The effect of temperature on average speed of wild-type (CC-1690) and mutant strain (CC-3913) of *Chlamydomonas reinhardtii*

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Abstract

The goal of this study was to examine the effects of temperature on the average speed of both wild-type (CC-1690) and mutant strain (CC-3913) of *Chlamydomonas reinhardtii*, a flagellated unicellular alga. Average speed of the wild-type and mutant *C. reinhardtii* were measured at 25^{o}C and 28.5^{o}C. Cell movements were filmed with DinoXcope camera attached to a compound microscope. Selected cell paths were calculated for their average speed using ImageJ with MTrackJ plugin. Data analysis (two-way ANOVA) indicated no statistical difference between the average speed (n=4) of wild-type and mutant *C. reinhardtii* (*p*-value of 0.36). Furthermore, even though greater rate of movement was observed at higher temperature, the results were not statistically significant for *C. reinhardtii*'s average speed (*p*-value of 0.09). The difference between the effects of increased temperature on wild type compared with mutant was also not statistically significant (*p*-value of 0.42). These findings can have broader implications in future studies regarding the movement pattern and physiological behaviour of *C. reinhardtii*.

Introduction

Chlamydomonas reinhardtii is a unicellular eukaryotic alga, which acquires its energy by means of photosynthesis. It is bi-flagellar, about 10 μm wide and green in appearance. The two flagella with axoneme, radial-spoke system, and inner and outer dynein arms are responsible for generating and regulating the motility of *C. reinhardtii* (Merchant *et al.* 2007). The wild-type *C. reinhardtii* is has flagellar-assisted movement and a short reproduction cycle. The mutant strain, however, has an insertion mutation at the *pf9* locus which prevents it from forming the inner dynein arms which are responsible for the flagellar, wave-like motion that helps in formation of the propulsive force (Myster *et al.* 1997).

In this study we examined the effects of temperature change on the motility in both the wild-type and mutant *C. reinhardtii*. Elevated temperature causes unicellular organisms to have a greater swimming velocity (Maeda *et al.* 1976). The results of this experiment could potentially be useful since *C. reinhardtii* is a widely used model organism for studying other flagellated

unicellular organisms and their interactions with the environment. For instance, *C. reinhardtii* is used extensively in the study of human sperm motility (Qin *et al.* 2015). The following hypotheses and predictions are the basis of this study:

$H_{0(1)}$: Temperature has no effect on the average speed of *Chlamydomonas reinhardtii*.
$H_{a(1)}$: Temperature has an effect on the average speed of *Chlamydomonas reinhardtii.*

Vítová *et al.* (2011) conducted an experiment with a temperature range of 15°C – 37°C and found that optimal growth conditions were at 28°C. It has been discovered in the past that there exists a correlation between temperature and flagellar assembly in *C. reinhardtii* (Huang *et al.* 1997). Effects of other abiotic factors (pH, light intensity, and nutrient concentration) on *C. reinhardtii*'s behaviour and mobility have been linked to the alteration of organism's physiological state due to the change in the environment (Wu *et al.* 2015). Thus, we predict the impact of temperature on C. *reinhardtii* will be due to the change in the organism's metabolism and physiological state.

$H_{0\ (2)}$: The average speed of wild-type *Chlamydomonas reinhardtii* is the same as that of the mutant *Chlamydomonas reinhardtii*.
$H_{a\ (2)}$: The average speed of wild-type *Chlamydomonas reinhardtii* is different from the mutant *Chlamydomonas reinhardtii*.

It was previously discovered that the mutant strain has impaired motility compared to the wild type due to the lack of functional flagella (Myster *et al.* 1997). We predicted that the effect of temperature change on the motility of *C. reinhardtii* will be greater in the wild type compared to that in the mutant.

$H_{0\,(3)}$: Temperature has the same effect on average speed in wild-type and mutant *C. reinhardtii.*

$H_{a\,(3)}$: Temperature does not have the same effect on average speed in wild-type and mutant *C. reinhardtii.*

So far, there has not been a study that has looked at the difference in effect of temperature and mutation on the motility of *C. reinhardtii*. Since this mutation directly affects its flagellar assembly and it can live in a wide range of temperatures, *C. reinhardtii* motility should be affected more by its mutation than temperature.

Methods

Culture

The wild-type *C. reinhardtii* (CC-1690) with wild-type alleles at the *NIT1* and *NIT2* loci, and the mutant (CC-3913) with an insertional allele at the *pf9-3* locus, were both grown for three weeks in a 17^{o}C environmentally controlled chamber in a modified version of Sager and Granick medium.

