Average speed of wild-type CC-1690 and mutant strain CC-3913 of *Chlamydomonas reinhardtii* in response to varying light intensities

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Abstract

Chlamydomonas reinhardtii responds to environmental stimuli such as light, carbon dioxide and oxygen with the goal of finding an optimal environment to grow. Their ability to move allows them to find a suitable environment. This study looks at the speeds that *C. reinhardtii* swim at when exposed to three different light intensities (10 Lux, 270 Lux, 500 Lux). For each, the mutant strain CC-3913 and the wild-type strain CC-1690, there were three replicates per light treatment. All of the replicates were exposed to the three different light intensities with their movement recorded using the DinoXcope. The videos were then projected onto CellTrack a program that calculated the average speed of *C. reinhardtii*. A two-way analysis of variance test was used to interpret the results. The test revealed three calculated p-values all of which indicated rejection of the null hypotheses, thus providing support for all three alternate hypotheses. For an improved future study, a way to control the heat emitted from the light sources should be enforced.

Introduction:

Chlamydomonas reinhardtii are single-celled green algae that inhabit terrestrial and aquatic environments (Harris 2001). The organism's prominent features include a large circular shaped chloroplast, which facilitates photosynthesis and an eyespot that senses light (Yoshimura 2011). In addition to these features there is a visually distinct feature that differentiates the mutant strain CC-3913 and wild-type strain CC-1690 of *C. reinhardtii*. This feature is the underdeveloped flagella on the mutant strain.

Under different light intensities, *C. reinhardtii* demonstrates positive phototaxis, which is movement towards light and negative phototaxis, which is movement away from light (Yoshimura 2011). When light hits *C. reinhardtii's* eyespot, a light-sensitive receptor protein called rhodopsin sends a signal to the flagellar membrane where calcium channels open. The opening of the calcium channels leads to high concentrations of calcium, which leads to the activity of kinase in the flagella, this generates movement.

This process is outlined in Figure 1, which is from King and Dutcher study on *C. reinhardtii* in 1997.

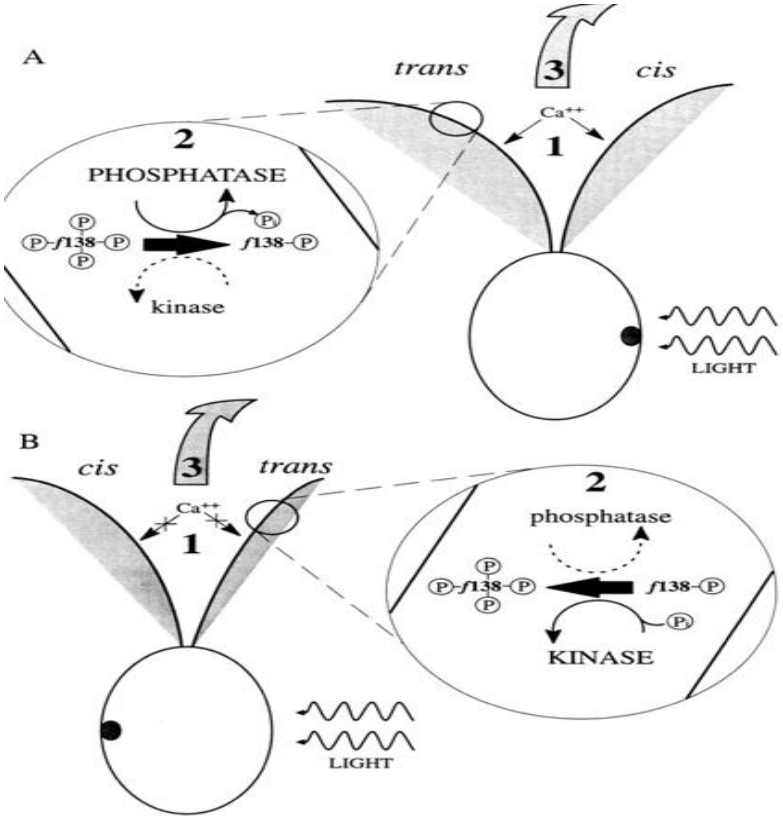

Figure 1. The top of the diagram labeled A, shows how *C. reinhardtii,* moves in response to light. The bottom of the figure, labeled B, shows how darkening conditions leads to decreases movement.

