

The effect of monochromatic light on *Drosophila melanogaster* larvae: As measured by distance travelled

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Abstract:

Drosophila melanogaster, a species of fruit fly, have photoreceptors in their eyes to help them distinguish light and navigate in their environment (Keene *et al.* 2012). In this experiment, we obtained two groups of *D. melanogaster* larvae: the wild type and the *rosy* mutant, to investigate in which wavelength of light they will travel the greatest distance. Blue, red and white light acted as the treatments. After 30 seconds of each treatment, we observed how far the larvae travelled from the starting point. The distance travelled by the wild-type larvae exposed to white light was not found to be statistically different from the distance travelled in either blue or red light. However, the distance travelled when exposed to red light was found to be significantly different when compared to blue light. In the case of the mutant group, the distance travelled while under the exposure to white light was not statistically different from the distance travelled when exposed to red light. However, the distance was found to be significantly lower when exposed to blue light compared to that of white light. Based on the wild-type *D. melanogaster* data, we failed to reject the first null hypothesis, exposure to blue light will decrease or have no effect on the distance travelled in 30 seconds by wild-type *D. melanogaster* larvae as compared to white light. We also failed to reject the second null hypothesis, exposure to red light will increase or have no effect on the distance travelled in 30 seconds by wild-type *D. melanogaster* larvae as compared to white light. For the mutant *D. melanogaster* data, we failed to reject the third null hypothesis (similar to the first null hypothesis for wild type) and we also failed to reject the fourth null hypothesis (similar to the second null hypothesis for wild type).

Introduction:

The objective of this study was to determine the wavelength of light in which the *Drosophila melanogaster* larvae moved the greatest distance. The importance of this investigation is to see what effects the presence or the absence of different pigments have on the *D. melanogaster* (Hearl and Jacobson 1984). Our in-lab observations of *D. melanogaster* larvae showed that *D. melanogaster* larvae are pale in colour with a distinct head and tail region, which agrees with the findings of Demerec and Kaufmann (1996). The eyes are located in the head region, which has small black dots and is more slender than the tail. In the larval eye of the *D. melanogaster*, there

lie twelve photoreceptors of which four photoreceptors express the blue-sensitive gene rhodopsin5, and eight photoreceptors express the green-sensitive gene rhodopsin6 (Keene *et al.* 2012). In a *D. melanogaster* larval eye, a light stimulus is first detected by one of the photoreceptor neurons, which transform specific wavelengths of light into neuronal information. This is then processed by second order neurons and then received and further processed by higher brain centers (Keene *et al.* 2012), as depicted in Figure 1. Fully functioning photoreceptors in the eye are therefore essential for the *D. melanogaster* larva to be able to navigate effectively in its environment (Keene *et al.* 2012). From this information we hypothesized that the *D. melanogaster* larvae should show different patterns of movement under exposure to various types of monochromatic light, and decided to test these differences by measuring the distance travelled. We decided to carry out our investigation in both the mutant and the wild-type *D. melanogaster* to determine if the *rosy* eye mutation, a mutation resulting in lower levels of the enzyme xanthine dehydrogenase (XDH) (Chovnick *et al.* 1976), has an effect on how the *D. melanogaster* perceived and behaved in different colours of light. The XDH deficiency results in different levels of eye pigmentation in the *D. melanogaster* eyes (Chovnick *et al.* 1976), therefore in our study we decided to have the same set of hypotheses for the mutant and wild-type *D. melanogaster*, which may or may not have a cascading effect on the photoreception abilities of *D. melanogaster*. The information we gained from this research can be extrapolated towards other organisms, such as humans, that contain pigments in their eyes (Bird *et al.* 1998).

The following are the hypotheses for our research:

Wild type

H₀: Exposure to blue light will decrease or have no effect on the distance travelled in 30 seconds by wild-type *D. melanogaster* larvae as compared to white light.

H_a: Exposure to blue light will increase the distance travelled in 30 seconds by wild-type *D. melanogaster*

larvae as compared to white light.

