The effect of exposure to varying light wavelengths during development on locomotor speed of adult *Drosophila melanogaster*

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Abstract

As a model organism, *Drosophila melanogaster* has been thoroughly researched. Variation in light wavelength has been demonstrated to affect rate of eclosion and development. Yet, the effect of visible light on the developmental rate, and its effect on the motor capabilities of these organisms have yet to be determined. In the present study, we demonstrated that exposure to increased wavelength during the development of *D. melanogaster* resulted in an increase in motor activity in sample replicates. Batches of *D. melanogaster* larvae were divided into treatments corresponding to that of blue (410nm), green (520nm), and red (680nm) wavelengths, and two controls of white-light and darkness. Motor abilities in these groups were determined after two weeks of growth. Results demonstrate a significant difference in locomotor activity between organisms grown in red wavelength treatments, recorded speed of 1.53 cm/s, and those exposed to green wavelength treatments, 1.01 cm/s. However elevated activity in the blue treatment group, 1.40 cm/s caused us to fail to reject our null hypothesis. Our results suggest that exposing *D. melanogaster* to optimal green wavelengths induces early eclosion and maturation, and thus leads to slowed motor activity relative to the sub-optimal (blue and red) treatment environments.

Introduction

Drosophila melanogaster (common fruit fly) is a mainstay organism in life science research. Such research has provided unique and fundamental insight into understanding various biological processes involving it and more complex, higher, organisms. *D. melanogaster* has demonstrated its usefulness in paralleling several aspects of organism behaviour and development, thus providing us with a better understanding of specific factors that may influence an organism's development (Beckingham *et al.* 2005). Exposure to light is one factor long suspected of influencing the development of *Drosophila melanogaster* since *D. melanogaster* is a particular organism that has the ability to detect light beyond the visible spectrum (Chadha 2008).

Light has been shown to impact several aspects of *D. melanogaster's* development, including having a significant role in establishing the organism's circadian rhythm and

neuroendocrine secretion levels (Saunders 1982, Subramanian *et al.* 2009). Saunders (1982) has suggested that circadian rhythm influences the development, behaviour and metabolic rate in many insects. Given that light wavelength increases the expression of circadian rhythm (Subramanian *et al.* 2009), we can infer that light of a specific wavelength may also influence *D. melanogaster's* development rate, as determined by measuring *D. melanogaster's* locomotor speed. Further evidence suggesting that exposure to light of a specific wavelength may influence *D. melanogaster's* locomotor speed, as an indication of its development, comes from studies that confirm that neuroendocrine secretion levels of this organism regulate its light avoidance behaviour and development rate. (Yamanaka *et al.* 2013).

According to previous research conducted, *D. melanogaster* is known to possess particular photoreceptors, R1-R6, which are responsible for motion detection, and R7-R8, which are responsible for colour vision (Yamaguchi 2010). Coordination between the R1-R8 photoreceptors provokes *D. melanogaster* to induce physical movement in response to colour. To expand on this, we designed our experiment to determine the effect of exposure to various specific light wavelengths (410 nm, 520 nm, 680 nm, dark light and white light) on the development of *D. melanogaster* as measured by their locomotive speeds once they reached maturity. Locomotor speed is an accurate indicator of development since previous studies have used *D. melanogaster* to model age-related locomotor impairment and have provided evidence that climbing speed declines with age (Rhodenizer *et al* .2008).

Additionally, previous studies performed have shown that *D. melanogaster* have the ability to detect differences in varying light wavelengths and they exhibit developmental delays when grown in longer wavelengths corresponding to red light (Wang *et al.* 2013). These developmental delays are expressed by agitation and decreased mating activity as well as

delayed eclosion (hatching) when maturation occurs in red light (Wang *et al.* 2013). Previous studies performed have shown that increased age leads to a decrease in locomotor speed of *D. melanogaster*, as measured by negative geotaxis, the automatic response of flies to move against Earth's gravitational vector (Rhodenizer *et al.* 2008). Thus, we formulated our alternate hypothesis, which states that exposure to increased light wavelength during development will result in increased locomotor speed of *D. melanogaster* as indicated by geotaxis, whereas our null hypothesis states that exposure to increased light wavelength during development will have no effect or result in decreased locomotor speed of *D. melanogaster* as indicated by geotaxis.

