Wild-type and mutant *ort*^{1} *Drosophila melanogaster* travel varying distances in response to different light intensity gradients

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Abstract

The objective of our study was to investigate whether different light intensity gradients had an effect on the movement of wild-type and *ort*^{1} mutant *Drosophila melanogaster.* It has been found that R1-R6 and R7-R8 photoreceptors, which are commonly found in wild-type *D. melanogaster*, are missing or are defective in the *ort*^{1} mutant *D. melanogaster*. This leads to a decreased ability to detect light and lowers phototactic response of the mutant *D. melanogaster.* We hypothesized: 1) light intensity gradients have an effect on the distance travelled towards light, 2) the presence of a mutation has an effect on the distance travelled, and 3) the effect of the light intensity gradients is different in wild-type and mutant *D. melanogaster*. We tested 36 mutant and 36 wild-type *D. melanogaster* at light intensity gradients of 0 lux, 0-500 lux, and 0-1000 lux, and measured the distances travelled toward the light source within a large test tube. We found that the wild type moved more in the 0 lux and 0-500 lux treatments, while the *ort*^{1} mutants moved more in the 0-1000 lux treatment. We conducted a two-way ANOVA and obtained p-values of H_{O1}:0.06, H_{O2}:0.17, H_{O3}:0. Although we observed similar trends as seen in the literature, due to these p-values, we failed to reject all three of our null hypotheses.

Introduction

Drosophila melanogaster, also known as the common fruit fly, has been studied extensively in many fields of science such as regenerative biology, pharmacology, and bioengineering (Jennings 2011). In addition to temperature, the overall fitness of *D. melanogaster* depends on the light intensity (De *et al.* 2013). The objective of this experiment was to investigate how *D. melanogaster* and the *ort*^{1} mutant move in different light intensity gradients.

D. melanogaster has photoreceptors that regulate voltage channels and changes in light absorbance (Juusola & Hardie 2001). Its compound eyes contains eight different photoreceptors (Figure 1) that are interconnected to help increase its visual sensitivity (Borst 2009). The six outer photoreceptors, R1-R6, are responsible for detecting motion and dim light conditions. The two inner photoreceptors, R7 and R8, are responsible for positive

phototactic behavior, an attraction towards light, and color sensitivity (Yamaguchi *et al.* 2010). Sarthy (1991) suggested that the *D. melanogaster* photoreceptors use histamine as a major neurotransmitter; the function of the neurotransmitter is to maintain vision in *D. melanogaster* and control phototactic responses (Gengs *et al.* 2002).

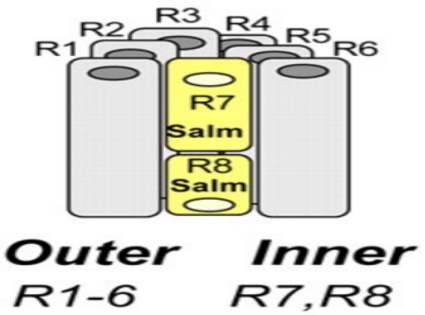

Figure 1. R1-R8 receptors of *Drosophila melanogaster* eyes: Xie *et al*. (2007) found that R1-R6 are outer receptors and R7-R8 are inner receptors.

O'Tousa *et al.* (1989) found that the ort^{1} mutant type of *D. melanogaster* lacks functional R1-R6 receptors due a deletion within the *ort* gene. They also discovered that the mutation does not influence the structure of photoreceptors, but affects the visual process instead. It was found that the mutant has an altered regulatory gene that affects the synaptic transmission between the R7 and R8 photoreceptors (Gengs *et al.* 2002). This alteration results in the mutant *D. melanogaster* having a lower phototactic response (Gengs *et al.* 2002).

These R1-R8 receptors are important for the visual system of *D. melanogaster.* The wild type has functional receptors that aid its visual perception. However, the mutant has abnormal receptors because of the deletion within the *ort* gene. Therefore, we suspected that lack of these receptors and the altered neurotransmission influence their movement toward light. Because of this, our experiment investigated how wild type and the mutant move in different light-intensity gradients. This experiment can contribute to future research because

we can apply this knowledge towards other insects with compound eyes that have histamine as a major neurotransmitter.

