Honors Committee

Dominican University

7900 West Division Street

River Forest, IL 60305

To the Honors Co-Directors and Committee Members:

This letter indicates the submission of my project titled " Testing the Lifespan of *Caenorhabditis elegans* Using Sulfur-containing Compounds" for the B.S. Honors with Distinction degree in Neuroscience for my graduation in May of 2016. My two Dominican faculty readers are Dr. Pliny Smith from the biology department and Dr. Ellen McManus from the English Department.

Sincerely,

Dalal Abuaqel

Testing the Lifespan of Caenorhabditis elegans *Using Sulfur-containing Compounds*

A Completed Project Submitted in Fulfillment of the Requirements for the Bachelor of Science Honors and Distinction Degree in Neuroscience for Graduation in May 2016

Submitted to the Honors Committee by:

Dalal Abuaqel

Abstract

Aging is an unavoidable, universal, and biological phenomenon affecting all multicellular organisms. Although different hypotheses have been put forward to explain the cellular and molecular mechanisms of aging, recent studies have made it progressively clear that it is indeed possible for organisms to have an increase in life span through pharmacological intervention. This study is focused on investigating the interaction of genes controlling the rate of aging in wild type and DAF-16 *Caenorhabditis elegans* (*C. elegans*) strains in order to understand the mechanisms of aging that could uncover new therapeutic approaches for the treatment of age related disease. In this work, I report that exposing *C. elegans* to sulfur-containing compounds increases the lifespan of *C. elegans*. These compounds work through a mechanism independent of insulin-like signaling and are not involved in increased resistance to free radicals.

Acknowledgements

I would like to give my sincerest thanks to Dr. Pliny Smith for letting me participate in his research study since the beginning of summer 2015. I am thankful for everything I have learned under Dr. Smith's guidance, for he has trained and challenged me to my greatest ability.

I greatly appreciate Dr. Pliny Smith and Dr. Ellen McManus for serving as my first and second readers. They have been integral in the process of revising and editing this paper, as well as forcing me to think critically and build upon my foundation for scientific curiosity.

Lastly, I would like to thank the Honors Committee for allowing me the opportunity to present the research I have conducted since the beginning of summer 2015.

Introduction

Background

Aging is an unavoidable, universal, and biological phenomenon affecting all multicellular organisms. Although different hypotheses have been put forward to explain the cellular and molecular mechanisms of aging, recent studies have made it progressively clear that it is indeed possible for organisms to have an increase in longevity. This study is focused on investigating the interaction of genes controlling the rate of aging. In particular, we used the free-living nematode, *Caenorhabditis elegans* (*C. elegans*), because it has a well-defined genetic component for longevity, it is simple to culture, and it has a short (two- week) lifespan. Our research has tested the hypothesis that sulfur-containing compounds increase longevity of *C. elegans* by reducing oxidative stress.

The goal of aging research is to find drugs that delay the onset of age-associated disease. Various studies performed on invertebrates, particularly *C. elegans*, have uncovered numerous genes that are involved in aging. Dr. Smith's lab at Dominican University is particularly interested in discovering the effects of adding sulfur-containing compounds on the lifespan of *C. elegans*, by using chemicals such as Dimethylsulfoxide, Dimethyl Sulfone, and Dimethyl sulfide.

Caenorhabditis elegans

Caenorhabditis elegans, also known as *C. elegans*, are small and free-living nematode worms. *C. elegans* are non-hazardous, non- infectious, non-pathogenic and non-parasitic organism. As demonstrated in Figure 1, *C. elegans* have a generation time of about 3.5 days and a life span of about two weeks; some strains have shorter or longer life-spans. They grow to a length of 1.3 mm and have a diameter if 80 um. Overall, adult C. elegans contain 959 somatic cells that are visible with a microscope(Jenson 2006).

They are also self- fertilizing hermaphrodites and each *C. elegans* worm has the ability to give rise to 300 offspring. They can be easily cultured in the laboratory on bacterial lawns grown on an agar substrate, and they also can easily produce a variety of mutagens. These characteristics make the animal very amenable to genetic analysis (Brenner 1974).

C. elegans share many biological traits that are essential characteristics in human biology. The organism is conceived as a single cell or zygote that soon undergoes a complex process of development. Just as in human biology, the worm starts with embryonic cleavage to produce more cells, progressing through morphogenesis and growth to an adult. As shown in Figure 2, it has nervous system with a "brain," that

is also known as the circumpharyngeal nerve ring (Brenner 1974). Just as humans, *C. elegans* have the ability to produce sperm and eggs and therefore to mate and reproduce (Jenson 2006). After reproduction, it begins to gradually age, lose vigor, and die.

As shown in Figure 3, before reaching adulthood, *C. elegans* pass through four larval stages: L1, L2, L3 and L4. If food is scarce, these animals could go through an alternative developmental sequence in which a resistant dauer larval form is produced in the L2 and L3 stages. These dauer larva can survive harsh living conditions, such as desiccation and lack of food, for long periods of time until conditions improve and food becomes available, at which point they moult and become normal adults (Cassada & Russel, 1975; Riddle et. al. 1981). Particularly, the dauer form may develop in response to various types of signals such as temperature, food supply, and population density.

In this particular experiment, we worked with two strains of *C. elegans*, the N2 wild type strain and N2 worms that have a mutation in DAF-16. The N2 strain was originally isolated from mushroom compost, and most studies on *C. elegans* use this single genetic background that comes from the N2 wild type strain (Dalley 1992). The wild type strain is less common in nature; however it has acquired over 40 years of different mutations from various laboratory cultivations. Wild type hermaphrodite strains have median life spans ranging from 11.8 days to 17 days, when grown on agar plates at 20 °C with *Escherichia coli* (Lakowski, 1996). Such differences have been attributed to environmental variation, specifically temperature, because growth conditions are known to affect lifespan.

DAF-16 functions as a transcription factor that acts in the insulin/IGF-1-mediated signaling (IIS) pathway that regulates dauer formation, longevity, fat metabolism, stress response, and innate immunity (Worm Base). DAF-16 proteins are expressed in most cells, including ectoderm, muscles, intestine and neurons, in late embryos, larvae and dauer larvae. As a forkhead box O (FOXO) transcription factor, DAF-16 plays a role in oxidative heat stress and starvation (Henderson and Johnson, 2001).

Longevity

Longevity refers to members in a population that are long-lived and have increased life expectancy. Aging is characterized by general physiological decline over time. The continued progression through life is the onset of various age-related weaknesses including neurodegeneration, cardiovascular disease, and cancer (Olshanky, 2007). Although environmental factors clearly contribute to the amplified prevalence of disease with age, recent studies govern both lifespan and health. Current aging research is aimed at accomplishing an increase in longevity, particularly, researchers are interested in understanding if certain chemicals or techniques could lead to an increase in lifespan. Substantial progress has been made using model organisms, especially the nematode *C. elegans*, to explain the various

genetic and biochemical pathways involved in aging and to identify strategies for therapeutic intervention in humans.

