Effect of temperature on distance travelled in wild-type and mutant *Chlamydomonas reinhardtii*

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Abstract

The objective of our study was to investigate the effects of elevated temperatures on the mean distances travelled by wild-type (CC-1690) and mutant (CC-3913) strains of *Chlamydomonas reinhardtii.* The average motility of the wild-type and mutant strains were measured at temperatures of 20°C, 25°C, and 30°C, with n=4. Movement of the cells were filmed using a DinoXcope camera connected to a Zeiss Axio compound microscope. Each replicate was observed and the length of movement was calculated during a five-second interval using ImageJ with MTrackJ plugin. A two-way ANOVA test was used to analyze the results, which indicated statistical significance with respect to the effect of increased temperature on the mobility of wild-type *C. reinhardtii* compared to *pf9-3 mt-* mutants (p=0.01). In addition, wild-type *C. reinhardtii* showed a general trend of increased movement with higher temperatures (p=0.000001) whereas the presence of the mutation *pf9-3 mt-* in the mutant *C. reinhardtii* had a negative effect on their overall movement (p=0.007). Overall, wild-type organisms travelled farther than *pf9-3 mt-* mutants did, which was likely due to defective inner dynein arms in mutant *C. reinhardtii* flagella. The findings from our study can be used in further studies to understand the movement of *C. reinhardtii.*

Introduction

Chlamydomonas reinhardtii is a small, photoreceptive, unicellular alga that swims by beating its two flagella in unison (Majima *et al.* 1975). It is highly adaptable and therefore able to survive in different freshwater environments around the world (Hemme *et al.* 2014). The *pf9-3 mt-* mutant strain occurs when 13 kb of DNA in the *Dhc1* gene are deleted, rendering the mutant unable to assemble the inner dynein arm (a motor protein) of the flagella (Myster *et al.* 1997). The inner dynein arm determines the specific pattern and magnitude of the waveform, whereas the outer arm provides the driving force to increase flagellar beat frequency (Porter & Sale 2000). Thus, the mutants which lack the inner arm will have difficulty providing direction to their movement. Myster *et al.* (1997) showed that the *pf9-3 mt-* strain had lower motility than the

wild-type due to these flagellar malfunctions. Figure 1 shows a cross section of the central strand of a flagellum, or an "axoneme", and its components.

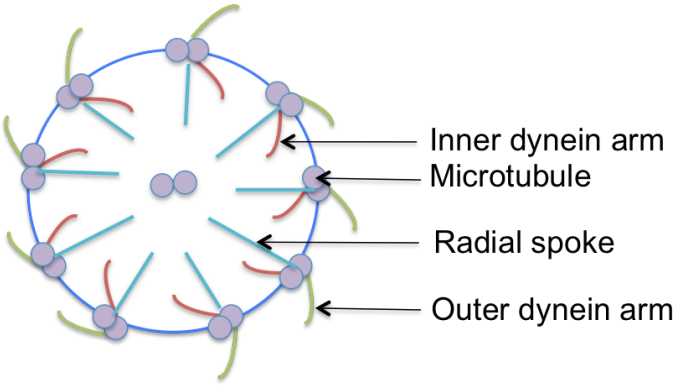

Figure 1. Cross section of a flagellar axoneme, showing the inner and outer dynein arms and radial spokes that relay signals from central microtubules to the arms.

The objective of our research was to determine if temperature has an effect on the activity levels of *C. reinhardtii* wild type and mutant *pf9-3 mt-*, and whether the effect of increased temperature varies between the two *C. reinhardtii* strains.

