Effect of Light Wavelength on the Phototaxis Response of Wild-type Oregon-R and Mutant *ort^1* *Drosophila melanogaster*

Samantha J. Mee, Ekum K. Romana, Akanksha R. Stevens, Guan Qun Zhao

Abstract

Drosophila melanogaster, whose common name is "fruitfly", is a universally accepted model organism in biological research. In comparison to the wild-type strain Oregon-R, the mutant *ort^1 Drosophila melanogaster* has a decreased phototaxis response in green (visible) light as well as in the ultraviolet region. Previous research has tested the behavior of *D. melanogaster* under light intensity and wavelength, from visible to ultraviolet light. However, the focus of this experiment is to observe and compare the effect of green light on the two strains: mutant *ort^1* and wild-type Oregon-R. We used three different light treatments, testing the effect of green, red, and white filters; we used 20 wild-type and 20 mutant replicates for each of these treatments and recorded the time it took for the specimen to reach the indicated marker. White light was the intended negative control to determine whether negative geotaxis or phototaxis was the dominant response for both the mutant and wild-type strains. In green light, the mean phototaxis response time was 38 seconds for wild-type *D. melanogaster* compared with 10 seconds for mutant *ort^1*. This difference was statistically significant ($p- < 0.05$).

Introduction

The wavelength preference of *Drosophila melanogaster* plays an important role in the overall fitness of the species (Chadha 2008), as the mating activities of the fly strongly correlate to the UV wavelength region (Sakai *et al.* 2002). Although responses to certain the regions of wavelength and light intensity have been heavily investigated, there is a lack of research on the phototaxis response of *D. melanogaster.* Phototaxis is an organism's attraction to light and negative geotaxis is an organism's response away from gravitational pull (Yoshimura 2011). An increased phototaxis response promotes movement towards a light stimulus (Yoshimura 2011). Understanding the phototaxis response of *D. melanogaster* under the visible light spectrum gives us better insight on their mating habits and living environments (Sakai *et al.* 2002).

Subramanian (2009) analyzed the amount of activity of *D. melanogaster* under different colours of light, similar colours to our own experiment, but he does not give any additional insight on the phototaxis response. Our experiment attempts to investigate and broaden the knowledge of the phototaxis response by studying both the wild type and mutant under green light. The wild-type strain that we used is known as Oregon-R; the mutant that we based our analysis on, known as *ort*^{1}, has defective histamine gated chloride channels (Iovchev *et al.* 2002). This mutation results in an abnormal motor recovery time at 40 °C, as well as a decreased phototaxis response in green and UV light (Iovchev *et al.* 2002). This decreased phototaxis under green light was the basis for our experiment as we examined the differences between the wild-type and mutant behaviours. It is important to study *ort*^{1} mutants, as this mutation could be attributed to a decrease in fitness and thus alter their behavior in the natural environment (Iovchev *et al.* 2002).

Overall, we addressed three hypotheses. Initially, we tested whether wavelength has an effect on the phototaxis of *D. melanogaster.* Our H_{O1} states that wavelength has no effect on phototaxis response of *D. melanogaster;* our H_{A1} states that wavelength of light has an effect on the phototaxis of *D. melanogaster.* Second, we compared our findings to determine whether the presence of mutant *ort*^{1} significantly changes the results. Our H_{O2} states that the presence of mutation *ort*^{1} has no effect on the phototaxis of *D. melanogaster*, and our H_{A2} is that the presence of mutation *ort*^{1} has an effect on the phototaxis of *D. melanogaster*. Third, we determined if the effect of wavelength is the same in wild-type Oregon-R and the mutant *ort*^{1}. Our H_{O3} states that the effect of wavelength on the phototaxis response of *D. melanogaster* is the same in wild-type

Oregon-R and the mutant *ort*^{1}, and our H_{A3} states that the effect of wavelength on the phototaxis response of *D. melanogaster* is different in the wild type and mutant.