Longevity in Caenorhabditis elegans

Diverse genetic and environmental factors regulate longevity in a broad range of organisms (Finch 1990), but characteristics of lifespan control that might be universal to animals have started to emerge. Particularly, in *C. elegans*, modulations of particular gene activities cause a profound increase in lifespan (Guarente and Kenyon 2000). Most notably, reduced activity of the *C. elegans daf-2*/ insulin-like signaling pathway causes more than two-fold lifespan increase (Kenyon et. Al, 1993). Signaling from the DAF-2/ insulin-like receptor antagonizes the forkhead (FOXO) transcription factor *daf-16*, the major effector of *daf-2*/ insulin like regulation of *C. elegan* lifespan (Lin et al. 1997). There are more than 30 insulin genes in this nematode that could possibly mediate input to the *daf-2* pathway through environmental cues, such as nutritional access or growth conditions (Wormbase).

Interestingly, the interaction between temperature and lifespan is of greater importance in roundworms and fruit flies than in warm-blooded animals. In *C. elegans,* research has established that lifespan varies inversely with temperature, for example 20^0C for top culture or 12^0C for shorter experiments. Decreasing temperature increases lifespan in every strain, but the extent of the increase varies widely between strains (Leiser 2009). Some strains are short or long-lived compared to wild type only at high temperatures, while others only show lifespan effects at lower temperatures.

The Sir2 histone deacetylase is another significant longevity determinant that regulates lifespan in various species (Kaeberlein et al. 1999). In yeast, Sir2 facilitates heterochromatin formation, and by reducing recombination at the rDNA locus, Sir2 helps to maintain genomic stability and extends lifespan (Imai et al. 2000). Similar to yeast, overexpression of the *C. elegans* SIR2 homolog sir-2.1 also extends lifespan (Tissenbaum and Guarente, 2001), although the molecular mechanism whereby sir-2.1 enhances longevity in nematodes is less clear. Genetic studies indicate that sir-2.1 works upstream of *daf-16* to regulate C. elegans lifespan (Tissenbaum and Guarente 2001).

Mutations inhibiting *C. elegans* germline proliferation also extend lifespan (Hsin and Kenyon 1999; Arantes-Oliveira et al. 2002). The germline produces a *daf-16*-dependent signal to regulate lifespan, but acts in the absence of the *daf-2* gene (Lin et al. 2001). Thus, *daf-16* might act as a major regulator of lifespan, capable of incorporating several distinct longevity signals.

Many genes have also been found to regulate *C. elegans* lifespan through pathways independent of *daf-16*. The feeding-defective eat mutants live slightly longer in a

daf-16-independent manner. The eat mutations are thought to extend lifespan through a mechanism resembling caloric restriction (Lakowski and Hekimi 1998). Mutations in *clk-1*, a gene required for coenzyme Q synthesis, also extend lifespan independently of *daf-16* and slow the rate of many physiological processes (Lakowski and Hekimi 1996). Similarly, reduced function of the mitochondrial oxidative phosphorylation machinery extends *C. elegans* lifespan independent of *daf-16* (Feng et al. 2001; Dillin et al. 2002; Lee et al. 2003b). A small-scale RNA interference (RNAi) screen for longevity regulators revealed that inactivation of particular mitochondrial electron transport chain (ETC) components increases *C. elegans* lifespan (Lee et al. 2003b).

Genome-wide RNAi screening has also been validated by the recovery of genes and gene classes previously described to regulate *C. elegans* life duration. It was found that RNAi of *age-1* as well as *akt-1*, both of which are components of the *daf-2*/insulin-like signaling pathway, caused robust lifespan extension (Hamilton 2010). Interestingly, although *akt-1* is a well-characterized component of the *daf-2* signaling pathway, an *akt-1* deletion mutant was previously reported to exhibit normal lifespan (Hertweck et al. 2004). The lifespan extension phenotype caused by *akt-1* RNAi might only be apparent when *akt-1* activity is reduced by RNAi knockdown, and not when *akt-1* function is completely lost, as in the case of a deletion mutant. An alternative hypothesis that nonspecific knockdown of additional gene(s) by the *akt-1* RNAi construct is also possible, though Blast analysis reveals additional genes with only 17 base pairs of sequence identity to *akt-1*.

C. elegans have a disconnected response to food constraint early in life, especially when it enters an alternative developmental stage, known as the dauer. Unlike the normal feeding state, the dauer can live for many months. Dauers are an alternative L3 state, and dauer formation is potentiated by food limitation and high temperature. The dauer state is induced by a constitutively produced dauer pheromone, whose concentration increases as the nematodes crowd together around the remaining food. The dauer differs from the adult in many ways. Its growth is arrested, and it contains intestinal granules that are thought to store food. Dauers appear dark for this reason. It is encased by a dauer-specific cuticle that is relatively resistant to dehydration. Dauers have reduced metabolic rates (O'Riordan and Burnell 1989, 1990) and elevated levels of superoxide dismutase, and are relatively resistant to oxidative stress (Anderson 1982; Larsen 1993; Vanfleteren 1993). They also have elevated levels of several heat shock proteins (Dalley and Golomb 1992). Animals that exit from the dauer state resume growth and have subsequent life spans that are similar to those of animals that have not arrested at the dauer stage (Klass and Hirsh 1976).

Environmental Gene Influence

Evolutionary models of aging propose that a trade-off occurs amongst the resources an organism dedicates to reproduction and development, and those devoted to

cellular maintenance and repair, such that an optimal life history always entails an imperfect ability to resist stress. Yet, since environmental stressors such as caloric restriction or exposure to mild stress can expand stress endurance and life span, it is possible that a genetic method could regulate the allocation of resources in response to a changing environment (Valle 2015).

It has been shown that nematodes carrying an integrated DAF-16:GFP transgene have the ability to grow and reproduce more slowly, yet are more stress resistant and longer lived than controls carrying the integration marker alone (Henderson 2005). The *daf-16* gene produces three different transcripts: a1, a2, and b, which encode a putative transcription factor with a fork head or winged helix DNA binding domain. It has been suggested that changes in the subcellular localization of DAF-16 by environmental cues allows for rapid reallocation of resources in response to a changing environment at all stages of life (Olshanky 2007).

Role of Sulfur-Containing Compounds in Longevity

Previous research has made it progressively clear that Dimethyl sulfoxide plays an essential role in understanding gene expression. Particularly, sulfur-containing compounds were chosen because they have the ability to react as either an oxidant or reducing agent. Also, previous research has demonstrated that Dimethyl sulfoxide increases life span as well. It was found that exposing *C. elegans* to DMSO in liquid extends lifespan up to 20% and that these compounds work through a mechanism independent of insulin-like signaling and dietary restriction (Frankowski 2013).