This research is important for studying flagellar and overall motility, especially when considering the *C. reinhardtii* mutant strain. Learning more about how abiotic factors such as temperature, affects this organism, allows us to predict how similar organisms may be affected. This is useful for investigating species that are less abundant or that are not as easy to isolate and study as *C. reinhardtii*, which is an ideal model organism due to its simple life cycle and fully sequenced genome (Kou *et al*. 2013). If the temperature does affect the mutants differently than the wild type, then perhaps there are certain temperatures where the motility of the mutant strain improves such that it appears to move as a wild type. If this improvement exists and occurs, then there may be temperature conditions at which mutants are more competent competitors for resources in the wild. Due to the nature of our comparisons, our hypotheses are as follows:

H_{01}: Temperature has no effect on the motility of *Chlamydomonas reinhardtii*.

H_{A1}: Temperature has an effect on the motility of *Chlamydomonas reinhardtii*.

H_{02}: Presence of the mutation *pf9-3 mt-* has no effect on the motility of *Chlamydomonas reinhardtii*.

H_{A2}: Presence of the mutation *pf9-3 mt-* has an effect on the motility of *Chlamydomonas reinhardtii*.

H_{03}: Increased temperature does not have a greater effect on the motility of wild-type *Chlamydomonas reinhardtii* compared to *pf9-3 mt-* mutants.

H_{A3}: Increased temperature has a greater effect on the motility of wild-type *Chlamydomonas reinhardtii* compared to *pf9-3 mt-* mutants.

We predicted that the presence of the *pf9-3 mt-* mutation would have an effect on the motility of *C. reinhardtii*, as research done by Myster *et al.* (1997) indicated that the mutants did not swim as rapidly as the wild type. Furthermore, we predicted that temperature would also have an effect on motility of *C. reinhardtii*, as Ukeles (1961) showed that growth rates of wild-type *C. reinhardtii* at different temperatures may be an indication of flagellar motility. Since *C. reinhardtii* use flagella for movement and mating, a decline in growth rate could indicate a decline in flagellar motility.

A decrease in available calcium will cause inactivation of flagellar axonemes in *C. reinhardtii,* causing the cells to swim in circular patterns Kamiya and Witman (1984). Since the mutant already swims in circular patterns and has a defective flagellum, we predicted that temperature change would have a greater effect on the wild-type *C. reinhardtii* than on the mutant.

Located in the cell membrane, transient receptor potential (TRP) channels transfer ions from the environment inside the cell. *C. reinhardtii* require 19 different TRP channels for efficient ion transfer, each of which respond to different environmental factors such as light, salinity, and temperature (Wheeler & Brownlee 2008). Among these are the TRPV channels, transporting only calcium, sodium, and magnesium cations to assist in overall cell motility (Clapham *et al.* 2005). Figure 2 is a proposed model explaining our prediction that increasing temperatures will increase motility. Higher external temperatures allow certain TRP channels to open in *C. reinhardtii*'s flagellar membrane, resulting in an influx of calcium (Ca^{2+}) ions (Bloodgood 2010; Sanderson *et al.* 1986). The excess Ca^{2+} will activate signalling pathways to increase flagellar beating and waveform frequency, resulting in elevated flagellar motion and swimming velocity (Bloodgood 2010; Sanderson *et al.* 1986).

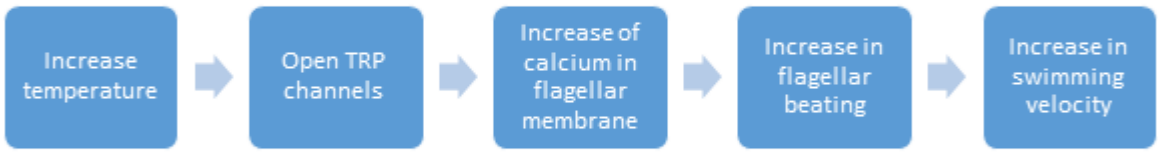

Figure 2. A proposed model. Increasing temperature will activate signaling pathways to increase swimming velocity (Bloodgood 2010; Sanderson *et al.* 1986).

