PROPOSAL FOR UNDERGRADUATE RESEARCH AND CREATIVE ENDEAVOR AWARD

Automated Acquisition of Lifespan-Related Data in Genetics Model *Caenorhabditis elegans*

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Purpose/Outcomes

One unifying characteristic of animals is that we all grow old, and older animals are more susceptible to disease (1). Further, age-related neurodegenerative diseases tend to have a strong genetic component (2), and modern genetic techniques have opened the doors to the characterization of genetic factors that affect aging and aging-related pathology. Over the last twenty-five years, various groups of scientists have used the model organism *Caenorhabditis elegans* to identify genes that have significant effects on lifespan, and many of those genes have near-identical functions in mammals (3-5).

While groups of researchers across the world have identified and characterized hundreds of individual genes that affect lifespan, the relationships between those genes remain poorly understood (6). For example, while mutations in two distinct genes may each individually shorten lifespan, there is limited literature documenting what happens when those two mutations act jointly in a single organism. By analyzing the effects of two or more mutations acting in unison, it is possible to obtain a more complete understanding of how aging emerges as the combination of various interconnected factors.

Aim 1: To create organisms with mutations in multiple aging-related transcription factor genes.

We hypothesize that transcription factors that mediate the responses to stress, and have broad and varying effects on downstream targets, will give the most immediate insight into the interactions between the genetic pathways that regulate aging. We will focus on genetic interactions with the gene *daf-16*, a well-studied FOXO transcription factor with significant implications for mammalian aging (7).

Aim 2: To record lifespan-related phenotypes, such as resistance to oxidative and heat stress, using an automated lifespan machine.

We hypothesize that different genetic interactions will differentially affect certain emergent phenotypes, such as the rate at which an organism dies upon exposure to oxidants or extreme temperatures. I will use a high-throughput lifespan acquisition platform based on flatbed scanners that my lab has recently built to measure survival under stressful conditions (see Methods and Research Design). This approach will allow me to obtain higher quality data (higher temporal resolution and larger population sizes) and reduce the time required to conduct experiments.

Preliminary Results/Progress

Since February 2016, I have worked in the Apfeld lab with the ultimate goal of creating the shortest-lived, yet healthy, strain of *C. elegans*. This proposal is a continuation of the broader project that, unlike previous proposals, focuses on publication-quality data acquisition.

To date:

(i) We have identified 13 transcription factor genes that affect lifespan and developed and validated PCR-based molecular genotyping assays for: *daf-16, daf-12, hlh-30, hsf-1, xbp-1, sbp-1*, and *nhr-49*.

(ii) I have outcrossed *hlh-30* and *nhr-49* six times each, a necessary step for double-mutant construction.

(iii) I have created three double mutants: *daf-16; daf-12*, *daf-16; hlh-30*, and *daf-16; skn-1/NT1;* and

(iv) I have measured lifespan and various other lifespan-related phenotypes of the *daf-16; daf-12* and the *daf-16; hlh-30* double mutants.

