UNIVERSITY OF CALIFORNIA SAN DIEGO

An Analysis of the Temperature Size Rule in Ectotherms

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Biology

by

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ABSTRACT OF THE THESIS

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Biologists have long been interested in the dynamic relationship between organismal development and environmental temperature. Known as Bergmann size clines, and in the lab the Temperature Size Rule (TSR), the vast majority of animals exhibit larger body sizes at cooler temperatures (~higher latitudes) and smaller body sizes are warmer temperatures (~lower latitudes). While this can be considered a rule for endotherms which have evolved thermogenesis, ectotherms, which are at the mercy of the temperature of their immediate environment, have unique and disparate responses. Using the humble

roundworm genus *Caenorhabditis*, I show that there is no robust pattern of developmental response to temperature. The temperature size rule, which has predominantly been described by the comparison between a high and a low temperature, can be better thought of as points along a continuum of response, which can be modeled as a concave distribution unique to genotypes, known as norms of reaction. I also look at changes in reproductive strategy as the main driving factor affecting the responses to temperature we observe. I propose a possible signaling pathway connecting changes in environmental temperature to changes in reproduction and longevity. Continuing prior research, I provide evidence in support of a Single Nucleotide Polymorphism (SNP) in a gene encoding a calcium dependent protease known as *tra-3* attenuating *C. elegans* ability to maintain larger body sizes as temperatures drop. I propose a reasonable mechanism by which the polymorphism may be exerting its effects.

INTRODUCTION

For every scientific revelation, a dozen new and pressing questions take its place in the never-ending cycle of the scientific enterprise. The discovery of DNA as the underpinning of biological life (Watson and Crick, 1953) was no exception. With this long-sought elucidation of molecular life came a swarm of new and unanswered questions. One branch of this surge is known as developmental biology. The field of developmental biology can be generally summarized as the study of the processes by which organisms change from a single cell into the diverse, yet highly conserved array of organisms we see today. Environmental effects on organism phenotype have been noted by biologists for well over a hundred years. (Darwin, 1859) Indeed, the dynamic relationship between environment and development provides a mechanism by which natural selection can dictate an organism's evolutionary path. While there are myriad environmental variables resulting in developmental changes, temperature has continually affected life since its puzzling beginnings and will undoubtedly impact the continually evolving trajectory of all organic life as we know it.

Temperature, and the continuity of its effect on biological life, has long been puzzled over and studied by biologists. More specifically, scientists have long sought to resolve the question of how temperature affects development

(Lillie and Knowlton, 1897). One pattern observed in nature, designated Bergmann's rule or Bergmann's size cline, is the observation that animals living in lower latitudes, thus at higher temperatures, are markedly smaller than their genus or species counterparts living in high latitudes, and thus at lower temperatures (Bergmann, 1847). Although Bergmann's rule continues to perplex scientists (Stillwell, R.C. ,2010; Watt, C. et al., 2010; Meiri S.,2011) it is clear that an observable relationship does exist between temperature and organism development. Both endotherms (animals which have acquired the ability to regulate their own body heat), and ectotherms (animals to which their internal temperature is beholden to that of their immediate environment), show correlative relationships between development and temperature. The endothermic responses to temperature can be explained by adaptations to minimize metabolic rates and decrease the ratio of surface area to volume, both of which affect total body heat conservation (Scholander, P. F. et al., 1950; Porter, W. and Kearney, M., 2009). In fact, the original proposal of Bergmann's rule in 1847 was restricted to endotherms (Bergmann, 1847). The generality of this rule has been transposed to and studied in ectotherms however, resulting in a multitude of findings both supporting and rejecting the overarching hypothesis (Pincheira - Donoso, 2010). The current scientific community still has no consensus on whether or not ectotherms even obey Bergmann's rule, much less an understanding of the mechanistic underpinnings.

Scientists studying ectotherms at different temperatures in the laboratory found that the majority of organisms developed a larger body size at colder

temperatures, supporting a Bergmann's size cline (reviewed in Atkinson 1994). Atkinson named this phenomenon the Temperature-Size Rule (TSR). While Bergmann's rule describes a general trend observed in nature, the Temperature-Size rule describes the ectothermic relationship of body size to temperature (although the rule itself colloquially covers a broad range of life history markers and their relationship with environmental temperature) as observed in a laboratory, summarized graphically as reaction norms (Angilletta and Dunham, 2003). These norms of reaction are representative of the range of an organism's phenotypic plasticity, that is the range of phenotypes that can result from a single genotype. The TSR has been studied in many organisms and settings (Angilletta et al., 2004; Atkinson et al., 2006; Diamond and Kingsolver, 2009; Arendt et al., 2011). While many endeavors have sought to model this relationship mathematically, (Berrigan and Charnov, 1994; Perrin, 1995; von Bertalanffy, 1960), general explanations remain insufficient and require more specific data (Atkinson and Sibly 1997; Angilletta et al. 2004).

This may be due to the fact that existing data is in many cases counterintuitive (Moussea, 1996), seeming to exclude any general explanations that may or may not exist. Nevertheless, many hypotheses have been proposed to explain this relationship. In broad terms, all hypotheses can be grouped into one of two categories: adaptive and non-adaptive

Non-adaptive

Any response that is considered to be non-adaptive is by definition unrelated to evolutionary fitness. The feeling that such a widespread and general relationship should have a just as widespread and general casual source seems fairly intuitive. Therefore, many theories have been proposed to explain the ectothermic response to temperature through non-specific, non-adaptive mechanisms.

Oxygen availability

One hypothesis that stands out as a potential contributor to the temperature response is the changing availability and metabolic consumption of oxygen at the cellular level in varying temperature environments. By using R_{max} as the radius at which oxygen concentration drops to zero in a cellular sphere (indicating the inability of a cell to function properly), Woods (1999) showed that as temperatures increases, the metabolic rate of oxygen consumption outpaces the increasing oxygen supply due to the differences in respective Q_{10}'s (change in a given variable per 10C). Since the diffusion coefficient of Oxygen is only weakly sensitive to temperature (Q_{10} = ~1.4) when compared to the sensitivity of oxygen consumption (Q_{10} = ~ 1.5 - 4.0), cells at higher temperatures should necessarily require a smaller $R_{max,}$ to counteract the decreasing ratio of oxygen

supply and demand, resulting in a decrease in overall size. These findings indicate a non-adaptive relationship between temperature and cellular size. Empirical evidence may suggest that while changes in oxygen availability create boundaries outside of which cellular growth and function is not possible, the range of responses observed in ectotherms are more likely due to a less generalizable response.

Variable temperature sensitivities of growth and differentiation

One widely used metric to test the Temperature Size Rule is size at maturity (organism size at the start of their reproductive cycle). An interesting and plausible argument that has been proposed to explain size differences at maturity is that of the uncoupled nature of growth and differentiation. If growth is measured as changes in biomass per unit time, and differentiation is measured as the diversification of cell types per unit time, one can look at the underlying mechanics of these individual processes to address how temperature may exert independent effects (Van de Have and de Jong, 1996). By utilizing protein synthesis as a proxy for growth, and DNA replication as a proxy for differentiation, Van de Have and de Jong (1996) put forth a well written first step in laying out a plausible mechanism by which a larger Q_{10} of differentiation relative to growth would result in smaller size at maturity, as growth (biomass accumulation) is simply outpaced by differentiation as temperature increases. This model has many parallels to the Disposable Soma Theory of Ageing

proposed by Thomas Kirkwood in 1977. More conceptual than mechanistic, the theory proposes that as total energy obtained is finite at any given time for a given organism, a tradeoff of energy allocation between reproduction and soma growth exists. The disposable soma theory of ageing is generally thought of as a conceptual framework for explaining the positive correlation between senescence and life span, an organism's tendency for a specific ratio of energy allocation may be influenced by its surrounding environmental temperature. While this theory requires a pseudo adaptive mechanism for shifting the energy balance in response to temperature, the independence of enzymatic kinetics of cell differentiation and growth proposed by Van der Have and de Jong provide a plausible non- adaptive mechanistic underpinning for how energy may be utilized discretely towards temperature dependent cellular processes.

Adaptive

Although both of the proposed non-adaptive mechanisms are plausible in their own right, and at minimum seemingly must play roles in constraining temperature responses, the connection between the Temperature-Size rule and an underlying genotype cannot be disregarded (Partridge et al., 1994; Partridge and Coyne, 1997). Even considering a genome that has evolved to include a larger body in colder temperatures and a smaller body in warmer temperatures as a response necessarily pushes one towards adaptive plasticity when considering the totality of an explanation for how temperature dependent responses can provide fitness advantages that can be selected for.

Warm Environments

In warm environments, the overarching response of organisms that obey the TSR is a decrease in time to maturity, maturity being defined as the ability to reproduce. Generally, reproduction in ectotherms is thought to follow a conserved trend wherein a smaller body size confers a smaller brood size, while a larger body size is correlated to a larger brood size (Roff, 2002). Whether or not this is a biological law is still unclear, however, a simple explanation would be that a larger organism is able to allocate more resources towards reproduction. Conversely then, an organism that is smaller at time of reproductive maturity would produce less progeny. At first glance this would seem to decrease an

organism's overall fitness. Consider, however, that larger organisms take longer to accumulate their size, delaying reproduction in colder environments. This line of reasoning has led many to propose what is known as the, "compound interest hypothesis" (Dixon, 1992; Partridge and French, 1996; Fischer and Fiedler, 2002; Atkinson et al., 2003). This theory focuses on the decrease in time to reproductive maturity in warm temperatures as a driving selective force, providing a stronger positive selection than any negative selective pressure provided by a decrease in progeny output. Therefore, organisms that are restricted to a particular breeding season (usually the warm season) benefit from completing multiple generations in as little time as possible, compounding the chance of viable offspring and generating a selectable advantage. This theory provides a conceptual framework for how natural selection could indirectly select for a smaller body size but does not provide a plausible mechanism for how this phenotype could arise. One theory proposed by Kindleman and Dixon (1992) describes a mechanism of differential energy assimilation shaping an organism's fecundity function. Increasing energy allocation towards gonadal growth and development reduces energy utilized towards overall soma growth. In short, organisms have adapted mechanisms to optimize both individual and population growth rates by regulation of their internal distribution of energy. This explanation closely parallels the framework of the Disposable Soma Theory of Ageing mentioned earlier and can be loosely conceptualized as the adaptive version of Van der Have and de Jong's uncoupled growth and differentiation. Senescence too, may be a possible adaptable mechanism (Kindleman and Dixon, 1992). As a

logical rule, an organism's reproductive cycle needs to occur before senescence makes reproduction inviable. If the rate of ectothermic senescence is increased with increasing temperature, selection would favor organisms who are able to reach reproductive maturity early enough to beat their biological clocks (Kindleman et al, 2001).