Dimethyl sulfoxide is an organic sulfur compound that is known as an industrial solvent and is used for multiple medical properties in the medical field. DMSO is an intermediate product of the global Sulfur Cycle, which distributes bioavailable sulfur for all animal and plant life (Martin, 2002). Sulfur compounds are found in all body cells and are indispensable for life. They are needed for a number of chemical reactions involved in the detoxification of drugs and other harmful toxins, and they have potential clinical applications in the treatment of a number of conditions such as depression, fibromyalgia, arthritis, interstitial cystitis, athletic injuries, congestive heart failure, diabetes, cancer, and AIDS (Martin, 2002). Among the sulfur compounds, DMSO is probably the one that has the widest range and greatest number of therapeutic applications ever shown for any other single chemical.

As the proceeding literature review shows, a great deal of research has been done on the mechanisms involved in increased longevity of *C.elegans*. However, there is a gap in knowledge in relation to understanding which compounds have the greatest effect on increased lifespan and the role these chemicals play in the expression of genes. This research project is focused on understanding the interaction of the DAF-16 gene and identifying a possible set of antioxidants that could increase lifespan.

Research Goal

The goal of this research project is to investigate the interaction of genes controlling the rate of aging and a set of possible antioxidant chemicals. Our research tests the hypothesis that sulfur-containing compounds increase longevity of *C. elegans* by reducing oxidative stress. We demonstrate that dimethyl sulfoxide(DMSO) has longevity promoting properties when inserted into the worm environment at 1.0% volume ratio.

This project also tests the interactions with the gene DAF-16 and sulfur containing environmental compounds, such as Dimethylsulfoxide (DMSO), Dimethyl Sulfone, and Dimethyl sulfide. Our hypothesis is that DMSO and related compounds will increase lifespan of a normally short-lived worm strain with a nonfunctional allele of the gene DAF-16. If DMSO acts as an antioxidant, rather than a signaling molecule, DAF-16 mutants incubated with DMSO should have longer life spans than their controls.

Materials and Methods

The experiment will use synchronized wild type and DAF-16 *C. elegans* cultures so that all worms have identical age at the start of testing. To prevent reproduction, the worms will be incubated with Fluorodeoxyuridine (FUdR) chemically. The use of FUdR for longevity analysis does not affect adult life span and removes the need to transfer worms every few days in order to separate them from growing larva. Each experimental condition will have approximately 100 worms, which will be divided onto four sets of plates. The number of living worms is counted and recorded each day for each plate until all worms have died.

Materials

The *C. elegans* were raised on standard *Escherichia coli* bacterial lawns on agar plates that were produced in the lab. They were incubated at 25^0C. The worms and sulfur-containing chemicals, such as Sulfide, Sulfone, and DMSO, were prepared in the lab as well. FUdR was used to inhibit DNA synthesis. In particular, it was used because of its ability to prevent a synchronous population of *C. elegans* from reproducing without otherwise interfering with the organism's post-maturational development and aging.

Preparing C. elegans *Strains*

Petri dish plates were selected to contain worms from previous strains already prepared by Professor Smith's lab. Four plates were selected from each strain, including both wild type and DAF-16 worms. 1 mL of H_2O was added to each petri dish to order to select the C. elegans, and then the H_2O was placed with the worms into fresh petri dishes that were previously prepared. The worms were then placed into a 25 degree Celsius incubator for two days. In essence, the temperature in which the *C. elegans* were placed determined when the worms were ready.

Bleaching and Preparing C. elegans Strains

The materials needed were prepared earlier, including NaOH and bleach. Each petri dish containing worms was then washed with 2.5 mL of H_2O, and the solution was then placed into a 15 mL conical tube. In order to eliminate the possibility of error, two sets of each strain were created; this means that there were a total of 4 conical tubes (2 tubes containing N2 Wildtype worms and 2 tubes containing DAF- 16 worms).

To make the bleach solution for a total of 4 conical tubes, 5 mL of bleach (2.5 mL of Saline hypochlorite) was mixed with 1.25 mL of 10 N NaOH and 6.25 mL of H_2O. These solutions combined created the master solution. In order to avoid any mistakes, the master solution ingredients were doubled or tripled.

After the bleach solution was complete, 2.5 mL of H_20 was added into each of the four conical tubes and then mixed into the solution. After mixing the solutions for a minute or two, more H_2O was added to dilute the worm solution. The mixed worm master solution was then centrifuged for approximately 3 minutes. Slowly, after it was done, the bleach and water was removed so that only the eggs that were visible at the bottom remained. This procedure was then repeated in order to remove the bleach another time, ensuring that most of the bleach was removed. Each of the 4 tubes was then suctioned to grab all of the eggs left and place them into the appropriate petri dishes. The plates were then checked to ensure that the worms were indeed placed on the petri dishes; they were then inserted into an incubator at 25 degrees Celsius.

Placing C. elegans *with Sulfur-Containing Components*

Before preparation of the chemicals, the bleached *C. elegans* plates were observed once again to ensure accurate amounts of *C. elegans* were available for plating. Lots of petri dishes were needed for each strain, as well as for each of the sulfur-containing compounds (control group, Dimethylsulfoxide group, Dimethyl Sulfone group, and Dimethyl sulfide group). Fluorodeoxyuridine, also known as FUdR, which is the inhibitor of DNA synthesis, was used to prevent a synchronous population of

C. elegans from reproducing without otherwise interfering with the organism's post-maturational development and aging.

Each of the chemicals used was prepared in the lab before the start of each experiment. In order to make the Dimethylsulfoxide, 2 mL of DMSO and 8 mL of H_2O were added and then filtered. For the Dimethyl Sulfone, 2 g of Sulfone and 10 mL of H_2O were added together then filtered. For the Dimethyl Sulfide, 2 mL of sulfide and 8 mL of H_2O were added together, then filtered. In order to make the Fluorodeoxyuridine, 40 mg of FUdR was combined with 10 mL of H2O; it was then filtered with a 0.22 μm syringe tip filter.

The chemicals were then distributed on sets of plates between the two strains (N2 wild type and DAF-16). The sets that the worms were distributed on were the control group, DMSO group, Sulfide group, and Sulfone group. When making the plates, 500 microliters of each chemical was added with 100 microliters of FUdR. Finally, the *C. elegans* and chemicals were distributed on the plates evenly and then the petri dishes were placed in an incubator at 25 degrees Celsius.

Analyzing the Data Collected

The data was analyzed using the Online Application for Survival Analysis (OASIS). OASIS is a one-stop tool for diverse statistical tasks involved in investigating survival data in a user-friendly manner. OASIS provides a uniform platform that is a fundamental application to facilitate effective statistical analyses of survival data in the ageing field. To visualize survival data, OASIS generates survival and log cumulative hazard plots that enable researchers to easily interpret their experimental results. It also provides various statistical tests including comparison of mean survival time, overall survival curve, and survival rate at specific time point.

The tests we generated from OASIS included the Kaplan Meier estimator, statistics for mean/median lifespan, and graphs that plot survival rates. As for statistical testing, a Log-Rank test was performed on the mean lifespan. The Log-Rank test was used to compare the survival distributions of the samples and to generate the p-values needed to determine whether the changes in lifespan are due to the DAF-16 gene or the antioxidant. A Fisher's Exact Test was also performed on the specific time points in order to test the statistical significance in the analysis of the contingency tables.