Initially, we examined the effect of light intensity gradients on *D. melanogaster*. Our H_{O1} states that light intensity gradients have no effect on the distance travelled towards light by *D. melanogaster*; our H_{A1} states that light intensity gradients have an effect on the distance travelled towards light by *D. melanogaster*. Next, we tested whether the presence of *ort*^{1} mutation has an influence on the distance travelled towards light. Our H_{O2} states that the presence of the *ort*^{1} mutation has no effect on the distance travelled in light intensity gradients by *D. melanogaster*; our H_{A2} states that the presence of the *ort*^{1} mutation has an effect on the distance travelled in light intensity gradients by *D. melanogaster*. Finally, we examined the effect of the light intensity gradient in *ort*^{1} mutants and wild-type *D. melanogaster*. Our H_{O3} states that the effect of light intensity gradients on the distance travelled by *D. melanogaster* is the same in the wild type and mutant; our H_{A3} states that the effect of light intensity gradients on the distance travelled by *D. melanogaster* is not the same in the wild type and mutant.

We predict that wild-type *D. melanogaster* will travel less distance in the dark environment (control) compared to the other two treatments because it has R1-R6 photoreceptors to detect low light intensities and it has a preference for activities, such as feeding, grooming, and resting, in a 5 to 10 lux environment (Rieger *et al.* 2007). Also, we predict that the *ort*^{1} mutant *D. melanogaster* will have overall less movement compared to the wild type in each light intensity gradient due to decreased phototactic response caused by the defective synaptic transmission (Gengs *et al.* 2002).

Methods

The apparatus for our experiment consisted of a ring stand, ring clamp, large test tube, cardboard divider, and an adjustable lamp (Figure 2). We first took a test tube, with

dimensions of four cm in diameter and 27 cm in length, and labelled it with one cm increments using masking tape from 0-27 cm. We placed a cotton ball into the closed end of the test tube and attached the test tube via a ring clamp onto a ring stand horizontally. Then we placed a lamp under the opening end (27 cm) of the tube. To ensure light would only strike this end, we constructed a divider out of cardboard to separate the lit end of the tube from the dark end of the tube.

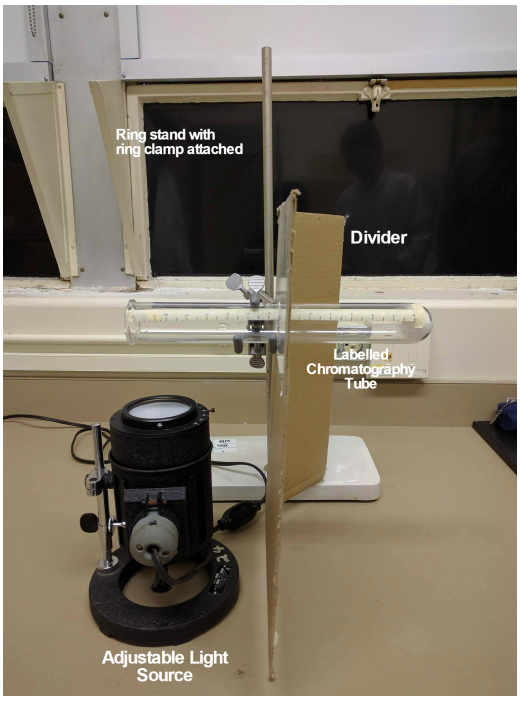

Figure 2. Setup for testing the distance travelled by wild-type and mutant *ort*^{1} *Drosophila melanogaster* in response to different light intensity gradients.

To assist with transferring of the *D. melanogaster* we obtained 12 *Drosophila* of the same genotype at a time, since each light intensity treatment had 12 replicates. The vial with the *D. melanogaster* and food medium was exposed to approximately eight seconds of carbon dioxide to temporarily anesthetize the *D. melanogaster*; this exposure time prevented most of them from moving for about one to two minutes. During this sedation period, we took a paint brush and swept the *D. melanogaster* into separate vials so that future transfer, once the *D. melanogaster* had woken up, was easier. Indication of the *D. melanogaster* waking up was when we observed twitching movement or *D. melanogaster* walking. We used a drinking

straw to trap the *D. melanogaster*, and then let the *D. melanogaster* walk up into the straw, covering both ends of the straw with our fingers so they couldn't escape. We then placed one end of the straw within our test tube and blew air into the straw so the *D. melanogaster* would start the experiment at the 0 cm point of the tube.