Treatments

We set up two temperature treatments, 25^{o}C and 28.5^{o}C, to test the effect of temperature on the motility of *C. reinhardtii*. To ensure equal temperature distribution, we transferred each cell type into an 8-mL test tube for acclimation at 30^{o}C and at 25^{o}C two hours before the experiment began. There were four test tubes in total, two tubes per treatment. To maintain the treatment temperature while filming, especially for the higher temperature treatment, we decided to conduct the experiment in two rooms, one room's temperature was not altered (25^{o}C) and the other was heated by a portable Thermowell heater (28.5^{o}C). We also measured the temperature

before each replicate to make sure the treatments were relatively constant throughout the experiment. We placed the heater near the microscopes and elevated it so that its midline was approximately at the level of the microscope stage (Figure 1). This ensured that the temperature around the microscope would be similar to that of the incubator where the cells were acclimatized. Furthermore, to ensure that the room was sufficiently warmed, we heated it for more than 30 minutes before the start of the experiment.

Figure 1. The setup of the Thermowell and the Axio Star microscopes in the 28.5°C room.

Filming

We used the DinoXcope to record videos on the Axio Star microscopes. We set up four microscopes, two in each room, one for each cell type, and one experimenter operating on each setup. We removed 20 μL of cells onto the haemocytometers. Haemocytometers were used because it was discovered that they have just enough depth for the cells to move freely but not too much so that they swim out of focus. To control for light intensity from the microscopes, we standardized their illumination by having all microscopes with the same maximum illumination setting. Similarly we measured the rooms' light intensities on the platform of the microscope to make sure there was not much variation between the two rooms.

We used the 10x lens to film cells in our field of view. We prepared an initial slide for the microscope, focusing so the following replicates could be immediately filmed without having to refocus. We also randomized an area on the slide and all subsequent replicates were filmed at the same area. Moreover, we left the stage and any other parts of the microscope untouched after the initial setup. We each had a haemocytometer which we cleaned and reused for each replicate.

Qualitative Measurement

We recorded any observable behaviour during the filming. We also compared the wild-type and mutant movement to see whether or not they followed the same pattern.

Quantitative Measurement

We filmed each replicate for 30 to 60 seconds. At the beginning of each replicate we measured the light intensity and also the temperature.

Data Analysis

From the 30-second video capture of a random group of cells, we took a two to six second snippet for analysis (the time depended on how long the cells moved within the frame). We chose the fastest moving cell to remove the effects of immobility (sticking, dormant, or dead cells) and to isolate for average speed of active flagella between mutant and wild type. One cell was selected from each replicate for mutant and wild type at 25^{o}C and 28.5^{o}C (n=4 per temperature treatment and strain type). We analyzed the data by a frame rate of one second and converted into a video file compatible with MtrackJ plugin software. We calculated the resulting distance using pixel units with MTrackJ "Measure" function (Figure 2).

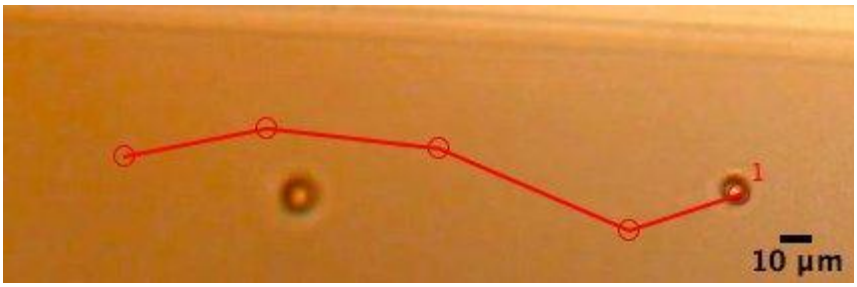

Figure 2. Example of a path travelled by *Chlamydomonas reinhardtii* in a 5 second frame. Path was traced using ImageJ with MtrackJ. The second cell in the frame is a different cell.

We did the calibration using the reference diameter of *C. reinhardtii* at 10 µm. We then converted the resulting path length from pixel/second (1) to µm/s (3) using the following equations:

Speed (pixel/sec) = Pixels Travelled (pixel unit) / Time Travelled (sec) (1)

Conversion = Diameter of reference cell in µm / Diameter of reference cell in Pixel Unit (2)

Speed (µm/s) = Speed (pixel unit/sec) x Conversion (µm/pixel unit) (3)

We calculated the average speed (n=4) and 95% confidence interval per treatment and *C. reinhardtii* strain (wild-type at 25^{o}C, wild-type at 28.5^{o}C, mutant at 25^{o}C, and mutant at 28.5^{o}C). We used the R data analysis software for a Shapiro-Wilk test on normality and a Grubbs test on outliers. We used Excel to conduct two-way ANOVA to test for the influence of temperature and mutation on the motility of *C. reinhardtii*. Furthermore, we graphed average speed against temperature for each cell type on a scatterplot graph with error bar sat 95% confidence interval.