As such, the objective of our experiment was to examine if the speed by which *C. reinhardtii* moved during positive and negative phototaxis, under different light intensities was different for the mutant and wild-type. Accompanying our objective were our three sets of hypotheses.

The null hypothesis for our first set is that light intensity has no effect on the average speed of *C. reinhardtii.* In contrast, our first alternate hypothesis is that light intensity has an effect on the average speed of *C. reinhardtii.* In the 1971 study conducted by Feinleib and Curry, the relationship between light stimuli and oriented

phototactic responses was studied. Results of the study suggested that *C. reinhardtii* take about one second for c. reinhardtii to switch from positive to negative phototaxis, thus altering speed.

The null hypothesis of our second set of hypotheses is that the presence of mutation has no effect on the average speed of *C. reinhardtii.* The alternate hypothesis of our second set of hypotheses was that the presence of mutation has an effect on average speed of *C. reinhardtii.* Our alternate hypothesis is supported by a 1984 study, which looked at the different beat-like projections of flagella in the mutant and wild-type strains (Segal *et al.* 1984). They discovered that their mutant strain of *C. reinhardtii* exhibited only backward motions whereas the wild-type exhibited both forward and backward movement.

The null hypothesis of our last set was that the effect of light intensity on average speed of *C. reinhardtii* is the same in wild type and mutant. The alternate hypothesis to this is that the effect of light intensity on average speed of *C. reinhardtii* is not the same in wild type and mutant. The 1982 study looked at flagella structure and function, which validates that the presence of well-developed flagella results in faster motion (Brokaw *et al*. 1982).

The results of our study are important because it can ignite further investigation on the behavior of *C. reinhardtii* and on its control process in regards to directional movement.

Methods

To determine whether or not light intensity had an effect of cell speed, we used three separate light intensities: a dark setting, a control or normal setting and a bright

setting. To set up the dark setting, we used a box covered in black plastic and placed it over top of our compound microscope to ensure no outside light would enter. There were holes cut at the top of the box to allow the exchange of the slides and to allow the eyepieces to come out. We used a light meter to measure the intensity inside the box and found the dark setting to be 10 Lux. For the control, we removed the box and just used the natural light in the room. Again, we used the light meter and found our control setting to be 270 Lux. For our third treatment, we used a lamp and placed it 60 cm away from the microscope to set the Lux at 500.

We used three replicates under each of the light intensities for our experiment. We took three samples from both the wild type and the mutant type solutions. From these samples, we took 20 µL using the micropipettes for each of the three settings. Figure 1 shows an example of how the replicates were done.

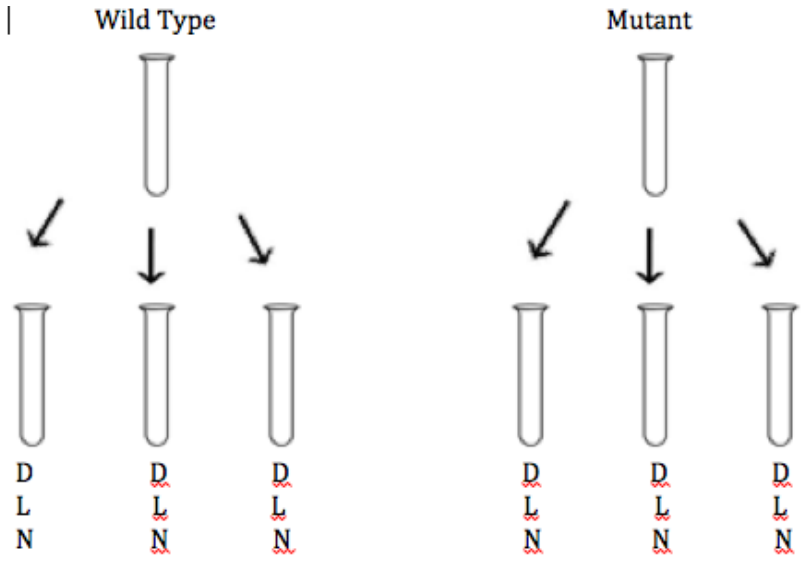

Figure 2. Sample of how the replicates were used. The letters represent the conditions each replicate was placed under. D represents the dark, L represents light added and N represent no added light.

