The effect of light exposure on *Drosophila melanogaster* survival

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ABSTRACT

The purpose of our study was to observe how exposure to light affects the survival of adult *Drosophila melanogaster*. We exposed groups of adult *D. melanogaster* to three different treatments: constant light (24L), constant darkness (24D), and a circadian cycle of 6 hours light and 18 hours darkness (6L18D). Our data showed no significant difference between the numbers of *D. melanogaster* still alive in each of the three treatments after 16 days, although the 24L treatment did experience earlier mortality than the other two treatments. The final counts for the 24L, 6L18D and 24D treatments were 1.8 ± 0.7, 2.6 ± 1.2 and 2.6 ± 0.5 individuals respectively. Based on our results we were unable to reject our null hypothesis that stated the survival of *D. melanogaster* decreases or is not affected by a decreased exposure to light. Although our research did not generate any significant differences among the treatments, our experiment yields useful information on how to set up future research on the survival of *D. melanogaster*.

INTRODUCTION

Drosophila melanogaster, also known as the common fruit fly, is a sexually reproducing organism that is known to be light dependent (Chadha 2008). It is important to study factors that affect *D. melanogaster* because they contribute to understanding disorders in humans such as aging and neurodegenerative diseases since their genome is similar to that of the human genome (Prasad and Hedge 2010).

D. melanogaster fitness and mating behavior is affected by the amount and type of light to which they are exposed (Sheeba *et al.* 2000). Previous experiments done on *D. melanogaster* have shown that the organism lives longer under dim lighting, but there has yet to be an explanation behind these findings (Allemand *et al.* 1973).

The effect of light exposure on *D. melanogaster* requires further research because data that have been gathered do not give statistically significant results (Sheeba *et al.* 2000). We felt it was appropriate to focus our experiment on exposure to light since the effects of exposure to light on survival have been studied to a lesser extent than other factors.

Figure 1. A wild-type female adult *D. melanogaster*, distinguishable by its red eyes and the bands on its abdomen.

It has also been found that *D. melanogaster* depend on light to stimulate their mating behaviour; exposure to less or more light changes the number of eggs they lay, and the number of eggs they lay play a role in their life span (Chada 2008). Mating speed, copulation, and fitness have also been measured under varying levels of light in previous experiments, and it was found that, of these, copulation speed was the only factor that varied under changing light exposures (Allemand *et al.* 1973).

Subsequently, because previous studies suggest *D. melanogaster* has a preference for exposure to less light (Rieger *et al.* 2007), we have chosen our alternate hypothesis to state that *D. melanogaster* survival increases as the organism's exposure to light decreases. Our null hypothesis states that *D. melanogaster* survival decreases or is not affected by decreased exposure to light. The objective of our research is to provide further evidence for whether or not a correlation exists between light exposure and *D. melanogaster* survival.

METHODS

For this experiment, Oregon-R wild-type *D. melanogaster* were used. We wanted the biological variation of the *D. melanogaster* to be minimized as much as possible; therefore, we collected individuals of approximately the same age on which to carry out our experiment. We did this by taking several different vials of wild-type *D. melanogaster*, and removing all the adults from the vials. We set the vials aside and then waited approximately 4 hours to collect the new adults that had just matured from the pupa stage; this procedure allowed us to have a group of *D. melanogaster* that were all approximately the same age with a variation of just a few hours.

