Extending Lifespan with Genetic Modification

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Abstract

Aging is a complex biological process that is characterized by different factors. One major factor are telomeres, which cause cell death and results in aging when they shorten. Telomeres are the end caps of the chromosome and are made of a repeated section of DNA. Their purpose is to maintain genomic and cellular stability, which involves protecting the genome from breaking down or mutating. The goal of this experiment is to learn how telomeres function in *Drosophila melanogaster* in order to better understand their aging process. In the experiment the expression of two genes that produce telomere complex proteins, *spindle*-E and *cav,* will be decreased to determine their effect on the life cycle and lifespan. I will measure the life cycle by recording the time it takes for an adult to eclose. The lifespan will be measured by counting how many fruit flies are alive and dead each day.

I found that there is an increase in life cycle compared to control and there's a mutation in eye phenotype of the UAS.Cav.RNAi. x ey-GAL4 population. There is a significant increase in lifespan of the crosses: UAS.Spn-E.RNAi. x Act5C-GAL4, UAS.Spn-E.RNAi. x ey-GAL4, UAS.Cav.RNAi. x Act5C-GAL4, and UAS.Cav.RNAi. x nos-GAL4. The reduction of *spindle-E* and *cav* in *D.melanogaster* appears to affect the ability to develop into adults. There are other telomere genes that can assist in telomere maintenance in the absence of another gene and that these genes have only an effect in specific tissues, which limits their effects in other regions of the organism.

Keywords: aging, telomeres

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Introduction:

In the United States of America the aging population of 65 years and older represent 14.1% of the population in 2013, which is 44.7 million people ("Aging Statistics"). By 2040 older people will be 21.7% of the population and by 2060 it is estimated that there will be 98 million older people ("Aging Statistics"). So it appears that the aging population is significantly increasing and aging research is becoming a larger scientific field.

Aging is a complex biological process that is characterized by different factors (Pusceddu *et al*. 2015). Some of those different factors are the accumulation of diverse deleterious changes occurring in cells, tissues and a decline in organ function (Tosato *et al.* 2007). Additional factors also include, deterioration of genomic integrity and genomic instability, the loss or diminished function of post mitotic cells, and a decline in the ability of (stem) cells to sustain replication and cell divisions (Pusceddu *et al*. 2015). As age increases the risk of age-associated diseases and death increases (Tosato *et al*. 2007). Aging is an intricate process that has multiple theories that try to explain why aging occurs. Out of the multiple theories being studied, a few have become well-known. For example, the telomere theory focuses on studying telomeres, which are the ends on a chromosome and are important in protection of the DNA in the chromosome that basically codes for all the functions in an organism.

Background:

There many different theories that try to explain the aging process. The theories include evolution, molecular, cellular and system theories.

The Evolutionary theory focuses on the buildup of mutations that affect health at older ages which are not selected against during natural selection (Weinert and Timiras 2003). The molecular theory involves the concept of gene regulation which concentrations on changes in the expression of genes that regulate development and aging (Weinert and Timiras 2003). The cellular theory consists of the 2 theories: cellular senescence-telomere and free radical. The cellular senescence-telomere theory centers on the increase in frequency of senescent cells, which may result from telomere loss, genomic damage, toxins, irradiation, oxidative stress, oncogene expression, tumor suppressor gene activation and epigenomic alterations (Weinert and Timiras 2003, Pusceddu *et al*. 2015). The free radical theory focuses on the process of oxidative metabolism, which produces highly reactive free radicals that can damage lipids, proteins and DNA (Weinert and Timiras 2003). The system theories consist of neuroendocrine and immunologic theories. The Neuroendocrine theory involves the alterations in the neuroendocrine ability to regulate homeostasis, which results in aging-related physiological changes (Weinert and Timiras 2003). The immunologic theory is the decline of immune function as age increases. It results in decreased incidence of infectious diseases, but increased incidence of autoimmunity, which is when the immune response of an organism targets its own cells and tissues (Weinert and Timiras 2003).

One of the main focuses of the cellular senescence-telomere theory are telomeres, which are the end caps of chromosomes and are nucleoprotein structures that are highly conserved (Pusceddu *et al*. 2015). When observing telomeres in humans they have a tandem repeat of six nucleotides, TTAGGG, in their double-stranded DNA

sequence (Pusceddu *et al*. 2015). The 3' end of the telomere has numerous G nucleotides on the single-stranded overhang, which can fold back to make a loop (T-looped structure) that acts as a protective cap at the end of the chromosome (Pusceddu *et al*. 2015). The purpose of telomeres is to maintain genomic and cellular stability and replication, which involves protecting the genome from degradation, unwanted recombination and chromosomal fusion (Pusceddu *et al.* 2015). The length of telomeres very between chromosomes, between species, and are influenced by age, heritability, telomerase repair, recombination, and timing of replication (Aubert and Lansdorp 2008). Telomeres are important and problems in the cell can occur with the loss, shortening or damaging of the telomeres.

Telomere loss can result from a problem in DNA replication and the processing of chromosome ends after replication (Aubert and Lansdorp 2008). Telomeres shorten because of oxidative damage to the telomere DNA, the failure to unwind or correctly process the multiple G-nucleotides at the end of the telomere DNA and by T-loops being removed during recombination. (Aubert and Lansdorp 2008).