The movement of the cells was measured by using a DinoXcope and a compound microscope. We replaced one of the eyepieces with the DinoXcope and plugged it into

the laptop. We allowed each of our samples to acclimate to the change in light intensity for two minutes before measuring the speed. Once acclimated, we picked a random part on the slide by not looking through the eyepiece and scrolling around the slide for five seconds. Once the five seconds were up, that particular spot was chosen as the testing site and recorded a 30-second video. We analyzed the video using the program CellTrack (Sacan et al., 2008) that allowed us to gather tracking data on the cells of our choice. From each sample, we chose three *C. reinhardtii* cells that were moving and focused on those as seen in Figure 2 below. We then used the program to gather tracking information for each cell and then we analyzed this information using CellTrack (Sacan et al., 2008) to find the average speed. We did this for all six of our replicates.

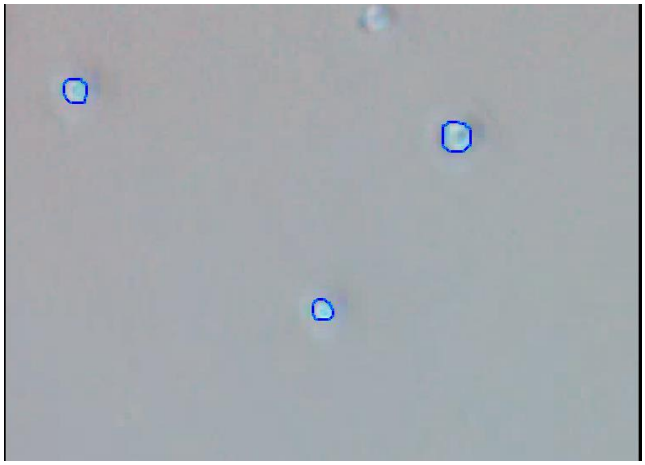

Figure 3. This is an image of us preparing one of our mutant type cells for tracking.

The other factor that we had to account for was temperature to ensure that the change in cell speed was not due to the temperature; however, we could not control the change. We measured the temperature with a thermometer and recorded it in degrees Celsius for each treatment. The temperature for the dark treatment was 25^0 C, 25.5^0C for the control and 27^0C for the third treatment. The same microscope and DinoXcope were

used for all replicates to ensure minimal change. To determine if there was a significant difference, we used a two-way Analysis of Variance (ANOVA) test.

Results:

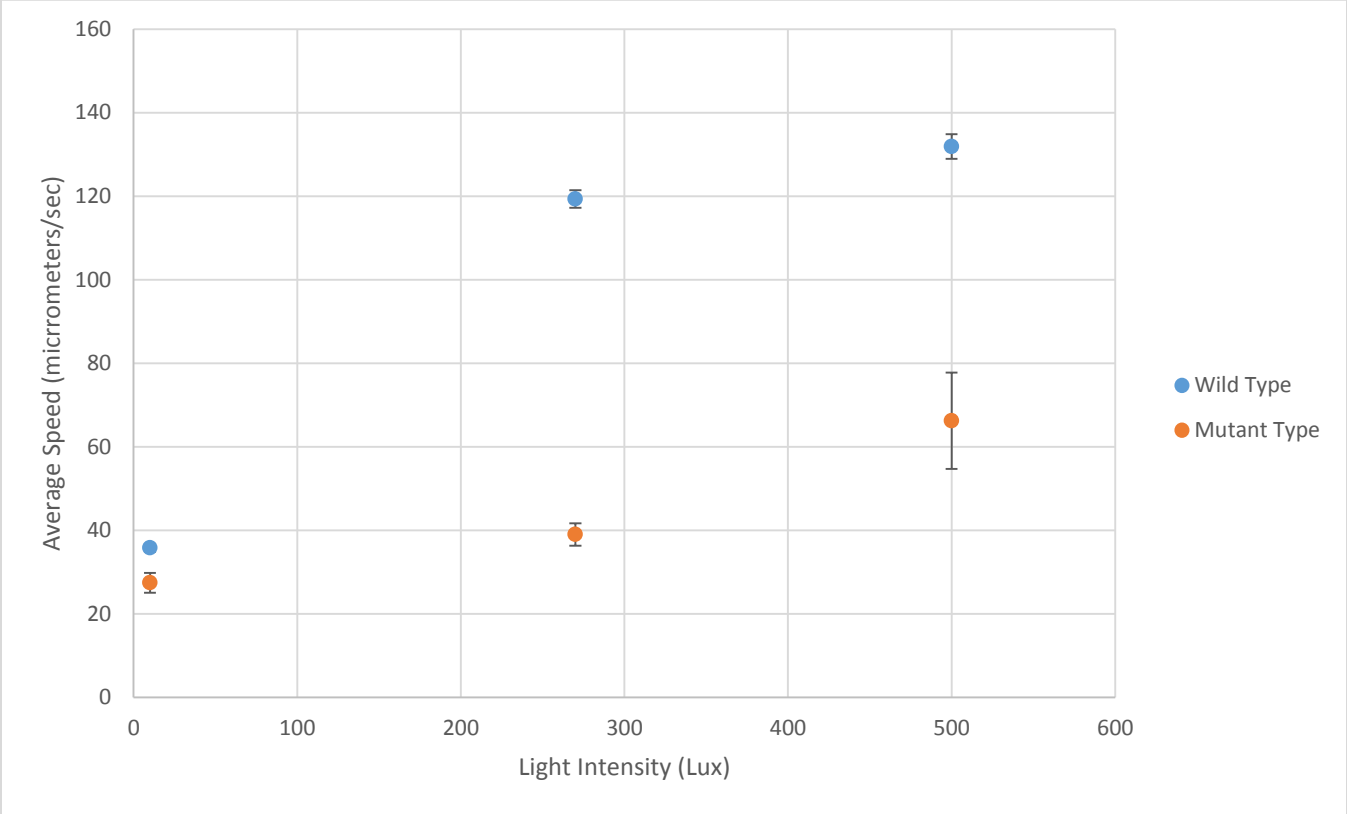

Figure 4. Average speed of mutant and wild type *C. reinhardtii* as a function of different treatments at various light intensities. At n=3, the speeds at the 95% confidence interval were, in µm/s, displayed as the error bars. The average speeds were displayed as the mean speed for that particular type of cell. The calculated p-values were 2.41×10^{-11}, $1.69 \times^{-11}$, and $2.57 \times^{-8}$.

As seen in Figure 3, there appears to be a positive trend as increasing light intensity leads to an increase of the average speed of *C. reinhardtii*. There was no overlap in confidence intervals between the mutant and wild-type cells.

We applied a two-way ANOVA test to differentiate between the effect of light intensity and also wild and mutant types while using the means. We found that the average speed of *C. reinhardtii* is significantly different over different light intensities as the calculated p-value was 2.41×10^{-11}.

The average speeds of wild type *C. reinhardtii* are significantly different than mutant *C. reinhardtii* as the calculated p-value was $1.69 \times ^{-11}$.

We also discovered that the effect of light intensity on average speeds of *C. reinhardtii* is significantly different for wild type and mutant as the calculated p-value was 2.57×10^{-8}.

Discussion

For our first hypothesis the p-value was much less than 0.05. Therefore, we were able to reject our first null hypothesis and provide support for its alternate hypothesis that light intensity has an effect on the average speed of *C. reinhardtii*. We observed this effect as *C. reinhardtii* had a faster mean speed as we increased the light intensity.

The scientific literature supports our observations regarding our first hypothesis. This is most evident as *C. reinhardtii* have an eyespot and chloroplast that they use in conjunction to sense light and facilitate photosynthesis. Since they require photosynthesis as a means to acquire nutrients, *C. reinhardtii* display a behavior called phototaxis which causes them to move toward light sources (Yoshimura 2011).

However, phototaxis can cause organisms to exhibit a negative response to environmental factors as well. In his research, Yoshimura (2011) also found that *C. reinhardtii* would move away from light sources if the intensity was too high. We also observed this negative phototaxis response in the practice run of our experiment when *C. reinhardtii* had very little to no movement in our bright light intensity treatment. Consequently, we reduced the light intensity for our bright light intensity treatment from 1980 Lux to 500 Lux in our final experiment.

The p-value for our second hypothesis was also much less than 0.05. As a result, we were able to reject our second null hypothesis and provide support for its alternate hypothesis that mutation has an effect on average speed of *C. reinhardtii*. As observed in Figure 3, the mutant-type *C. reinhardtii* demonstrated a much slower average speed at each treatment compared to the wild-type of *C. reinhardtii*.