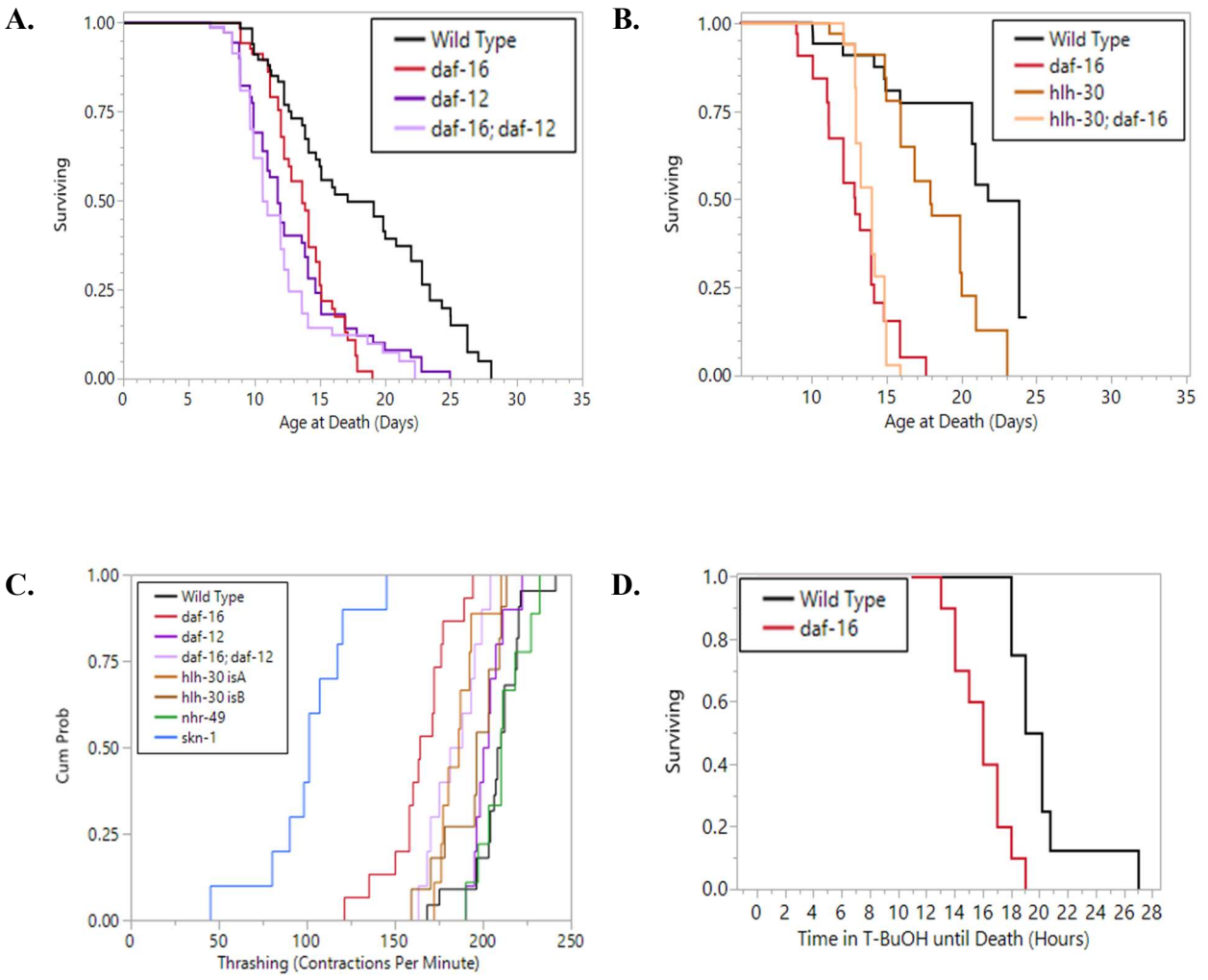


Figure 1: *C. elegans* lifespan and related phenotypes. Panel A shows that *daf-16; daf-12* does not appear to live any shorter than either *daf-16* or *daf-12.* Panel B shows that *daf-16; hlh-30* mutants seem to live as short as *daf-16*. Panels C-D show health related phenotypes; *skn-1*

thrashes less frequently in water than wild type (C), and *daf-16* is less resistant to oxidative stress (D).

