The effect of light wavelength on phototactic response in *Drosophila melanogaster* wild-type and *ort*^{1} mutant

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Abstract

Different spectral wavelengths of light exist in our natural environment that can influence an organism's behaviour. Some organisms may show a preference for different areas with certain wavelengths while others may completely avoid such regions. We were interested in the phototactic response to certain spectral wavelengths of light, in both the wild-type and *ort*^{1} mutant of *Drosophila melanogaster*. We performed two separate experiments for the wild-type and mutant strains, using green, red, and blue acetate filters at spectral wavelengths of 500, 680, and 410 nm, respectively. We constructed a setup with four t-tubes and measured the time spent by an organism in each of the three regions, in order to test if spectral wavelength preference was present. We found that for both the wild-type and mutant strains there was a significant difference between the percentages of time spent in each of the three different colour regions. For the wild-type strain, we found that the mean percentage of time spent in the green was the highest, followed by red. We found that the blue region had the lowest percentage of time, indicating the lowest preference. For the mutant strain, we found that the highest mean percentage of time was in the green region, followed by blue, and then red. We used the Kruskal-Wallis one way analysis of variance with an alpha *p*-value of 0.05 and found the wild-type had a *p*-value of 0.0226 and the mutant had a *p*-value of 0.028; therefore, we rejected the null hypothesis and found support for our alternate hypothesis; there is an effect of spectral wavelength on phototactic response in wild-type and mutant *D. melanogaster*.

Introduction

The principle objective for this experiment was to determine the effect of spectral wavelength on phototactic behaviour, which is the movement toward a light source (Fischbach 1979), in populations of the wild-type and *ort*^{1} mutants of *Drosophila melanogaster. D. melanogaster,* commonly known as the fruit fly, has a wild-type genome of approximately 180Mb with four chromosomes: X/Y, 2, 3, and 4, with approximately 13601 genes (Wixon & O'Kane 2000, Beckingham et al. 2005). The *ort*^{1} mutation is a deletion mutation of 569 nucleotides located in the *helA* gene (Attrill et al. 2016). The *helA* gene codes for histamine, which acts as a neurotransmitter that interacts with photoreceptors, and is a vital component for invertebrate vision, especially for *D. melanogaster*. The gene codes for histamine-gated chloride

channel subunits which are located in insect optic lobes (Zheng et al. 2002). The characteristics for this mutation are the following: defective optomotor response, defective visual behaviour, and defective phototactic behaviour, such as exhibiting lower positive phototactic behaviour in green and UV light (Bulthoff 1982; Gao et al. 2008; Rister et al. 2007).

Bausenwein, Dittrich and Fischbach (1992) studied various colour wavelengths and the differences between wild type and mutant, which led to a good literature foundation for our experiment. We were interested in expanding this research with *D. melanogaster,* which could be helpful to our understanding of how photoreceptors operate and how mutations can alter vision perception.

Hypotheses

For the wild-type *D. melanogaster,* our null hypothesis is that changes in spectral wavelength have no effect on wild-type *D. melanogaster* positive phototactic behaviour. Our alternate hypothesis is that changes in spectral wavelength have an effect on wild-type *D. melanogaster* positive phototactic behaviour.

For the mutant *D. melanogaster* our null hypothesis is that changes in spectral wavelength has no effect on positive phototactic behaviour in ort^1 mutant *D. melanogaster*. Our alternate hypothesis is that changes in spectral wavelength have an effect on positive phototactic behaviour in ort^1 mutant *D. melanogaster.*

Predictions

Adult wild-type *D. melanogaster* have specific neurons which contribute to phototaxis in response to colour wavelength as opposed to light intensity (Gong 2012). Specifically, the DM8 neurons promote the positive phototactic behaviour of *D. melanogaster* towards UV/green light preference (Gong 2012). In addition to preference for green light, due to the R8 photoreceptor

cells in *D. melanogaster* , blue light will also be somewhat preferred but less so than green (Bausenwein, Dittrich & Fischbach 1992). Literature was not found for a direct comparison of preference for red light over other light wavelengths in *D. melanogaster* preference; however we predict that *D. melanogaster* will exhibit the lowest preference towards red light since there is a larger spectral wavelength difference between red and green compared to blue and green. Thus we predict it will be the least preferred out of the three wavelengths. Our prediction for the relative ranking of time spent in spectral wavelength from most to least would be green (500 nm), blue (410 nm) and red (680 nm).