Cold Environments

It may seem logical to assume that the compound interest hypothesis should apply generally, regardless of temperature, and thus always select for the shortest possible time to maturation. There are, however, other considerations and hypotheses that may explain why an increased time to maturation (as seen by organisms that obey the TSR) and delayed but increased growth may be a selective advantage. Considering the costly nature of reproduction and importance of maximizing chances of survival, especially in single lifetime brood organisms (organisms that have only one distinct reproductive cycle during their lifetime), organisms may have evolved a strategy of increasing offspring investment time in response to changing temperatures. The effects of temperature on juvenile mortality can be classified into both direct and indirect effects. Direct effects are considered to be the effects of temperature on development, physiology, and behavior. Indirect effects can be biotic or abiotic; for example, changes in local resources, predators, competitors, or parasites, or effects on salinity or pH (Angeilletta et al., 2004). If indeed colder environments

provide a harsher juvenile environment, resulting in higher rates of juvenile mortality, the selective advantage may lie in investing more into reproduction, mitigating the increase in juvenile mortality (Sibly and Atkinson 1994; Atkinson 1995, 1996).

While all of the proposed theories I outlined above seem to be plausible in at least some observed responses to temperature, there has yet to be a proposed theory that is sufficiently generalizable to explain the vast range of temperature responses observed across ectothermic organisms.

Possible genetic mechanisms: *tra - 3*

The current body of literature exploring the genetic roots of the TSR leaves many questions yet to be answered. In *C. elegans*, one door that has been opened as a possible genetic link is a single nucleotide polymorphism (SNP) in *tra-3* (Kammenga et al., 2007). *tra-3*, or more specifically, a F96L mutation within the *tra-3* gene resulting from the nucleotide polymorphism, was shown to have a statistically significant association with whether two strains of *C. elegans* obeyed the TSR. *C. elegans* strain N2 contains an adenine base, while *C. elegans* strain CB4856 (also referred to as the Hawaiian strain) contains a guanine base, resulting in a change in the translated amino acid phenylalanine (F) to leucine (L) in the 96th position. *tra-3* is a member of the calpain protein family, which acts a calcium induced protease (Barnes and Hodgkin, 1996). In *C. elegans* it appears to also operate in the sex determination pathway,

specifically regulating hermaphroditic versus male sex determination (Ellis and Schedl, 2007). The research further suggests that this alteration in outcome may in part be due to a change in the protein's ability to bind calcium, which has been shown to increase in cytosolic concentration in response to lowered environmental temperatures (Shuttleworth and Thompson, 1991; Shiels and Vornanen, 2002), providing a possible link between changes in temperature and a corresponding molecular mechanism.

Study System

Isolated from Algerian soil and first described by French librarian Emile Maupas in the1900's, the nematode *C. elegans* did not come to prominence until Sydney Brenner's proposal of *C. elegans* as a model system for genetic study in the 1960's. Brenner had success using molecular genetics in this simple organism to study cellular development and the worms could be easily managed in large numbers. Interestingly, Brenner originally proposed to use *C. briggsae* as the model. However, perhaps by chance, *C. elegans* was used instead and thus became the model system for developmental study that we use today. There are a few key features that make this humble worm an excellent organism for the study of cellular and organism development. One of the most intriguing attributes of *C. elegans* is that they are eutelic and thus the entire lineage of every cell could be traced to provide a strictly controlled developmental fate map (Sulston

et al., 1983). It is also important to note here that the transparency of the *Caenorhabditis* body heavily contributed to not only the success of the early fate maps and lineage traces but generally provided the ability to track cellular movement and changes in the living organism as it developed. Another aspect of the *Caenorhabditis* genus that enabled rapid adoption by the biological community is the simplicity of its organs and organ systems. Lacking a respiratory system, circulatory system, and many of the other organ systems of more complex animals, *C. elegans* are broadly composed solely of a GI tract and reproductive structures. Notwithstanding its simplistic anatomy however, the genome of *C. elegans* contains approximately the same number of coding genes and shares many molecular pathways with humans. Another attribute that makes C. *elegans* an especially good choice for this study and good choice for developmental or genetic study generally is their hermaphroditic nature. Since *C. elegans* are either male or hermaphrodite, they provide a useful mechanism for maintaining uniform genetics through self-fertilization, while also facilitating the transfer of genetic markers through males (Brenner, 1973). For our purposes, the genetic stability provided by self-fertilization in *C. elegans* negated the potentially confounding and hard to control for variable of mating and genetic crossover. Hermaphroditic *C. elegans* are truly somatic females that have evolved spermatogenesis, allowing them to either self-fertilize or outcross. Hermaphrodites are able to reproduce asexually by producing sperm early in life, which can then later fertilize oocytes (Nayak et al. 2005; Baldi et al. 2009). In my opinion however, a large reason *C. elegans* were adopted so readily as a

developmental model system is simply the ease and practicality of maintenance in the lab. *C. elegans* are quite small, adults measuring on average around 1 mm long. They are extremely fertile laying hundreds of eggs throughout their reproductive life. Their generation time is only about 3 days which makes them useful when tracking development and heritable changes. *C*. *elegans* can be cultured by the hundreds on a single petri dish containing agar and a bacterial *E. coli* strain OP50 for food and can also be frozen and thawed without killing them (probably in part due to their simple anatomy). The ease of maintaining and storing large numbers in a lab make them accessible for use to a wide range of laboratories virtually regardless of funding. This facilitated the generation of an extremely large shared data set focused on all aspects of *C. elegans* form, function, and development.

Table 1. All strains used in this research

strain	species	locality of origin	Phylogeographic group	latitude	approx. elevation(m)
AF16	C. *briggsae*	Ahmedabad, India	tropical	23°01'N	50
HK104	C. *briggsae*	Okayama, Japan	temperate	34°40'N	30
N2	C. *elegans*	Bristol, England	temperate	57°27'N	36
CB4856	C. *elegans*	Hawaii, USA	tropical	24°20'N	1
JU1568	C. *elegans*	Ivry-sur-Seine, France	temperate	48°48'N	48
ECA593	C. *elegans*	Santa Barbara, Ca	temperate	34°N	12
JU1373	C. *tropicalis*	la reunion, France	tropical	21°06'N	1

Here, I empirically quantified the temperature driven outcomes of nematode development using the ectothermic model system *C. elegans* and related species. I gathered data from the nematode to add to the small body of existing TSR data in hopes of further elucidating this seemingly convoluted temperature response. I also test the breath of the *tra-3* polymorphism and its dependence as a marker for TSR outcome using strains N2 and CB4856, as well as the closest phylogenetic neighbors to both N2 and CB4856 carrying the alternative polymorphism. As the data revealed itself, I decided to further test *C. elegans* strains N2 and CB4856 in two more temperatures, allowing me to model their reaction norms and gain a better understanding of their individual responses and their relation to the *tra*-3 variation.

MATERIALS AND METHODS

Maintaining *Caenorhabditis* strains

I used seven strains of *Caenorhabditis* for this research (see Table 1). Four strains of *C. elegans:* N2, CB4856, JU1568, and ECA593; two strains of *C. briggsae*: HK104 and AF16; and a single strain of the species *C. tropicalis,* JU1373. I maintained worms at room temperature (~20°C) on 9 mm petri dishes containing NGM lite agar medium. Plates were seeded with *Escherichia coli* strain OP50 throughout to ensure adequate nutrients for the worms. I transferred worms to new 9 mm dishes every 5-7 days, or as deemed necessary to prevent starvation and maintain population growth. When plates became contaminated, I bleached them using a standard protocol. This cycle of transfers and bleaching was repeated continuously throughout the experiment or as necessary to maintain uncontaminated populations of worms.

Measuring lifetime growth rates

I tracked Individual worms and recorded their body size for the duration of their life cycle at a range of temperatures (12°C, 16°C, 24°C, 27°C, as well as 30°C specifically for C. *briggsae* strain AF16). In order to ensure a synchronized life cycle across replicates, I transferred ~10-20 gravid worms onto a 3 cm plate and allowed them to lay eggs for a period of 1-2 hours. Eggs were picked onto

individual 3 cm petri dishes and placed in an incubator at the designated temperature. The following day I checked each replicate to determine if the egg had hatched into the L1 (larval 1) stage. I photographed embryos that hatched successfully every 24 hours until they died using a Canon EOS camera. In order to standardize image size across plates, I added 100 μm Cospheric microspheres to each plate. I used these beads as a reference measurement to convert a measured pixel length into an area in micrometers, taking care not to touch the microscope optic to ensure the reference frame remained unchanged between images of the worms and their corresponding beads. Once worms had reached reproductive potential and began to lay eggs, I transferred each worm to a new 3 cm plate daily for the duration of their reproductive span. Once the worms and beads were photographically recorded, I used an image processing program running on MATLAB to measure the pixel lengths of both beads and worms. As the glass beads were fairly irregular and non-uniform, I recorded approximately 5-10 beads per dish and calculated the average diameter in pixel length. I then compared the calculated mean pixel length to the manufactured bead diameter of 100 μm to determine the pixel length corresponding to 100 μm. I used this as the conversion factor to convert the measured worm pixel length into a value in micrometers. This length in micrometers was then used to calculate the area of the worm in micrometers. I processed a subset of replicates multiple times to ensure precision in the image processing software (see appendix). Worms were tracked and photographed from egg hatching to death to provide total life span data. Worms that bagged (died due to offspring hatching

within their body cavity), walled (exhibited searching behavior to the point of crawling up the walls and drying out), or died during the transferring process were excluded from data sets.

Measuring lifetime fecundity

Worm's reproductive cycles began anywhere from 2-10 days post-hatching, and in rare cases even later. The duration of egg laying lasted anywhere between 2-13 days. Once worms entered their reproductive cycle and began laying eggs, they were transferred to new plates daily. I counted the number of eggs laid by each replicate each day and recorded the data into an excel file.

Calculating Biomass Measurements

The measurements pertaining to the biomass accumulation were derived using values from Byerly *et al.* (1976), who used a conversion factor for egg mass of .035 μg per egg, and a conversion factor of a 1400 μm length worm having a mass of 4.3 μg. While both of these are very general and may miss subtle differences in individual mass of eggs in different temperatures or variation in length-width dimensions in worms, I am mostly interested in looking for general relationships between biomass distribution and therefore using these conversion factors are sufficient.