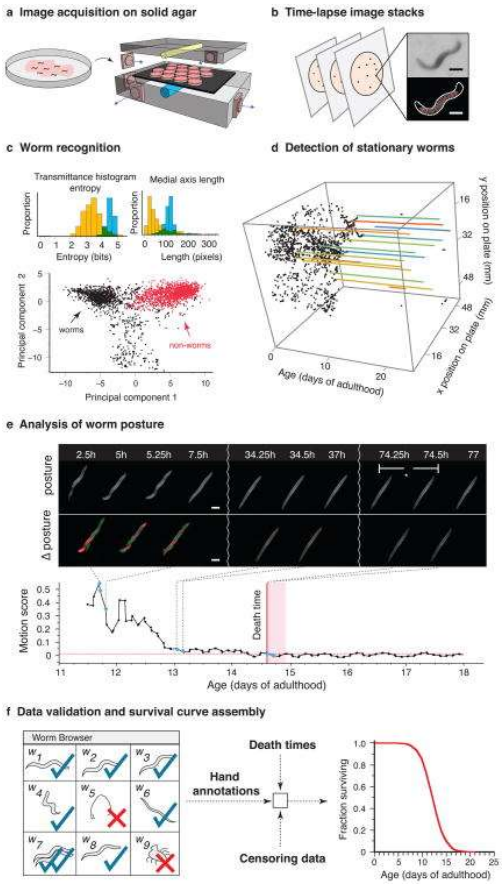
Significance and Originality

The study of aging is at the forefront of scientific research due to its relevance to the rapidly aging population and the understanding of the diseases related to aging, such as Alzheimer's disease. This project will create a niche within the broad field by focusing on the interactions between specific transcription factors that regulate aging in *Caenorhabditis elegans*. While many of these individual genes are well understood individually, there is little to no published information about their interactions.

Methods and Research Design

In the past eight months, Dr. Apfeld and I have identified thirteen genes of interest: *daf-16, daf-12, skn-1, nhr-49, xbp-1, sbp-1, daf-3, hlh-30, hsf-1, atfs-1, jun-1*, *egl-27,* and *nhr-8.* Each of these genes act as transcriptional regulators that have a documented effect on lifespan (3, 7-19).

In order to ensure that a specific mutant does not have any undesired mutations, it must be repetitively mated to wild-type and re-isolated in a process known as outcrossing. This project requires six outcrosses per mutant, each of which can take over 8 weeks. At this time, six strains have been fully outcrossed. One aspect of this project will be to complete outcrossing the remaining strains.



The process of creating double and triple mutants will vary from mutation to mutation. Genes located on the X chromosome, such as *daf-12*, are relatively easy to cross with mutations on autosomal chromosomes due to the haploid X chromosome in male worms. Mutations that are located very near each other, however, will require rare crossover events. Each double or triple mutant cross will be confirmed using single-worm PCR and/or distinguishable phenotypes.

One major focus of the spring semester will be measuring resistance to oxidative and heat stress via an automated lifespan machine, as described in Stroustrop et al. 2013 (20). I will measure oxidative stress resistance on each strain with varying concentrations of the oxidant tert-butyl hydroperoxide as well as resistance to heat stress at varying temperatures. With

both phenotypes, hundreds of worms will be assayed at once through a computer algorithm which utilizes a flatbed scanner to produce precise, consistent data.

Shown above is Figure 1 from Stroustrup et al. 2013, which describes the method by which the lifespan machine obtains data (20).

Dissemination

By the end of this project I will produce and store new strains of *C. elegans* and extensive data from observations about health, stress resistance, and reproduction of mutant worms. I will then continue this project into the summer and plan to submit my findings for presentations at the Genetics Society of America's 21st International *C. elegans* conference in June of 2017. I will also plan to apply to multiple Northeastern presentation opportunities, such as the RISE 2017 poster session. I hope to continue this project throughout my time at Northeastern eventually collect enough data to be the primary author of a publishable research paper.

Evaluation

This project can be evaluated on the basis of scientific rigor and acceptance to present at academic conferences. I will consider it a success if I am able to analyze lifespan, oxidative, and heat stress resistance data in a clear and concise manner.

Budget

The money from this award will be used for general supplies for this project. A sample distribution of expenses is shown below.