We also predict that mutants will spend an equal amount of time in different wavelength areas of light. The reason for this prediction is that mutant *D. melanogaster* have difficulty in photoreception, which significantly hinders their phototactic response (Gao 2008). With the deletion mutation, ort^1 mutant *D. melanogaster* should therefore not have a preference for a certain light wavelength because it is unable to differentiate between them (Benzer 1967).

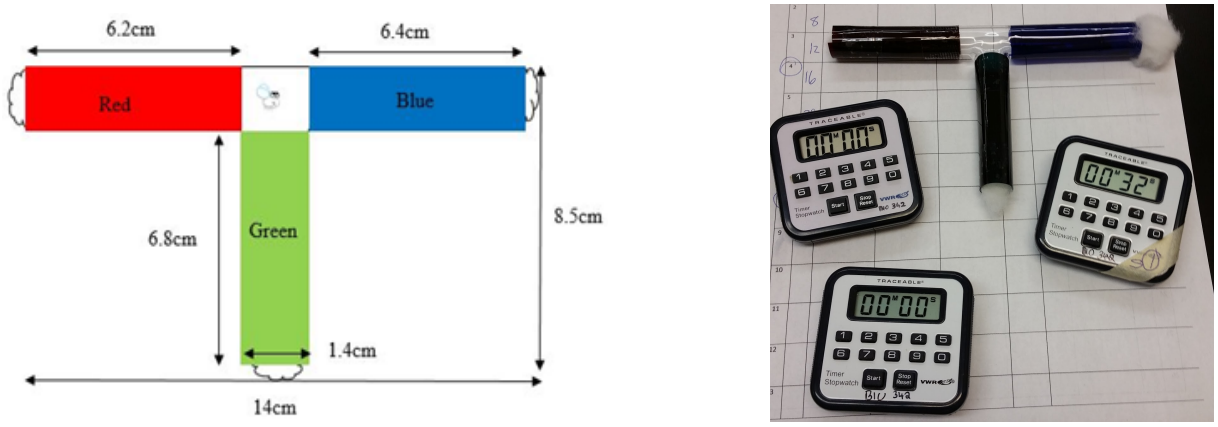
Methods

Materials and Setup

We used four glass t-shaped connection tubes in this experiment. We wrapped the three arms of the tube once in one of three coloured filters, leaving an exposed square section in the middle of the tube, which acted as our entry area (Figure 1a). Room light, 903 lux, was the only light source. The three coloured filters were red, green and blue, each with the following lux intensities: 18 lux, 31 lux, and 9 lux, respectively. The wavelengths for the coloured filters were 680 nm for red, 500 nm for green, and 410 nm for blue. To reduce experimental bias, we randomly ordered the coloured filters for each t-tube, resulting in four different orientations. The openings of two of the three sides of the t-tube were closed with cotton balls, while the third one

was left open for insertion of our subjects. The t-tubes were set above a white piece of paper to easily observe the *D. melanogaster*.

Each experimenter used four stopwatch timers to keep track of the time the *D. melanogaster* spent in each portion of the t-tube (Figure 1b).



a. b.

Figure 1a). One orientation for the coloured filters on a t-tube with the dimensions of the t-tube. Cotton was placed at the end of the tube to plug the openings. b). Experimental setup with the 3 timers used to keep track of the time one *D. melanogaster* spent in each arm of the t-tube

D. melanogaster Oregon R strain were bred in the laboratory and kept in vials with their food medium on the bottom of the vial. *D. melanogaster* of various life stages lived within the vials: the larvae were in the agar, the pupae were along the edges of the vial and the adults remained still along the wall or were flying around in the empty spaces within the vials. We used 25 wild-type and 25 ort^1 mutant *D. melanogaster*.

Procedure

We used carbon dioxide (CO_2) to anaesthetize the *D. melanogaster,* and they were exposed to CO_2 for 10 seconds. Once the *D. melanogaster* were anaesthetized, we used a small paintbrush to transfer each *D. melanogaster* to an empty vial plugged with a cotton ball.