Methods

We obtained 12 mL each of mutant and wild-type *C. reinhardtii.* We had three treatments of 20°C (the control), 25°C, and 30°C; the water bath at 20°C was maintained using ice, the higher temperatures were maintained with standard hot water baths. These temperatures were chosen based on the physiological requirements and growth temperature of *C. reinhardtii,* as previous literature indicates growth of the cells ceases above temperatures of 32°C. To generate

our replicates, we pipetted a 1 mL sample of *C. reinhardtii* into a microcentrifuge tube, and repeated this procedure until 24 samples were obtained: 12 wild-type and 12 mutant *C. reinhardtii,* for a total of four replicates per treatment (Figure 3).

We submerged the *C. reinhardtii* samples in the water baths for 10 minutes to become adjusted to the temperature (Figure 4*)*. After five minutes elapsed, we placed another centrifuge tube in the water bath for 10 minutes, set on a second timer. Staggering each replicate by five minutes allowed us to prepare the slide and observe each replicate under the microscope for approximately three minutes.

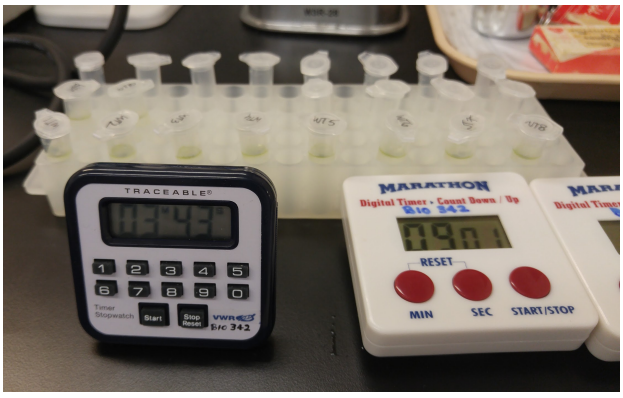

Figure 3. Stopwatches were used to ensure all replicates were acclimating for the correct amount of time (10 minutes). Also present are some examples of the microcentrifuge tubes we used to hold our replicates.

Figure 4. Microcentrifuge tubes with 1 mL of either mutant or wild-type *C. reinhardtii* were submersed in water baths set to the treatment temperatures (20°C, 25°C and 30°C) for 10 minutes prior to observation.

When the ten-minute acclimation period elapsed, we retrieved the centrifuge tube and pipetted 20 µL of the *C. reinhardtii* sample onto a microscope slide (Figure 5). We observed *C. reinhardtii* using a Zeiss Axio dissecting microscope, and recorded a 15-second video on DinoCapture 2.0 software, using a DinoXcope ocular attachment (Figure 6). We recorded multiple videos to account for some alga not remaining in the frame for a minimum of 10 seconds. In addition to the video, we recorded qualitative observations regarding the motility of the cells, such as speed, direction, and type (rolling/spinning/drifting/linear) of movement.

To quantitatively track *C. reinhardtii* movement, we used ImageJ and the plugin MTrackJ to track *C. reinhardtii* movement over a five-second period. We summed the distance covered in each period and calculated a total length in pixels, an example is shown below in Figure 7.

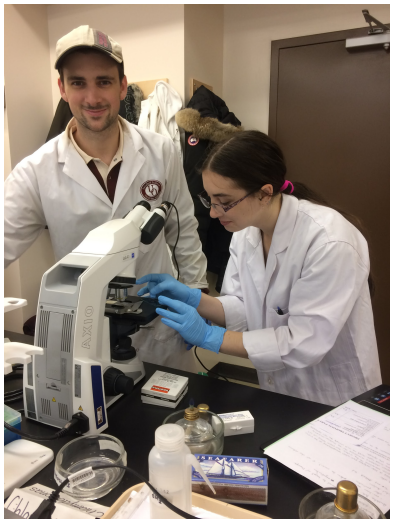

Figure 5. Preparing a slide with *C. reinhardtii* for observation.

Figure 6. Locating and tracking *C. reinhardtii* using a DinoXcope.