We conducted our experiment at three different light intensity gradients with 0 lux being the control and the other two light intensity gradients being treatments at 0-500 lux and 0-1000 lux. For 0 lux, once the *D. melanogaster* was at the 0 cm end of the tube, one individual turned off the lights, started the timer, and called out every 15 seconds until one minute had passed, so a recording could be made. Two other group members covered the opening of the tube with cotton, observed the *D. melanogaster* in the dark using the night-vision mode of a camcorder (Sony Handycam DCR-SR200), and made recordings of the *D. melanogaster*'s position at the time intervals aforementioned. The last group member took qualitative notes and wrote down the measurements. After each replicate ended, the *D. melanogaster* were disposed in a "morgue", which was composed of soapy water, and then the next replicate was conducted. We repeated this procedure with 24 *Drosophila*, 12 wild type and 12 mutants for each light intensity gradient, at 0-500 lux and 0-1000 lux. We used an adjustable lamp to create these light intensity gradients. We rolled a die to ensure that direction of the tube was randomized before every replicate. Temperature and light intensity were also measured before every replicate to ensure that the light intensity and temperature were constant.

We calculated the means for the distance travelled from the starting point by averaging the distance recorded for each 15 second interval for both the wild type and ort^1 mutant. We did this for each of the three treatment levels and then we performed a two-way ANOVA test in order to analyze our data. The two-way ANOVA test, the calculations for the mean distances, and the 95% confidence intervals for the distances were all done using

Microsoft Excel. The two factors we tested for are whether or not a light intensity gradient has an effect on the distance travelled by the *D. melanogaster* and whether or not a presence of mutation had an effect on the distance travelled by the *D. melanogaster*. We plotted one scatterplot with 95% confidence intervals that has both the wild type and ort^1 mutants for each of the three treatment levels.

Results

At the 0 lux treatment, the activities of the mutant and wild-type *D. melanogaster* were very similar. Both samples of the *D. melanogaster* had minimal movement and displayed fairly similar distances travelled within the test tube. The mean distances travelled with 95% confidence intervals for the first treatment of 0 lux were 4.64 cm ± 3.53 cm for the wild type and 3.00 cm ± 1.82 cm for the ort^1 mutant (Figure 3). Activities of both the wild-type and the mutant ort^1 *D. melanogaster* increased at the 0-500 lux treatment. Both sets of *D. melanogaster* were moving faster and had more vertical movement within the tube. We observed that the wild-type *D. melanogaster* travelled closer to the light source at 0-500 lux with the mean distance travelled with 95% confidence intervals 11.36 cm ± 4.87 cm and 4.50 cm ± 3.02 cm for the ort^1 mutant (Figure 3). Compared to the activity of the *D. melanogaster* at 0-500 lux, at 0-1000 lux the activity decreased for the wild-type *D. melanogaster*, but the ort^1 mutant travelled farther distances (Figure 3). Although the ort^1 mutant *D. melanogaster* had a higher distance travelled at 1000 lux, all the *D. melanogaster* moved slower and seemed hesitant to move towards the light source compared to 0-500 lux. The mean distances travelled with 95% confidence intervals for the first treatment of 0-1000 lux were 7.27 cm ± 4.71 cm for the wild type and 9.02 cm ± 4.54 cm for the ort^1 mutant (Figure 3).

From the two-way ANOVA, the p-value for the effect of a light intensity gradient on distance travelled was 0.06, the p-value for the effect of the presence of a mutation on

distance travelled was 0.17, and the p-value for the difference in distance travelled between both wild type and mutant was 0.10.