H₀: Exposure to red light will increase or have no effect on the distance travelled in 30 seconds by wild-type *D. melanogaster* larvae as compared to white light.

H_a: Exposure to red light will decrease the distance travelled in 30 seconds by wild-type *D. melanogaster* larvae as compared to white light.

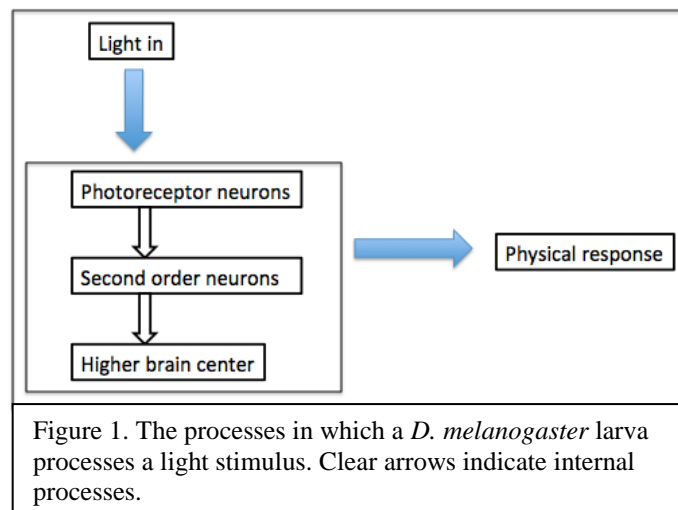
Mutant

H₀: Exposure to blue light will decrease or have no effect on the distance travelled in 30 seconds by mutant *D. melanogaster* larvae as compared to white light.

H_a: Exposure to blue light will increase the distance travelled in 30 seconds by mutant *D. melanogaster* larvae as compared to white light.

H₀: Exposure to red light will increase or have no effect on the distance travelled in 30 seconds by mutant *D. melanogaster* larvae as compared to white light.

H_a: Exposure to red light will decrease the distance travelled in 30 seconds by mutant *D. melanogaster* larvae as compared to white light.



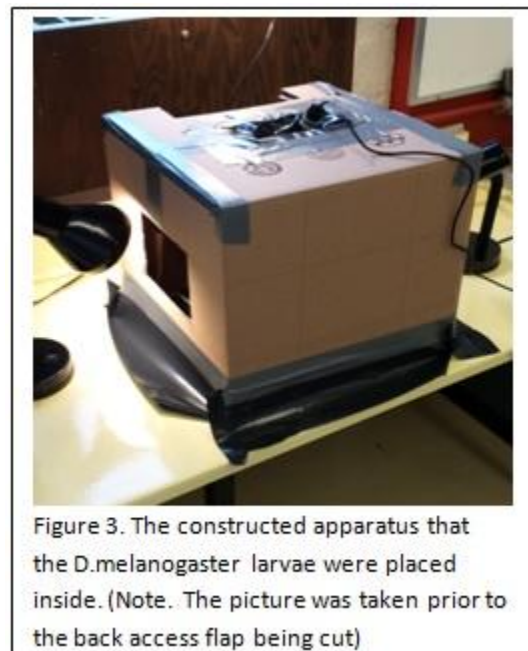
Methods:

For this experiment, the wild type and mutant *rosy D. melanogaster* were grown on an oatmeal medium. Three different light treatments were set up in our experiment to observe differences in movement under monochromatic light conditions. We used blue acetate paper to simulate blue light, red acetate paper to simulate red light and uncovered openings to



simulate white light. White light was used as a control as it is believed to be the primary mode of photon exposure for both the wild-type and mutant *D. melanogaster* larvae. For each of the treatment groups, five replicates were set up, for a total of thirty replicates (fifteen for wild type and fifteen for mutant). Larvae were removed from a vial (Figure. 2) in which they were growing and placed into a petri dish (60 mm in diameter) with just enough 18% glucose solution to help separate them from the growth medium. Larvae were separated from the growth medium and solution one at a time using wire loops and transferred to the centre of another petri dish (60mm in diameter) which had a layer of agar along the bottom for the larvae to move around on.