Results

Qualitative observation made during the experiment indicates noticeable differences in movement patterns between 28.5^{o}C and 25^{o}C. Mutant and wild-type *C. reinhardtii* at 28.5^{o}C were observed to be moving linearly or in a zigzag pattern. Overall, the mutant appeared to move

more in a zigzag pattern. It was observed that wild-type *C. reinhardtii* at 25°C were moving more circularly and erratically. In all video captures at the two temperatures, some cells remained stationary with little movement. Furthermore, at our magnification with the 10X objective lens, the bi-flagellar characteristic was not seen.

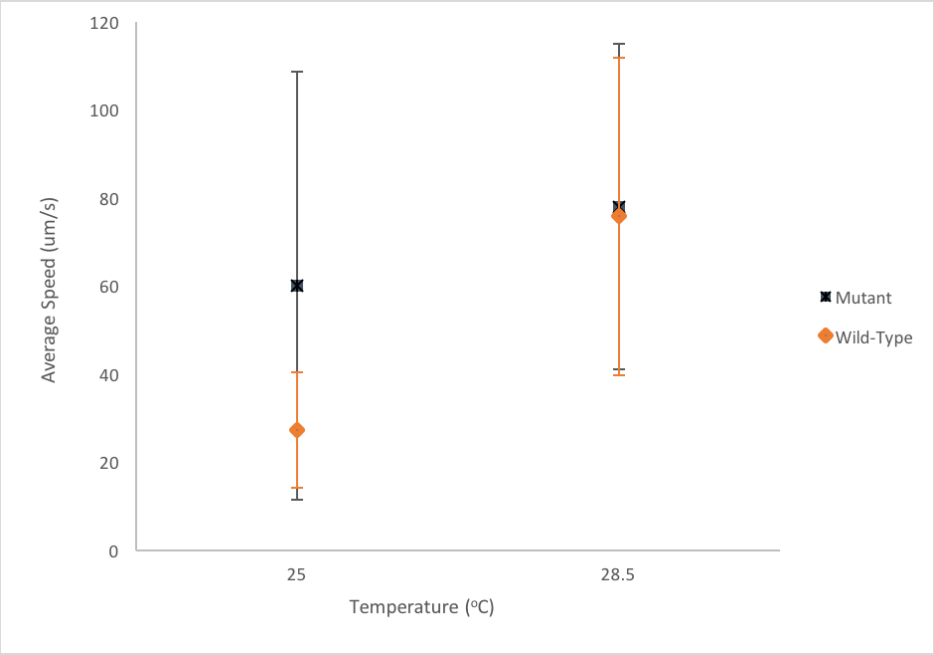

Figure 3. The effect of temperature at 25°C and 28.5°C on average speed in µm/s (n=4 per group) of *Chlamydomonas reinhardtii* wild type and mutant. Error bars indicate 95% confidence intervals.

Figure 3 shows overlapping 95% confidence intervals between temperatures and average speed for wild-type and mutant *C. reinhardtii*. Higher average speed was noticeable in the mutant (60 ± 48 µm/s at 25°C, and 78 ± 38 µm/s at 28.5°C) compared to the wild type (28 ± 13 µm/s at 25°C, and 76 ± 37 µm/s at 28.5°C).

Results of the two-way ANOVA indicated a *p*-value of 0.09 for the effects of higher temperature on average speed of *C. reinhardtii*, a *p*-value of 0.36 for average speed between mutant and wild-type *C. reinhardtii*, and a *p*-value of 0.42 for the effects of temperature on

mutant and wild-type *C. reinhardtii*. Although not statistically significant, there is a trend of higher average speed at higher temperature in both wild type and mutant.

Discussion

We fail to reject the null hypothesis that temperature has no effect on the average speed of *C. reinhardtii* (p_1=0.09). Furthermore, we fail to reject the null hypothesis that the average speed in the mutant and wild-type *C. reinhardtii* is the same (p_2=0.36). Additionally, the effect of higher temperature on average speed of *C. reinhardtii* was not statistically significantly between wild type and mutant (p_3=0.42).

Temperature affects motility of *C. reinhardtii* and other flagellated unicellular organisms. Arrhenius law states that chemical reactions are temperature-dependent. Raising the temperature from 5°C to 37°C results in an increase of the velocity of microtubule gliding (Böhm *et al.* 2000). This corresponds to an increased motility. Although our data were not statistically significant, we did observe an increased average speed at higher temperature.