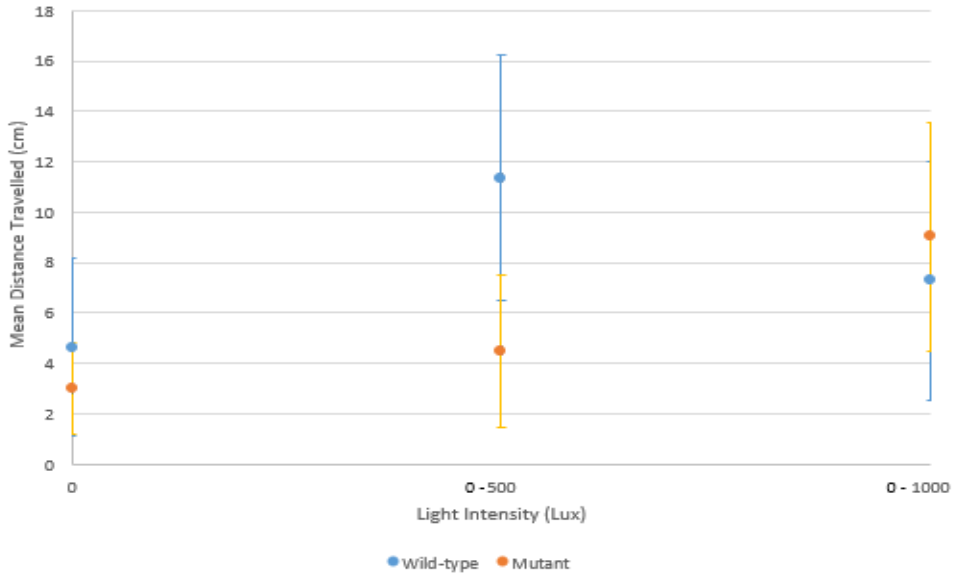

Figure 3. Mean distances travelled by wild-type and mutant *ort*^{1} *D. melanogaster* in centimeters at 0 lux, 0-500 lux, and 0-1000 lux. Bars represent 95% confidence intervals. Sample size for each treatment, n = 12. H_{O1}: p-value 0.06, H_{O2}: p-value 0.17, H_{O3}: p-value 0.10.

Discussion

Based on the results obtained from the two-way ANOVA test, we failed to reject all three null hypotheses, H_{O1}, H_{O2}, and H_{O3}, and could not provide support for their respective alternative hypotheses, H_{A1}, H_{A2}, and H_{A3}. This is because the p-values for each hypothesis, 0.06, 0.17, and 0.10, respectively, were greater than the 0.05 significance level. This indicates that the light intensity gradient has no significant effect on the distance travelled towards light by *D. melanogaster*. Also, this shows that the presence of the *ort*^{1} mutation has no significant effect on the distance travelled in a light intensity gradient by *D. melanogaster*. Finally, this shows that the effect of a light intensity gradient on the distance travelled by *D. melanogaster* has no significant difference between the wild type and mutant.

At the 0 lux treatment (control), *D. melanogaster* had the lowest movement compared to the other two treatments (Figure 3). This trend agrees with our prediction and shows consistency with Rieger *et al.*'s (2007) experiment. They set up various light intensity

gradients, such as 50 to 1000 lux, 5 to 100 lux, and 0.5 to 10 lux, and found that *D. melanogaster* showed preference for resting activities under low-light environment (5 to 10 lux). Parsons (1974) also observed that the movement of *D. melanogaster* was at a minimum once they reached the dark region of the box. The results obtained in our experiment are consistent with what is found in nature, where *D. melanogaster* can be commonly seen feeding on decaying fruits in shaded areas (Rieger *et al.* 2007).

The wild-type *D. melanogaster* were the most active and travelled the longest distance towards the light in the 500 lux treatment (Figure 3). Both Parsons (1975) and Kempinger *et al.* (2008) also observed that wild-type *D. melanogaster* were more active in high light intensity. Parsons (1975) set up a light intensity gradient from 10 to 590 lux inside a box and found that *D. melanogaster* favored the 590 lux intensity. *D. melanogaster* was shown to display phototactic behavior and try to escape towards the light source when startled (Kain *et al.* 2012; Rieger *et al.* 2007). In contrast, the ort^1 mutant only showed a minor increase in distance travelled towards the light. Gao *et al.* (2008) reported that the ort^1 mutant displayed a lower phototactic response due to the mutated *ort* gene. The ort^1 *D. melanogaster* has malfunctioning histamine chloride channels, which are responsible for vision control and phototactic response (Gengs *et al.* 2002). Gao *et al.* (2008) also found that the motor response of *D. melanogaster* is not affected by mutation in the *ort* gene. Therefore, the short distance travelled by the ort^1 mutant is due to a visual defect (Gao *et al.* 2008).