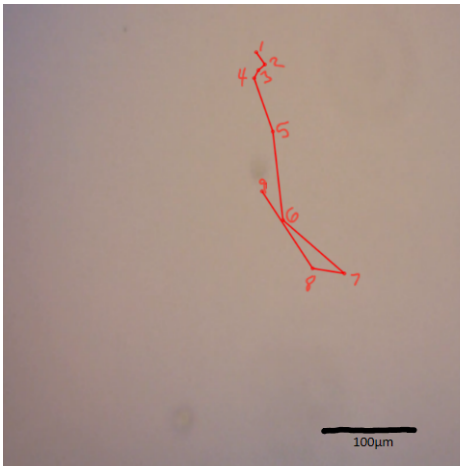

Figure 7. An example of how MTrackJ was used to track the distance travelled by *C. reinhardtii* over a five-second period.

To reduce sources of error, we kept the light intensity constant while samples were observed under the microscope, and ensured all group members were following the same criteria for slide preparation and video capture. We analyzed the data using Google Sheets and the XLMiner Analysis ToolPak by two-factor ANOVA with replication, after removing anomalies.

Results

There were four replicates for each treatment, including the control. The wild-type *C. reinhardtii* travelled a mean distance of 320.0 ± 184.8 µm at the control temperature (20°C), with

a standard deviation (SD) of 188.6 µm. At the first treatment level of 25°C, the wild-type strain travelled a mean distance of 489.5 ± 73.9 µm with a SD of 75.4 µm. At the second treatment level of 30°C the wild-type strain travelled a mean distance of 650.1 ± 462.4 µm with a SD of 471.8 µm, if all 4 replicates are included in the analysis. There was one wild-type replicate at 30°C that remained stationary for the duration of the observation period (omitted from Figure 8). Without the anomaly, the mean distance travelled by wild-type strain at 30°C was 866.7 ± 258.5 µm with a SD of 228.5 µm.

The mutant *C. reinhardtii* travelled a mean distance of 135 ± 94.5 µm at the control level, with a SD of 96.5 µm. At 25°C, the mutants travelled 31.2 ± 52.3 µm with SD of 53.4 µm. Finally, mutants travelled an average of 111 ± 59.4 µm with SD of 60.8 µm at 30°C.