By understanding the effects of varying light wavelengths on *D. melanogaster's* locomotor speed, an ideal environment for development may be produced to further evolutionary studies. In this experiment we attempt to determine if varying wavelength of light during *D. melanogaster* development could result in differences in locomotion speed in response to negative geotaxis. To the best of our knowledge, no previous study has explored the effect of specific light wavelengths on development of wild type *D. melanogaster*.

Methods

To begin our experiment, we collected wild type *Drosophila melanogaster* from five stock vials containing adult flies and larvae grown from a standard cornmeal medium. We removed and discarded the adult flies after anaesthetizing them with carbon dioxide gas and proceeded to extract the larvae using a sterile loop. We then transferred them into a 60mm Petri dish half filled with 18% sucrose solution, which aided to dissolve the growth medium, thereby separating the larvae. After separation, the larvae were transferred via a sterile loop into their respective replicate vials. Our experiment consisted of 3 treatments (410 nm, 520 nm, 680 nm)

and 2 controls of clear and dark (no light), with 4 replicates each, for a total of 20 replicates. Each replicate vial contained 5-6 larvae and approximately the same medium volume. These treatments were made by covering the 4 replicate vials of each treatment, collectively, with acetate paper corresponding to the 410 nm, 520 nm, 680 nm wavelengths and the clear control, as well as a black garbage bag for the dark control. We then placed all treatments on top of the refrigerator, where the temperature remained fairly constant and was measured to be 26 degrees Celsius. We positioned the treatments in a manner that they all received an equal amount of light, except for the dark control, which was kept at the back, in order to minimize the amount of light it received.

Figure 1. Experimental set up above the refrigerator. Note the light source above and the positioning of each treatment.

Before taking measurements for our experiment, we observed our organism, *D. melanogaster*, in each replicate vial of each treatment approximately every three days. This allowed us to take temperature measurements, ensure that they were growing as expected and that any extraneous sources such as mould had not contaminated our treatments. Three days after we set up our treatment, we added paper towels around the garbage bag dark treatment to decrease the light intensity the sample received and also secured the acetate covering on the treatments with additional tape in order to minimize light contamination of unwanted wavelengths.

Ten days after we set up our various treatments, we marked each of the testing vials at a height of eight centimeters using masking tape to ensure identical measurements amongst all treatments. We began by determining the average recovery time of an adult *D. melanogaster*

Figure 2. Experimental set up above the refrigerator. Each treatment was wrapped with acetate and secured with a rubber band and tape.

after being exposed to carbon dioxide gas. We first treated all adult *D. melanogaster* with carbon dioxide gas while inverting the vial, allowing them to fall down to the lid once they were anaesthetized. Once all the *D. melanogaster* were anaesthetized, we transferred one *D. melanogaster* into our testing vial and after it had recovered from the carbon dioxide gas treatment and had become active, we tapped the testing vial, causing the *D. melanogaster* to fall down to the bottom of the testing vial. We then proceeded to record the amount of time it took for each *D. melanogaster* to make its way to the top of the vial, just below the eight-centimeter mark. We discarded the *D. melanogaster* into the morgue once it had been tested. We collectively decided that if a particular *D. melanogaster* took longer than ten minutes to become active, we would discard it and immediately transfer it into the morgue. We then observed *D. melanogaster* from each treatment under a light microscope to determine if there were morphological differences among the five treatments. We repeated all the above procedure for all replicates in all treatments.

After the data was collected, we calculated the mean, standard deviation and 95% confidence intervals for the ascension time of *D. melanogaster* for each treatment to determine if there were significant differences amongst the treatments. To ensure that the difference between treatments were significant, we also performed the student's *t*-test.

Results

The mean, standard deviation, 95% confidence intervals for the ascension times of each treatment were calculated. Results greater than two standard deviations away from the mean were considered outliers and discarded.

Sample calculations involved:

Speed

$$s = \frac{\Delta d}{\Delta t} = \frac{8cm}{9.16s} = \mathbf{0.87}\,\frac{\mathbf{cm}}{\mathbf{s}}$$

Mean

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i = \frac{1}{4}(1.20 + 1.57 + 1.53 + 1.70) = \mathbf{1.53}\,\frac{\mathbf{cm}}{\mathbf{s}}$$