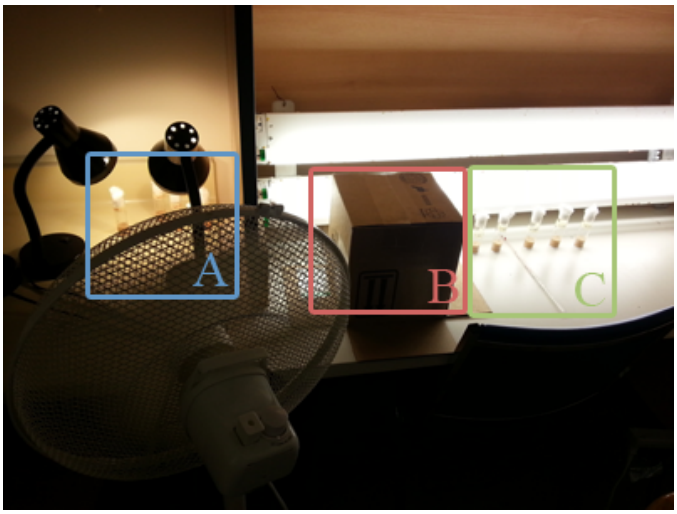

Figure 2. Our experimental set up of the three different treatments: A was 24 hours light, B was 24 hours darkness, and C was 6 hours light & 18 hours dark

Three different light and dark treatments were set up in our experiment: 24 hours a day of constant exposure to light (24L), 24 hours a day of constant darkness (24D), and finally a circadian cycle of 6 hours light and 18 hours dark (6L18D). According to Reiger *et al.* (2007) D. *melanogaster* are usually found hiding in dark places away from the light when in the wild, therefore we treated the 6L18D treatment as our control. For each of the treatments, five replicates were set up, for a total of fifteen vials (which were all filled with agar medium for the *D. melanogaster* to feed on). From the number of adult *D. melanogaster* that were available after we removed all the older flies, we were able to place four individuals into each replicate vial: 2 males and 2 females. The vials were set up this way to minimize any stress that could have been caused due to different interactions between male and female individuals (e.g. competition for mating). The sexes of the *D. melanogaster* were identified by examining each individual and

looking for the characteristics on their abdomen that were different between the two sexes: thin bands on females (see Figure 1), and a solid black area on the ends of males.

For the three different treatments, the vials were all set up in a small, windowless room, in order to keep the temperature as constant as possible. To account for the increase in temperature due to the heat produced by the lamps used for the 24L treatment, we placed a fan in front of the experimental area, and had it constantly fanning the vials, as shown in Figure 3, over the data collection period. No fan was required for the other two treatments because no extra heat was produced in their respective experimental areas. The temperature inside the room that we used was usually between 24 and 27°C, with the optimal temperature for *D. melanogaster* being 25°C (Bonnier 1961, Haji and Lee 2011).

For the data collection process, we returned to the lab on every Monday, Wednesday and Friday at 12:00 P.M. during our data collection period of 16 days, and counted the number of *D. melanogaster* that were still alive in each vial. Individuals were identified as dead if they were observed to have not been responsive to us picking up the vial and moving it around (live individuals would fly around when the vial was moved). If we observed any pupae on the sides of the vials, we transferred the adults into new vials with fresh agar medium. This was done to ensure that we were only working with the original *D. melanogaster* individuals we started off with at the beginning of the experiment.

A total of eight sets of were collected for each treatment over the 16 days of the experiment. We analyzed the data by calculating the mean and 95% confidence intervals for each treatment for all eight data sets.