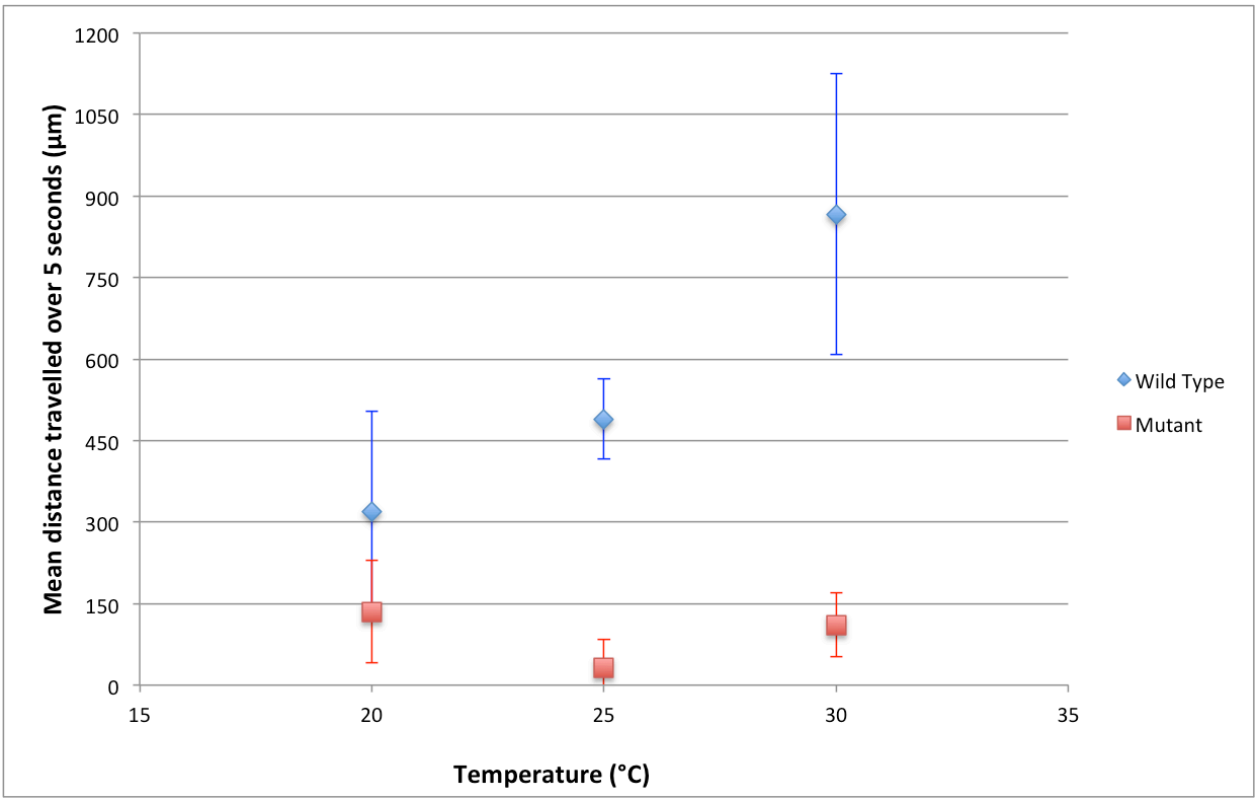

Figure 8. Mean distance travelled (µm) over 5 seconds by wild-type and mutant *C. reinhardtii* after being exposed to either 20°C, 25°C or 30°C for a period of 10 minutes. Distance was measured using a DinoXcope, ImageJ and MTrackJ plugin. n=4, error bars are 95% Confidence Intervals.

As shown in Figure 8, the wild-type *C. reinhardtii*, moved further in the five second time span than the mutants did, on average. As temperature increased, the wild-type organisms covered a longer distance in five seconds, but this trend does not appear in the mutants. The mutants moved the farthest distance at the control temperature, and they moved the least at 25°C. The mutants often remained stationary, moved in loops or rotated without covering much distance. They also appeared to float or drift on the microscope slide before moving, whereas the wild-type strain was actively swimming from the start of the observation time. The wild-type algae moved mainly linearly, apart from the one anomalous replicate at 30°C that remained stationary. After omitting this anomaly, there was still higher variation in the 30°C treatment measurements than at the lower temperatures. The mutant results generally had less variation than the wild-type measurements.

A two-way ANOVA statistical test produced $p<0.05$ for all three of our hypotheses. For H_{01}, $p=0.000001$, for H_{02} the p-value was 0.007 and $p=0.01$ for H_{03}.

Discussion

We rejected all three null hypotheses, as the two-way ANOVA statistical test produced p-values of less than 0.05 for each hypothesis tested. Thus, we were able to lend support to our alternate hypotheses; that temperature did have an effect on the mobility of *C. reinhardtii*, as did the presence of the *pf9-3mt-* mutation. Furthermore, increasing the temperature had a greater effect on the mobility of wild-type *C. reinhardtii* compared to the *pf9-33mt-* mutants.