Calculating body size at maturity

Body sizes at time of maturity were calculated by first using the measured rates of egg laying to estimate a precise time when the first egg was laid. If the first 24-hour cycle recorded in which eggs were noted is considered day n, day n + 1 was used to calculate a rate per hour by dividing by 24. This rate was then used to determine the hours needed to lay the amount of eggs recorded on day n. The body size growth over than 24 period was then calculated and using a similar derivation, then the body size at the particular time of first egg laying as shown above was calculated. While this method is less precise as it assumes perfectly linear relationship of both egg laying and organism growth over the 24-hour period, I saw no obvious contradictions between my data and prior data.

RESULTS

In this experiment I aimed to measure and record the effects of environmental temperature on various life history traits in several species of *Caenorhabditis* to elucidate the relationship between ectotherms and temperature. The nematode model system was chosen for this experiment due to the large body of literature focused on its molecular development, as well as its practical usefulness in laboratory settings. Seven members of the *Caenorhabditis* genus were used (see Table 1). Two strains from the species *C. briggsae* were used: a temperate strain (HK104) and a tropical strain (AF16). A species *C. tropicalis* (JU1373) was used as well simply due to the fact that there has not

been much literature focused on this species, it is a hermaphroditic species, decreasing potentially confounding genetic variables, and it has a name that would lead many to assume it is potentially well adapted to a warmer environment. The remaining four strains all come from the species *C. elegans* and were chosen in part based on previous research by Jan Kammenga that showed a potential correlation between a single nucleotide polymorphism (SNP) and developmental differences in response to temperature (Kammenga et al., 2007). The strains N2 and CB4856 (also known as the Hawaiian strain), were chosen initially due to the breadth of current comparative literature on these two strains, as well as their appearance in Kammenga et al., 2007. Two further strains were chosen based on their phylogenetic position in relation to the *tra-3* SNP of interest (see appendix). *C. elegans* strain JU1568 is the closest phylogenetic neighbor to strain N2 that has the SNP that correlates with CB4856. The strain ECA593 was chosen for precisely the opposite reason; it is the closest phylogenetic neighbor to CB4856 with the nucleotide seen in strain N2. A high temperature of 27°C and a low temperature of 16°C were used for each strain (C. briggsae strain AF16 was the only strain tested in 30°C as a high temperature) and further testing of strains CB4856 and N2 at 12°C and 24°C were performed after preliminary data was collected.

Body Size Development

One of the initial interests of this experiment was to see how body size development was affected by temperature. Body size development in high and

low temperatures can be classified into one of two groups or trajectories: nested and crossing (Figure 1). All *C. elegans* species show a crossing trajectory resulting in a smaller final body size in warmer temperatures and a larger final body size in colder temperatures (Figure 2). It should be noted however that *C. elegans* strain ECA593 is rather unclear in terms of its growth trajectory, although the trajectory is crossing, it seems as though a larger warm temperature body size was reached overall. This is especially interesting considering this was the only *C. elegans* strain to show non-compliance with the TSR at these temperatures. Despite, however, showing the same general growth trajectories, they vary in final size. Both the N2 strain and its phylogenetic neighbor, despite carrying the ancestral *tra*-3 polymorphism, grow to be approximately 70000-80000 microns^2. This is much larger than the other two strains; CB4856 and its closest phylogenetic neighbor (ECA593) containing the N2 like nucleotide in *tra*-3, which both only grew to an average size of 50000-60000 microns^2. It is also interesting to note that the growth of worms is slowed in all cases, resulting in a much steeper growth curve in all warm temperatures. When looking at the *C. briggsae* and *C. tropicalis* strains, both the tropical *C. briggsae* strain AF16 and the *C. tropicalis* strain JU1373 display a nested growth pattern, resulting in a final body size in cooler temperatures that is smaller than their final body sizes in warm temperatures. While the temperate strain of *C. briggsae* HK104 retains the crossing growth trajectory (Figure 3), all three strains develop to approximately a similar final body size in warm temperatures of 50000 microns^2. In the case of AF16 and JU1373, their growth in warm temperatures does not increase too

result in the nested pattern, instead their growth in cooler temperatures decreases. This is in contrast to all *C. elegans* strains, whose final body size in both temperatures seem to increase or decrease proportionally. There does not seem to be any clear pattern relating compliance with the TSR and growth trajectories.

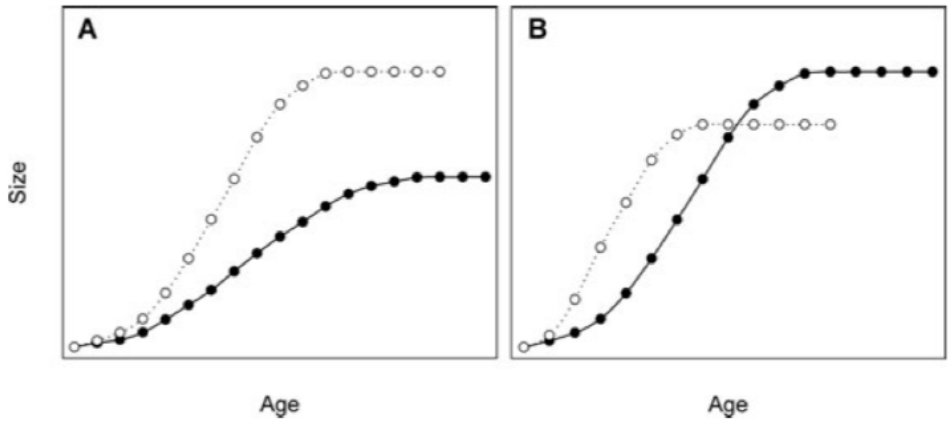

Figure 1. Simple cartoon showing a growth trajectory for both warm (light) and cold (dark) temperatures. Two trajectories are depicted, a nested trajectory (A) and a crossing trajectory (B). Image taken from Arendt (2011).

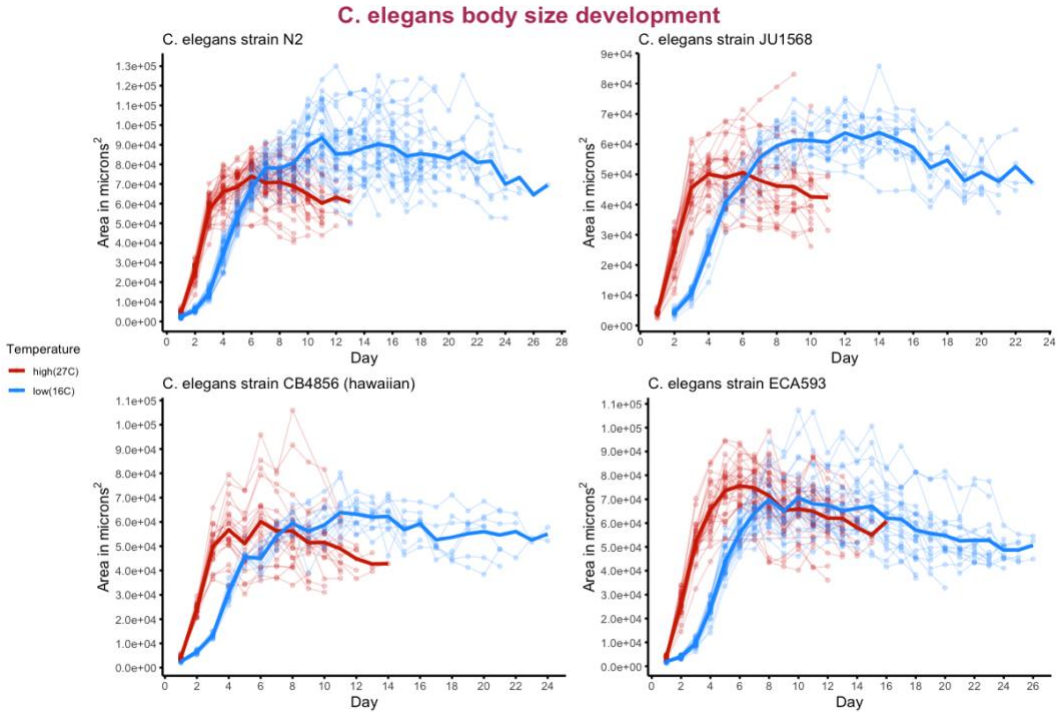

Figure 2. *C. elegans* average body size development across their lifetimes comparing warm (27°C in red) and cold (16°C in blue) temperatures. The y-axis shows body size as measured in microns^2 every 24 hours. All strains show a crossing trajectory, resulting in a larger final body size in cooler temperatures.

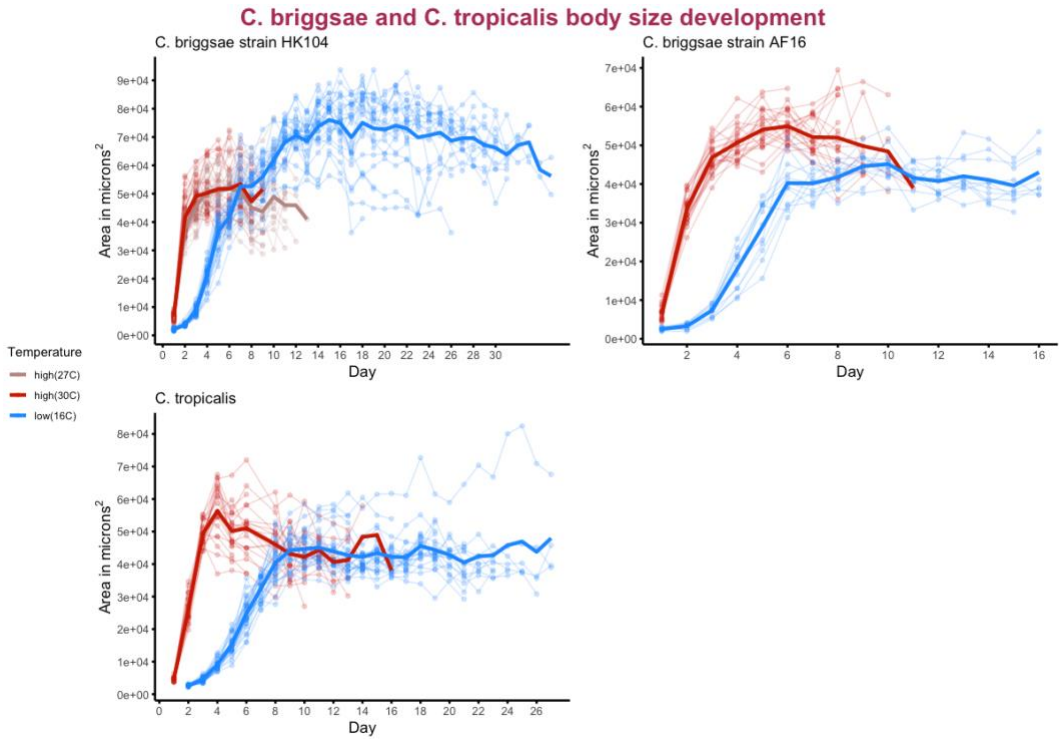

Figure 3. C. *briggsae* and C. *tropicalis* average body size development across their lifetimes comparing warm (30°C in red) and cold (16°C in red) temperatures. A third temperature of 27°C was used for C. briggsae strain HK104 as 30°C seemed to be outside the range of a viable living environment. The y-axis shows body size as measured in microns^2 every 24 hours. *C. briggsae* strain AF16 and C. *tropicalis* show nested patters, while *C. briggsae* strain HK104 shows a clear crossing trajectory. Compliance with the TSR seems to be independent of any correlation with growth pattern trajectories.

