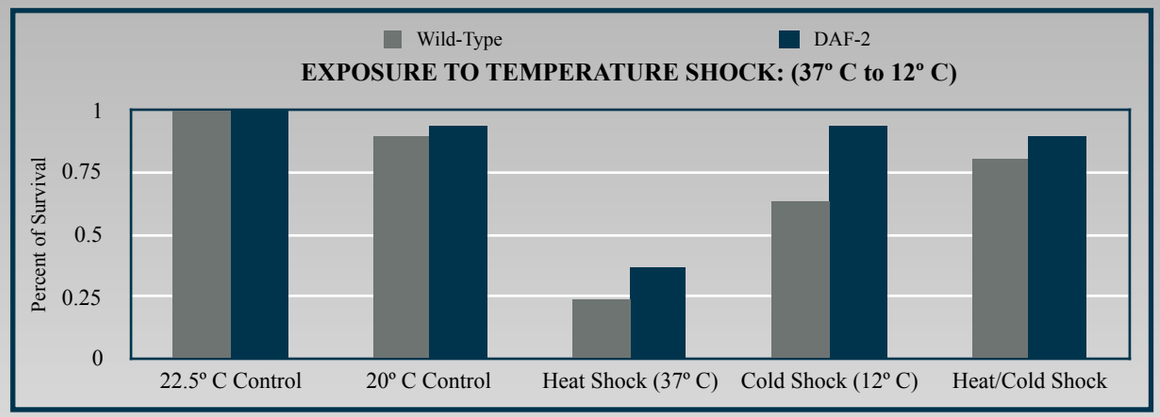
SURVIVABILITY RATES IN WILD-TYPE AND DAF-2 *C. ELEGANS* EXPOSED TO ACUTE HEAT AND COLD SHOCK

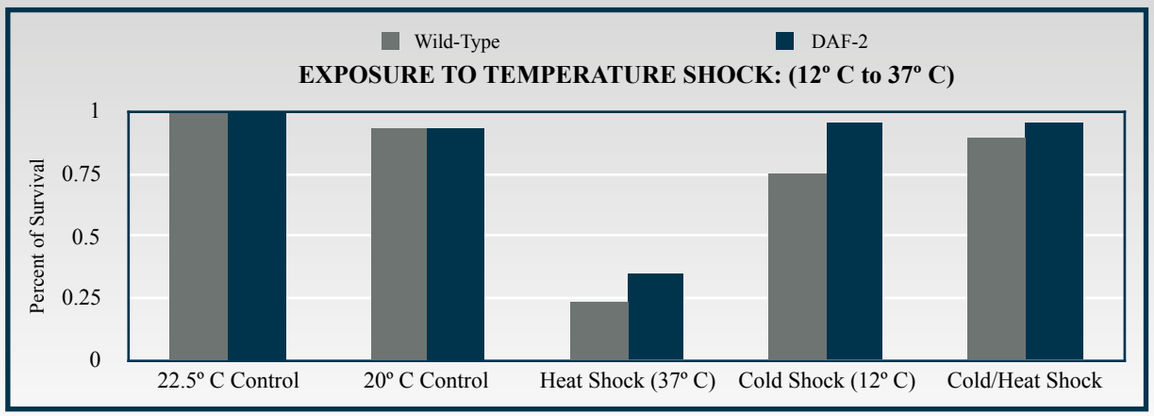
Saia Patel, Crossroads Academy
Mentors: Kelly Salmon (Ph.D candidate), Peter Faletra, Ph.D.
New Hampshire Academy of Science

ABSTRACT

Adjustments in temperature are a prominent environmental stimulus to many organisms and affect the life processes of almost all organisms. Environmental temperatures that diverge from optimal physiological temperature have been shown to exert physiological stresses and death in organisms including invertebrate organisms such as the nematode *Caenorhabditis elegans*. *C. elegans* is a model organism widely used in scientific studies because of its short lifespan, transparent body, sequenced genome, and availability of a wide array of mutant strains. *C. elegans* have been subjected to various environmental stresses and tested for their effects on the organism's development physiology, development, and fecundity. Shock was defined as the divergence from the optimal in vitro temperature (20° C). Three categories of shock were established; heat shock (37° C), cold shock (12° C), and heat shock followed by cold shock (37° C —12° C). Heat shock and cold shock, effects on the organism have been investigated, but responses to heat shock followed by cold shock has not been tested. In this investigation, wild types and DAF–2 (e1370) *C. elegans* survival was measured following heat shock (37° C for 12 hours) and cold shock (12° C for 12 hours) and heat shock followed by cold shock (heat/cold shock). DAF–2 worms are thermotolerant, hypoxia–resistant, and longer–lived and were hypothesized to tolerate sub-optimal culture temperatures better than wild type worms. Heat shock resulted in 36% v.s. 23% survival for the DAF–2 and the wild–type worms, respectively. Cold shock, resulted in 93% v.s. 63% survival rate for the DAF–2 and the wild–type worms, respectively. Heat/cold shock resulted in 90% v.s. 80% survival for the DAF–2 and the wild–type worms, respectively. These results support previous work that the DAF–2 worms in vitro have a higher survival when exposed to both colder and hotter temperatures than wild type worms. The high survival rate in the DAF–2 worms for the heat/cold shock treatment contrasted with the heat shock is surprising. One possible explanation is the short time period (30 seconds) between the change from heat to cold compared to the much larger time that it requires to quantify the death rate after exposure to the heat/cold shock treatment. The results suggest that DAF–2 worms in vitro have a higher survival than wild type worms when exposed to heat quickly followed by cold.