Results

The significant question asked in these experiments was whether sulfur-containing compounds would have an effect on lifespan in *C. elegans*. Previous pilot

experiments performed during the summer of 2015 on the N2 *C. elegans* strain suggested that worms put in contact with sulfur-containing compounds do indeed survive longer. This experiment looked solely at the effect of these chemicals on various types of *C.elegans* strains, such as N2 and DAF-16.

For a total of four experiments, each experiment used approximately 500 to 1000 worms. Each experiment had a control, DMSO, dimethyl sulfide, and dimethyl sulfone group, as well as FUdR in order to halt reproduction. Specifically, experiment 1, 2, and 3 tested the both N2 and DAF-16 *C. elegans* strains, while experiment 4 tested only the N2 strain. The program used to analyze the data was the online application for the survival analysis of lifespan assays (OASIS) (http://sbi.postech.ac.kr/oasis/).

Experiment 1: N2 Versus DAF-16 Strains

This experiment analyzed the two common *C. elegans* strains, the N2 and the DAF-16 strains. After doing multiple experiments on the N2 strain during the summer of 2015, it was evident that these sulfur-containing compounds were indeed extending *C. elegans* lifespan (P-value< .05). However, in order to confirm these predictions it was necessary to compare these chemicals' effects on different *C. elegans* strains in order to test the validity of the results.

As shown in Figure 4, it was evident that the DAF-16 strain had increased mortality when compared to N2 strain (P-value <.05). In this experiment, on average the DAF-16 strain began to decrease in lifespan around the sixth day, while the N2 strain began to deteriorate around the eighth day. It was also apparent that the DAF-16 and N2 strain that was mixed with the DMSO died relatively quicker compared to those mixed with the other sulfur-containing chemicals (P-value < .05). In this experiment, the Dimethyl Sulfone N2 and DAF- 16 strains survived the longest (P-value < .05).

Experiment 2: N2 Versus DAF-16 Strains

This experiment was very similar in that it investigated the two common *C. elegans* strains, the N2 and the DAF-16 strains. In essence, Experiment 1 was performed again in order to confirm if Sulfone was the sulfur-containing compound that had the greatest effect on *C. elegans* lifespan.

Similar to Experiment 1 and as shown in Figure 5, the DAF-16 strain had increased mortality compared to the N2 *C. elegans* strain (P-value < .05). Also, the DAF-16 strain began to show a decrease in lifespan around the fourth day, while the N2 strain experienced this decrease around the eighth day. It was evident that the control DAF-16 were the quickest to die. However the Dimethyl Sulfide DAF-16

strain died relatively quicker (day 15) compared to Experiment 1 (day 17). The strains that had the most prolonged mortality were the Dimethyl Sulfone N2 and DAF-16 once again. (P-value < .05).

Experiment 3: N2 Versus DAF-16 Strains

Similar to Experiments 1 and 2, this experiment also researched the two common *C. elegans* strains, the N2 and the DAF-16 strains. We repeatedly performed this experiment in order to confirm whether Dimethyl Sulfone was indeed the factor that had the greatest effect on *C. elegans* lifespan.

Similar to the previous experiments, as shown in Figure 6 the DAF-16 strain had increased mortality compared to the N2 *C. elegans* strain (P-value < .05). Also, the DAF-16 strain began to decrease in lifespan around the fifth day, while the N2 strain experienced this decrease around the eighth day. It was evident that the control DAF-16 were the quickest to die. However, the Dimethyl Sulfone DAF-16 strain died relatively quicker (day 16) compared to Experiment 1 (day 17). The strains that had the greatest extended mortality were the Dimethyl Sulfone N2 and DAF-16 once again (P-value < .05).

Experiment 4: N2 Strain

This experiment focused on the single N2 *C. elegans* strain. This experiment was performed in order to confirm whether Dimethyl Sulfone was indeed the factor that had the greatest effect on *C. elegans* lifespan. We also wanted to confirm whether there were other correlations witnessed between the experiments, dependent on a single strain.

As shown in Figure 7, this experiment was particularly interesting because the N2 Dimethyl Sulfone strain had the greatest decrease in lifespan (P-value < .05). This was interesting because compared to the other experiments, the Dimethyl Sulfone-containing petri dishes seemed to have the most increased mortality rate compared to the other chemicals. The strain that had the greatest extended mortality was the Dimethyl Sulfide N2 Strain (P-value < .05).

Analysis of First Three Experiments Combined

The first three experiments focused on the two common *C. elegans* strains, the N2 and the DAF-16 strains. However, in Figure 9 all of the results from the first three experiments were combined in order to confirm whether dimethyl sulfone was indeed the factor that had the greatest effect on *C. elegans* lifespan.

As shown in Figure 9, the DAF-16 strain had a small increase in mortality compared to the N2 *C. elegans* strain (P- value < .05). It was evident that the control DAF- 16 was the quickest to die., However the DMSO DAF-16 strain died relatively quicker (day 17). The strain that had the most prolonged mortality was indeed the dimethyl sulfone N2 strain once again (P-value < .05).

Conclusion

As seen in this research, the sulfur-containing compounds do extend lifespan in the nematode worm *C. elegans* (Figure 8). In particular, this research suggests that the compound associated with the greatest decrease in mortality was the dimethyl sulfone-containing compound. Current aging research aims at accomplishing increased longevity, in which lifespan extension in humans is accompanied by an expansion in the quality of life (Olshansky 2007). Substantial progress has been made using model organisms, especially the nematode *C. elegans*, to understand the various genetic and biochemical pathways involved in aging in order to identify strategies for therapeutic intervention in humans.

This experiment concludes that dimethyl sulfone does extend the lifespan of the N2 strain in *C. elegans*. The DAF-16 strain did experience an increase in lifespan as well. However the P-values were not as significant when compared to the N2 strain. Since the p-values are significant, this suggests that the increase in *C. elegans* lifespan was not caused by antioxidants, but rather a result of DAF-16 gene expression.

DAF-16 functions as a transcription factor that acts in the insulin/ IGF-1-mediated signaling (IIS) pathway that regulates dauer formation, longevity, fat metabolism, stress response, and innate immunity (Worm Base). DAF-16 proteins are expressed in most cells, including ectoderm, muscles, intestine and neurons, in late embryos, larvae, and dauer larvae. As a forkhead box O (FOXO) transcription factor, DAF-16 plays a role in oxidation, heat stress, and starvation (Henderson and Johnson 2001).

The goal of aging research is to find drugs that delay the onset of age-associated disease. Various studies performed on invertebrates, particularly *C. elegans*, have uncovered numerous genes that are involved in aging. This study confirms that there is indeed a correlation between sulfur-containing compounds and longevity in *C. elegans*. This study also confirms that dimethyl sulfone plays a principal role in decreasing the rate of mortality. However, more studies need to be performed in order to confirm these conclusions.

Diverse genetic and environmental factors regulate longevity in a broad range of organisms (Finch 1990), but characteristics of lifespan control that might be universal to animals have started to emerge. Particularly, in *C. elegans*, modulations of particular gene activities cause a profound increase in lifespan (Guarente and Kenyon 2000). There are numerous causes that may have led to an increase in longevity, and in the near future this lab will try to uncover some of those causes.