Fecundity

Fecundity was recorded by counting the amount of eggs on individual plates in a 24-hour period. All strains displayed similar general patterns of changes infecundity. In cooler temperatures, *C. elegans* strains were able to not only increase the per day output of eggs, but the duration of the reproductive cycle as well, drastically increasing the total brood output in the cooler temperatures as compared to the warm (Figure 4). In *C. briggsae* and *C. tropicalis* strains, the amount of eggs laid per day decreased in cooler temperatures but was seemingly counteracted by an increase in the duration of the reproductive cycle (Figure 5). All four *C. elegans* strains, as well as the tropical *C. briggsae* strain (AF16) laid more eggs in cooler temperatures than they did in warm temperatures, with average brood sizes of no less than 200 progeny. The two strains that did not produce more eggs in the cooler temperatures were the tropical strain of *C. briggsae* (HK104) and *C. tropicalis* (JU1373). In is interesting to note that neither of these strains obeyed the TSR when looking at body size at maturity. Temperatures of 27°C resulted in no *C. elegans* strains producing on average more than 100 progeny in their lifetime, while both *C. briggsae* strains produced between 100-150 progeny on average and *C. tropicalis* produced slightly over 100. The additional temperatures of 12°C and 24°C used to test C. *elegans* strains N2 and CB4856 show that as temperatures continue to cool, the reproduction continues to decrease in output per day while also continuing to increase in duration (Figure 6).

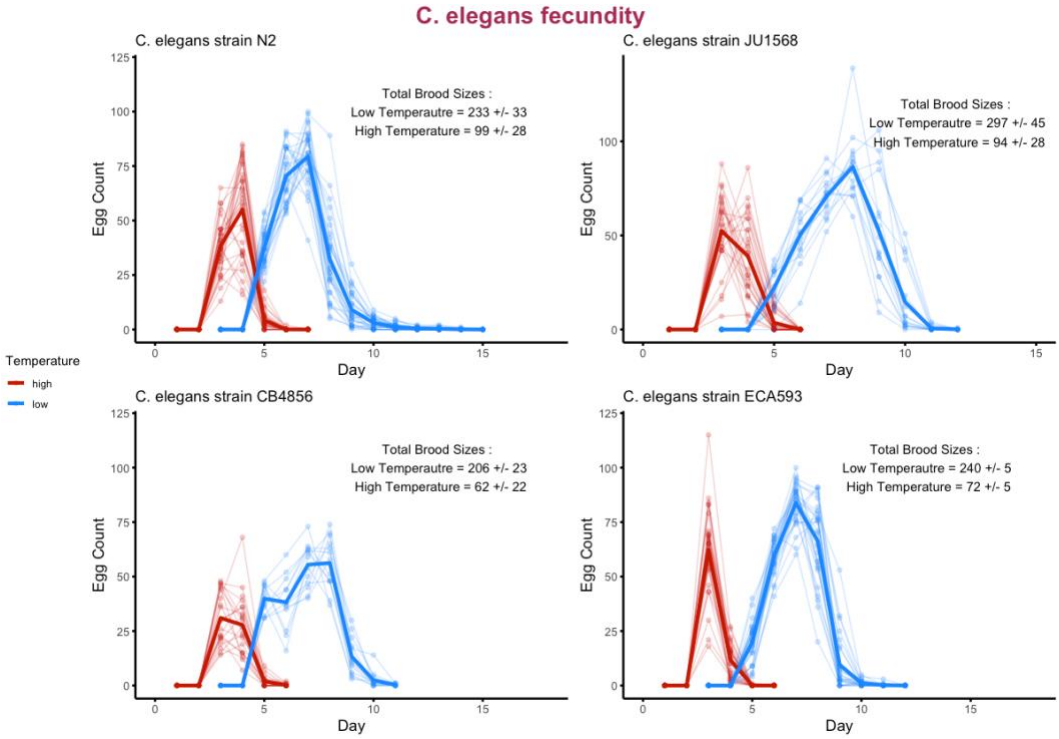

Figure 4. *C. elegans* duration and average size of reproductive cycle in warm (27°C in red) and cold (16°C in blue) temperatures. Average total brood sizes are shown. The y-axis shows the amount of eggs laid per 24 hours. All strains show not only an increase in eggs laid per day, but an increase in the duration of the reproductive cycle. This is emphasized by the drastic increase in progeny output (~162 eggs) in all strains in cooler temperatures.

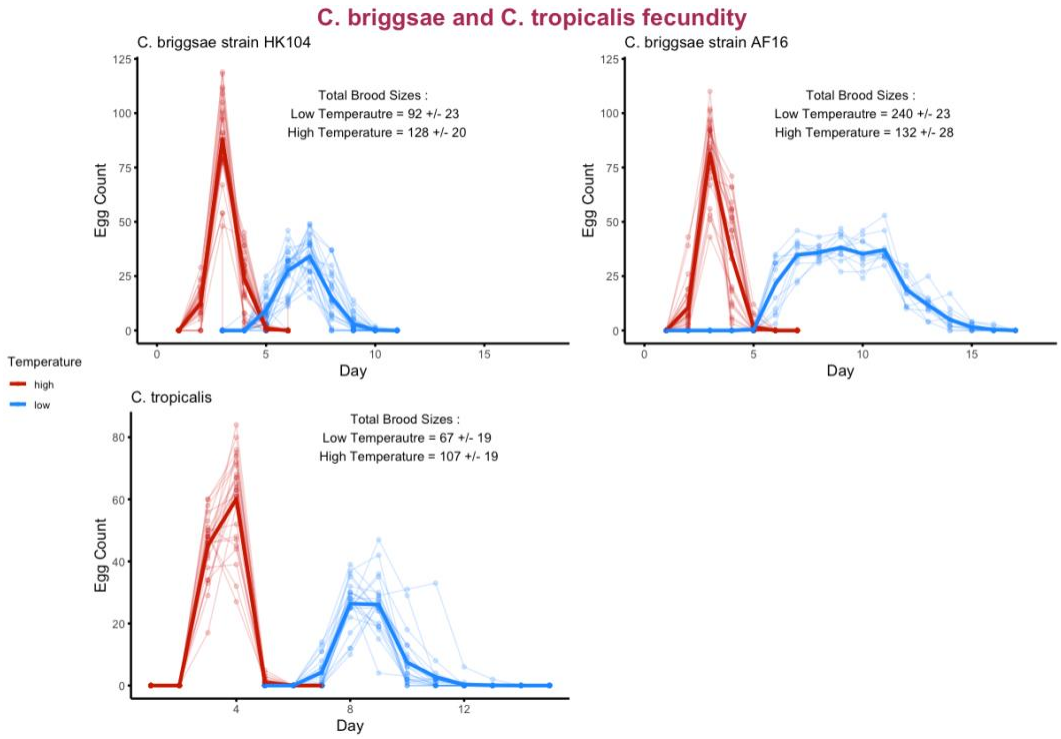

Figure 5. *C. briggsae* and *C. tropicalis* duration and average size of reproductive cycle in warm (27°C in red) and cold (16°C in blue) temperatures. Average total brood sizes are shown. The y-axis shows the amount of eggs laid per 24 hours. Unlike *C. elegans* strains, *C. briggsae* and C. *tropicalis* strains are not able to maintain a high per day output of eggs in colder temperatures. Although the reproductive duration is increased, only the tropical strain of *C. briggsae* (AF16) is able to increase the duration of the reproductive cycle long enough to produce a larger total brood sizes in cooler temperatures as compared to warm.

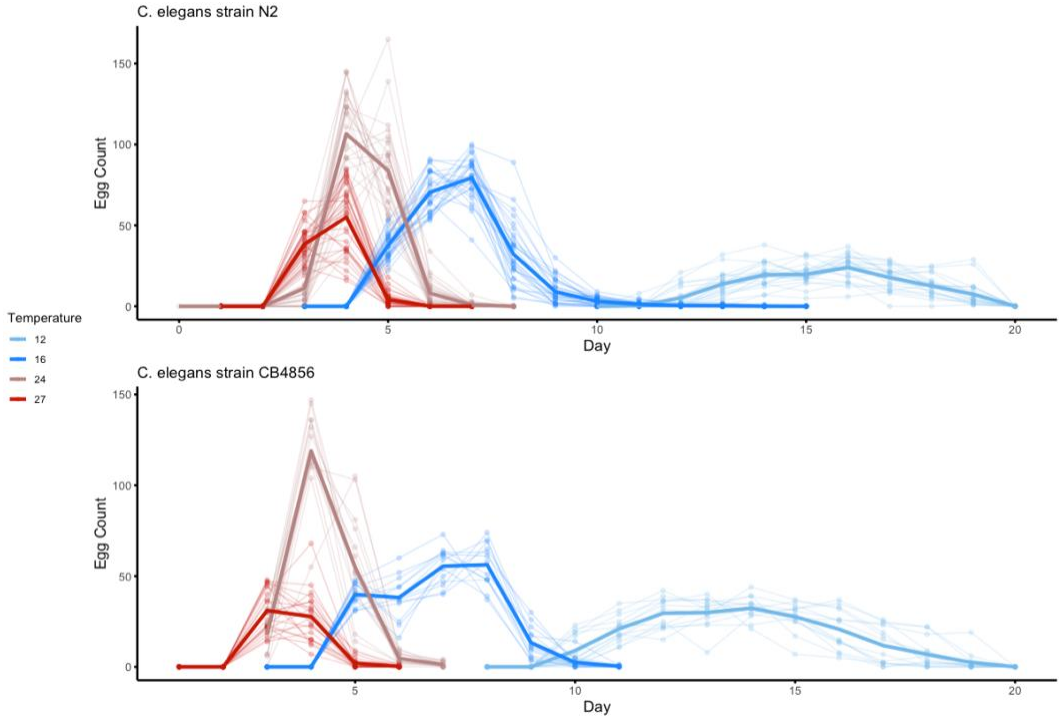

Figure 6. Two strains of *C. elegans* (N2 and CB4856) duration and average size of reproductive cycle across a range of temperatures (12°C, 16°C, 24°C, 27°C). Both strains show a pattern of increased duration of reproductive cycle as temperatures cool, and decreased duration as temperatures warm. The amount of eggs laid per day is maximized at 24°C and decreases as temperatures either warm or cool.