Generally, at higher temperatures, wild-type *C. reinhardtii* moved faster, covering a longer distance in five seconds than at lower temperatures. This is supported by a study done by Majima *et al.* (1975) in which the mobility of *C. reinhardtii* increased with elevated temperature. A sudden change in temperature causes an immediate effect on the swimming velocity of *C.*

reinhardtii; if the temperature is increased or decreased, swimming velocity increases or decreases respectively (Majima *et al.* 1975). As Figure 2 shows, a change in temperature may alter the transient receptor potential channels (TRPs), thus possibly resulting in the observed effect on mobility. When the physical temperature of *C. reinhardtii* increases, thermo-TRP ion channels are activated in the flagella, which transport calcium and other ions across the cell membrane (Brauchi *et al.* 2006). According to our model (Figure 2), we expect an increase in temperature to cause an influx of calcium ions into the flagella and a subsequent increase in motility for both the wild-type and mutant *C. reinhardtii,* as both have functioning TRP channels receptive to temperature. However, many of the *C. reinhardtii* mutants swam in a circle, making the average speed difficult to determine since the path travelled was nonlinear. The increase in temperature did not affect the direction of travel in the mutant, based on qualitative observations, since mutants were swimming in circles in all three temperatures. This is because an increase in temperature does not restore flagellar motility, and flagellar beating and waveforms remain asymmetric, so the cell continues to swim in a circle (Kamiya & Witman 1984).

Wild-type organisms also travelled farther overall than *pf9-3mt-* mutants did. This is because the *pf9-3mt-* mutation causes defective inner dynein arms in *C. reinhardtii* flagella, which are important in creating symmetric flagellar waveforms for coordinated movement (Myster *et al.* 1997). Without functioning inner dynein arms, the *pf9-3mt-* mutant swims much slower compared to the wild-type *C. reinhardtii*, and therefore does not travel as far in a given time (Myster *et al.* 1997). Additionally, kinesin-like proteins in the flagella are also affected by temperature; an increase in temperature causes these kinesin-like proteins to become more active in intraflagellar transport and bidirectional movement, both of which increase the motility of *C. reinhardtii* (Kozminski *et al.* 1995).

Since there was more variation seen in the 95% confidence intervals (CIs) for the mutant data than for the wild type, we suspect that the presence of the mutation impacted motility more so than temperature; the mutant organisms were not affected to the same extent by increasing temperatures, but the wild-type organisms' motility was greatly affected (Figure 8).

Despite observing a significant difference for all three of our hypotheses, there were a few potential sources of error that may have impacted our results. Firstly, not all of the stopwatches used were of the same brand which may have caused them to have been calibrated differently from each other. This could have allowed one replicate to spend more time in the water bath compared to another. For example, the third replicate at 30°C displayed anomalous behaviour in that it remained stationary throughout the observation period. We know that the organism was alive as we could see its vacuole moving, but if the stopwatch assigned to this replicate wasn't calibrated in the same way as the stopwatches for the other 3 replicates, this alga may have been negatively impacted by a longer adjustment time to the higher temperature. If we don't include this anomalous result, the 95% CI for treatment 2 decreases to ± 258.5 μm, which is similar to the other results.

Additionally, we had to allow the mutants a few seconds to adjust to being placed on a slide before we could observe them and track their movement, as we noticed that many of them were 'drifting' or floating as they settled. However, we did not notice this effect with the wild-type organisms as they were actively swimming as soon as they were placed on the observation slides. Due to this slight difference in adjustment time, the temperature of the mutants may have decreased more rapidly towards room temperature than for the wild types, resulting in a lack of a visible trend in the mutant data. This effect would have been more pronounced at 30°C due to the

greater temperature gradient between the microscope slide and the algae, which may be why the data for that temperature are more similar to the control data than at 25°C.

Conclusion

In summary, we rejected all three null hypotheses and provided support for our alternative hypotheses with all of our p values being less than 0.05. Our experimental results suggest that increased temperature affects the movement of *C. reinhardtii*. They also indicate that increased temperature has an effect on the motility of wild-type *C. reinhardtii* but not the *pf9-3mt-* mutant strain. This was likely due to the *pf9-3mt-* mutation, which causes defective inner dynein arms in *C. reinhardtii* flagella and therefore causing the mutant to swim considerably slower compared to the wild type.

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