Standard Deviation

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2} = \sqrt{\frac{1}{4}[(1.20 - 1.53)^2 + (1.57 - 1.53)^2 + (1.63 - 1.53)^2 + (1.70 - 1.53)^2]}$$

$$= \mathbf{0.22}\,\frac{\mathbf{cm}}{\mathbf{s}}$$

Variance

$$v = \sigma^2 = 0.22^2 = \mathbf{0.05}$$

95% Confidence Interval

$$\bar{x} \pm t\frac{s}{\sqrt{n}} = 1.53 \pm 1.96\frac{0.22}{\sqrt{4}} = 1.53 \pm 0.22 = [\mathbf{1.31}, \mathbf{1.75}]$$

Student's t-test

$$t = \frac{\overline{x_1} - \overline{x_2}}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Where *s* is the combined standard deviation of the two samples:

$$s^2 = \frac{\sum(x - x_1)^2 + \sum(x - x_2)^2}{n_1 + n_2 - 2} \rightrightarrows s = \sqrt{\frac{0.199 + 0.131}{4 + 4 - 2}} = 0.23$$

$$t = \frac{1.53 - 1.03}{0.23\sqrt{\frac{1}{4} + \frac{1}{4}}} = \mathbf{3.07}$$

According to the table for *t*-distribution, the *t* value at 6 degrees of freedom and 2-sided 95% is 2.447. Since the calculated *t*-value is greater than the theoretical *t*-value, we can conclude the means of the two samples are significantly different.

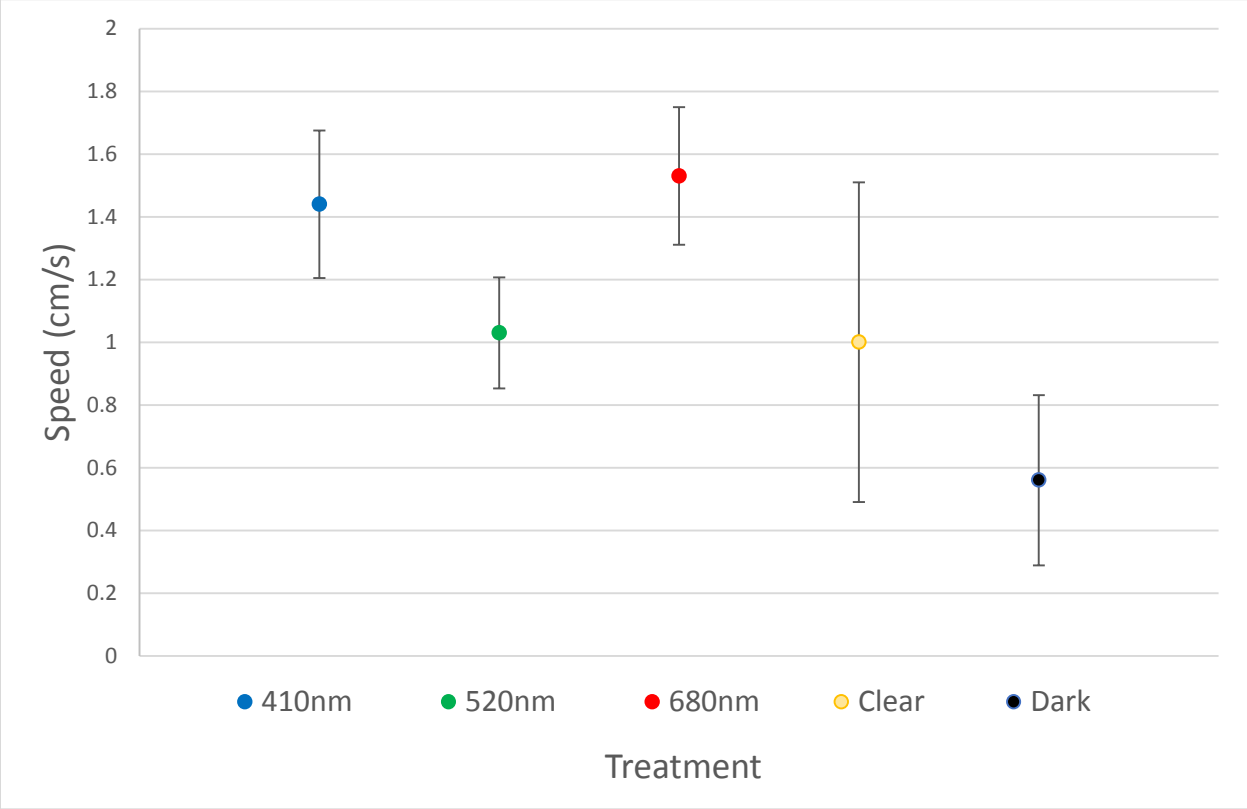

Figure 3. Average speed in cm/s measured for *D. melanogaster* to ascend 8 cm up a vial as a function of their various treatments. At n=4, the speed at 95% confidence were, in cm/s, [1.17, 1.64], [0.83, 1.18], [1.31, 1.75], [0.56, 1.58], [0.29, 0.83] with means of 1.40, 1.01, 1.53, 1.07, 0.56 for *D. melanogaster* treated with 410nm, 520nm, 680nm, light control and dark control respectively.