Methods

We studied the phototaxis response by observing *D. melanogaster's* behaviour inside a test tube with a light source at the bottom. If phototaxis were greater than negative geotaxis, the specimen would remain near the light source longer, located at the bottom of the test tube (Figure 1). Green and red wavelengths were tested along with white light (negative control) for wild type and mutant (Figure 2).

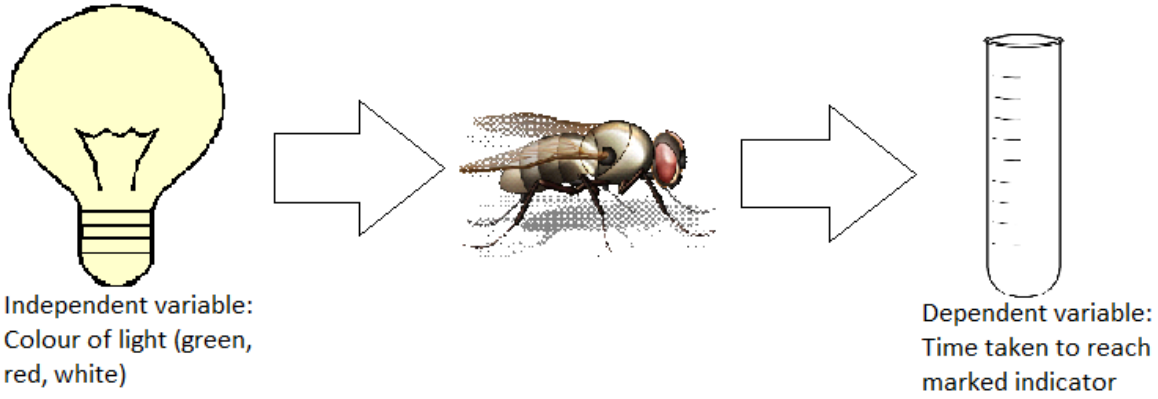

Figure 1. Diagram illustrating the independent and dependent variables that we tested in our experiment.

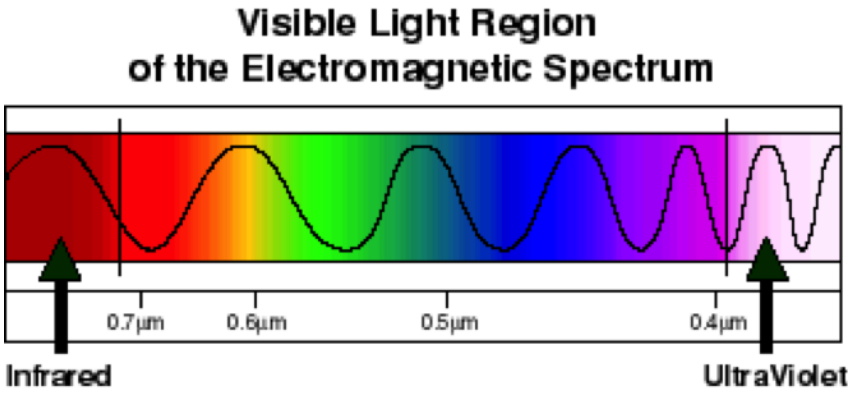

Figure 2. The 'Visible Light Spectrum'. Wavelength ranges for the three treatments correspond to the spectrum for green, red, and white light sources.

Prior to our project, we measured the recovery time from immobilization for both the mutant *ort*^{1} strain and wild-type Oregon–R *D. melanogaster.* The mutant strain is known to have abnormal motor recovery time at 40°C as well as decreased phototaxis in green and UV light (Iovchev *et al.* 2002). The recovery time of the wild-type-strain of *D. melanogaster* from CO_2 treatment was roughly five minutes, whereas the *ort*^{1} did not recover within an hour of monitoring, thus confirming a longer recovery period. Furthermore, we placed both strains of *D. melanogaster* in a 40°C incubator for seven minutes. The wild type recovered nearly instantaneously from the temperature incubation; however the mutant strain took approximately ten minutes to recuperate from the elevated temperature. We considered these findings prior to our experiment, to ensure that the data collection occurred within a reasonable time frame.