RESULTS

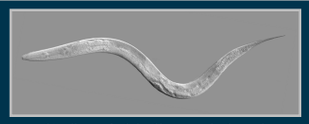




INTRODUCTION & HYPOTHESIS

Adjusting to changing temperatures is essential for survival. Extreme temperature shifts have been shown to cause metabolic and reproductive responses in organisms. In previous experiments, *C. elegans* were placed under heat shock ranging from 28° C to 31° C. Although able to survive the heat stress, the germ line and somatic gonad were damaged. A study involving acute cold shock showed that *C. elegans* lacking the G–protein coupled receptor FSHR–1 are more successful recovering from shorter sub-lethal cold shocks and immune to longer cold shocks. Temperature–related stress has been shown to be affected by the insulin/IGF1–like receptor pathway. Little is known of the death rates in *C. elegans* when exposed to extreme temperature shifts. I tested the death rates of *C. elegans* exposed to heat shock (37° C), cold shock (12° C), and heat shock (37° C) directly followed by cold shock (12° C). The DAF-2 worms were hypothesized to tolerate varying sub-optimal culture temperatures better than wild-type worms.

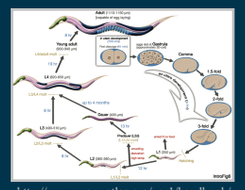
MODEL ORGANISM



http://wp.wpi.edu/qntl/resources/c-elegans/

Caenorhabditis elegans is a transparent nematode that is about 1 mm in length. Partly due to the fact that its whole genome is sequenced, it is a widely-used test subject and model organism. Many of the genes in the C. elegans genome have similar counterparts in humans, which make them useful models for human diseases. It has a rapid life cycle and exists mostly as a self-fertilizing hermaphrodite. The worms are best grown between 15º C and 25º C, with 20º C being optimal. An increase of 10º doubles their rate of development and reproduction for more than eight hours above 25° C is not possible. C. elegans also enter a "chill coma" in temperatures less than 15º C. During this state, the worms are temporarily paralyzed and take one hour to fully emerge from the coma after being exposed to regular temperature.

Mutant strains of *C. elegans* can be produced with specific genes turned on or off. DAF-2 worms are known to be thermotolerant, hypoxia-resistant, and longer-lived than wild-type worms. In these worms, the DAF–2 gene, which encodes for the insulin–like growth factor (IGF-1) receptor, is turned on. Mutations in the DAF-2 gene has been demonstrated to double the lifespan of the worms containing the gene by Cynthia Kenyon, a molecular biologist.



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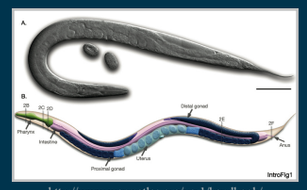

http://www.wormatlas.org/ver1/handbook/anatomyintro/anatomyintro.htm

DISCUSSION [RESULTS]

The results suggest that the DAF–2 worms demonstrate a substantially greater survivability rate in the heat/cold shock and individual heat and cold shocks in comparison to the wild–type worms. Overall, DAF–2 worms are substantially more thermotolerant than the wild–type worms. In the 22.5° control, both strains of worms had a 100% survival rate. In the 20° control, the wild–type worms had a 93.33% survival rate and the DAF–2 worms had a 90% survival rate. This might be due to the fact that the worms were raised at 22.5° C (room temperature) rather than their more ideal 20° C environment. In the heat shock, the wild–type worms had a 22.33% survival rate and the DAF–2 worms had a 36.66% survival rate. In the cold shock, the wild–type worms had a 63.33% survival rate and the DAF–2 worms had a 93.33% survival rate. The worms had a significantly higher survival rate during the cold shock compared to the heat shock. During extremely cold temperatures, worms go into a chill coma where they are temporarily paralyzed.

METHODS & MATERIALS

A room temperature control (22.5° C) and an ideal temperature control (20° C) were used. Wild–type and DAF–2 worms were kept at 22.5° C on plates seeded with *Escherichia coli*. L4 larvae were picked to fresh seeded NGM plates and allowed to develop at 20 °C for 4 hours except for the room temperature controls, which were placed at 22.5° C. Heat shock plates and heat/cold shock plates were then transferred to 37° C for heat shock assays, which lasted for two hours. At the end of two hours, heat shock plates were observed for dead worms and heat/cold shock plates were transferred to 12° C in the refrigerator for cold shock assays along with cold shock plates for two hours. At the end of the cold shock period, worms were removed and transferred to room temperature to recover. Observation for survival numbers could not be performed immediately upon return to room temperature because when *C. elegans* are exposed to cold temperatures, they enter a chill coma that inhibits motion. *C. elegans* emerge from the chill coma an hour after return to room temperature with dead worms counted using the touch assay.

Groups / Strains	22.5º C Control	20º C Control	37º C Shock	12º C Shock	37º C-12º C Shock
Wild-Type	100%	90%	23%	63%	80%
DAF-2	100%	93%	36%	93%	90%

CONCLUSIONS

Exposure to extreme cold after extreme heat may counteract the effects of the heat. I do not understand why this occurred, but it would be interesting to research this phenomenon. In the future, I would repeat this experiment but substitute 37° C with a lower temperature and further lower the temperature of the cold shock to see if the worms could survive below 12° C. Another possible experiment would be a study in epigenetics, the heritable modifications in gene expression that emerge from changes in chromosomes without alteration of the DNA sequence. I would track the DAF–2 worms that survive the extreme cold shock and determine if they could carry that tolerance throughout generations. Upon further research, I also discovered DAF-2 encodes for the IGF–1 receptor in *C. elegans*. Although DAF–2 worms have the DAF gene, they are not exactly the same, but they do have a similar function. A study was published a year ago that diabetes places individuals at greater risk for heat–related illness due to an impaired capacity to dissipate heat.

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