Survival

Survival in all strains was increased in colder temperatures with an average lifespan of around ~25 days for all strains. At warmer temperatures the duration of life was reduced, showing an average lifespan of only ~12 days (Figure 7).

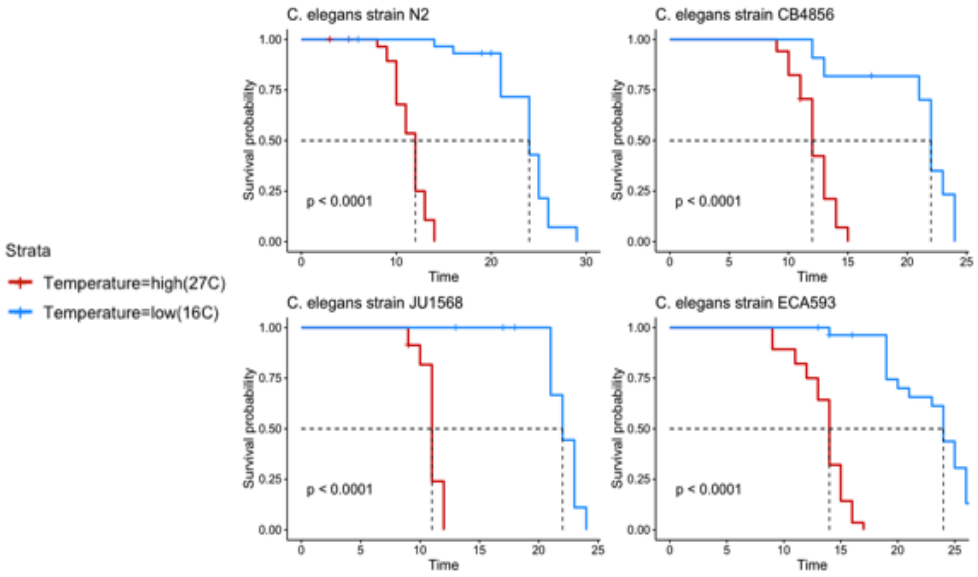

Figure 7. Survival curves for all C. elegans strains tested. Colder temperatures (16°C in blue) provide an increase in longevity while warmer temperatures (27°C in red) seem to speed up all life processes which results in a shorter overall lifespan. 50% population survival is denoted by the dotted black line. Time is reported in days.

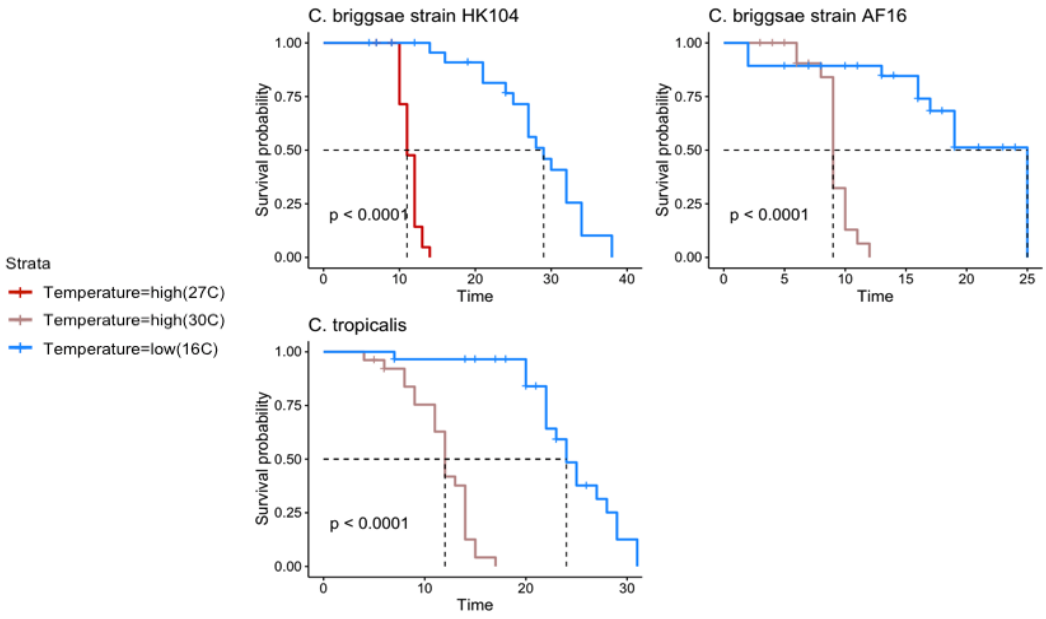

Figure 8. Survival curves for *C. tropicalis* and *C. briggsae* strains. Colder temperatures (16°C in blue) provide an increase in longevity while warmer temperatures (27°C in red and 30°C in brown) seem to speed up all life processes which results in a shorter overall lifespan. 50% population survival is denoted by the dotted black line. Time is reported in days.

Size at time of Maturity

The size at time of maturity was calculated for all strains at a cold temperature of 16°C and a warm temperature of 27°C or 30°C. A positive slope correlates with compliance of the TSR, while a negative slope indicates a smaller body size at time of maturity in cold temperatures, and thus non-compliance (Figure 8A). Looking at the *C. briggsae* and *C. tropicalis* strains, both the temperate strain of *C. briggsae* (HK104) and the *C. tropicalis* strain (JU1373) show a negative slope. The tropical strain of *C. briggsae* (AF16) shows a positive slope, obeying the TSR. *C. elegans* strain N2 exhibited a strong positive slope while also being the largest in size overall in both warm and cold temperatures. The *C. elegans* strain JU1568 that was chosen as a close phylogenetic neighbor to *C. elegans* strain CB4856 (Hawaiian) while still retaining the *tra-3* allele identical to N2, displays almost an identical slope to that of N2, although has an overall body size that is much smaller in both temperatures. CB4856, which was chosen as a participant originally due to its non-compliance of the TSR shown by Kammenga *et al.* (2007) displays a positive slope, seemingly obeying the rule, while the *C. elegans* strain ECA593, which was chosen based on its own phylogenetic proximity to N2 while retaining the mutated *tra-3* sequence shows a negative slope. My results that CB4856 obeys the TSR are contrary to the results shown by Kammenga *et al.* (2007). This prompted further testing using the same temperatures used by Kammanga *et al*. The data from these two new temperatures (12°C and 24°C) show CB4856 displaying a negative slope which indicates non-conformity to the TSR, confirming the results from Kammenga *et*

al. (2007), as well as providing a pseudo control for my methods of calculation of body sizes (Figure 8B). N2 still displays a strongly positive slope indicating compliance. Interestingly, although N2 displays an almost identical slope, the body size in the cooler warm temperature of 24° is larger than that of 27°, while the colder temperature of 12° compared to 16° is smaller. Conversely, CB4856 shows an approximately similar body size at 24° compared to 27°, but a drastically smaller size at 12° as compared to 16°. This would suggest that there is a temperature dependency mechanism at play.

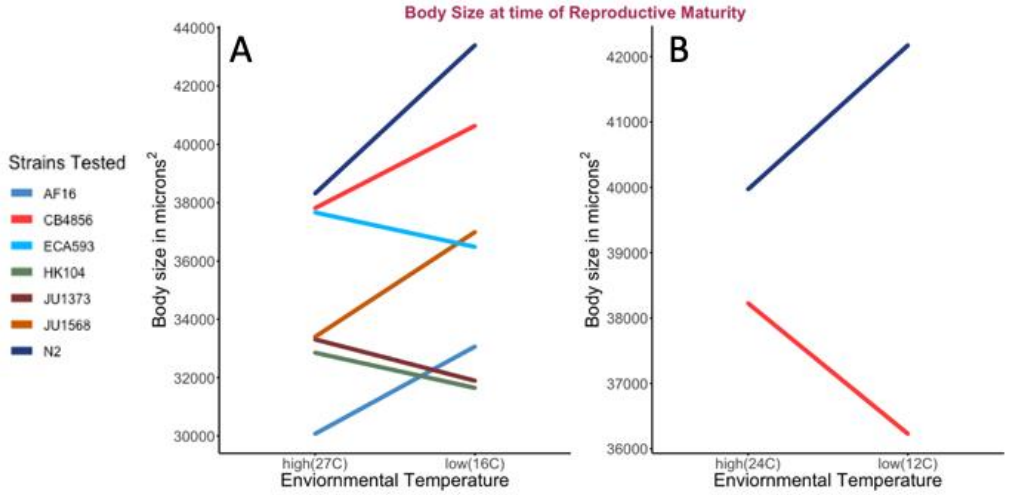

Figure 9. Body sizes at time of maturity for all strains. (A) Graph depicting body sizes for all strains in a high temperatures of 27°C (30°C for strain AF16 and JU1373)and a low temperature of 16°C. Three strains show non-compliance with the rule by showing a negative slope: *C. briggsae* strain HK104, *C. elegans* strain ECA593, and C. *tropicalis.* (B). Graphs depicting body sizes for two C. *elegans* strains (N2 and CB4856) in a high temperature of 24° and a low temperature of 12°C. Strain CB4856 shows non-compliance with the rule with a steeply negative slope, while N2 retains a strong positive slope showing compliance.

Reaction Norms

Reaction norms are graphical representations of the range of phenotypic outcomes given a single genotype. I modeled two reaction norms for two *C. elegans* strains; N2 and CB4856, using a local regression fit with 4 separate temperatures trials with no less than 20 samples per trial. The first shows body size at time of reproductive maturity as a product of temperature (Figure 9A), the second shows total brood size as a product of temperature (Figure 9B). Both graphs show unique bell distributions for each strain. The peak of the distribution can be considered the local optimum and correlates with the temperature at which body size or brood size will be largest. Both brood sizes and body size are largest around 20°C. Moving away from the local optimum in either direction results in lower than maximum phenotypic 'output'. The first plot showing Body size at maturity clearly shows a difference in the plasticity of both strains' phenotypes. N2 is far more capable of retaining a larger body size as temperatures drop, while CB4856 shows a much steeper slope between 12°C and 16°C, indicating a much smaller body size at lower temperatures, providing extra evidence of the nature of CB4856's non-compliance with the TSR. When looking at the brood size reaction norms, there is once again a noticeable difference in the phenotypic responses of these two strains as temperatures cool. CB4856 seems to be much more apt to retain larger brood sizes as the temperature drops compared to N2. It is interesting to note that this seems to be an almost inverse response when compared to the reaction norm for body size at maturity.