To test the effect of different monochromatic light colour environments on the mutant and wild-type *D. melanogaster* larvae, we constructed an apparatus using a cardboard box (Figure. 3) which was placed over a light microscope to observe larvae movement (Figure 4). The box had five window slits cut open; two of equal size on each side of the box, one of larger size



going from the top of the box to the front, one on top of the box just big enough for the ocular lenses to come through and one in the back for access to light microscope stage. Three sheets from each colour of acetate paper were cut out big enough to go over each of the side windows and the top/front window. From a black garbage bag two covers were cut out to go over the back access window and the ocular lens to prevent any extra light from getting through, and a skirt was manufactured from the garbage bag and applied to the bottom of the box for the same reason. Lamps were placed around the windows to ensure just enough light was getting through to observe larvae movement. To ensure light intensity was kept relatively constant we adjusted lamp distances from the windows to ensure the same lux (540 lux) throughout the experiment.

When collecting the data, we used a DinoScope to record video footage of the larvae moving around the petri dish for thirty seconds. The larvae were also observed through one ocular lens to make qualitative observations like head movements. After footage was recorded for all treatments, analysis of the larval movement was performed by measuring the total distance travelled from the centre of the larval body; as calculated with a 10 mm: 1 cm grid paper on which the petri dish was overlaid. For those larvae that moved outside of the frame during the thirty second intervals, we estimated the distance travelled by extrapolating the speed with which it was travelling when it exited the frame.

Upon complete collection of the data, we analyzed it by conducting a two-sided t-test using 95% confidence intervals and plotting side-by-side box plots.

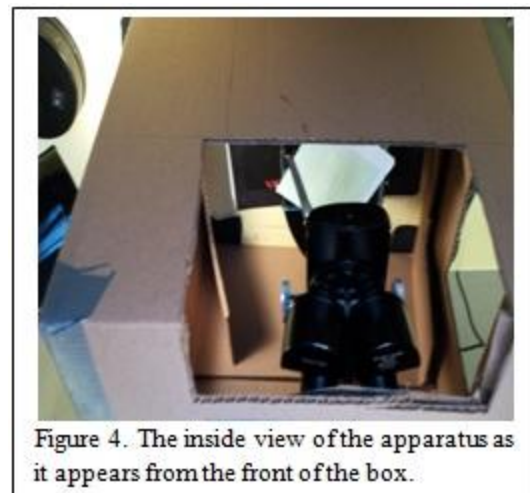
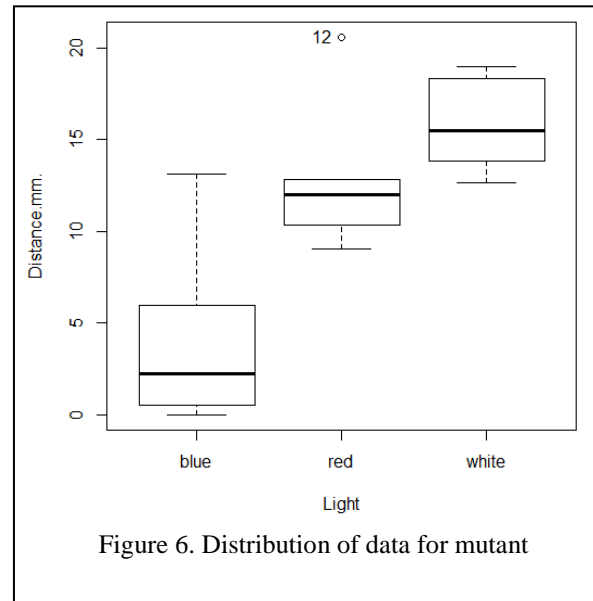
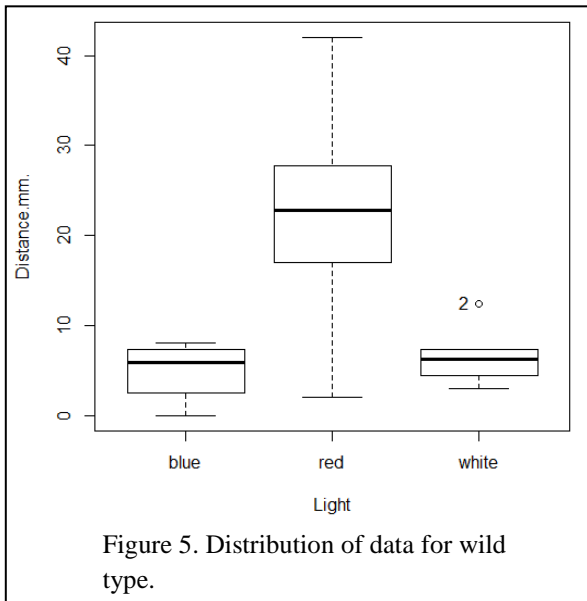