RESULTS

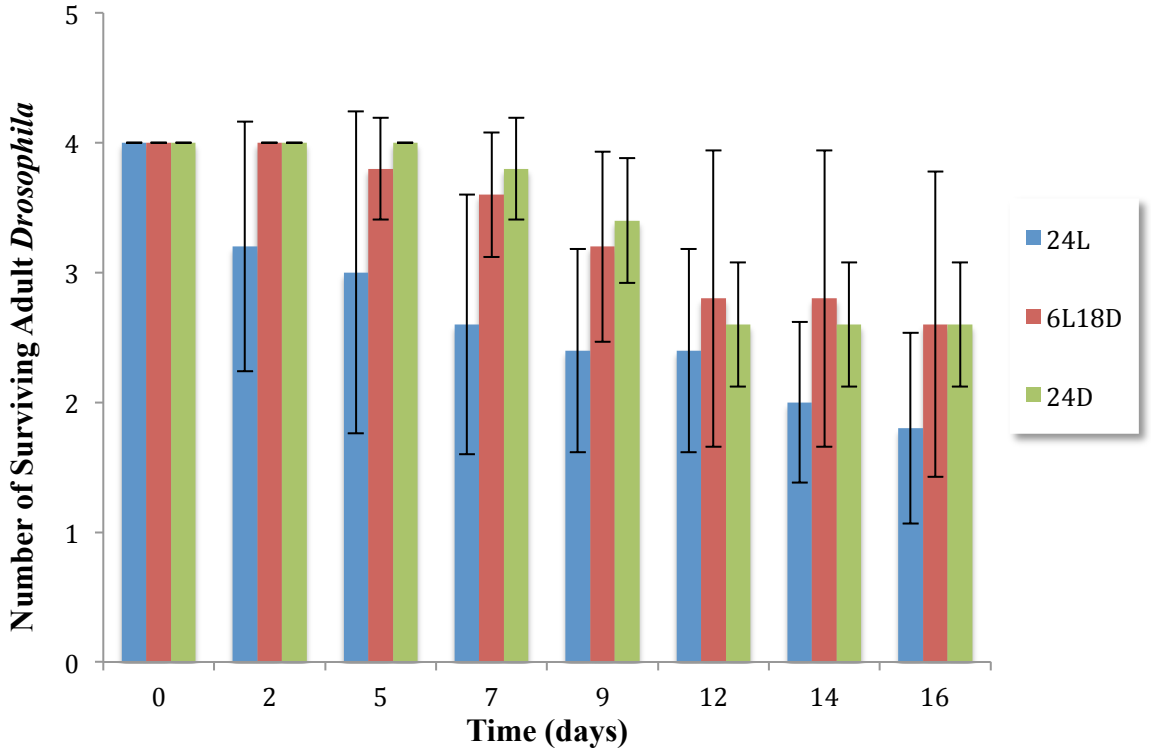

Figure 3. The effect of 24 hours light (24L), 6 hours light & 18 hours dark (6L18D), and 24 hours dark (24D) circadian cycles on adult *Drosophila melanogaster* survival over a period of 16 days at a temperature of 25.5±1.5°C. Bars represent mean number of individuals, error bars represent 95% confidence intervals, n=5.

For the three different treatments - 24 hours light, 6 hours light & 18 hours dark, and 24 hours dark -, there was no significant difference in the number of *D. melanogaster* still alive in each treatment when comparing the number of individuals for each day. As Figure 3 indicates, all three treatments had overlapping 95% confidence intervals of their means. There was however, a trend showing an earlier mortality in the 24L treatment when compared to the 6L18D and 24D treatments. The 24L treatment also had the least number of surviving *D. melanogaster* at the end of the experiment as shown in Figure 3. After 16 days, the 24L treatment had a mean number of 1.8±0.7 *D. melanogaster* individuals. This was lower than the 6L18D and 24Dtreatments, which had means of 2.6±1.2 and 2.6±0.5 respectively at the end of the data collection period. Although

Figure 3 shows similar survivorship levels for the 6L18D and 24D treatments, the 24D treatment had better survivorship until day 9. In addition, the 24D treatment has less variation in data as it had smaller 95% confidence intervals than that of the 6L18D treatment throughout the experiment.

Sample Calculations – 24 hours light (24L) treatment, day 16

$$\boldsymbol{mean} = \bar{\boldsymbol{x}} = \frac{replicate\ 1 + replicate\ 2 + replicate\ 3 + \cdots + replicate\ n}{n} = \frac{2+3+1+2+1}{5} = \mathbf{1.8}$$

$$\boldsymbol{standard\ deviation} = \boldsymbol{\sigma} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_1 - u)^2} = \sqrt{\frac{(2-1.8)^2 + (3-1.8)^2 + (1-1.8)^2 + (2-1.8)^2 + (1-1.8)^2}{5}} = \mathbf{0.83666003}$$

$$\mathbf{95\%}\ \boldsymbol{confidence\ interval} = 1.96 \times \frac{\sigma}{\sqrt{n}} = 1.96 \times \frac{0.83666003}{\sqrt{5}} = \mathbf{0.73336485}$$