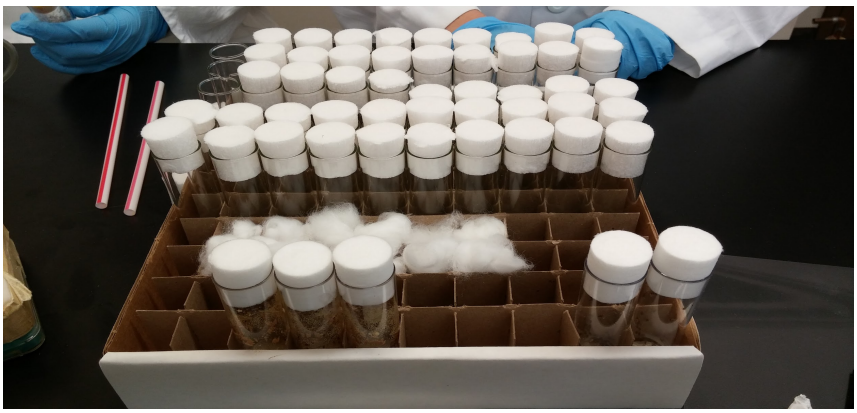


Figure 2. *D. melanogaster* transferred to 50 glass vials, one fly per glass vial. 25 glass vials for wild type and 25 glass vials for mutant ort^{1} *D. melanogaster*.

Each experimenter then took one vial and anaesthetized the *D. melanogaster* again, exposing *D. melanogaster* to the minimum amount of CO_2, with a maximum exposure of five seconds. We then immediately transferred the *D. melanogaster* into the t-tube and placed it in the centre of the exposed square section utilizing a straw or paintbrush (Figure 3). The side of the t-tube used to insert the fly was then plugged with the same cotton ball that plugged the other two sides.

Figure 3. Experimental setup with a wild-type *D. melanogaster* in the t-tube.

We started a five minute (300 seconds) countdown using our primary timer the moment the *D. melanogaster* began to exhibit decisive movement towards one direction of the t-tube. Fully recovery was defined as a definitive directional movement exhibited by the *D.*

melanogaster. Stationary movement, such as cleaning, was not considered full recovery. If the fly still did not reach full recovery after ten minutes in the t-tube, we started the five minute timer regardless.

We used three additional timers to time the portion of time spent in each arm of the tube. Every time the *D. melanogaster* entered a coloured area, we started the corresponding timer and stopped the timer the moment the *D. melanogaster* left the previous area. We continued as such until the five minutes were up. We then recorded the total time the *D. melanogaster* spent in each coloured arm of the t-tube, the sex, determined by examining the abdomen, and any additional qualitative observations.

When the five minutes were up, we anaesthetized the *D. melanogaster* again, removed it from the t-tube and placed it in the morgue. This process was repeated for the 25 ort^1 mutant *D. melanogaster*.

Statistical Analysis

The statistical analysis was completed on Excel 2013 using the online statistic engine Vassarstat (Lowry 2001). We utilized the non-parametric test Kruskal-Wallis one way analysis of variance, because our data were not normally distributed.

Results

We found that the average percentage of time spent in an area differed with the three spectral wavelengths tested for *D. melanogaster* wild type. Out of a total of five minutes, *D. melanogaster* wild type spent a mean 29 $\pm$ 14% of that time in the 680 nm red area, 34 $\pm$ 13% in the 500 nm green area, and 11 $\pm$ 11% in the 410 nm blue area (Figure 4). For the wild type, 25 replicates were completed, n = 25.

A nonparametric test Kruskal-Wallis one way analysis of variance was used with an alpha value of $p \leq 0.05$, and the resulting p-value was 0.0226, which is less than the alpha value (Figure 4). Since $p < 0.05$, the mean percentages for time spent in different spectral wavelengths are significant.