Majima *et al.* (1975) observed an abrupt velocity change on onset of a temperature change. An increase in velocity from a positive temperature change was only maintained for a few minutes until stationary velocity was observed. Reported velocity at an abrupt increase in temperature was 170 μm/s and tapered towards a stationary velocity of 120 μm/s for wild-type *C. reinhardtii* in five minutes. Therefore, our two-hour incubation time may have allowed *C. reinhardtii* to adapt to the treatment temperature and a lower stationary velocity. Further analysis in determining onset average speed and prolonged average speed at time intervals could be conducted to validate this effect. An increased average speed is observed; however, the insignificance may also be due to small temperature difference.

We failed to reject our second null hypothesis which states that there was no difference in motility between mutant and wild-type *C. reinhardtii*. It was also observed that mutant was moving faster than wild type, which was moving erratically or circularly. However, the mutant strain (CC-3913) has an impaired motility due to lack of a proper flagella. The mutation in the *Dhc1* gene is responsible for the improper formation of I1 inner arm (Myster *et al.* 1997). The I1 inner arm is one of the main components of flagella which helps *C. reinhardtii* bend the microtubules of the flagella to create waveform motions. The waveform is important for *C. reinhardtii* to generate propulsion from pushing off fluid (Bayly 2011). Similarly, Porter *et al.* (1992) also found that a mutant with a non-functional *Dhc1* gene at the *pf9* locus is associated with improper formation of I1 inner arm and slower velocity, but its flagellar beat frequency and axonemal ATPase activity are nearly identical to those of wild type. In comparison to reported swimming velocity published by King and Dutcher (1997), the *pf9-3* mutant velocity was reported to be 72.3 ± 14.1μm/s and wild type was reported to be 150.3 ± 20.3 μm/s. Additionally, Myster *et al.* (1997) reports *pf9-3* mutant velocity as 63.2 ± 8.7 μm/s. This suggests that our wild type was moving slower. A possible source of error could have arrived by either slow preparation or delayed onset of filming to allow for cells to begin sticking. Additionally, since different haemocytometers were used across experimenters, they may have introduced varying degree of stickiness and cell movement speed.

Bola *et al.* (2014) reported that there was a statistically significant difference between the speed of wild-type and mutant cells at all light intensities. Our results at an average of 158 Lux are similar with respect to mutant average speed as Bola *et al.* (2014) reported at light intensity of 500 Lux. Differences in our results may be due to the fact that light reading may not have been sufficiently controlled in our experiment. Another source of error could be observer bias, as

with four experimenters, there may be inconsistencies in the way each experimenter measured light intensity on the stage (under the arm of the microscope, or on the sides of the stage exposed to room lights). Flagella of the mutant *C. reinhardtii* never beat with a symmetrical waveform when stimulated with high light intensity, while in these conditions, the wild type swims backward with a symmetrical waveform flagellar beat (Kamiya *et al.* 1985).

Variation was also expected due to sharing of one heater with two microscopes in the heated room at 28.5^{o}C. Temperature was limited to 28.5^{o}C with one heater. A 30^{o}C temperature room to match incubation temperature would have been ideal. Uneven distribution of heat may have contributed to temperature differences in the heated room and temperature differences among cells in the test tube. One possible solution is to work in an environmentally controlled room so the temperature is uniform. Additionally, the room temperature pair was filmed in the area within the grid of the haemocytometer and the higher temperature pair was filmed outside the grid where there were no lines.

To reduce sources of error and to improve accuracy of distance travelled in micrometers, a stage micrometer could be included in the calibration using DinoXcope software calibration feature. Additionally, we could have analyzed across multiple moving *C. reinhardtii* cells using a more advanced cell motility tracking software. Another way to increase accuracy of data is by increasing cell tracking length over a longer period of time by viewing at a smaller magnification. Alternatively, capturing a greater number of frames per second would allow for increased accuracy in calculating distance travelled because small directional changes in distance would better be accounted for at higher frames per section. This will provide a more accurate picture of moving trends and average speed over a longer duration.

Conclusion

The goal of this study was to investigate the effects of temperature and mutation on *C. reinhardtii'*s average speed. We found that temperature had no statistically significant effect on *C. reinhardtii's* average rate of movement. We also found that there was no statistically significant difference between the average speed of wild type and mutant. This could potentially be due to our experimental errors as well as our small sample size. The difference between the effects of temperature on the speed of wild type and mutant *C. reinhardtii* was also not statistically significant. These results suggest that the behaviour and physiological mechanisms of *C. reinhardtii* may adapt quickly to a relatively small temperature change from 25^{o}C to 28.5^{o}C and further investigation into higher temperature is required to determine the temperature effects on average movement of wild-type and mutant *C. reinhardtii*.

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