Telomeres are important in aging because of multiple factors. The faster than normal shrinking of telomeres are possibly associated with multiple age-related conditions, like cardiovascular disease, type 2 diabetes, neurodegenerative diseases and premature aging syndromes (Pusceddu *et al*. 2015). It is known that telomeres are responsible for constant DNA damage signals in senescent cells, which can be detected in vivo (Aubert and Lansdorp 2008). The lack of telomerase affects telomere length because they help build up the telomere when it starts to shorten, and this lack of

telomerase also is related to inherited genetic disorders caused by gene mutations because telomerase fails to elongate or maintain telomeres and results in telomere shortening (Aubert and Lansdorp 2008).

There are multiple organisms used in science to help study various topics. For studying aging and telomeres some of the organisms used are *Nothobranchius furzeri, Drosophila melanogaster,* and *Caenorhabditis elegans.* These organisms are also considered to be model organisms, which are easy to maintain, have a short life span, similarities to humans, genome is well known, and they are not expensive.

The model organism that was used in this experiment was *Drosophila melanogaster. Drosophila* telomeres use a collection of retrotransposons that maintain chromosome length (Mason, Frydrychova, and Biessmann 2008). The telomere DNA in *Drosophila* has different combinations of proteins that control chromatin structure (Mason, Frydrychova, and Biessmann 2008). The terminal repeats in *Drosophila* are made of telomere-specific non-long terminal repeat retrotransposons, which include *HeT-A, TART,* and *TAHRE* (Mason, Frydrychova, and Biessmann 2008). *Drosophila* telomeres are similar to those of other organisms because they both have multiple proteins that are important for chromosome stability and elongation, which are found in the chromosome cap (Mason, Frydrychova, and Biessmann 2008). Loosing sequence specificity causes the telomere associated sequence to move to the end of the chromosome and loosing telomerase results in the loss of telomeric repeats, which in *Drosophila* also includes the loss of a TRF-2 like protein that has the purpose of stabilizing T-loops (Mason, Frydrychova, and Biessmann 2008).

The telomeres of *Drosophila* have the same function of telomeres in other eukaryotes (Mason, Frydrychova, and Biessmann 2008). The chromosome end in *Drosophila* are needed for stability, but the maintenance of telomeres in *Drosophila* are not completely understood (Mason, Frydrychova, and Biessmann 2008).

Due to the importance of telomeres in maintaining genomic and cellular stability, and having a role in various diseases they need to be protected. Within *Drosophila* there are multiple genes whose function is to assist in protecting telomeres. The focus of this project will be on the genes *caravaggio (cav)*, *spindle-E (spn-E), modigliani (moi),* and *verrocchio (ver). Caravaggio* produces the protein Cav, which is one of the major components of caveolae membranes and has a possible role in the functioning of hematopoietic stem cells (Bai *et al*. 2014). The loss of the Cav protein has a possible effect on genes that have a role in cell cycle control and cellular senescence (Bai *et al*. 2014). *Caravaggio* also encodes HOAP (Heterochromatin Protein 1(HP1)/Origin Recognition Complex (ORC)-associated protein), which is one of the components that forms the complex Terminin (Raffa *et al*. 2010). Terminin is the equivalent of shelterin in humans, with both having the function of protecting the telomeres (Raffa *et al*. 2009). The gene *spindle-E* has the function of controlling the flow of telomere elements to the ends of chromosomes (Casacuberta and Pardue 2006). It also has a role in the RNA interference (RNAi)-based silencing mechanism within *Drosophila*, which controls telomere maintenance within the germline (Savitsky *et al*. 2006). *Spn-E* is essential for correct localization of mRNA and proteins that are part of establishing the axis formation in embryos and *spn-E* also encodes a DEAD-box helicase, which is part of the RNAi

machinery (Casacuberta and Pardue, 2006; Savitsky *et al*. 2006). *Verrocchio* is a gene that codes for an oligonucleotide/oligosaccharide-binding fold-containing protein and it is a component of terminin that is essential for telomere fusion prevention (Raffa *et al*., 2010). *Modigiliani* is a gene that codes for the protein Moi, which is only present at the telomeres (Raffa *et al.* 2009). It is necessary to prevent telomere fusion and it forms the terminin complex with HOAP (Raffa *et al*., 2009).

In order to study the effects of the telomere specific genes *caravaggio* and *spindle-E* gene expression will be knocked down to observe if the lack of these genes will affect the survival of *D. melanogaster*. To knockdown gene expression the method of double-stranded RNA-mediated interference (RNAi) is used to decrease or knockdown gene expression (Agrawal *et al,* 2003). This process uses a sequence-specific RNA degradation process, which involves the binding of RNA nucleases with double stranded RNA of the target gene (Agrawal *et al.* 2003). It is then cleaved into fragments called single interfering RNA (siRNA) and injected into the organism (Agrawal *et al*. 2003). The siRNA binds to the RNA-induced silencing complex (RISC), then the corresponding single-stranded mRNAs are broken down resulting in the silencing of the target gene (Agrawal *et al.* 2003).