Item	Unit Cost	Estimate Units Per Week	Weeks in Spring Semester	Total Cost
Petri Dishes + NGM	0.5	100	15	750
5'-Fluoro-2'deoxyuridine (FUDR)	0.036	40	15	21.6
PCR Tubes (Strips of 8)	0.5	30	15	225
GoTaq Master Mix	0.3944	15	15	88.74
Agarose	0.4	5	15	30
Total				1115.34

References

1. **Citation:** Gardner, ID. 1980 The effect of aging on susceptibility to infection. Rev Infect Dis. 2:801-810.
   Commentary: Early paper formally characterizing the increased risk of infection with age.

2. **Citation:** Bertram, L. and Tanzi, R. 2005 The genetic epidemiology of neurodegenerative disease. J Clin Invest. 115:1449-1457.
   Commentary: A review characterizing the genetic components of neurodegenerative disease. Somewhat outdated, but a great introduction.

3. **Citation:** Kenyon, C., Chang, J., Genesch, A. R., and Tabtlang, R. 1993 A *C. elegans* mutant that lives twice as long as wild type. Nature 366:481-464.
   Commentary: Here, Dr. Kenyon reveals that *daf-2* mutants live twice as long as wild type, and shows that it requires the activity of *daf-16*, the transcription-factor-encoding-gene that we now know is repressed by *daf-2*.

4. **Citation:** Holzenberger, M. et al. 2003 IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421:182-187.
   Commentary: One of the papers that reported the conservation of the basic lifespan-enhancing characteristic of daf-2 mutants in mice.

5. **Citation:** Uno, M. and Nishida, E. 2016 Lifespan-regulating genes in *C. elegans.* npj Aging and Mechanisms of Disease 2:16010.
   Commentary: Detailed and approachable review detailing aging mechanisms and their conservation across species.

6. **Citation:** Zhou, K., Pincus, Z. and Slack, F. 2011 Longevity and stress in Caenorhabditis elegans. Aging 3:733-753.
   Commentary: For this proposal, this paper serves as a credible source which explicitly states that the relationship between longevity phenotypes remains unclear. It will also be very important as this project progresses. It highlights problems in replicability in oxidation studies and, most significantly, describes multiple short-lived mutants, such as *daf-18*, and is organized based on chemical pathways.

7. **Citation:** Hesp, K., Smant, G. and Kammenga, J. 2015 *Caenorhabditis elegans* DAF-16/FOXO transcription factor and its mammalian homologs associated with age-related disease. Experimental Gerontology 72:1-7.
   Commentary: This paper describes *daf-16* and FOXO and goes further to highlight the relevance of *daf-16* research to humans that gives a compelling argument in favor of the importance of *C. elegans* research.

8. **Citation:** Lithgow, G. and Fisher, A. 2006 The nuclear hormone receptor DAF-12 has opposing effects on Caenorhabditis elegans lifespan and regulates genes repressed in multiple long-lived worms. Aging Cell 5:127-138.

Commentary: This paper is the basis on which I propose making mutant combinations with *daf-12* mutants. It describes *daf-12*'s interactions with other key genes and describes *daf-12* as the first gene with loss-of-function and gain-of-function alleles that shorten and lengthen lifespan, respectively.

9. **Citation:** Taylor, R. and Dillin, A. 2013 XBP-1 Is a Cell-Nonautonomos Regulator of Stress Resistance and Longevity. Cell 153:1435-1447.
   Commentary: I found this article while trying to learn about ER stress, a concept that was ill-defined, or not defined at all, in other papers. The introduction provides crystal-clear background on the endoplasmic reticulum (ER) unfolded protein response and the role of *xbp-1* on ER stress and therefore lifespan.