D. melanogaster were raised on a cornmeal medium that included agar, dextrose and yeast extract (Iovchev *et al.* 2002). Given that incubation and CO_2 promoted a longer recovery time, we considered using an ice bath as an immobilization method. However, we decided not to use the previous methods because the recovery time exceeded ten minutes for both mutants and wild-type strains. Recovery time is the amount of time taken to regain full motility from the immobilization method (CO_2, incubation or freezing). Additionally, controls such as test-tube size, type of light source, termination time, the height above light source and the height to indicated marker were determined in this pilot study.

We used twenty replicates for each wavelength treatment (green light, red light, and white light) for both *ort*^{1} and Oregon-R *D. melanogaster*. Initially, we transferred twenty *D. melanogaster* specimens from the vial in which they were raised to twenty empty individual medium-sized test tubes. Each of the four stations consisted of a light

source, clamp and timer (Figure 3). Green and red acetate filters were placed over the white light source (Figure 4). Each of the four experimenters performed five replicates under each treatment to minimize error in the data. We clamped the test tube so that the bottom of the test tube was 4 cm from the light source; the indicated marker on the test tube was 7.5 cm from the bottom of the tube (Figure 3).

Initially, we clamped a test tube containing a single specimen of wild-type Oregon-R *D. melanogaster* 11 centimeters above the light source with a green acetate filter. Next, we tapped the test tube until the fly dropped to the initial starting point at the bottom; once the fly fell to the bottom, the timer was started. We observed the fly until it reached the indicated marker and the time (seconds). If the fly did not reach the indicated marker within a ninety second time period, we wrote the termination time (90 seconds) and an "X" in the data tables for clarity. We repeated this process for all twenty replicates with wild type in green light.

We repeated the procedure with 20 flies in red light. We repeated this procedure with the wild-type strain one more time for the white light. Twenty replicates of mutant ort^1 under the three treatments were performed the same way.

We used a two-way ANOVA analyze our data. We calculated 95% confidence intervals of the means for each treatment for wild type and mutant.

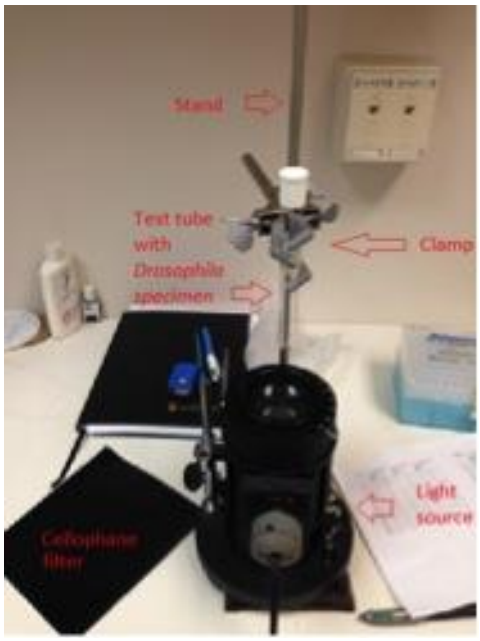

Figure 3. Experimental set up used for each treatment (green, red and white light). The test tube is 4 cm from the light source and the indicated marker is 7.5 cm from the bottom of the test tube.