Along with using RNAi to alter gene expression in this project we used the GAL4/UAS system, which is used for target gene expression in *Drosophila* with one parent fly expressing the UAS aspect and the other parent fly expressing the GAL4 aspect of this system resulting in offspring with the desired gene expression (Duffy, 2002). The target gene, which is the responder in this system, is controlled through the presence of

the UAS element and GAL4 activates that gene's transcription (Duffy, 2002). GAL4 is considered the driver in the system since the responder is expressed in the pattern of the GAL4 gene (Duffy, 2002). The RNAi expressed genes (*spn-E* and *cav*) in this experiment are also controlled through UAS and are crossed with Act5C, nos, ey, *ver*, and *moi* genes, which are the GAL4 drivers. This results in decreased levels of *spn-E* and *cav* being expressed in the regions were Act5c, nos, ey, *ver* and *moi* are expressed. The genes *ver* and *moi* are used to focus decreased *spn-E* and *cav* expression in the telomeres. Act5c, nos, ey are used to focus decreased *spn-E* and *cav* expression in various regions of the body within *D. melanogaster*.

nos (nanos) is a gene that is expressed during oogenesis and it is used to drive the expression of the RNAi UAS gene in the germ cells of *Drosophila* (Doren *et al.* 1998). ey is also referred to as eyeless and it is a gene that is involved in eye morphogenesis in *Drosophila* and is used in this experiment drive the expression of the RNAi UAS gene in the eye tissue of *Drosophila* (Quiring *et al.* 1994). Act5C is also known as Actin5C and it is a gene that codes for the filament-forming proteins that are a component in the cytoskeleton and its additional functions include maintenance of protein location in the cell, cell mobility, muscle contraction and chromatin remodeling (Flybase Consortium 1999). Act5C is expressed in somatic cells only and will drive the expression of the RNAi UAS gene throughout the organism (White-Cooper, 2012). To determine if there is an effect on *Drosophila* when knocking down *cav* and *spn-E* a control is required and for this experiment it will be the progeny of Oregon R and Canton S, which are both an inbred wild type strain and will produce a wildtype control (McGraw *et al.* 2009).

To analyze the survival of the control compared to the RNAi UAS-GAL4 crosses the two sample Kolmogorov-Smirnov test is used. The Kolmogorov-Smirnov test (KS test) is a nonparametric test that is not based on the assumption of normal distribution. It determines whether a set of observations are from the same distribution or if they are from a different distribution (Lilliefors, 1967). This test is used due to its ability to be used with small sample sizes and it is more powerful than the chi-square test for any sample size (Lilliefors, 1967). This test determines the maximum difference between two observed sample distributions (Lilliefors, 1967). If the maximum difference exceeds the critical value, then the two observed sample distributions are not from the same distribution (Lilliefors, 1967). The α used is 0.05 and the critical value is determined with the equation in the appendix using the program Mathematica. The survival of the control and the survival of the telomere crosses are compared. If the maximum difference between control and the telomere cross is greater than the calculated critical value then there is a significant difference in the survival of the telomere cross.

Objective:

Based on the information previously mentioned, the goal of my experiment is to learn how telomeres function in *Drosophila melanogaster* in order to better understand the aging process with in *D. melanogaster*. To accomplish this goal I will attempt to determine the effects of telomere specific genes on the lifespan and life cycle of *Drosophila melanogaster*.

Methods:

The fly stocks used to produce the crosses were ordered from Bloomington Drosophila Stock Center with stocks already genetically modified with RNAi and UAS/GAL4.

Crosses:

Oregon R x Canton S (Control)

UAS-SpnE.RNAi x Act5C-GAL4

UAS-SpnE.RNAi x ver-GAL4

UAS-SpnE.RNAi x moi-GAL4

UAS-SpnE.RNAi x ey-GAL4

UAS-SpnE.RNAi x nos-GAL4

UAS-Cav.RNAi x Act5C-GAL4

UAS-Cav.RNAi x ver-GAL4

UAS-Cav.RNAi x moi-GAL4

UAS-Cav.RNAi x Ey-GAL4

UAS-Cav.RNAi x nos-GAL4

Drosophila melanogaster was reared on standard laboratory fly medium (per 3 liter batch/ 300 vials: 218g corn flour, 38g nutritional yeast, 30g agar, 64g sucrose, 126g D-glucose, 3g tegosept, 30mL 95% ethanol) at a constant temperature (~22 degrees Celsius).

For one cross, 5 virgin females were collected from either of the designated parental genotypes being crossed. Five males were collected from the other fly genotype in the cross.

The males and females were placed in a vial labeled with their genotypes and the date of the cross. This was repeated for the other crosses (11 total including wildtype). The parental fly generation was moved to new vials every day for 5 days. The vials were monitored for eggs and for when an adult first eclosed (recorded the date of eclosing, how many adults eclosed and the phenotypes). The F1 generation was moved to new vials every day. The F1 vials were monitored each day for how many flies died and how many were alive, which was recorded on data sheets. Once enough vials were collected to produce an F2 generation the F1 generation was moved every other day and then just moved at the sign of larvae or pupae. These steps were repeated for the F2 generation, using the vials from the F1 generation that had eggs. For the F2 generation 5 vials were selected from the collection of old F1 vials for each cross (vials with evidence of larvae) and then moved the eclosed flies into new vials and recorded the same information collected for the F1 generation. Newly eclosed flies were continuously collected until no more eclosed. The adult flies were moved into new vials every other day and then only moved at the sign of larvae and pupae.

To analyze the survival of the telomere cross compared to the control Excel is used to record the number of flies alive, dead and cumulative dead over a span of days. The survival is determined for each day of its lifespan with (total cumulative dead - number dead on day)/total cumulative dead = survival rate. The maximum difference in the calculated survival of the control and the telomere cross is determined and the Mathematica equation is used to determine the critical value.