The mechanisms that determine the lifespan of an organism are still largely a mystery. Future directions of Dr. Smith's lab at Dominican University are to investigate the mechanisms involved in such increased rates of longevity. Additionally, the lab will focus on understanding and mapping why specific chemicals lead to a decrease in mortality in some strains when compared to other chemical and other *C. elegans* strains.

Discussion

Recent progress in understanding longevity has resulted in the identification of increasing numbers of compounds that extend lifespan in *C. elegans*. These lifespan extending compounds include metabolites and synthetic compounds, as well as natural products (Alberts 2002). As a consequence of the seminal discoveries demonstrating that lifespan can be modulated by genes, it became clear that lifespan might also be extended using chemicals (Johnson, 1990; Kenyon et al., 1993).

There are two fundamentally different approaches to identifying compounds that have a desired biological effect on organisms (Carretero, 2015). These two approaches are often referred to as forward and reverse pharmacology (Bartfai, 2006). Forward pharmacology approaches, also called phenotypic screens, screen for compounds that elicit a desired phenotype, like the extension of lifespan.

Reverse pharmacology circumvents the problem of target identification by screening for compounds that bind to, or inhibit, the function of a specific protein target. Reverse pharmacology screens are largely done in vitro and offer the ability to screen very large chemical libraries (+500,000) (Bartfai, 2006). Targets are validated based on prior knowledge, such as genetic studies in model organisms or gene association studies in humans affected by a disease. However, target validation, or choosing the protein target against which to develop a drug, also poses considerable difficulties (Pankevich et al., 2014).

As the process of aging is not easily replicated in vitro, most lifespan extending compounds have been identified by simply testing whether or not a given compound extends lifespan in a model organism (forward pharmacology). Thus far, most compounds that have been tested for their ability to extend lifespan had prior known pharmacology. Initially, these compounds were developed to inhibit a specific target, independent of their effect on aging. Only later were they tested for their ability to extend lifespan in *C. elegans* or other organisms. Thus, at its current state, the pharmacology of aging is a hybrid of forward and reverse pharmacology (Bartfai, 2006).

Early studies in *C. elegans* used extremely high concentrations of chemicals, creating the impression that worms were especially resistant. However, today many of the lifespan extending compounds are effective at concentrations in the lower micromolar range (Luciani et al., 2011; Ye et al., 2014). When compared to cell culture, these concentrations still seem high, but compared to mouse studies they are not. Drug injections are generally conducted at concentrations of 5–200 mg/kg, resulting in an internal concentration in the lower micromolar range (Hayashi and McMahon, 2002). As concentrations for *C. elegans* are indicated for the external culture medium, the internal concentrations are likely to be lower.

Antioxidants were some of the first compounds to be tested for their ability to extend lifespan (Harman, 1972; Melov et al., 2000; Benedetti et al., 2008). Antioxidants that extend *C. elegans* lifespan have been identified; specifically our experiment's results focus on an increase in lifespan caused by Dimethyl sulfide. Our findings initially supported to the idea that oxidative stress causes aging. Surprisingly, antioxidants blocked the lifespan extension of many hormetic agents, clearly suggesting that oxidative stress was required for the lifespan extension. Therefore, evidence exists that antioxidants extend lifespan through extension by hormetic agents. These findings suggest that our results are accurate by confirming the conclusion that Dimethyl sulfide has the ability to extend lifespan through a DAF- 16 dependent mechanism, thus affecting gene expression through oxidative stress.

In this particular study, we did not focus on understanding what changes in gene expression were occurring. However, in the future we would like to test these changes in order to try to understand the impact of sulfur- containing compounds, particularly Dimethyl sulfide, on gene expression. One of the most direct ways to see what a gene does is to do a test on an organism in which the gene is missing. Mutations interrupt cellular processes, and this could help us understand how the DAF-16 gene functions in response to sulfur-containing compounds. Some tests that could be performed to understand these factors would be Northern blot or serial analysis of gene expression (SAGE). Both of these techniques make it possible to identify which genes are turned on and which are turned off within cells. Subsequently, this information can be used to help determine what circumstances or substances trigger expression of various genes such as the DAF-16 gene.

Since *C. elegans* have similar biological traits that are essential characteristics in human biology, the goal of aging research is *C. elegans* is to find drugs that delay the onset of age-associated disease in humans. Although it may seem that there are many benefits to extending human lifespan, there are also many negative implications as well. Increased life extension could lead to overpopulation, resource strain, and greater susceptibility to diseases (Partridge, 2009). There are also many ethical issues that arise from slowing the aging process, such as creating an unnatural process or creating intergenerational problems because those with extended lifespans might be able to maintain their positions in society in a way that makes it more difficult for younger people to establish themselves (Partridge, 2009).

Figures

Figure 1: The average lifespan of *C. elegans* is 2-3 weeks, which is an extremely rapid life cycle. At 25 °C, embryogenesis, the period from fertilization to hatching, occurs in a total of 14 hours. This figure demonstrates how postembryonic development occurs in four larval stages (L1-L4) that last approximately 34 hours. . (Credit: Diagram provided by Jorgensen and Mango, Nature Reviews Genetics 3:356-369)

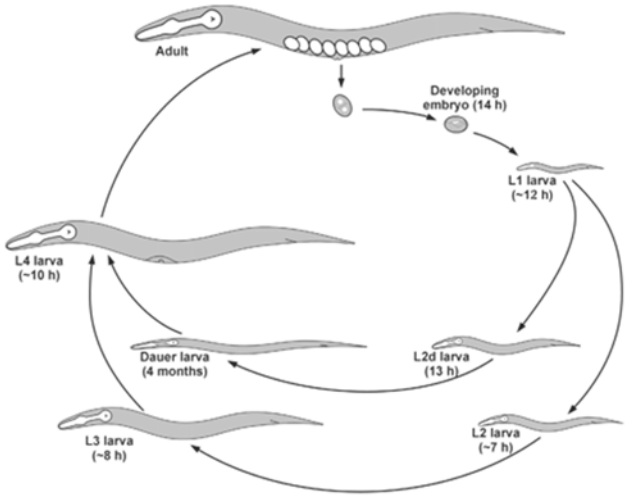


Figure 2: The nervous system is the most complex organ in *C. elegans*. A *C. elegans* hermaphrodite has a total of 302 neurons that belong to two distinct nervous systems known as the somatic and small pharyngeal nervous system. Particularly, as shown in the figure, this Epiflorescent image shows the large collections of neurons around the nerve ring and cell bodies of the tail ganglia. (Credit: Worm Atlas)