DISCUSSION

Based on the results of our experiment, we failed to reject our null hypothesis, which stated that, the survival of *D. melanogaster* decreases or does not change when exposure to light is decreased. Although our data failed to support our alternate hypothesis with significant differences in the number of surviving adults between 0 and 16 days for each of the three treatments, there was an earlier mortality observed in the *Drosophila* that were exposed to light for the entire duration of the experiment (24L treatment). Our data do show that there is a general trend in which there were fewer *D. melanogaster* individuals still alive in the 24L treatment when compared to the two other treatments starting from day 2 (Figure 3).

Allemand *et al*. (1973) observed that *D. melanogaster* survive for longer and experience mortality later when exposed to less light, and our data do show similar trends, but we do not have significant evidence of this (i.e. overlapping 95%CI). This observation could possibly be due to the *D. melanogaster*'s preference for carrying out activities such as feeding and copulating at lower light intensities (approximately 5 to 10 lux) or in the dark (Allemand *et al*. 1973, Rieger *et al.* 2007,). Production of eggs in sexually mature female *D. melanogaster* has also been linked with an increased rate of survivorship and longer life span (Chadha 2008). If *D. melanogaster* are more active in the dark or at lower light intensities as Allemand *et al.* (1973) and Reiger *et al.* (2007) found, by feeding and mating more actively, the *D. melanogaster* should be able to produce more eggs, and in turn have a higher survivorship rate. We did observe that the appearance of larvae and pupae in the vials was earlier in the 6L18D and 24D treatments. Larvae and pupae were first observed in all three different treatments on day 7: in all five of the 24D replicates, in four of the 6L18D replicates, and in only two of the 24L replicates. When comparing these qualitative observations with the quantitative data we collected (Figure 3), we can see that there does appear to be a correlation between production of offspring and increased survival. Moreover, *D. melanogaster* have been observed to lay eggs on a circadian cycle that is affected by both exposure to light, and temperature changes (Kannan *et al*. 2012), and they have been found to switch to temperature regulation for their egg laying cycles when kept under conditions of constant darkness (i.e. 24D treatment). Under conditions of constant light (i.e. 24L treatment), *D. melanogaster* egg laying patterns were unregulated, and the number of eggs they lay decreased (Kannan *et al*. 2012), and as mentioned before, reproductive output has been related to life span (Chadha 2008).

As described in our methods section, we attempted to minimize the biological variation of the *D. melanogaster* by using wild-type individuals of the same strain, and of approximately the

same age (differing by 4 hours at the most). We did however, run into some issues with attempting to place 2 adult males and 2 adult females into each of our replicate vials. As a result of using individuals that had just matured from their pupae stages, it was quite difficult to distinguish between males and females, because the bands on their abdomen were fairly lighter than if they had been older. This problem can be fixed in future experiments by possibly using other methods to distinguish sex such as identifying sex combs.

Another major issue that we ran into when we were setting up our experiment was obtaining enough adult *D. melanogaster* of the same age to use. Because we only had 4 individuals in each replicate, performing the statistical analysis on our collected data yielded extremely large 95% confidence intervals as shown in Figure 3, and as a result, we were unable to find significant differences in the number of surviving *D. melanogaster* between the three different treatments. If this experiment were to be repeated in the future, one improvement that should be made is increasing the number of individuals used for each replicate.

Our data failed to support the hypothesis that *D. melanogaster* survive for longer when exposed to less light; however, the trends shown in our data, and the relation between life span and reproductive output could be useful for future research on the life span of *D. melanogaster*.

CONCLUSION

The findings from our experiment failed to reject the null hypothesis, and failed to support the alternate hypothesis; there is insufficient evidence from our experiment to support that *D. melanogaster* survives longer under decreased exposure to light. Nonetheless, our experiment yields useful information on how to set up future research on the survival of *D. melanogaster*.

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