Results:

After monitoring the two generations various phenotypes have been observed, with a variation of eye color, wing type and head morphology.

Cross	Phenotype
UAS-SpnE.RNAi x Act5C-GAL4	Wings: CyO (curly) and wildtype; Eyes: white (males), wildtype
UAS-SpnE.RNAi x ver-GAL4	Wings: wildtype; Eyes: wildtype, yellow, white
UAS-SpnE.RNAi x moi-GAL4	Wings: wildtype; Eyes: wildtype, white, yellow
UAS-SpnE.RNAi x ey-GAL4	Wings: CyO and wildtype; Eyes: wildtype, yellow
UAS-SpnE.RNAi x nos-GAL4	Wings: wildtype; Eyes: wildtype
UAS-Cav.RNAi x Act5C-GAL4	Wings: wildtype; Eyes: wildtype
UAS-Cav.RNAi x ver-GAL4	Wings: CyO and wildtype; Eyes: wildtype
UAS-Cav.RNAi x moi-GAL4	Wings: CyO and wildtype; Eyes: wildtype, yellow, white
UAS-Cav.RNAi x ey-GAL4	Wings: CyO and wildtype; Eyes: small eyes with smaller mouths
UAS-Cav.RNAi x nos-GAL4	Wings: CyO; Eyes: wildtype, yellow, white
Oregon R x Canton S	Wings: wildtype; Eyes: wildtype

Table 1: Phenotypes of the crosses for the F1 generation.

The major change in phenotype occurred in the UAS-Cav.RNAi x ey-GAL4 offspring, which have a mutation in the formation of the eyes and mouth. The mouth and the eyes appear to have become smaller and this phenotype occurred in the offspring that had wildtype wings. Another interesting phenotype is in the offspring of UAS-Cav.RNAi x nos-GAL4, which only have CyO wings, which could be an indicator of how successful or viable the cross was.

Figure 1: A comparison of UAS-Cav.RNAi x ey-GAL4 offspring, with a noticeable difference in eye and head morphology of the top two flies compared to the bottom two flies.

Cross	Average life cycle
UAS-SpnE.RNAi x nos-GAL4	12.2
UAS-SpnE.RNAi x ey-GAL4	12
UAS-SpnE.RNAi x moi-GAL4	12.2
UAS-SpnE.RNAi x ver-GAL4	12
UAS-SpnE.RNAi x Act5C-GAL4	12.66666667
UAS-Cav.RNAi x Act5C-GAL4	12.2
UAS-Cav.RNAi x ver-GAL4	12
UAS-Cav.RNAi x moi-GAL4	12
UAS-Cav.RNAi x ey-GAL4	12.2
UAS-Cav.RNAi x nos-GAL4	12.6
Oregon R x Canton S (Control)	11.6

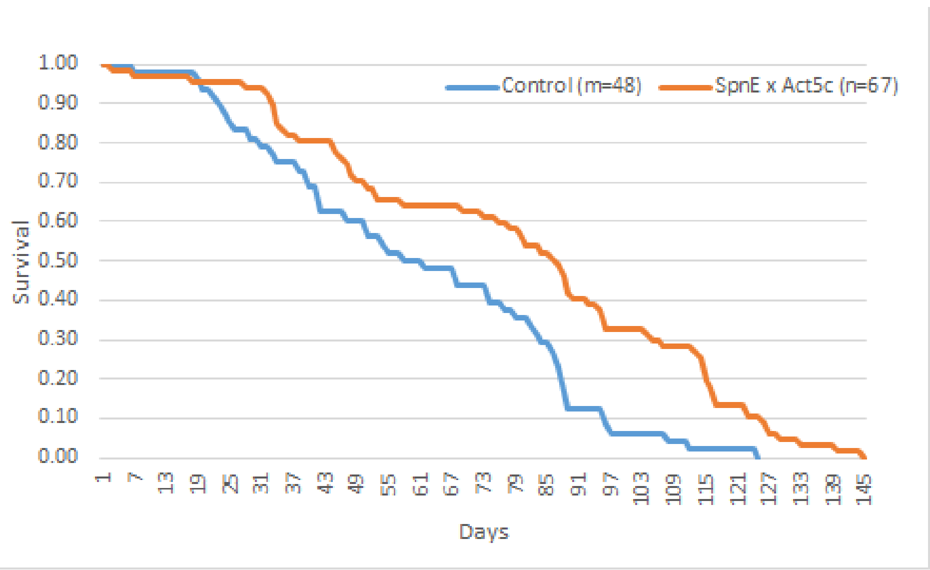
Table 2: The average life cycle of F1 generation in days.

The life cycle was determined from when the parental flies were put into the same vial to when the first adult eclosed. The average life cycle was obtained by averaging the life cycle of the 5 vials per cross. The control had the shortest average life cycle of 11.6 days and the cross UAS-SpnE.RNAi x Act5C-GAL4 had the longest average life cycle of 12.66666667 days.

Cross	Average life cycle
UAS-SpnE.RNAi x nos-GAL4	12.8
UAS-SpnE.RNAi x Ey-GAL4	12.2
UAS-SpnE.RNAi x moi-GAL4	13.4
UAS-SpnE.RNAi x ver-GAL4	13.4
UAS-SpnE.RNAi x Act5C-GAL4	15
UAS-Cav.RNAi x Act5C-GAL4	13.2
UAS-Cav.RNAi x ver-GAL4	13.4
UAS-Cav.RNAi x moi-GAL4	13
UAS-Cav.RNAi x Ey-GAL4	12.6
UAS-Cav.RNAi x nos-GAL4	12.2
Oregon R x Canton S (Control)	12.8

Table 3: The average life cycle of F2 generation in days.