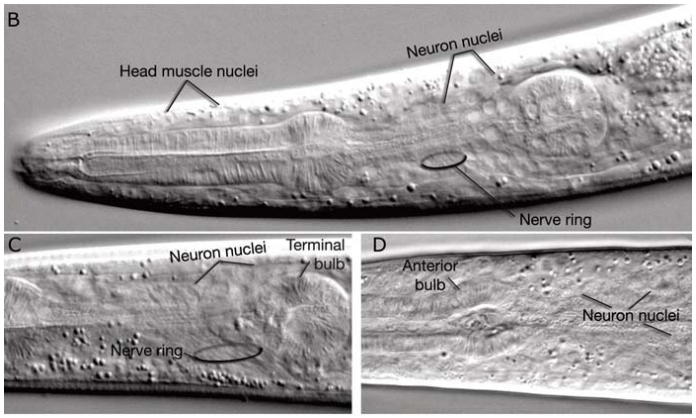


Figure 3: This figure illustrates the anatomy of an adult hermaphrodite *C. elegans*. **A.** Schematic drawing of the nematode worm. **B.** Image of an adult male *C. elegans.* **C.** The enlarged distal gonad of the nematode. D. The adult male tail, in ventral view. (Credit: Worm Atlas)

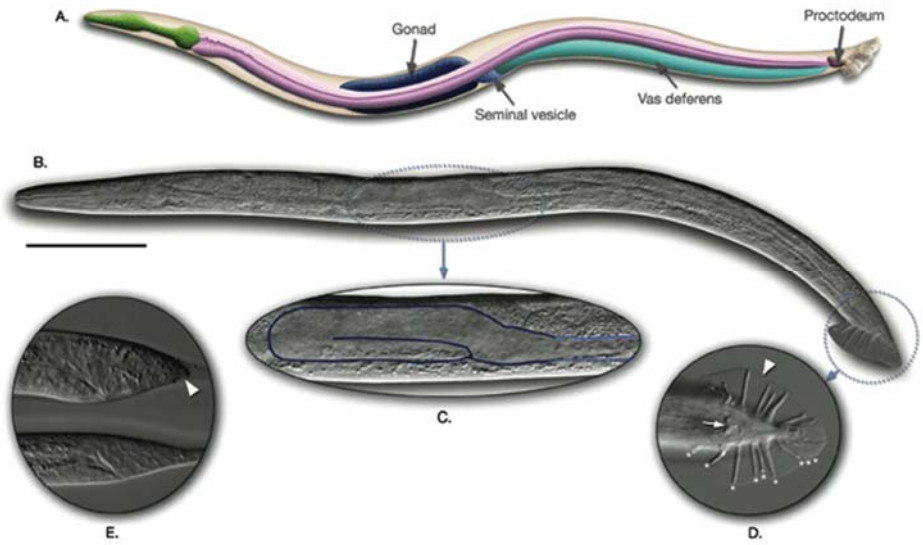


Figure 4: Results from Experiment 1, which clearly depict that *C. elegans* have an increase in longevity in both strains. The DAF-16 strain had increased mortality compared to the N2 *C. elegans* strain. It is also evident that Dimethyl Sulfone contributes greatly to a decrease in mortality as well. Graph was generated using OASIS.

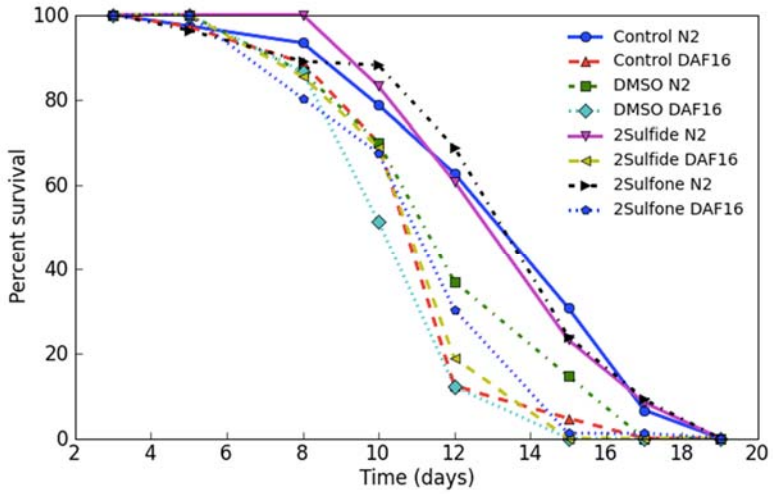


Figure 5: Results from Experiment 2, which clearly depict that *C. elegans* have an increase in longevity in both strains. The DAF-16 strain had increased mortality compared to the N2 *C. elegans* strain. It is also evident that Sulfone contributes greatly to a decrease in mortality as well. Graph was generated using OASIS.

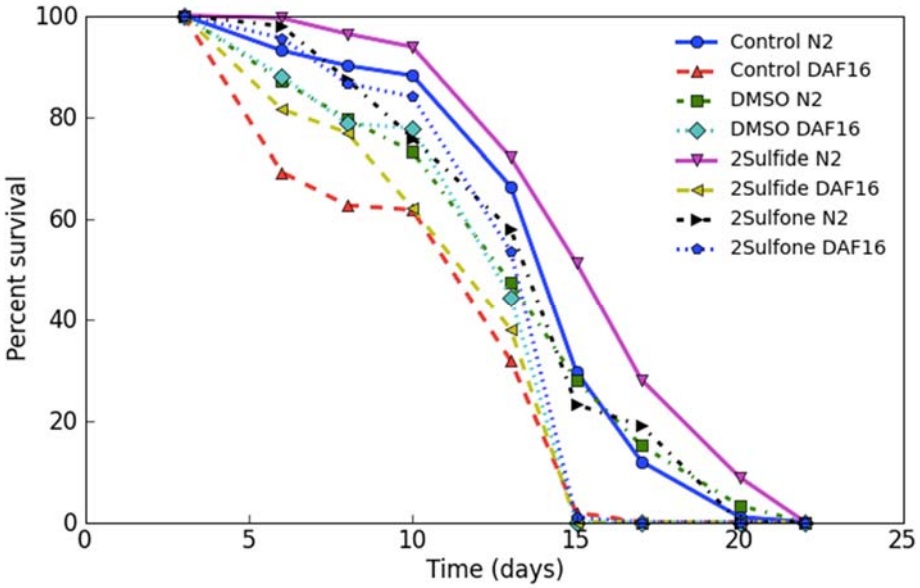


Figure 6: Results from Experiment 3, which clearly depict that *C. elegans* have an increase in longevity in both strains. The DAF-16 strain had increased mortality compared to the N2 *C. elegans* strain. It is also evident that Sulfide contributes greatly to a decrease in mortality as well. Graph was generated using OASIS.