Since Brokaw *et al.* (1982) found that the presence of functioning flagella enable faster motion, it makes sense that the presence of mutation caused lower speeds in *C. reinhardtii* because each of our mutant types were missing developed flagella. When Plummer *et al.* (1978) induced paralysis in *C. reinhardtii*, they observed a loss of function in radial spokes and dynein in the axonemes *C. reinhardtii*. In turn, the loss of function in these parts disrupted the sliding and bending process that facilitates doublet-microtubule interaction in cells (Plummer *et al.* 1978). It is these doublet-microtubule interactions that are largely responsible for flagella formation and activity (Plummer *et al.* 1978).

Additionally, the p-value for our third hypothesis was also less than 0.05. This lead us to reject the third null hypothesis and provide support for its alternate hypothesis that effect of light intensity on average speed of *C. reinhardtii* is not the same in wild-type and mutant. Even though both the wild type and mutant type of *C. reinhardtii* had increased speed as light intensity increased, the wild type was still noticeably more active and faster at each light intensity.

Furthermore, the results of our third hypothesis agree with research that was previously completed. In a similar experiment, Kuchka and Jarvik (1987) observed some *C. reinhardtii* that were moving noticeably slower than the others because they all had

shorter or no flagella at all. Upon further investigation they found that all the slower *C. reinhardtii* had multiple gene mutations that were causing the change in flagella function (Kuchka and Jarvik 1987).

Even though there was a strict attention to detail during the methods and data collection, there are some sources of uncertainty and variation that must be taken into consideration. For example, it is possible to create contamination when transferring our organisms from the given flasks and tubes to the microscope slides via micropipette. This includes exposure to any other chemicals or substances in our laboratory environment that may have had an effect on the behavior of *C. reinhardtii*. As well the presence of air bubbles formed from the placement of the coverslip to some of the microscope slides may have affected the speed of the *C. reinhardtii*. We noticed that when a large air bubble was present, our organism had less space to move in because they tended to avoid moving close to the air bubble. This may have been because they preferred the medium that they were already in.

While the program CellTrack is more accurate than trying to measure the speed of *C. reinhardtii* manually, there are still some limitations associated with using it to analyze the video data that was recorded (Sacan *et al.* 2008). Oftentimes, the program would fail to identify some *C. reinhardtii* cells so the cells had to be identified manually. This can be attributed to the cells being quite small. Also, our results may be affected by sampling bias because the *C. reinhardtii* cells that were moving very fast could not be easily recorded for analysis as they moved out of the area being recorded. Therefore, many of the cells that were recorded may represent *C. reinhardtii* cells that are slower.

Although we found a significant statistical difference for all three of our hypotheses, there is some uncertainty associated with biological variations between individual *C. reinhardtii* cells. For example, some cells may have experienced different levels of energy during the time that they were being observed. Aside from the differences caused by mutation, this may have been caused by factors such as fatigue, age, sex, genetic differences or size. Since the mutant type cells had a more irregular and asymmetrical movement pattern, they tended to circle around a smaller area compared to the wild types (Brokaw *et al.* 1982). In contrast the wild type were noticeably quicker and had a more symmetrical movement pattern (Brokaw *et al.* 1982).

Additionally, there were some environmental factors that could have also caused uncertainty in our experiment. We were able to keep light intensity constant for each treatment as outlined in the methods section. However, the higher light intensity treatments had slightly higher temperatures mainly due to the warmth emitted from the lamp that we used. Even though the difference in temperature was a mere one or two degrees Celsius, this may still cause variation of speed in *C. reinhardtii* (Majima and Oosawa 1975). Future experiments may want to improve on our own experiment by maintaining a more constant temperature between treatments. This may be obtained by using temperature controlled water baths.

While increased light intensity also increases average speed of *C. reinhardtii* there are other abiotic environmental factors that can be tested. A similar study to perform in the future is how the inorganic nitrogen levels affect the average speed of *C. reinhardtii* cells. This would be a compatible study because inorganic nitrogen is a main nutrient source for photosynthetic organisms (Fernandez and Galvan 2007).

Conclusion

We rejected all three sets of our null hypotheses. It was found that an increased light intensity led to an increased cell speed for both wild-type and mutant *C. reinhardtii.* Furthermore, a significant difference was found between the wild-type and mutant cell speeds at all light intensities, which was proven by two-way ANOVA test.

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