The life cycle was determined from when the eclosed F1 adults were placed in the vials to when an adult eclosed from the F1 produced eggs. The average life cycle was obtained by averaging the life cycle of 5 vials per cross. The crosses UAS-Cav.RNAi x nos-GAL4 and UAS-SpnE.RNAi x Ey-GAL4 had the shortest average life cycle of 12.2 days and the cross UAS-SpnE.RNAi x Act5C-GAL4 had the longest average life cycle of 15 days. The control had an average life cycle of 12.8 days. So there was a larger difference in life cycle between control and UAS-SpnE.RNAi x Act5C-GAL4.



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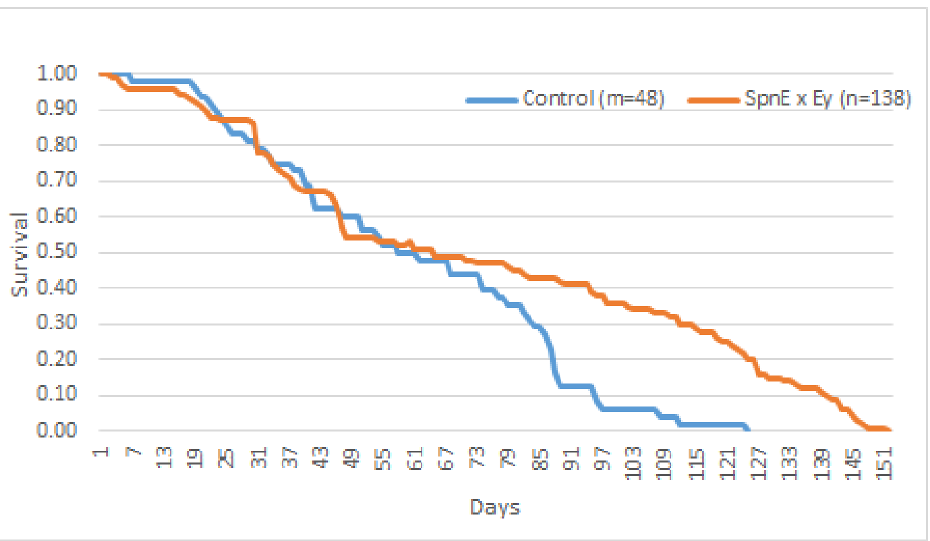

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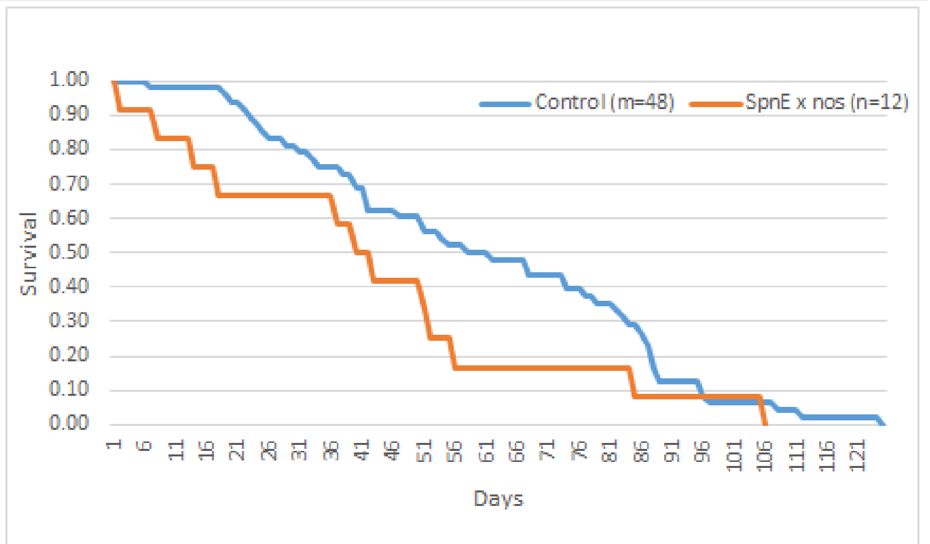