The means and 95% confidence intervals for each treatment in cm/s were 1.40 ± 0.24 , 1.01 ± 0.18, 1.53 ± 0.23, 1.07 ± 0.51 and 0.56 ± 0.28 seconds and variances were 0.06, 0.03, 0.05, 0.27, 0.08 for 410 nm, 520 nm, 680 nm, light control and dark control respectively. No visible trend of increasing locomotor ability with increasing wavelength was found.

Confirmation from the student's *t*-test concludes that the difference in results between the 680 nm and the 520 nm measurements were significant.

Discussion

Our results demonstrate a significant increase in motor speed, of 1.01 cm/s in the green (short) wavelength treatment group to 1.53 cm/s seconds in the red (long) wavelength treatment. However, this trend does no continue in the blue light treatment group, which does not exhibit significant difference in speed from the red or green treatment groups. As blue light possesses the shortest wavelength, we hypothesized that it would result in the slowest motor speed; however its corresponding motor speed of 1.40 cm/s is greater than that of the green treatment group. Therefore, we reject our alternative hypothesis and fail to reject our null hypothesis which states that an increase in treatment wavelength results in decreased or no effect in motor activity. Despite our data not cohering with the theory that increased wavelength results in greater motor speed, the data suggests a trend in optimal growth wavelength (the green treatment wavelength) significantly influencing motor speed.

D. melanogaster has various photoreceptors that it uses for brightness and colour detection (Yamaguchi 2010). The R1-6 receptors have been shown to be strongly associated with colour vision (Yamaguchi 2010). In a study by Tang and Guo (2001) *D. melanogaster* was shown as not possessing the photoreceptive capacity for detecting red light. In a subsequent

study *D. melanogaster* was also shown to exhibit a specific preference for green and blue wavelength stimuli, as indicated by phototaxis (Yamaguchi 2010). These findings support the suggestion that *D. melanogaster* has the ability to actively process sensory light input and perceive differences in certain colours (Heisenberg and Buchner 1977).

Despite demonstrating a capacity for colour distinction and preference, the growth of *D. melanogaster* at a particular visual spectrum has not been shown to lead to any evident gross morphological change that could then be linked to a change in motor or visual properties (Marko and O'Grady 2008). We do suggest, however, that there is evidence that wavelength affects *D. melanogaster's* eclosion (hatching) and thus maturation which would, in turn, impact their motor capabilities. This relationship between maturation and motor speed was shown by Rhodenizer *et al.* (2008) who demonstrated that *D. melanogaster* locomotor speed significantly decreased with increasing *D. melanogaster* age, meaning younger flies tended to exhibit the greatest motor speed.

Research on the predatory flower bug *Orius sauteri* has shown that the maturation time (days) is significantly longer in red and blue light treatment samples, compared to white and green light samples (Wang *et. al.* 2013). This prolonged time to eclosion (hatching) and thus maturation in the red and blue wavelength treatments suggests an adaptive preference for growth in their natural yellow-green environments (Wang *et. al.* 2013). Researchers Wang *et al.* (2013) hypothesize that it is the absence of the yellow-green wavelengths in the blue and red conditions that lead to the delayed development in their sample organisms; highlighting that when grown in red light *O. sauteri* demonstrated the longest time for eclosion and were therefore the slowest in achieving maturation.

D. melanogaster, too, exhibits a preference to the yellow-green wavelengths as shown in a study by Sakai *et al.* (2002). These researchers observed peak mating behaviour in *D. melanogaster* when grown in a yellow-green light (500-600 nm). This implies that the green and white-light treatments are representative of optimal (natural) wavelength environments whereas the red and blue treatments are not. In Figure 3, the results show a significant difference in the red motor speeds compared to green motor speeds. This suggests that exposure to red light is associated with increased locomotor speed (at the time of testing), which in itself may correlate to delayed eclosion and maturation.