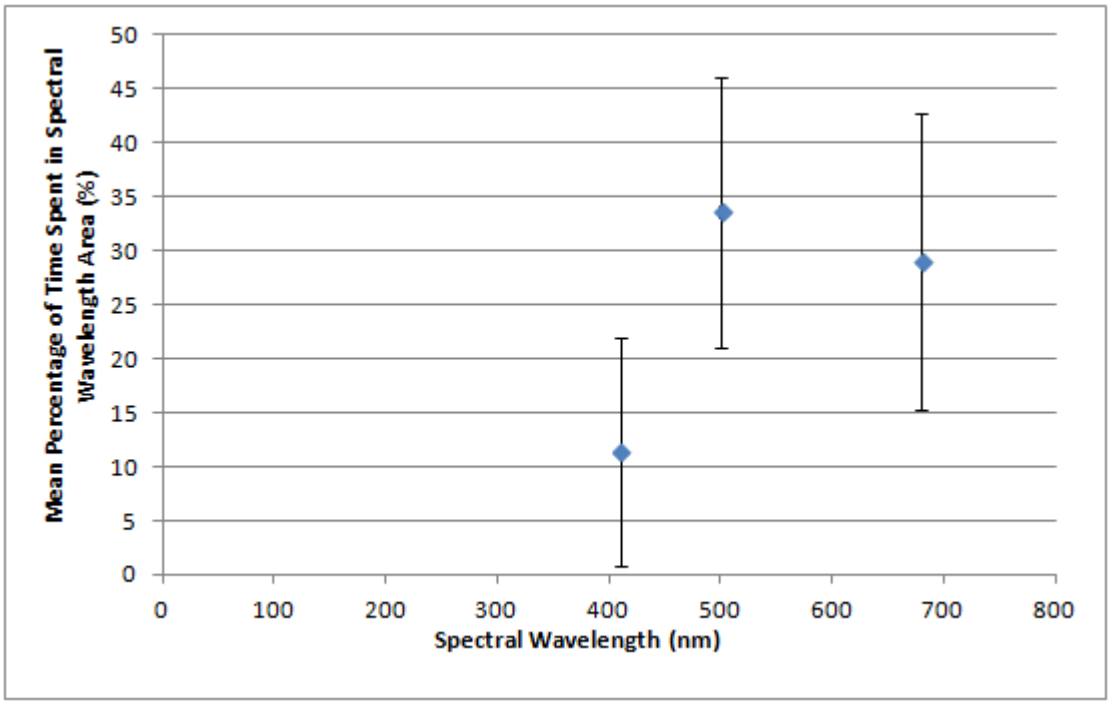


Figure 4. Mean percentage of time spent in spectral wavelength area (%) for *D. melanogaster* wild type. The points represent the mean percentage time spent in the three arms of the t-tube, each covered in a different coloured filter (n=25). Error bars represent 95% confidence intervals. The non-parametric test Kruskal-Wallis one way analysis of variance was used with a p-value of 0.0226 ($p \leq 0.05$).

For *D. melanogaster* ort^1 mutants, we also found that the average percentage of time spent in an area differed with the three spectral wavelengths tested. *D. melanogaster* ort^1 mutant spent a mean 14 $\pm$ 9% of the total five minutes in the 680 nm red area, 33 $\pm$ 12% in the 500 nm green area, and 17 $\pm$ 11% in the 410 nm blue area (Figure 5). For the ort^1 mutant, 25 replicates were also utilized, however only 18 replicates were used for the data analysis, resulting in n =18. Seven replicates were omitted due to the *D. melanogaster* not exhibiting definitive directional movement.

The mean percentage of time spent in the three spectral wavelength area were compared with Kruskal-Wallis one way analysis of variance with an alpha value of $p \leq 0.05$. The resulting *p*-value was 0.028 (Figure 5), thus there is a significant difference between the mean percentages for time spent in the different wavelength areas of the t-tube.