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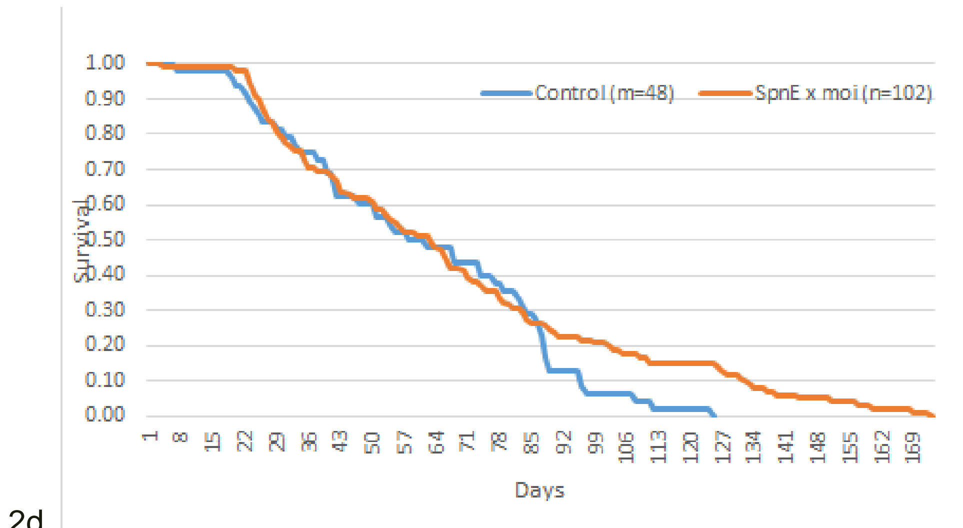
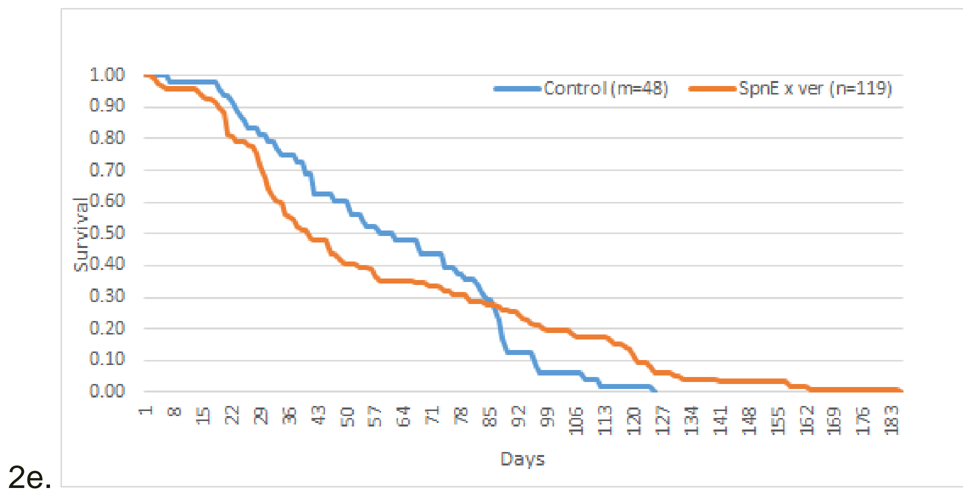


2d.

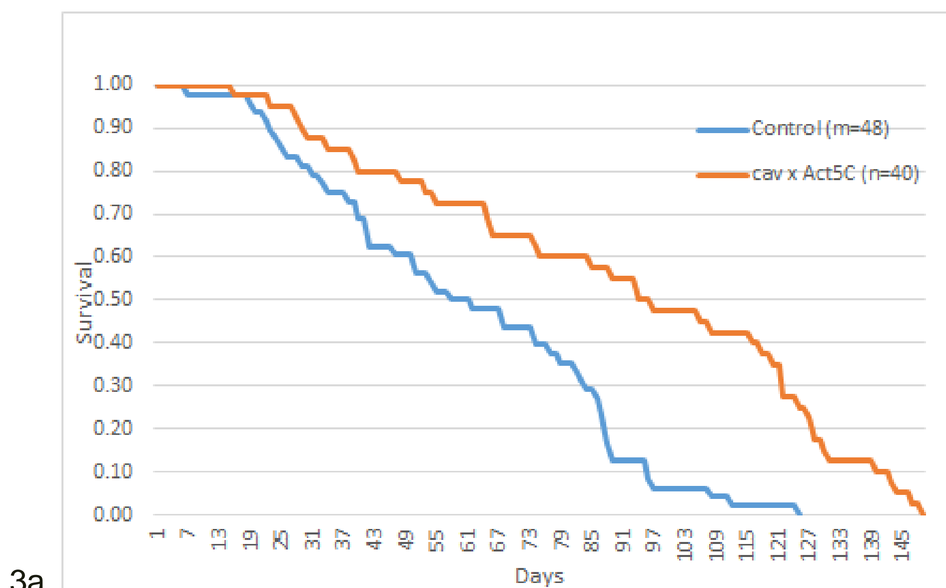


2e.

Figure 2: Comparison of the survival of the control and UAS-SpnE.RNAi crosses over multiple days.

Figure 2a shows that UAS-SpnE.RNAi x Act5C-GAL4 had a larger rate of survival than the control due to the cross surviving longer and the survival curve being above the curve of the control. Figure 2b shows that UAS-SpnE.RNAi x ey-GAL4 had a similar survival curve to the control at the start of the experiment, but the cross lived longer and had a larger rate of survival later in the experiment. Figure 2c shows that UAS-SpnE.RNAi x nos-GAL4 had a decreased rate of survival compared to the control. Figure 2d shows that UAS-SpnE.RNAi x moi-GAL4 had a similar survival curve to the control at the start of the experiment, but the cross lived longer and had a

larger rate of survival later in the experiment. Figure 2e shows that UAS-SpnE.RNAi x ver-GAL4 had a slightly decreased survival curve compared to the control at the start of the experiment, but the cross lived longer and had a larger rate of survival later in the experiment.



