, to ensure all the pigment came out of the paper. Perkin-Elmer 203 Fluorescence Spectrophotometer was used to measure the emission intensity of each sample. The sensitivity control was set to "4", the selector was set to "x10", the exciting wavelength was set to 375nm, and the emission wavelength was set at 515nm. This stayed consistent throughout each sample testing.

Statistical Analysis Section

A One-Way Analysis of Variance (ANOVA) was used to test the difference between the orange pigments from each treatment vial. A separate One-Way ANOVA was run for the blue-green pigments among the different treatment vials. Two separate ANOVA tests were used to test differences between the retention factors in each band (orange and BG) between each vial. Tukey tests were ran as the post hoc test.

RESULTS

Qualitative Results: In visually observing the chromatogram strips under UV lamp light (254nm), each strip of paper had clearly separated into two different pigment bands: an orange band and a blue-green band. The presence of these two bands, separated in the same order in all chromatograms, showed that even though developed in different light treatments, the fruit flies developed the same pigments.

Quantitatively, when comparing the orange emission intensity among the treatment vials via the One-Way ANOVA, there was an overall significant difference ($F=3.385$, $p=0.025$) between the eye pigments that were developed in different light vials. The BG emission intensity was also significantly different across the light treatments ($F=3.892$, $p=0.014$). This data suggests that among the different lighted vials, the amounts of each class of

pigments produced were dependent on the light environment the flies were developed in.

When comparing the retention factors between the vials of the orange band, there was no significant difference (p=0.099, F=2.207). When looking at the RF between the vials of the blue-green band, there was a significant difference (p=0.050, F=2.781).

The post hoc tests (Tukey) showed the relationships between the emission intensity data for each possible vial combination. A trend was noted that the pigments from flies in the UV vials and their combinations showed lower p values than other light treatments did, although in most cases not <0.05 (Blue vs. UV; p=0.057; See Figure 1). Within the BG pigment band, flies that developed in the UV environment had higher intensities than those that developed in the green environment (p=0.006), while differences in intensity between the blue and UV treatments were marginally insignificant (p=0.079).

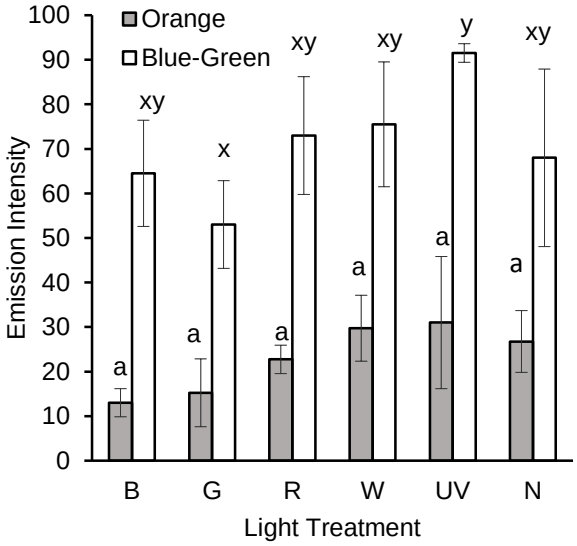

Figure 1. Comparisons among the mean values of the orange band emission intensity and the blue-green band emission intensity. Error bars show standard deviation of the average values. Letters denote statistically significant differences. No significant differences between light treatments in the orange band. One significant difference (p=0.006) between UV and Green vials within the BG pigment treatments.

DISCUSSION

The purpose of this research was to determine if there were any differences in the eye pigments among *Drosophila melanogaster* that are developed in different light treatments.

In the Tukey test, the bands from the vials that were very close to being significantly different (mentioned in results section) were not under 0.05 due to the lack of sample size. According to the sample size test, we would have needed 11 replicates per vial to see a statistically significant difference. This is an attainable number of replicates for future replications of this project. Due to the lack of the power from low sample sizes, a lot of the values that were higher than 0.05 were not seen as significantly different.

As discussed in the results section, the eye pigments of the flies raised in UV light showed to have the greatest significant difference in emission intensity. In this experiment, the UV light vial was the shortest wavelength between all vials. It was a “UVA” light bulb, meaning its wavelength emitted was ~320-400nm. Research by Stark, Walker, and Harris looked at the different functions of the photoreceptors R1-R8 in *Drosophila melanogaster*. This study revealed that the R7 photoreceptor is a UV receptor that contains the rhodopsin pigment that absorbs around 370 nm, and interconverts with metarhodopsin which absorbs around 470 nm (Stark et al, 1976). If *Drosophila* are raised in pure UV light, there may have been an increase of rhodopsin production due to the elevated R7 sensitivity.

Most species of invertebrates, including fruit flies, are sensitive to light ranging from red to UV (Salcedo et al, 2010). UV vision is mediated by rhodopsin and is important for foraging, navigation, and mate selection (Salcedo et al, 2010). A potential explanation of the data may be that more rhodopsin was developed in the eye pigments of flies raised in only UV light. Rhodopsin is a purple visual pigment so higher levels of the purple class of pigments within the orange and BG bands may have increased during development in UV light.

This may be an opportunity for a potential project to study the increased level of rhodopsin, and whether it would be passed down genetically to offspring of the flies raised in UV light. *Drosophila* eye development may be relatively plastic and dependent on developmental environment to some degree. Increased rhodopsin levels would help in UV vision, which is needed for foraging, navigation, and mate selection, all of which are important for survival in *Drosophila melanogaster.*

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