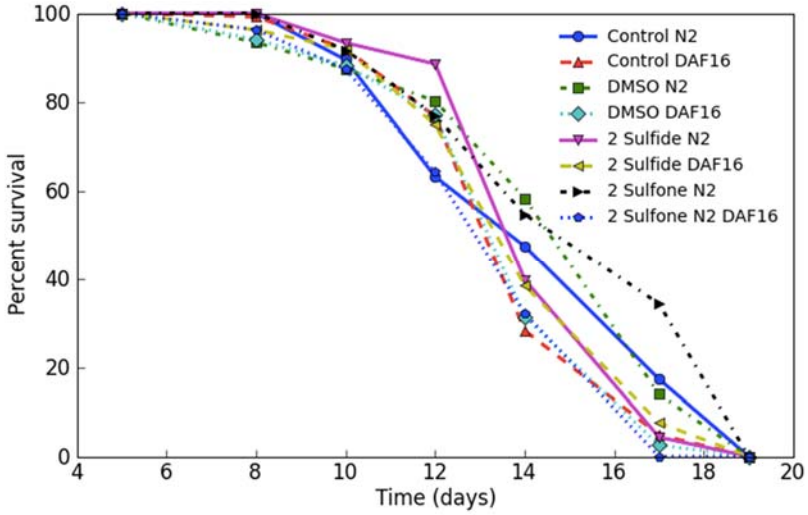


Figure 7: Results from Experiment 4, which clearly depict that *C. elegans* have an increase in longevity in the N2 strain. This graph, however, demonstrates that Sulfone has the opposite effect compared to all the other experiments; it leads to a decrease in *C. elegans* longevity. Graph was generated using OASIS.

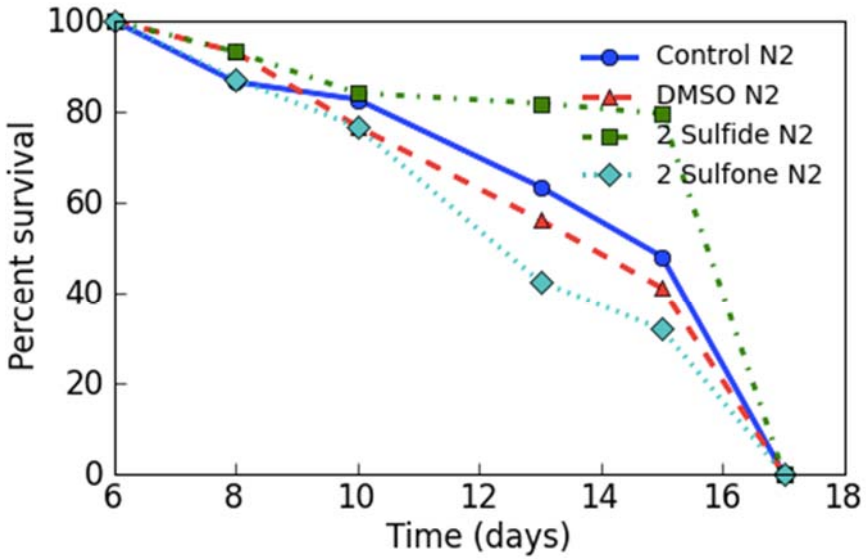


Figure 8: A comparison of all the data collected. Top left: Experiment 1. Top right: Experiment 2. Bottom left: Experiment 3. Bottom right: Experiment 4. As shown, sulfur-containing compounds do indeed increase lifespan in *C. elegans.* In particular, this research study and the data below suggest that the compound that had the greatest decrease in mortality was the Sulfone-containing compound. Graph was generated using OASIS.

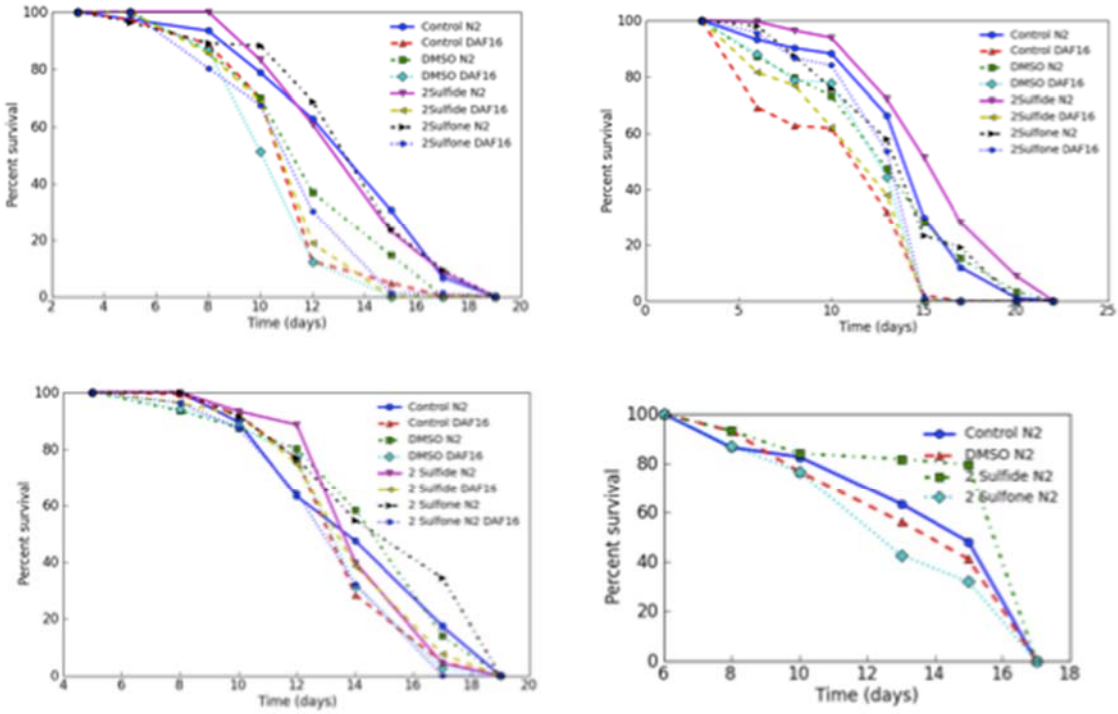
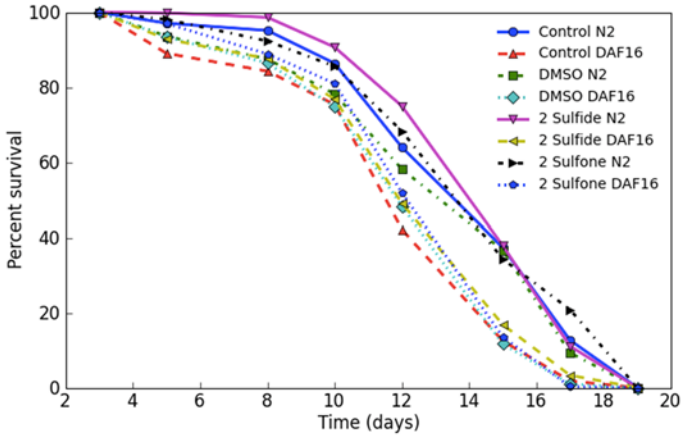


Figure 9: A comparison of all the data collected for the first three experiments, since they were similar. As shown, sulfur-containing compounds do indeed increase lifespan in *C. elegans* the greatest when compared to the other compounds. In particular, this research study and the data below suggest that the compound that had the greatest decrease in mortality was the dimethyl sulfone-containing compound. Graph was generated using OASIS.