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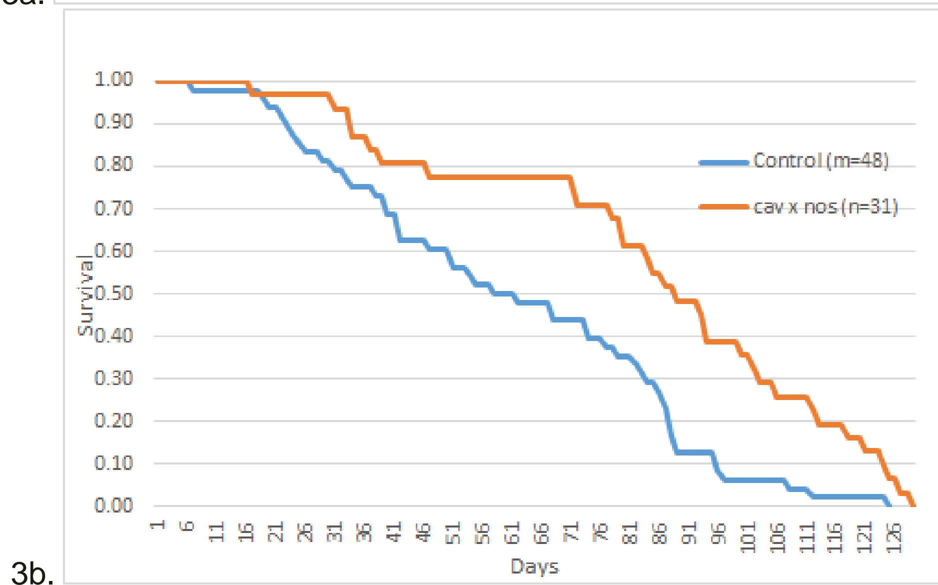

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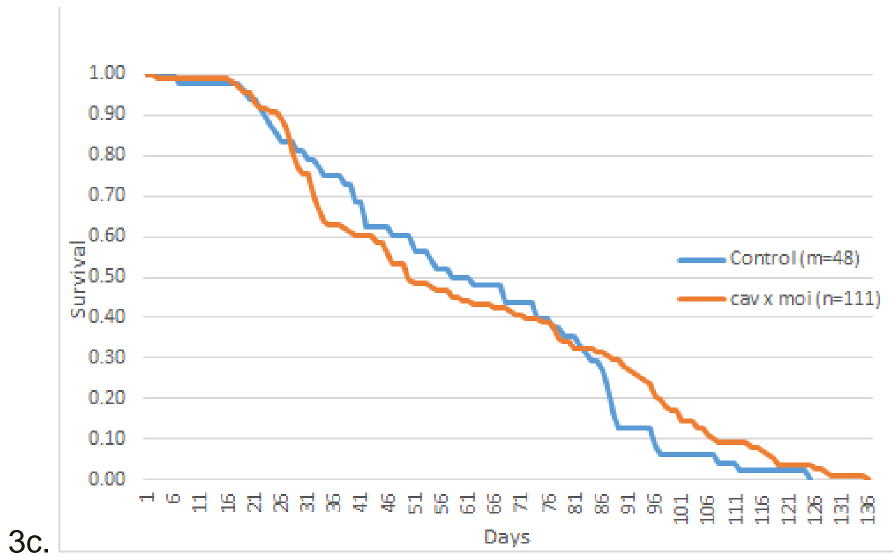
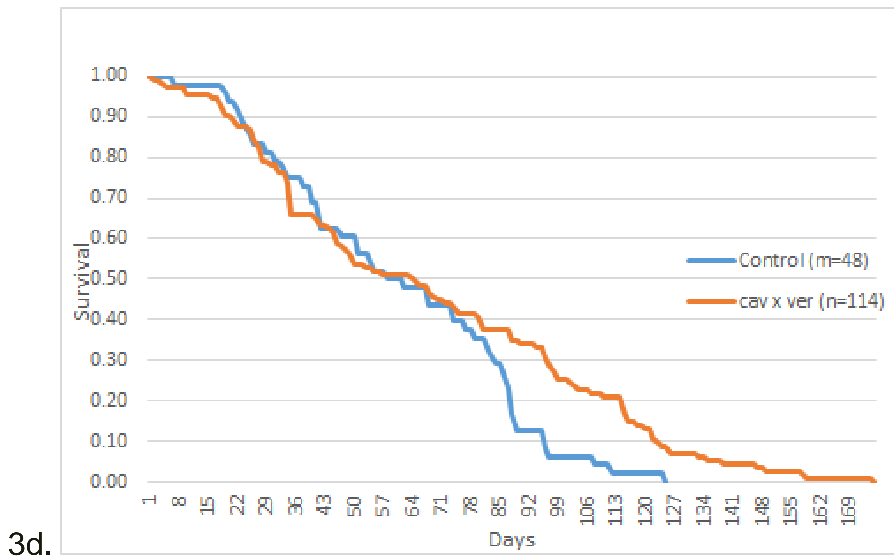


3c.