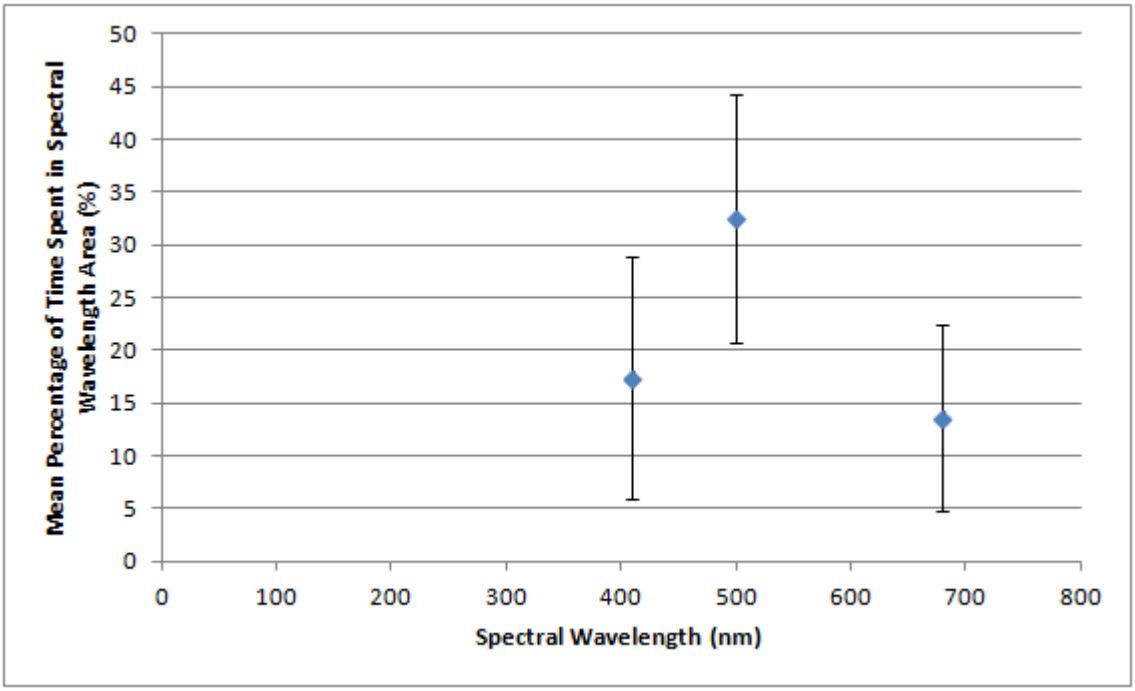


Figure 5. Mean percentage of time spent in spectral wavelength area (%) for *D. melanogaster ort*^{1} mutant. The points represent the mean percentage time spent in the three arms of the t-tube, each covered in a different coloured filter (n=18). Error bars represent 95% confidence intervals. The non-parametric test Kruskal-Wallis one way analysis of variance was used with a *p*-value of 0.028 ($p \leq 0.05$).

Discussion

Drosophila melanogaster wild type

The use of the Kruskal-Wallis statistical test allows us to determine whether the mean percentage times spent in the different wavelengths were significantly different from each other. The *p*-value for mean percentage of time spent in a tested area was 0.0226 which is less than $p = 0.05$. Therefore, we reject the null hypothesis and support the alternate hypothesis, that there is a significant difference in the time spent in each of the three spectral wavelength regions.

Our prediction for the wild-type *D. melanogaster* was that there will be a positive phototaxis exhibited for the green light spectral wavelength of 500 nm. We measured positive phototaxis based on the average length of time spent in a coloured light region; a longer time spent would correlate to a positive phototaxis for that spectral wavelength. Our results show that there is a higher mean percentage of time spent in the green light region of 500 nm at 34 $\pm$ 13%, compared to the red 680 nm and blue 410 nm light regions at 29 $\pm$ 14% and 11 $\pm$ 11% respectively (Figure 4) which support our prediction.

D. melanogaster shows a positive phototaxis towards green light due to the DM8 photoreceptor detection of the green light and the innate preference towards that wavelength (Gao et al. 2008). Furthermore, *D. melanogaster* also exhibits a strong optomotor response, which is modulated by the strength of stimulus light detected by the DM8, R1-R6 and R8 photoreceptors towards green light (Hecht & Wald 1934, Heisenberg & Buchner 1977). Contrarily, the phototaxis towards blue 410 nm and red 680 nm light regions is lower compared to the phototaxis for green light. The lower preference for blue light could be due to the toxic effects of the shorter wavelengths in blue light (Hori 2014) as well as the retinal damage induced in the presence of blue light (Stark, Walker & Eidel 1985). The lower preference for red light could be due to the *D. melanogasters'* insensitivity to the red 680 nm spectral wavelength (Hanai, Hamasaka & Ishida 2008).

The detection of light and wavelength-sensitive phototactic behaviour of *D. melanogaster* can be attributed to their inability to self-regulate body temperature. The *D. melanogaster* ectoderm cannot retain heat and thus they must rely on the external environment to provide heat (Dillon et al. 2012). Using their central nervous system to detect temperature, *D. melanogaster* will have a strong tendency for phototaxic behaviour as they find the optimal wavelength to

maintain their body temperature at a sustainable level (Dillon et al. 2012). In combination with R8 photoreceptor mentioned previously, *D. melanogaster* will be guided towards the green-light wavelength from their innate biological urge.

Drosophila melanogaster ort^{1} mutant

For the *ort*^{1} mutant *D. melanogaster* the *p*-value for mean percentage of time spent in a tested area was 0.028, which is less than $p = 0.05$. Therefore, we rejected the null hypothesis and provided support for the alternate hypothesis, which is that there would be a significant difference in the time spent in each of the three spectral wavelength regions.