References

Bartfai T. (2006). From lab bench to Wall Street. Faseb J. 20, 1578–1580. 10.1096/fj.06-0802ufm

Baumeister, Ralf, Barbara Meissner, and Maren Hertweck. "(Dipeptide) Nutrition, Ageing, and C. Elegans." GBM Annual Spring Meeting Mosbach 2004 2004.Spring (2004): n. pag. Web.

B. Dorman, Jennie, Bellie Albinder, Terry Shroyer, and Cynthia Kenyon. "The Age-1 and Daf2 Genes Function in a Common Pathway to Control the Lifespan of Caenorhabditis Elegans." *Geneses Society of America* (1995). Print.

Braeckman, Bart P., and Jacques R. Vanfleteren. "Genetic Control of Longevity in C. Elegans." *Experimental Gerontology* (2006): 90-98. Print.

Brenner, P. "Biochemical Genetics of Coenorhabditis Elegans." Mgg Molecular & General Genetics Molec. Gen. Genet. 135.1 (1974): 39-44. Web.

Brenner, Sydney. "College of Biological Sciences." What Is C. Elegans? University of Minnesota, 2005. Web. 14 Jan. 2016.

Carretero, Maria, Rafael L. Gomez-Amaro, and Michael Petrascheck. "Pharmacological Classes That Extend Lifespan of Caenorhabditis Elegans." Frontiers in Genetics. Frontiers Media S.A., 2015. Web. 2 Feb. 2016.

Cassada, Randall C., and Richard L. Russell. "The Dauerlarva, a Post-embryonic Developmental Variant of the Nematode Caenorhabditis Elegans." Developmental Biology 46.2 (1975): 326-42. Web.

Collins, James J., Kimberley Evason, and Kerry Kornfeld. "Pharmacology of Delayed Aging and Extended Lifespan of Caenorhabditis Elegans." *Experimental Gerontology* (2006): 1032-039. Print.

Crews, Douglas E. "Evolutionary Perspectives on Human Longevity and Frailty." Longevity and Frailty Research and Perspectives in Longevity: 57-65. Web.

Dalley, Brian K., and Miriam Golomb. "Gene Expression in the Caenorhabditis Elegans Dauer Larva: Developmental Regulation of Hsp90 and Other Genes." Developmental Biology151.1 (1992): 80-90. Web.

Finch, C. E. "Genetics of Aging." Science 2.435 (1997): 407-11. Print.

Frankowski, Harald, Silvestre Alavez, Patricia Spilman, Karla A. Mark, Joel D. Nelson, Pamela Mollahan, Rammohan V. Rao, Sylvia F. Chen, Gordon J. Lithgow, and H. Michael Ellerby. "Dimethyl Sulfoxide and Dimethyl Formamide Increase Lifespan of C. Elegans in Liquid." Mechanisms of Ageing and Development 134.3-4 (2013): 69-78. Web.

Henderson, Samuel T., and Thomas E. Johnson. "Daf-16 Integrates Developmental and Environmental Inputs to Mediate Aging in the Nematode Caenorhabditis Elegans." Current Biology 11.24 (2001): 1975-980. Web.

Jensen, Victor L., Marco Gallo, and Donald L. Riddle. "Targets of DAF-16 Involved in Caenorhabditis Elegans Adult Longevity and Dauer Formation." *Experimental Gerontology* (2006): 922-27. Print.

Kaeberlein, Matt. "Hypertrophy and Senescence Factors in Yeast Aging. A Reply to Bilinski Et al." FEMS Yeast Research FEMS Yeast Res 12.3 (2012): 269-70. Web.

Kimura, K. D. "Daf-2, an Insulin Receptor-Like Gene That Regulates Longevity and Diapause in Caenorhabditis Elegans." *Science* (1997): 942-46. Print.
Klass, Michael, and David Hirsh. "Non-ageing Developmental Variant of Caenorhabditis Elegans." Nature 260.5551 (1976): 523-25. Web.

Lakowski, B., and S. Hekimi. "Determination of Life-Span in Caenorhabditis Elegans by Four Clock Genes." Science 272.5264 (1996): 1010-013. Web.

Li, Zhu-Heng, Xiao-Li Hou, Jing-Bo Xu, and Yun-Biao Wang. "Effect of Temperature on Response of Caenorhabditis Elegan under Arsenic Stress." Chinese Journal Of Analytical Chemistry (Chinese Version) 41.4 (2013): 602. Web.

Martin, R. Bruce, Regina I. Hedrick, and Alice Parcell. "Thiazoline and Oxazoline Hydrolyses and Sulfur-Nitrogen and Oxygen-Nitrogen Acyl Transfer Reactions." The Journal of Organic Chemistry J. Org. Chem.29.11 (1964): 3197-206. Web.

Murphy, Coleen T., Steven A. Mccarroll, Cornelia I. Bargmann, Andrew Fraser, Ravi S. Kamath, Julie Ahringer, Hao Li, and Cynthia Kenyon. "Genes That Act Downstream of DAF-16 to Influence the Lifespan of Caenorhabditis Elegans." *Nature* (2003): 277-83. Print.

O'Halloran, Damien M., David A. Fitzpatrick, and Ann M. Burnell. "The Chemosensory System of Caenorhabditis Elegans and Other Nematodes." Chemical Ecology Wageningen UR Frontis Series (n.d.): 71-88. Web.

Olshansky, Brian. "C. Elegans and Volatile Anesthetics." Integrative Medicine (2007): 323-35. Web.

Partridge, Brad. "Issues Magazine." Living Forever: The Ethical Implications of Human Life Extension. Australian Issue, 2009. Web. 28 Mar. 2016.

Pun, Pamela Boon Li, Jan Gruber, Soon Yew Tang, Sebastian Schaffer, Raphael Lee Sheng Ong, Sheng Fong, Li Fang Ng, Irwin Cheah, and Barry Halliwell. "Ageing in Nematodes: Do Antioxidants Extend Lifespan in Caenorhabditis Elegans?" *Biogerontology* (2009): 17-30. Print.

Rea, Shane L., Natascia Ventura, and Thomas E. Johnson. "Correction: Relationship Between Mitochondrial Electron Transport Chain Dysfunction, Development, and Life Extension in Caenorhabditis Elegan." PLoS Biology PLoS Biol 6.1 (2008). Web.

Tissenbaum, Heidi A., and Leonard Guarente. "Increased Dosage of a Sir-2 Gene Extends Lifespan in Caenorhabditis Elegans." Nature 410.6825 (2001): 227-30. Web.

Warnhoff, Kurt, John T. Murphy, Sandeep Kumar, Daniel L. Schneider, Michelle Peterson, Simon Hsu, James Guthrie, J. David Robertson, and Kerry Kornfeld. "The DAF-16 FOXO Transcription Factor Regulates Natc-1 to Modulate Stress Resistance in Caenorhabditis Elegans, Linking Insulin/IGF-1 Signaling to Protein N-Terminal Acetylation." *PLOS Genetics PLoS Genet* (2013). Print.

Wolkow, C. A. "Regulation of C. Elegans Life-Span by Insulinlike Signaling in the Nervous System." *Science* (2000): 147-50. Print.