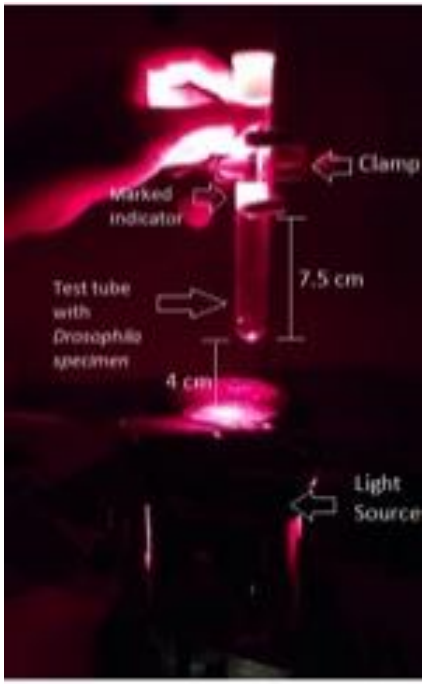

Figure 4. Experimental setup under the red light treatment. The same set-up was used for the green and white light treatments, however the light colour differed due to the acetate filter placed over the light source.

Results

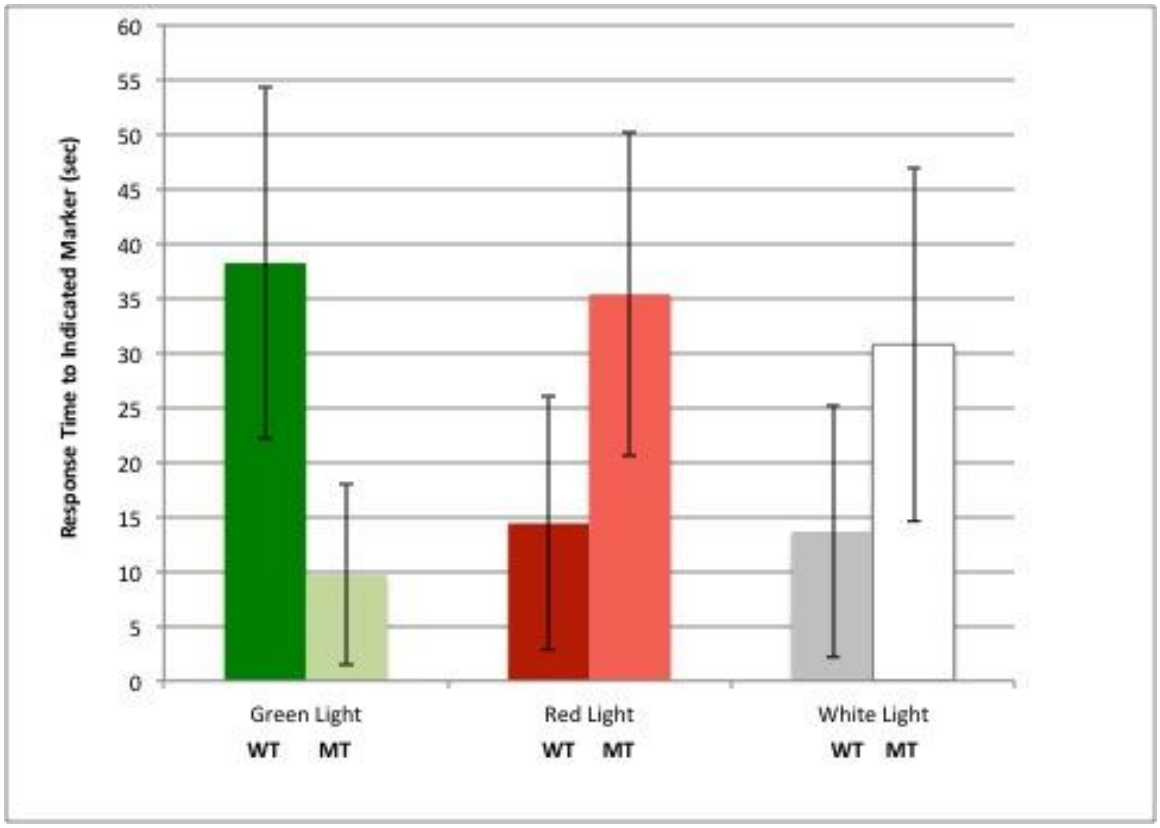

Figure 5. The effect of light colour on the response time (seconds) of *D. melanogaster* (seconds). Wild type (WT) and mutant (MT) are indicated for each light colour. The sample size for each treatment, n = 20. Error bars represent 95% Confidence Intervals. The calculated p-values for H_1, H_2 and H_3 are $p > 0.05$, $p > 0.05$ and $p < 0.05$ respectively.