Figure 4. The inside view of the apparatus as it appears from the front of the box.

Results:



Upon analysis of the data, it can be seen that the distribution regarding distance travelled of the wild type in red light is roughly symmetric about the mean, while this is not the case for all other treatments (seen in Figure 5 and Figure 6). There is an apparent trend in which both the mutant and the wild type performed poorly when exposed to blue light; that is, they did not travel far relative other treatments. Further investigation revealed non-overlapping 95% confidence intervals for the mean distance travelled in blue light and red light for the wild type, and in blue light and white light in the mutant (shown in Figure 7 and Figure 8). A two-sided t-test was carried out at the 95 % confidence level and in both cases, a significant difference was found.

Each box plot distribution shows one outlier: 12.4 mm by wild type in white light, 20.6 mm by mutant in red light (seen in Figure 5 and Figure 6). These were not removed from calculations as they do not reverse or change correlational outcomes and in fact, might help to give a more complete view of the true ratios as our experiment resolved few data points.

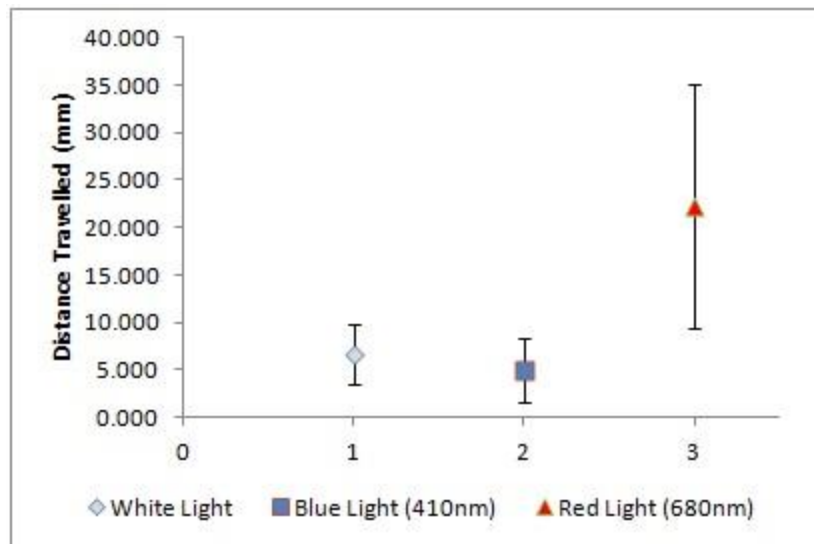


Figure 7. Mean distance travelled with 95% confidence intervals for wild type. n=4 for blue, n=5 for white light and red light