Contrary to our predictions, the ort^1 mutants travelled a greater distance than the wild-type *D. melanogaster* in the light intensity gradient of 0 to 1000 lux (Figure 3). Since this mutant has a deletion in the *ort* gene, the lack of R1-R6 photoreceptors causes the mutant to have difficulties detecting the difference in light intensity (Yamaguchi *et al.* 2010). Also, Gao *et al.* (2008) stated that at high light intensities, mutant *D. melanogaster* could elicit phototactic responses. This explains the mutant *D. melanogaster* travelling further in 1000

lux compared to the other two treatments. De *et al.* (2012) found that wild-type *D. melanogaster* will seek shelter to rest and hide when the light intensity and temperature in the environment are too high. Also, Rieger *et al.* (2007) suggested that *D. melanogaster* have the tendency to avoid bright light intensities to prevent the damage caused by UV light. Wild-type *D. melanogaster* possesses functional R1-R6 photoreceptors that can detect low light intensities (Gong 2012). Therefore, it could choose a more favorable environment, and the ort^1 mutant cannot.

It has been reported that *D. melanogaster* carry functional variations in response to light both between and within species (Kain *et al.* 2012). They also found that within certain species, such as *Drosophila simulans*, some have a strong attraction towards bright environments, while some have a strong preference towards the dark environment. Even though the individuals are genetically identical, they display opposite behaviors (Kain *et al.* 2012). Parsons (1975) also observed that when *D. melanogaster* are in a light intensity gradient, not all *D. melanogaster* will move towards the high light intensity. Parsons (1975) suggested that some *D. melanogaster* are naturally negatively phototaxic and will not be attracted towards a light source. The internal difference within *D. melanogaster* could contribute to the variation in the data collected.

We believe uncertainty within our experimental setup arose from two main sources. The first is that moisture formed within the test tube where *D. melanogaster* were tested. As we would transport *D. melanogaster* using the straw method mentioned previously, when air was blown into the straw, the air itself would cause the test tube to fog up. This fog made the inside of the tube slippery for *D. melanogaster* to walk on, and we noticed that sometimes *D. melanogaster* would slip as they would try walking up the walls of the tube. The presence of this moisture may have physically prevented *D. melanogaster* from walking across the whole length of the tube. The second source of uncertainty we believe was the effect of carbon

dioxide on each individual *D. melanogaster*. We noticed that although each treatment group was subject to the same time of exposure to carbon dioxide, some *D. melanogaster* seemed to become active faster, while other *D. melanogaster* remained groggy after the same time duration. The effect of the carbon dioxide may have physically altered the movement and response of the *D. melanogaster* to the light intensities.

Conclusion

The results from our study showed that both light intensity gradient and the presence of ort^1 mutant have no significant effect on the distance travelled towards light by *D. melanogaster*. Furthermore, our data found that there is no significant difference on the distance travelled towards light between wild-type and mutant *D. melanogaster.* However, the trend obtained from our data showed some support for our prediction that wild-type *D. melanogaster* travels the least in dark environments as they prefer resting activities under dim light environments.

Acknowledgements

We would like to thank Professor Carol Pollock and Professor Celeste Leander for all of their advice on experimental design, statistical analysis, and feedback provided throughout this project. We would also like to thank the course technician, Mindy Chow, for the feedback provided on our experimental setup as well as providing us with the materials required to conduct this experiment. We would also like to thank our teaching assistant, Jordan Hamden, for assistance with any questions we had, specifically on statistical analysis. Finally, we would like to thank the University of British Columbia for providing us the opportunity and environment to perform this experiment.

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