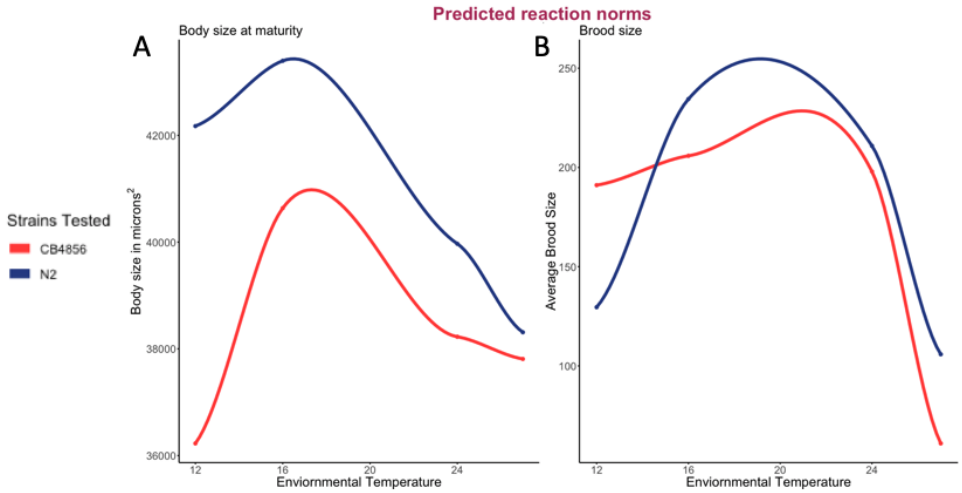

Figure 10. Two reaction norms for *C. elegans* strains N2 and CB4856. (A) Reaction norm depicting the relationship of body size to temperature. CB4856 is unable to retain a large body size as the temperature cools. N2 is not only larger generally but retains body size with dropping temperatures. (B) Reaction norm depicting the relationship between total brood size and temperature. CB4856 is able to maintain a higher brood size as temperatures cool when compared to N2. The local optimum for both graphs and strains is around 20°C.

Biomass Accumulation

Using conversion factors from Byerly *et al.* (1976), I was able to graph the accumulation of soma biomass and embryonic biomass across worms' lifespans. Looking at the accumulated mass per day figures, there is a clear pattern an energy balance allocation 'trade-off' that occurs in all strains and in all temperatures. As the organism begins to allocate energy towards reproduction, thus producing eggs, the distribution of energy towards soma growth (and presumably maintenance) as measured by accumulated biomass is seen to have an almost perfect opposing decrease. In warm temperatures the inverse relationship is stark, however in cooler temperatures, the decrease in soma biomass accumulation is much more gradual, indicating a slower change in energy allocation strategy. It is also interesting to note that in cooler temperatures, there is a large initial increase in soma growth in the first 24 hours, the next 24 hours however, show much less growth, followed by growth again increasing in subsequent 24 hours periods until the start of the reproductive cycle. When looking at the cumulative biomass accumulation graphs it is clear that there are essentially two opposing 'strategies' that result in clearly different overall allocations of resources. The first is to allocate the majority of resources towards reproduction, resulting in a much larger accumulation of cumulative egg biomass when compared to soma biomass. This strategy is clearly indicted in all *C. elegans* strains at low temperatures as well as the tropical strain of *C. briggsae* (AF16). Conversely it seems that in warmer temperatures *C. elegans* do not allocate nearly as much energy towards reproduction, because cumulative

egg biomass remains below cumulative soma biomass in almost all cases except for strain JU1568. Warmer temperatures seem to confer an almost opposite reaction in *C. briggsae* strains and *C. tropicalis*, with cumulative egg biomass surpassing cumulative soma biomass. The cumulative soma remains approximately constant throughout all strains and all species at ~3 μg. The tropical strain *C. briggsae* (AF16) is the only strain to clearly allocate more energy towards reproduction in both warm and cold temperatures.

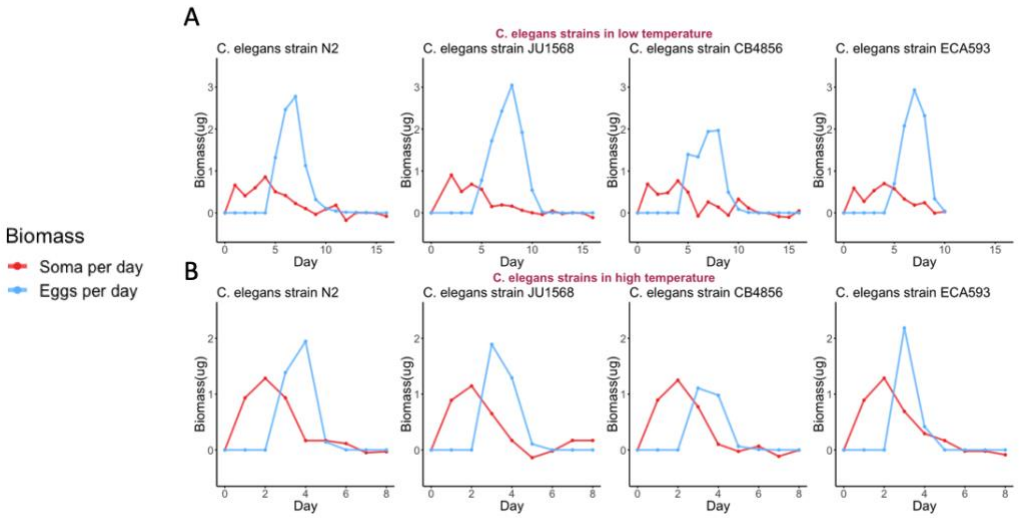

Figure 11. (**A**)Biomass accumulation per day in all *C. elegans* strains at low temperatures (16°C). The x axis shows accumulated biomass per 24 hours. All strains show a clear shift in energy allocation at the start of their reproductive cycles. However, in cooler temperatures the decrease in soma biomass accumulation seems to be much more gradual. All strains also display and usual pattern starting on the second day of decreasing Soma growth followed by a subsequent increase again and a gradual decrease as energy is allocated towards reproduction. (**B**) Biomass accumulation per day in all *C. elegans* strains at high temperatures (27°C). The x axis shows accumulated biomass per 24 hours. All strains show a clear shift in energy allocation at the start of their reproductive cycles.

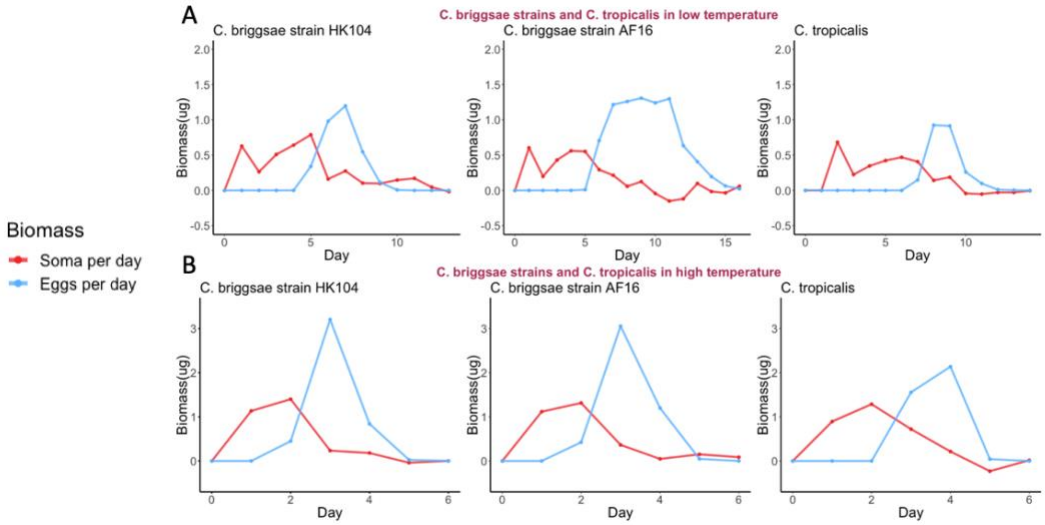

Figure 12.(**A**)Biomass accumulation per day in *C. briggsae* strains and *C. tropicalis* strains at low temperatures (16°C). The x axis shows accumulated biomass per 24 hours. All strains show a clear shift in energy allocation at the start of their reproductive cycles. However, in cooler temperatures the decrease in soma biomass accumulation seems to be much more gradual. Similar to *C. elegans* strains (and perhaps even more drastic), all strains also display the usual pattern starting on the second day of decreasing Soma growth followed by a subsequent increase again and a gradual decrease as energy is allocated towards reproduction. (**B**)Biomass accumulation per day in *C. briggsae* strains and *C. tropicalis* at high temperatures (27°C). The x axis shows accumulated biomass per 24 hours. All strains show a clear shift in energy allocation at the start of their reproductive cycles.

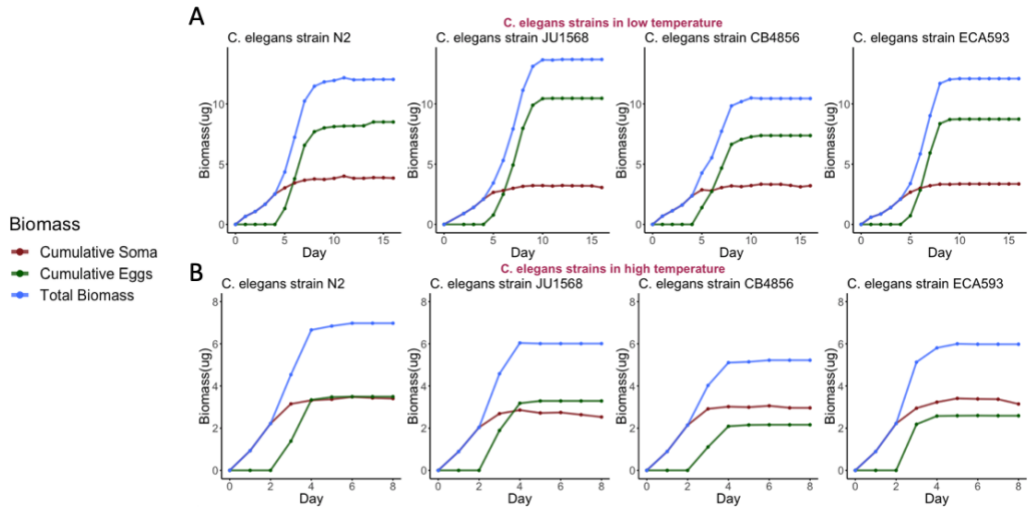

Figure 13. (**A**) Cumulative biomass accumulation all *C. elegans* strains at low temperatures (16°C). All strains show much more an investment in reproduction than in soma development in both total output of an investment in reproductive energy allocation and when compared to soma biomass allocation. The total biomass accumulation is approximately equal between all strains at ~12 μg. Soma biomass is approximately equal between strains and between temperatures (~3 μg). (**B**) Cumulative biomass accumulation in all *C. elegans* strains at high temperatures (27°C). All strains show less of an investment in reproductive energy allocation when compared to soma biomass allocation. The total biomass accumulation is approximately equal between all strains at ~6 μg. Strain JU1568 is the only strain to invest more energy into reproduction than into soma development.