10. **Citation:** Gilst, M., Hadjivassiliou, H., Jolly, A., and Yamamoto, K. 2005 Nuclear Hormone Receptor NHR-49 Controls Fat Consumption and Fatty Acid Composition in *C. elegans*. PLoS Biol 3:e53.
    Commentary: Gilst et al. make a handful of key observations about genes with a mutated *nhr-49* allele. They found two main phenotypes of the knockout: high-fat content and shortened lifespan. Interestingly, they find that those two phenotypes could be attributed to distinct pathways, and *nhr-49* more broadly influences the expression of thirteen genes.

11. **Citation:** Tullet, J. et al. 2008 Direct Inhibition of the Longevity-Promoting Factor SKN-1 by Insulin-like Signaling in *C. elegans*. Cell 132:1025-1038.
    Commentary*:* In this paper, researchers connect the detoxification response mediated by *skn-1* with *daf-16*, the aforementioned key player in the insulin response pathway. It also includes a table (Table 1) that describes the shortened lifespans of three separate *skn-1* mutants. This data does not include a *daf-16;skn-1* double mutant.

12. **Citation:** Steinbaugh, M. et al. 2015 Lipid-mediated regulation of SKN-1/Nrf in response to germ cell absence. eLife 4:e07836
    Commentary: Clarifies the relationship of *sbp-1* to aging and the IGF pathway.

13. **Citation:** Shaw, W. et al. 2007 The *C. elegans* TGF-β Dauer Pathway Regulates Longevity via Insulin Signaling. Curr Biol. 17:1635-1645.
    Commentary: This paper has clear lifespan data for *daf-3* and shows that it is both independently short lived and independent of the longevity induced by *daf-2* mutations.

14. **Citation:** Lapierre, L. et al. 2013 The TFEB orthologue HLH-30 regulates autophagy and modulates lonevity in Caenorhabditis elegans. Nat Commun. 4:2267
    Commentary: *hlh-30* is shown here to have a role in modulating lifespan—although it does not have a shortened lifespan, it is essential for the lifespan-extending qualities of multiple other mutations. Further, this group speculates about the connection between autophagy and lifespan extension.

15. **Citation:** Biard, N. et al. 2014 HSF-1-mediated cytoskeletal integrity determines thermotolerance and life span. Science 346:360-3.
Commentary: Heat shock transcription factors are very important in stress resistance and lifespan; therefore, *hsf-1* is essential to this proposed project. This recent Science paper highlights the shortened lifespan of *hsf-1* mutants and their weakened thermal stress resistance.

16. **Citation:** Tauffenberger, A., Vaccaro, A. and Parker, J. 2016 Fragile lifespan expansion by dietary mitohormesis in *C. elegans*. Aging 8:50-57.
Commentary: This recently-published work describes the lifespan of worms exposed to *atsf-1* RNAi but further explains the role of *atsf-1* in the broader mitochondrial unfolded protein response process.

17. **Citation:** Uno, M. et al. 2013 A fasting-responsive signaling pathway that extends lifespan in *C. elegans*. Cell Rep. 31:49-91.
Commentary: Infers relationship between JUN-1/FOS-1 and DAF-16.

18. **Citation:** Xu, X. and Kim, S. 2012 The GATA Transcription Factor *egl-27* Delays Aging by Promoting Stress Resistance in *Caenorhabditis elegans*. PLOS Genetics.
Commentary: Finds that *egl-27* is required for the lifespan-extending effects of IIS pathway, and extends lifespan when overexpressed.

19. **Citation:** Thondamal, M. et al. 2014 Steroid hormone signaling links reproduction to lifespan in dietary-restricted *Caenorhabditis elegans*.
Commentary: Characterizes many genes, including NHR-8, which appears to be critical to mTOR and dietary restriction-mediated longevity.

20. **Citation:** Stroustrup, N. et al. 2013 The *C. elegans* Lifespan Machine. Nat Methods 10:665-670.
Commentary: This paper describes in great detail the workings of the lifespan machine, and the supplement describes how the machine can be built. Strostrup, the paper's first author, is currently working with the Apfeld lab to help re-build the machine on site.