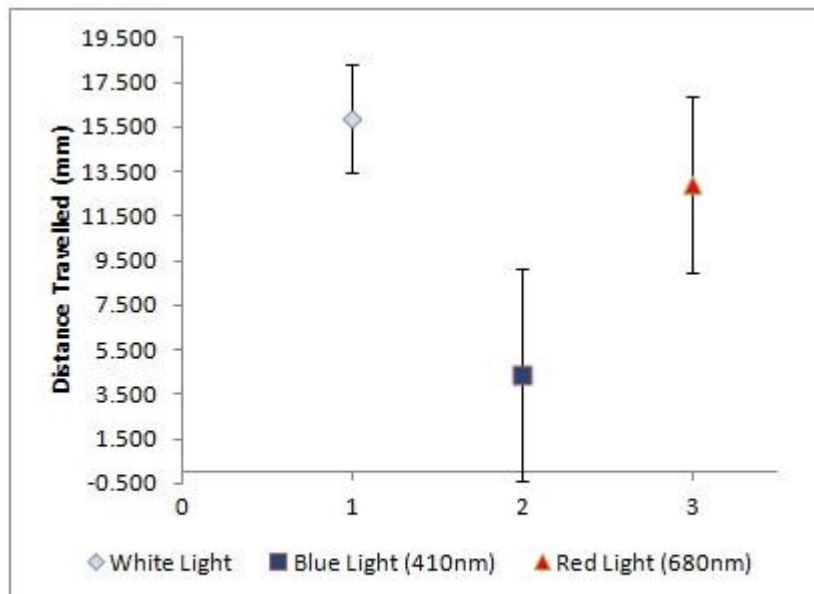


Figure 8. Mean distance travelled with 95% confidence intervals for mutant. n=5 for all treatments.

Sample Calculations:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad \text{Where } s \text{ is square root of the pooled standard deviation; } s^2 = \frac{\sum(x-x_1)^2 + \sum(x-x_2)^2}{n_1 + n_2 - 2}$$

t-test for wild type mean distance blue and red:

$$t = \frac{22.307 - 4.933}{5.695 \sqrt{\frac{1}{4} + \frac{1}{5}}} = 4.548, \quad \text{where } s = \sqrt{\frac{3.511^2 + 14.654^2}{7}} = 5.695$$

t-test for mutant distance white and blue:

$$t = \frac{15.853 - 4.630}{2.150 \sqrt{\frac{2}{5}}} = 8.451, \quad \text{where } s = \sqrt{\frac{2.760^2 + 5.419^2}{8}} = 2.150$$

Discussion:

Following the analysis of the wild-type *D. melanogaster* data, we failed to reject the first null hypothesis and therefore could not support the first alternate hypothesis. We also failed to reject the second null hypothesis and therefore could not support the second alternate hypothesis. The distance travelled by wild-type larvae exposed to white light was not found to be statistically different to the distance travelled by wild-type larvae exposed to either red or blue light. However, the distance travelled by larvae was found to be significantly greater when exposed to red light than when exposed to blue light.

Our findings about the wild-type larvae were inconsistent with the literature. Warrick *et al.* (1999) found that larvae were only weakly responsive or unresponsive when tested at the wavelength 650 nm (red light). In contrast, larvae were found to be strongly responsive to white light and maximally sensitive to indigo light at a wavelength of 420 nm. In another study, Xiang *et al.* (2010) tested wavelength-dependent photo-avoidance of wild type larvae and found them to be most sensitive to blue, violet and ultraviolet light. In contrast the larvae were found to be largely unresponsive to red and green light (Xiang *et al.* 2010). The above studies are in agreement with proposed models of the visual system of *D. melanogaster* larvae. Keene *et al.* (2011) found that rapid light avoidance behaviour is dependent on blue-sensitive photoreceptors and the absence of green-sensitive photoreceptors did not alter the visual response, hence *D. melanogaster* larvae are maximally sensitive to blue light. The deviation of our results from those present in the literature may be attributed to experimental differences. Xiang *et al.* (2010) measured larval head turns and Warrick *et al.* (1999) measured the proportion of larvae in the

light and dark quadrants of a petri dish to analyze photo-behaviour. The total distance travelled may not be an effective means of analyzing the responsiveness of larvae to specific wavelengths.