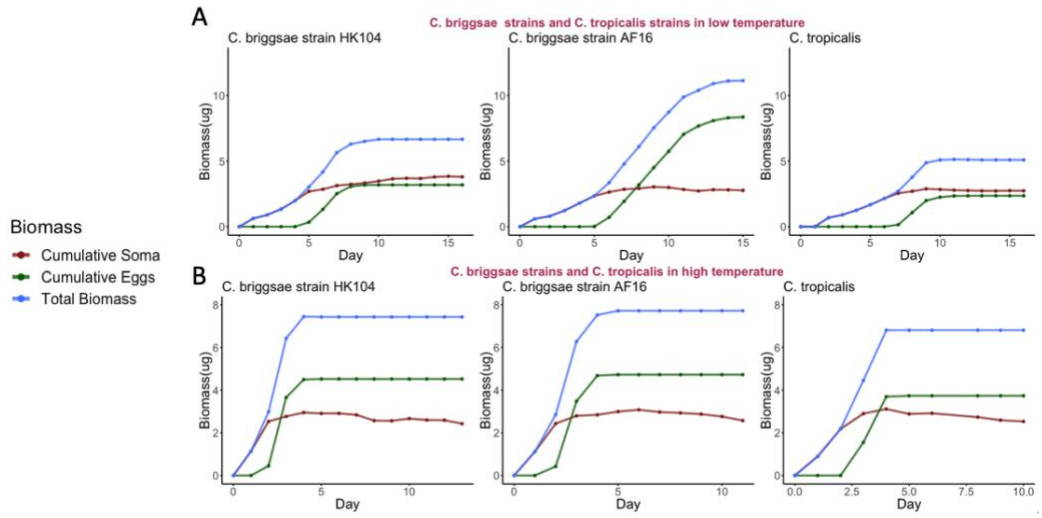

Figure 14. (**A**)Cumulative biomass accumulation in *C. briggsae* strains and *C. tropicalis* at low temperatures (16°C). *C. briggsae* strain HK104 and *C. tropicalis* have not only greatly reduced total accumulation but invest less energy into reproduction than into soma growth. These two strains were non-conformers to the TSR as well. Interestingly soma biomass accumulation remained about the same in all temperatures across all strains at ~3 μg. (**B**)Cumulative biomass accumulation in *C. briggsae* strains and C. *tropicalis* at high temperatures (27°C). When compared to *C. elegans* strains at high temperatures, it is clear that *C. briggsae* and C. *tropicalis* all invest much more energy into reproduction while actually having a lower total accumulated biomass of approximately ~ 8 μg. Soma accumulation remains the same between all strains at high temperatures at ~3 μg.

DISCUSSION

Broadly speaking, the goal of my project was to investigate the rather interesting puzzle of ectothermic phenotypic responses to different temperatures. Caenorhabditis seemed like the obvious choice, a highly robust system that has proven itself to have desirable traits for genetic control as well as practicality in the lab. The prior research involving *tra-3* and its potential influence on phenotypic response became very interesting and much of the later work done was in the direction of testing the validity of *tra-3* as causal link between clear changes *C. elegans* ability response to colder temperatures. I found that the range of phenotypic responses varies drastically between species and even between different strains of the same species. I further gathered evidence in support of a polymorphism in *tra-3* having a strong relationship with *C. elegans* phenotypic response to temperature. Using multiple temperatures, I was able to model reaction norms for the relationship of temperature to both body size at time of maturity and total brood size. I also used accumulated biomass to illustrate the potential differences in trade-off strategies of energy allocation towards soma development and reproduction.

Temperature Effects on Fecundity

Broadly speaking, the results of this experiment indicate that temperature has the largest effect on the duration of the reproductive cycle, independent of reproductive output. The general trend observed is that as temperatures cool, reproduction duration increases, as per day embryonic production drops. As temperatures warm, however, the duration of the reproductive cycle is decreased in spite of a similarly decreasing rate of per day embryonic output. Some strains show detrimental decreases in fecundity in lower temperatures such as the temperate strain of *C. briggsae* (HK104) and the species *C. tropicalis*. This may indicate that these temperatures are bordering on the edges of viability for these particular species and are in fact seriously damaging the organism rather than exerting influence within their respective reaction norm. Any effects on an organism's fertility must be the result of changes in (1) sperm produced, (2) oocytes produced, or (3) the fertilization of sperm and egg, including changes in gamete viability. While we don't know what the immediate upstream effects are that result in the observed changes, further experimentation can be done to distinguish these potential causes (see section: *continuing experimentation*).

Temperature effects on survival

It has long been observed that many organisms' life spans can be markedly increased or decreased in response to various environmental influences. Decreased temperature within an organism's reaction norm has previously been shown to increase longevity in many organisms (Korpelainen, 1986; Stommes et al., 2005; Conti, 2008). The results of this experiment are consistent with these previous findings. In all cases the lowered environmental temperature resulted in an increased life span. In order for the life span of an organism to be increased, there must be an increase in the organism's ability to either grow or maintain soma. Considering total energy as the sum of energy expended on growth and maintenance and energy expended on reproduction, decreases in temperature may facilitate a shifted energy balance or allocation mechanism towards soma growth and maintenance as shown by this research (see Figures 11-14).

Possible mechanism

While there is no clear mechanism that underlies exactly how temperature has effects on either fertility or lifespan, multiple discrete lines of research may provide a general picture of a possible signaling pathway linking temperature to both reproductive fertility and longevity.

Calcium is understood to be at least one of the direct links between environmental temperature and organism response, as it has been shown that levels of intracellular calcium increase with decreasing temperatures due in part to changes in various calcium channel activation (Shuttleworth and Thompson, 1990; Knight and Knight, 2000; Shiels et al., 2002).

In *C. elegans* specifically, much work has been done mapping the nervous system, or connectome (White et al., 1986). The functions and locations of virtually every neuron is now known. A subset of Amphid Sensory neurons in *C. elegans* that detect light and pheromones, known as ASJ neurons, have been shown to respond to temperature both through calcium imaging and cGMP signaling impairment in ASJ neurons resulting in abnormal cold habituation (Ohta et al., 2014). The downstream effects of temperature derived changes in ASJ activity are elevated levels of insulin. Insulin further acts on the intestine and various other neurons to promote organism changes (Ohta et al., 2014). Parallel to these findings, it has been shown that a *daf*-2/*daf*-6/FOXO pathway affects longevity, reproduction, and larval arrest (Gems et al., 1998). A link between insulin and Daf-16 has been established independently by Lin et al. (2001) and Sonada et al. (2016) utilizing the insulin receptor family member *daf* -2. Interestingly, both Hsin and Kenyon (1999) and Sonada *et al.* (2016) further established a connection between the reproductive system and longevity in *C. elegans*. Specifically, mutations in sperm affected *C. elegans* ability to respond to cold temperatures (Sonada et al., 2016). A further link was established by Kenyon (2010) who showed that a decrease in germline stem cells resulted in

upregulation of *daf*-16/FOXO, both of which further act downstream in endodermal tissue to activate “lifespan – extending genes”. The mechanism by which ‘lifespan – extending genes’ function still remains unclear. All of these independent pieces of research do paint a cogent and plausible picture of what could potentially be part of the signaling mechanism. This pathway has not been studied in the context of both reproduction and survival and I believe further experimentation in this regard would yield interesting answers (see section: *continuing experimentation*).

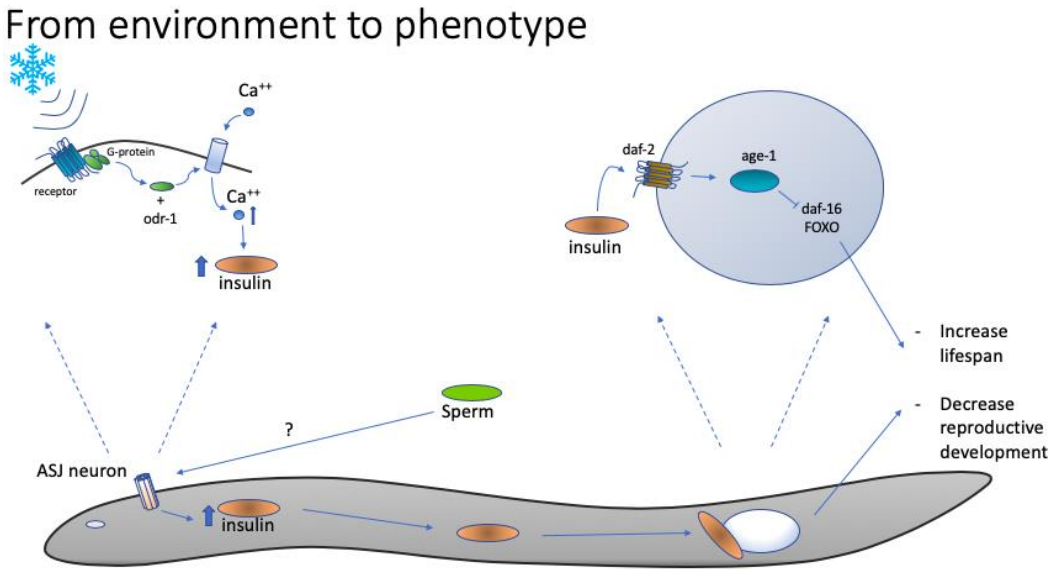

Figure 15. Simplified schematic of current cold adaptive pathway as noted by current literature. The effect of sperm on ASJ neurons is still not completely known.