Our prediction for the mutant *ort*^{1} *D. melanogaster* was that they would not exhibit a significant preference towards one spectral wavelength over the other, as their mutation affects communication to their photoreceptors (Gao et al. 2008) and therefore would spend a relatively equal amount of time in each of the three coloured light regions. We originally thought that because of *D. melanogasters'* decreased photodetection capabilities, they would not be able to efficiently distinguish between the three different colours and their presence inside each of the colours would be simply due to chance. Contrary to our prediction, the mutant *D. melanogaster* did exhibit a preference for green light with a higher 33 $\pm$ 12% of the total five minutes spent in the green 500 nm light area compared to the lower 14 $\pm$ 9% and 17 $\pm$ 11% spent in the red 680 nm area and blue 410 nm area respectively.

Gao et al. (2008) found that the *D. melanogaster ort*^{1} mutant exhibits a lower positive phototaxis to green light compared to wild-type *D. melanogaster* due to its photoreceptor mutation. There is a current method that can be used to compare mutants in ongoing studies. Utilizing a two-dimensional digitization, it is possible to compare sets of neurons in different mutant strains of *D. melanogaster*, which tend to possess reduced visual lobes or have a visual

perception deficit (Bausenwein, Dittrich & Fischbach 1992). This method could also be used to compare the resulting phototactic behaviour after exposure to various colour wavelengths (Fischbach & Dittrich 1989).

Being a complex behavioural response, phototaxis is only exhibited accurately when the following process occurs: light is absorbed by a receptor cell, which produces a neural stimulation, and is transmitted and integrated into the central nervous system (Benzer 1967). A comparison with other input results and then an appropriate motor signal is produced, which results in phototactic behaviour. If there is a deficit in any one of these steps then there can be an altered or an elimination of the phototactic response, and negative phototactic behaviour will be observed (Benzer 1967). Our results showed a significant preference for green light compared to blue and red light, this could be perhaps not be due to a positive phototaxis towards green light since *ort*^{1} mutant are not as sensitive to green light (Gao et al. 2008), but rather a negative phototaxis towards red and blue light, resulting in what appears as a positive phototaxis for green light by default. More research needs to be done in this field in order to find out whether *D. melanogaster* shows both positive and negative phototactic behaviour.

Drosophila melanogaster wild-type and *ort*^{1} mutant

We found that our 95% confidence overlap for both the wild-type and mutant strains for all three spectral regions. However, we still reject the null hypothesis due to the significance of the Kruskal-Wallis statistical test.

For both the wild-type and mutant experiments, we rejected the null hypothesis and provided support for our alternate hypothesis which stated there would be a significant difference between the percentage of time spent in each of the three different colour regions.

Sources of uncertainty

Differences in human reaction time were a major influence of uncertainty in our experiment. In addition to having to alternate between three stopwatches, we had to observe the movement of the *D. melanogaster* and react accordingly by starting one stopwatch and stopping another. The rapid movements of the *D. melanogaster* were at times difficult to follow and resulted in large uncertainties in the timing process. Biological uncertainty was another factor to consider when doing our experiment. We observed qualitatively that there were instances where the *D. melanogaster* would remain static in the control region whereas in other instances, *D. melanogaster* would fly and switch from region to region erratically.

Other biological traits such as size, relative age and sex of the organism were not considered in our experiment but may have contributed to our results, for example larger male *D. melanogaster* move more than smaller *D. melanogaster* (Partridge, Ewing & Chandler 1987). The dimensions of the t-tubes were quite narrow and larger test subjects will have a more difficult time navigating between the different regions.

Light intensity is an important extraneous variable that could have influenced our results. The coloured film distorted the wavelength of light entering the tube but at the same time reduced the intensity of light. Room light in the control region was 903 lux which is not optimal for the *D. melanogaster* since the preference of both the mutant and the wild type alike is seven lux (Rieger et al. 2007). In comparison, regions where the t-tube was covered with an acetate filter had an average lux of 19. *D. melanogaster* may be inclined to move to a coloured region since the light intensity is much lower.

Conclusion

Wild-type and mutant *D. melanogaster* have a significant preference for the green spectral wavelength when compared to blue, and red light. This resulted in our rejecting our two null hypotheses, and providing support for our two alternate hypotheses, where changes in spectral wavelength did have an effect on wild-type *ort*^{1} mutant *D. melanogaster* positive phototactic behaviour. Although the results agree with our prediction for wild-type, it contradicted our prediction for the mutant. This discrepancy in our prediction for the mutant could be a result of the biological variation of different *D. melanogaster* but could also be due to the large amount of variation that resulted from a variety of extraneous variables.

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