From our experiment we determined the mean times for wild-type *D. melanogaster* in green, red and white light respectively were 38 seconds, 14 seconds and 14 seconds. As seen in Figure 5, the mean times for mutant *D. melanogaster* in green, red and white light were respectively 10 seconds, 35 seconds and 31 seconds. Furthermore, the confidence intervals for the wild type were 38 +/- 16 seconds, 14 +/- 12 seconds and 14 +/- 11 seconds respective to green, red and white light. Additionally, the confidence intervals for the mutant were 10 +/- 8 seconds, 35 +/- 15 seconds and 31 +/- 16 seconds respective to green, red and white light. The respective p-values for green red and white light treatments were 0.766, 0.761 and 2.58×10^{-4}.

Figure 5 shows that the mean response time in the green light treatment was faster in the mutant strain, when compared to the wild-type flies. However, the mean response time under red and white light was slower when testing the mutant than the response time exhibited by the wild-type strain. The variance in our data was larger in the treatment with green light for wild-type flies compared to mutant flies. On the other hand, the variance displayed for wild-type flies under both red and white light was smaller than the variance exhibited by the corresponding mutant flies. The 95% C.I. for the mutants in the green light was smaller than the wild-type flies in green light. As well, the 95% C.I.s for the mutants in the red and white light were larger than the wild type. The confidence intervals in Figure 5 had a large region of overlap between the red and white light for the wild-type flies; overlap also occurred in the confidence intervals for the red and white treatments in the mutant data.

Discussion

Based on the data obtained during our experiment and its subsequent analysis, we failed to reject H_{O1}: Wavelength has no effect on the phototaxis response of *D. melanogaster* because the p-value calculated was larger than 0.05, and we were unable to provide support for H_{A1}. We also failed to reject H_{O2}: Presence of the *ort*^{1} mutation has no effect on the phototaxis response of *D. melanogaster* as the calculated p-value was greater than 0.05. As shown in Figure 5, there was a substantial overlap in the 95% C.I.s under white and red light columns.

While there was no significant difference in the behavior exhibited by *D. melanogaster* (of both wild type and mutant) under the different wavelengths of light tested, nor a significant difference in the response of a mutant across the different

treatments, we were able to reject H_{03} as the calculated p-value was less than 0.05. This allowed us to provide support for the corresponding alternate hypothesis, H_{A3}: the effect of wavelength on the phototaxis response of *D. melanogaster* is different in the wild type and mutant. We found that the time it took to travel from the bottom of the test tube to the indicated marker, under all wavelengths of light tested, to be significantly different between the wild-type and mutant *D. melanogaster*. The difference in time taken is even greater when exposed to green light, which is evident in Figure 5, where we see no overlap between C.I.s of the means of the two groups.

Contrary to the findings of Washington (2010), who studied the features of colour vision pertaining to phototaxis, we found that our results were inconsistent with the literature because we were unable to reject our first null hypothesis. Based on experiments using a "T-Maze", it was argued that there is a different response displayed in *D. melanogaster* in regards to light from two wavelength regions (Washington 2010). Washington (2010) looked at the earlier phase of development, focusing part of his research on the larval stage. Larvae exhibit a photophobic response and prefer darkness compared to light (Gong 2009). Although the response exhibited in the larvae is opposite to what is shown in the adult stage, Washington's (2010) findings help to explain how the choice of one wavelength over another can ultimately depend on the intensity of those lights. The data obtained by Washington (2010) proposed that R1-6, which is the main photoreceptor group found in the retina that is responsive to low light intensity as well as color vision (Salomon *et al.* 1982), may contribute to the intensity-dependence of phototaxis response observed in the study.