tra-3 and its effects on the TSR

As mentioned earlier, *tra*-3 has been shown to have effects on *C. elegans* ability to regulate size at time of maturity. Specifically, an F96L polymorphism found in the Hawaiian strain (CB4856) attenuates its ability to retain a larger body size as temperatures drop (Kammenga et al., 2007). The gene *tra*-3 encodes an atypical calpain regulatory protease, that while lacking calcium binding EF hands, retains calcium dependent proteolytic activity. Membrane bound *tra*-2A has been shown to be the substrate cleaved by *tra-3* (Sokol and Kuwabara, 2000). *tra-2A* is known to have functionality in the *C. elegans* sex determining pathway, acting as an inhibitor of *fem-1*, *fem-2*, and *fem-3* proteins, which are themselves acting to inhibit the functionality of *tra-1*, a promotor of feminization (Kuwabara et al., 1992). Thus, an increase in *tra-2A* activity would act to increase feminization of the worm. A mechanism by which *tra-2A* may exert its effects was proposed by Kuwabara, Okkema, and Kimble (1992). *Tra-2A* has intracellular *fem* binding regions, that when activated by an extracellular ligand, or possibly simply in the absence of its upstream inhibitor *her*-1(Doniach and Hodgkin, 1984), sequesters *fem* proteins locally along the membrane, effectively decreasing the concentration of intercellular *fem* inhibition on *tra-1*, increasing *tra-1* feminizing potential (Kuwabara et al., 1992). It is interesting to note that as well as acting as an inhibitor of *tra-1*, *fem* genes also promote spermatogenesis in hermaphrodites (fem-I (Nelson *et al.* 1978; Doniach and Hodgkin, 1984), fem-2 (Kimble *et al*, 1984; Hodgkin,1986) and fem-3 (Hodgkin, 1986)). This model has the potential to

explain the effects of a *tra*-3 mutation shown by Kammenga *et al.* (2007). The resulting peptides of cleavage of *tra-2A* by *tra*-3 are essentially non membrane bound intercellular *tra-2A*, since it has been shown that the cleaved fragment retains its the *fem-3* binding region (Doniach, 1986; Kuwbara and Kimble, 1995; Mehra et al., 1989; Sokol and Kuwabara, 2000). Assuming that free cystolic *tra-2A* has a higher probability to bind *fem* proteins and promote spermatogenesis (simply by not being localized in the membrane), it would follow that increased cleavage of *tra-2A* by *tra-3* would result in an increase in feminization and a decrease in spermatogenesis. Further following this chain of reasoning, if calcium dependent proteolytic activity in *tra-3* was affected by the F96L mutation, it would inhibit *tra-3* response to increased intercellular calcium, effectively decreasing *tra-2A* cleavage (see figure 20). When looking at the reaction norms for body size, it is clear that N2 retains the ability to maintain body size at colder temperatures, resulting in a longer left tail skew of its concave distribution. This is interesting in its own right, as functional *tra-3* would predict this type of phenotypic response. However, when compared to the reaction norm of brood sizes, it seems that CB4856 is actually better adapted to maximize progeny output in decreasing temperatures. Considering the frequency of the F96L mutation is approximately .50 in *C. elegans*, and the ancestral polymorphism is the nucleotide guanine carried by CB4856. It would suggest that mutated *tra-3,* as expressed in N2, results in a decrease in progeny output in cold environments, which would potentially decrease overall fitness. This would

necessarily require a corresponding second affect afforded by this mutation that would provide and even greater increase in fitness.

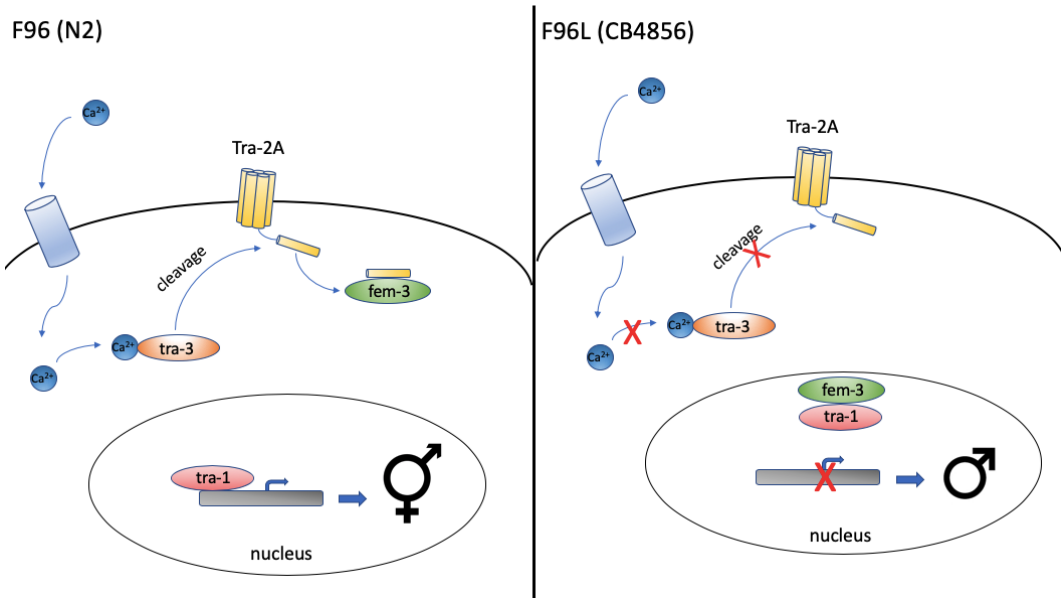

Figure 17. schematic of the possible downstream effects of *tra*-3. (left) ancestral nucleotide maintaining the binding capability of tra-3, allowing the downstream sequestration of *fem*-3. (right) polymorphism in *tra*-3 decreasing calcium's binding capacity, having the downstream effect of tra-1 inhibition though *fem*-3.

What is ‘compliance’ with the TSR

Much prior research has looked at the TSR as whether or not an organism grows larger or smaller in response to temperature. While this may be a suitable approach for smaller organisms that seem to have a linear reaction norm within their thermal boundaries, my data indicates that for species within the genus *Caenorhabditis*, the reaction norms follow a concave distribution, indicating that there is a local optimum for a given phenotypic response. When reacting to changes in temperature then, in either warming or cooling conditions, any movement away from the local optimum will result in a decrease in phenotypic output (i.e. lower brood size, body size, or reproductive rate). In the case of this type of norm of reaction, it seems that compliance or non-compliance of the TSR is simply a result of temperatures chosen to compare. In both *C. elegans* strains N2 and CB4856, there are temperature comparisons that will result in seeming compliance and seeing non-compliance with the TSR based on the reaction norm models (see figure 9). It seems that a better conceptual approach to phenotypic response to temperature should be to consider the reaction norm as a whole rather than discrete temperature points along the distribution.

An evolutionary approach to the TSR

How an organism may generate a response to any sort of environmental temperature is a question aimed at the mechanistic aspects of physical bodies. Questions of this nature usually fall into the category of anatomy and physiology. However, one can also ask why an organism may respond the way it does, and further ask how such a response has come to be engrained in the nature of the organisms as we see them today. Questions along these lines are best answered through the lens of selective pressures and evolutionary theory. As stated earlier, there are a multitude of potential selective pressures and mechanisms, both adaptive and non-adaptive, leading to the observed phenotypic responses to temperature that have been proposed. The data collected here affords some potential elucidations that may implicate some theories over others. The results obtained from biomass measurements lend support to Thomas Kirkwood's proposal in 1977 of the Disposable Soma Theory of Ageing. His theory proposed that there is a natural 'trade-off' of an organism's finite energy resources towards reproduction and soma development. Furthermore, organisms will naturally adopt the most efficient strategy of energy partitioning that affords the highest levels of fitness. All cases show an approximately equal decrease in soma biomass accumulation for an increase in reproductive capacity, while total body mass stays relatively linear. My results in high temperatures environments indicate that most strains mobilize virtually all available energy towards reproduction, whereas in low temperature environments, the energy trade off seems to shift in favor of

soma growth and development. During the reproductive cycle, worms continue to noticeably grow. This may also lead to the increases in longevity seen in all strains in cold temperatures. While this shows that the strategy of energy partitioning changes in response to different environmental temperatures, it is unclear whether or not this is due to changes in energy allocation as proposed by Kirkwood (1999), or due to differences in the temperature sensitivities of growth and differentiation as proposed by Van de Have and de Jong (1996), who suggest that the cellular machinery of these two processes have differing sensitivities to temperature, leading to an uncoupling effect between growth and differentiation. I also noted above multiple lines of evidence that point towards a potential signaling mechanism responsible for allocating energy preferentially towards earlier reproduction. A signaling pathway responsible for these changes would support Kindleman and Dixon's (1992) idea of differential energy allocation, as simple differences in temperature sensitivities would not require any evolved mechanism.

The effects of temperature on reproduction and body size are generally thought of as two separate processes. Considering the inverse nature of energy allocation towards reproduction and growth, the data indicate that a decrease in energy allocation towards soma growth and maintenance seem to occur in response to an increase in reproductive energy allocation. It seems reasonable to suggest that the effects seen on body size development may only be indirect effects, resulting from direct changes in reproductive strategy.

The question of why a delayed and prolonged reproductive cycle in colder temperatures would constitute a selective increase in fitness however, remains unclear. Based on my personal observations that a high proportion of eggs laid at lower temperatures were noticeably larger, this may indicate increases in the investment towards each embryo, potentially reducing juvenile mortality in colder temperatures. Cold temperatures provided a difficult environment for offspring from the beginning of embryogenesis. A longer period of germination may provide noticeable changes in overall brood viability. This would result in a selective advantage of potential increases in viable offspring due to a delayed and prolonged reproductive cycle (see *continuing experimentation)*.

CONTNUING EXPERIMENTATION

While there are virtually endless directions that one could continue this research if so inclined, I have outlined some of the lines that I think would be most interest.

1. As noted above, I think it would be a fruitful use of time to test for differences in juvenile mortality using temperature shift experiments. If indeed there is selective pressure to invest more time in embryos produced in colder environments, this could be tested by moving just-oviposited embryos from warm temperatures to cold temperatures and

monitoring resulting offspring development as compared to embryos in a continuously cold environment.

2. The effects that temperature has on fecundity are fairly drastic. I think it would be very helpful to understand what mechanisms are directly causing the changes seen. I am not actually sure how one could test this, let alone if we currently have the technological precision and knowhow to perform such an experiment.
3. Tra-3 was shown to have strong correlative effects on C. *elegans* ability to regulate body size in dropping temperatures. With the functionality and practicality of CRISPR, I think it would not only be cheap, but it would be very informative to see if a directed tra-3 mutation in N2 would result in a reaction norm similar to that of CB4856, a natural carrier of the mutation.
4. While much of my work focused on tra-3 and therefore *C. elegans*, the small amount of data I did collect from the strains of *C. briggsae* and the *C. tropicalis* strain was very interesting. The *C. briggsae* strains has very different reactions to temperature, as well as *C. tropicalis* seemingly not complying with the TSR. It would be very interesting to see what their full reaction norms looked like and one could use the very same methods I used in this work. I think this would be especially interesting as there are no differences in *tra-3* among these three organisms, therefore any differences would have to be due to another mechanism.

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