Following the analysis of the mutant *D. melanogaster* data, we failed to reject the third null hypothesis and therefore could not support the third alternate hypothesis. We also failed to reject the fourth null hypothesis and therefore could not support the fourth alternate hypothesis. The distance travelled by larvae exposed to white light was not found to be statistically different to the distance travelled by larvae exposed to red light. However, the distance travelled by larvae was found to be significantly lower when exposed to blue light than when exposed to white light. Interestingly, exposure to blue light produced the least distance travelled for both the mutant and wild-type larvae.

There is an absence of literature that explicitly analyzes the behavioural response of rosy mutant larvae at specific wavelengths. However, Xiang *et al.* (2010) found that class IV dendritic arborisation neurons line the body wall of larvae and function in sensing light. These sensory neurons are critical in understanding larvae photo-behaviour as larvae spend the majority of their time with their heads digging into food, thus the larval eye is rarely exposed to light (Xiang *et al.* 2010). Moreover, dermal receptors are the primary sensors at high light intensities, which were used in our experimental setup (Xiang *et al.* 2010). Class IV dendritic neurons were found to be most responsive to blue light and unresponsive to red light (Xiang *et al.* 2010). Hence, our results for both the mutant and wild-type are inconsistent with the literature. However, as larvae are primarily exposed to white light in nature, we can hypothesize that larvae exposed to blue light may require some time to acclimatize to the new light conditions. This may have caused the distances observed in blue light to be lower. Further research into the adjustment periods of larvae to different light conditions is required.

There were a number of sources of error that may have caused the discrepancy between our results and those presented in the literature. Firstly, we attempted to maintain the light intensity at a constant value of 540 lux in each trial. However, the box shifted a number of times during the recording of the larvae, which may have caused light intensity to vary from trial to trial. Warrick *et al.* (1999) found that red light at greater light intensities could evoke the same behavioural response in *D. melanogaster* larvae as blue light at lower light intensities. This may have been the reason why our wild-type *D. melanogaster* larvae were more motile in red light than in blue light. Secondly, very few replicates were used in our experimental setup. This may have led to the large confidence intervals observed for a number of the distances. Lastly, there were a number of times when the larvae moved out of the frame of the video and the distance travelled was extrapolated from the initial observed speed. If the larvae did not maintain the initial observed speed, the calculated distances may have been inaccurate.

Biological factors may have also led to our results deviating from the literature. We attempted to reduce biological variation by using *D. melanogaster* larvae of the same strain that were of about the same age. However, it is possible that some larvae were more mature than others. Keene *et al.* (2012) claim that *D. melanogaster* larvae strongly avoid light from the first-instar stage to the early third-instar stage, but photo-avoidance rapidly decreases in the third-instar stage. Consequently, differences in the visual behaviour between larvae due to age may have introduced significant biological variation.

Conclusion:

From the investigation, it was determined that for the wild-type *D. melanogaster* larvae, we failed to reject the first null hypothesis and therefore could not support the first alternate hypothesis. We also failed to reject the second null hypothesis and therefore could not support

the second alternate hypothesis. The distance travelled by wild-type larvae exposed to white light was not found to be statistically different to the distance travelled by wild-type larvae exposed to either red or blue light. However, the distance travelled by larvae was found to be significantly greater when exposed to red light than when exposed to blue light. For the mutant *D. melanogaster*, we failed to reject the third null hypothesis and therefore could not support the third alternate hypothesis. We also failed to reject the fourth null hypothesis and therefore could not support the fourth alternate hypothesis. The distance travelled by larvae exposed to white light was not found to be statistically different to the distance travelled by larvae exposed to red light. However, the distance travelled by larvae was found to be significantly lower when exposed to blue light than when exposed to white light. Interestingly, exposure to blue light produced the least distance travelled for both the mutant and wild-type larvae. The importance of this investigation is to see what effects the presence and absence of different eye pigments have on *D. melanogaster* (Hearl and Jacobson 1984). The information we learn from this research can be extrapolated towards other organisms, such as humans, that contain pigments in their eyes (Bird *et al.* 1998).

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