This finding points to the possibility that exposure to an optimal wavelength (yellow-green) provides the larvae with a more favourable growth environment, which could translate into earlier eclosion, and thus differences in maturity. As maturity is correlated to motor capabilities (Rhodenizer *et al.* 2008), we therefore suggest that the absence of this optimal green-yellow wavelength is what led to the increased speeds observed in the blue and red treatment groups. As speed is an indicator of age, we can deduce that the sub-optimal wavelengths of the red and blue treatments possibly led to delayed eclosion and maturity, with the most significant impact seen in the red treatment group.

Another example of how the rate of eclosion and maturation affects mobility can be seen in the dark treated samples, which possessed the lowest mean speed. Much research has been done regarding the growth of *D. melanogaster* in varying light and dark conditions (Yamanaka *et al.* 2013). Research has shown that, through neuroendocrine control, larvae can detect and actively avoid light when finding a pupariation site, meaning larvae prefer growth in darker conditions (Yamanaka *et al.* 2013). We suggest that light avoidance and early pupation correlates

to decreased motor activity, as was observed in our dark grown samples. These conditions appeared to be the most favourable for metamorphosis and resulted in earlier maturation.

In view of the literature, we then propose that the effect of specific wavelength on the maturation of *D. melanogaster* could theoretically impact their motor capabilities. We suggest that at the time of testing, the test subjects themselves could have been at different stages of maturation, leading to the differences in the observed speed.

Similarly, examples exist in the literature regarding the impact of wavelength and light on circadian rhythm; a process that influences the development, activity and behaviour of *D. melanogaster* (Helfrich and Englman 1983). A strong correlation has been shown between circadian rhythm and the behaviour and metabolism of insects; where any alteration of this pattern is reflected in the processes it controls (Saunders 1982). Subramanian *et al.* (2009) have shown that when *D. melanogaster* is exposed to various specific wavelengths, there is a significant consequence to circadian rhythm where red wavelengths result in a shortened activity period within a 24 hour light cycle, and short wavelengths lengthened the period of activity. Despite there being no literature on the specific effects of such an increase or decrease in circadian cycle with relation to *D. melanogaster*, it is possible that their behaviour and metabolic rates may be affected, - all of which would influence their motor processes.

Variations in experimental technique may have led to discrepancies in our data, namely in the recovery time of *D. melanogaster*, which may have been influenced by the duration of CO_2 exposure time (MacAlpine *et al.* 2011). Furthermore, we did not control for gender in our experiment. This may have contributed to error, where research has shown that females and males have different activity levels, developmental rates and exhibit sexual dimorphism (Faucher *et al*. 2006, Tompkins *et al.* 1982). Evidence of such errors can be seen in Figure 3, where large

discrepancies between the magnitude of standard deviations amongst treatments exist. Additionally, logistical limitations (in particular, the equipment and measuring apparatus that we used) may have led to errors involving controlling light intensity and wavelength, accurately measuring locomotor velocity and controlling for light-dark cycles. The lack of controlled environments, as seen by the different number of starting larvae within each replicate, could also possibly affect development. These errors may have collectively impacted the accuracy and confidence of our conclusions.

With a better controlled methodology in testing and improvements to the environmental set-up, a more thorough examination could be conducted to further determine the extent of wavelength-dependent development and motor response. The use of a more precise measuring apparatus could better determine speed over greater distances and time periods, and improvements to housing treatments would control for light availability and intensity, wavelength and the 24 hour light-dark cycle. Larger treatment sizes would also decrease variance about the mean and result in clearer trends for analysis. In addition to improved methodology, we suggest that future experimentation determining the extent of wavelength-dependent development affecting motor response, also account for the rate of maturation and eclosion within samples. A trend of delayed eclosion in the sub-optimal (red and blue) treatments would further support the hypothesis of optimal (green-yellow) wavelength environments encouraging early eclosion and maturation relative to suboptimal wavelength environments, the effects of which would be observed in the relative motor speeds of each treatment sample.

Conclusion

Compared to the 520 nm treatment, the locomotor speed of *D. melanogaster* increased when treated with 680 nm. However, significant difference was not found between the 520 nm and the 410 nm treatments. Thus, we have failed to reject our null hypothesis, which stated that exposure to increased light wavelength will result in decreased or have no effect on the locomotor speed of *D. melanogaster* as indicated by geotaxis. Our alternative hypothesis, which stated that exposure to increased light wavelength will result in increased locomotor speed of *D. melanogaster* as indicated by geotaxis, was not supported by our results.

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