While we tried to minimize the influence of intensity by measuring the distance from the light to the bottom of the tube, a number of errors could have occurred. First,

our light sources were not consistently turned off after every trial; this would have led to a warmer light if not turned off before the next replicate and result in heat as an extraneous variable, which may in turn create noise in our results. Second, we failed to use a light meter to measure the intensity of each light source and thus the intensity could have varied slightly between each experimenter. Additionally, we used four different light sources simultaneously in the experiment room; interference may have arisen from the light source adjacent to each experimenter.

Following the analysis of the mutation found in the *ort*^{1} gene in *D. melanogaster*, we found no significant difference in the phototaxis response between the light treatments tested. Gao *et al.* (2008) declared that although a phototaxis behavioral response is common and seen in a variety of insects, there is a preference exhibited when comparing different wavelength regions, and this preference can differ among species being tested. As an example, green light is often depicted as being a nutrient rich source (Storz and Paul 1998), and thus *Daphnia magna* (a water flea) are attracted to this wavelength region but on the other hand, avoid the harmful UV light.

As we failed to find a significant difference in the preference of light chosen in the mutant *D. melanogaster*, results are consistent with Gao *et al.* (2008). Previous studies indicated that the photoreceptor neurons, located in the retina, are histamine-regulating receptors, a characterization known as histaminergic. The receptor and target site most influenced by histamine is the ort (*ora transientless*) gene. It was stated that in order to exhibit a green light preference, *D. melanogaster* must contain the histamine gated chloride channel ort; mutations in the *ort*^{1} gene return a defect, in which the information obtained from the visual receptors R1-6 fail to transmit (Gao *et al.* 2008). To distinguish between different wavelengths, an organism must have at least

two visual receptors and a method of comparing the outputs of the receptors (Washington 2010). In Washington's study (2010) that was carried out, the mutant flies, when compared to the wild type, expressed a weaker phototaxis response towards green light by up to three orders of magnitude (Gao *et al.* 2008). When testing negative geotaxis, through the white-light treatment, mutations found in the ort^1 gene would not cause a defect in the motor response of *D. melanogaster* (Gao *et al.* 2008), and thus would result in a similar response to the other wavelengths. This is consistent with the data we obtained, as seen in Figure 5.

In the analysis of the phototaxis behavior based on wavelength, between both the wild-type and mutant *D. melanogaster*, we found a significant difference between the timed-responses. The largest difference was exhibited in the green light response. As previously discussed by Gao *et al.* (2008), a stronger phototactic response should be visible in wild-type flies. In contrast, mutant *D. melanogaster* exhibited a weaker response in the wavelength range, due to the defective ort^1 gene.

Discrepancies between the results we obtained and those found in the literature studied could be attributed to the sources of error found in our experiment. One source of error that could have contributed to the inconsistencies in our data was in our method of initiating the experiment, when we tapped the test tube to get the fly to the bottom. Occasionally, we would have to knock the tube vigorously in order to get the fly to the bottom; this may have shocked the fly and thus changed their reaction time. The biggest source of error would lie in biological variation, in the age of *D. melanogaster*. Although we attempted to use flies of the same age, the red light treatment flies were older and may have exhibited a delayed response because of this.

Conclusion

This experiment tested the phototaxis response to varying regions of the wavelength spectrum; the mutant *ort*^{1} and wild-type Oregon-R *D. melanogaster* were exposed to green, red and white light. The data from our experiment suggest that wavelength has no effect on the phototaxis response of *D. melanogaster*. Furthermore, the data suggest the presence of the *ort*^{1} mutation has no effect on the phototaxis response of *D. melanogaster*. However, the analysis provides support to the third hypothesis in which the effect of light wavelength on the phototaxis response of *D. melanogaster* is not the same in the wild type and mutant – the mutant *ort*^{1} *D. melanogaster* have characteristic a decreased phototaxis response to green light.

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