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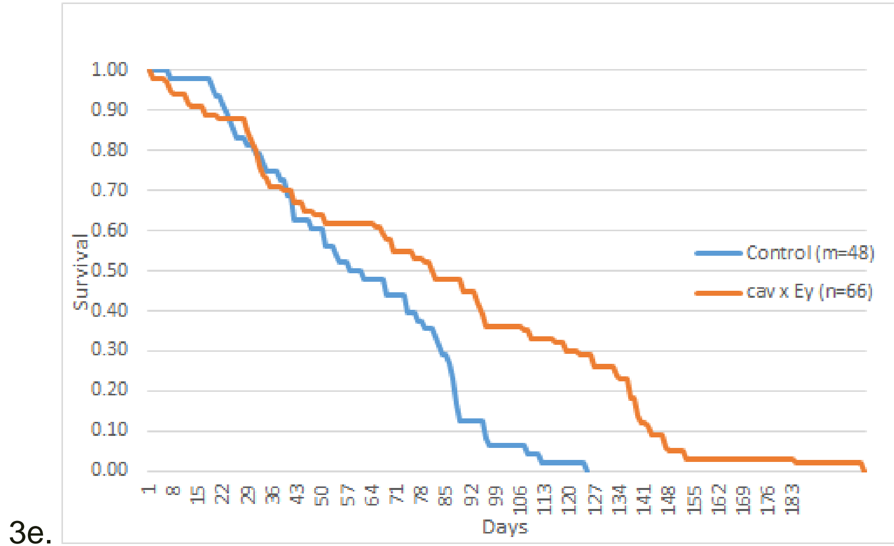
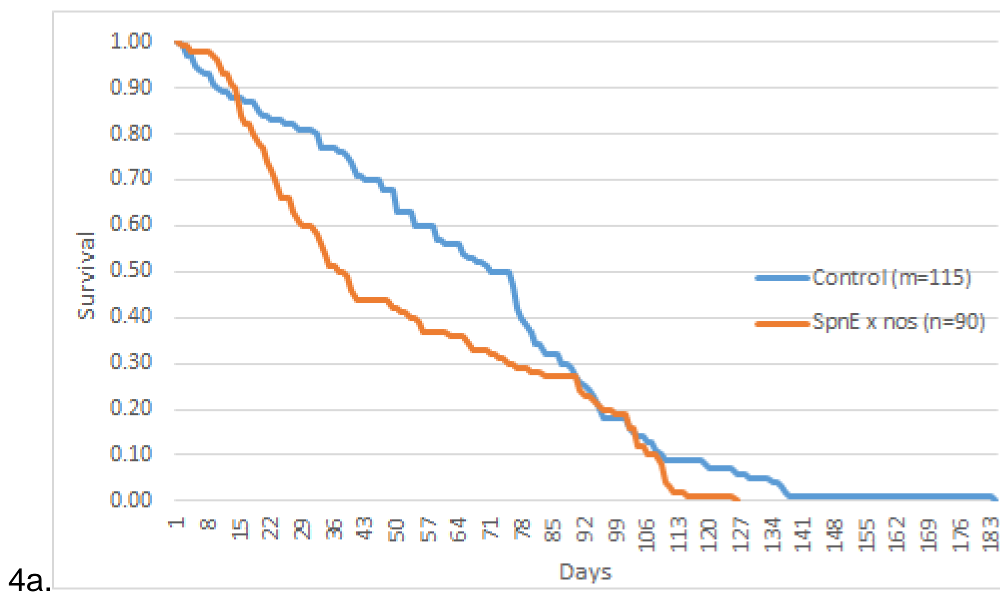


3e.

Figure 3: Comparison of the survival of the control and UAS-Cav.RNAi crosses over multiple days.

Figure 3a shows that UAS-Cav.RNAi x Act5C-GAL4 had a larger rate of survival than the control due to the cross surviving longer and the survival curve being above the curve of the control. Figure 3b shows that UAS-Cav.RNAi x nos-GAL4 had a larger rate of survival than the control due to survival curve being above the curve of the control. Figure 3c shows that UAS-Cav.RNAi x moi-GAL4 had a similar survival curve compared to the control. Figure 3d shows that UAS-Cav.RNAi x ver-GAL4 had a similar survival curve to the control at the start of the experiment, but the cross lived longer and had a larger rate of survival later in the experiment. Figure 3e shows that UAS-Cav.RNAi x ey-GAL4 had a similar survival curve to control early in the experiment. However, for more than half the lifespan the survival rate is larger than the control and the population survived longer than the control.



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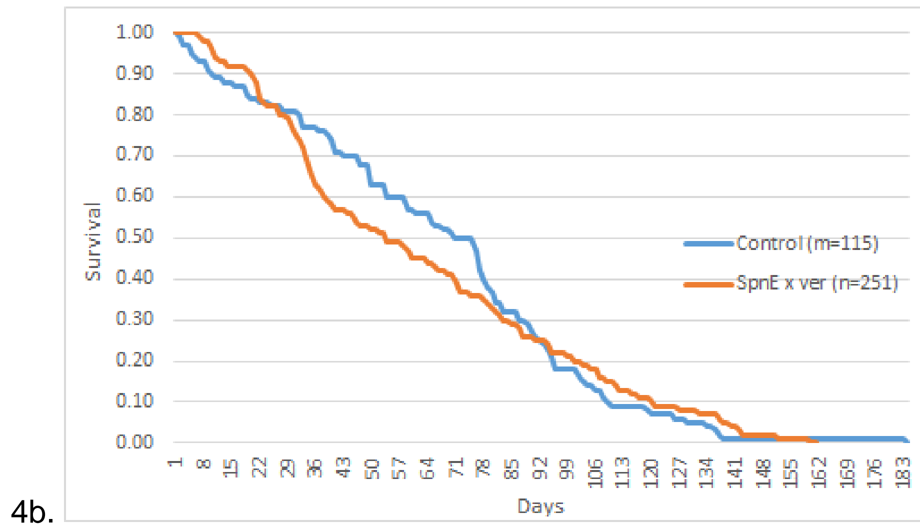

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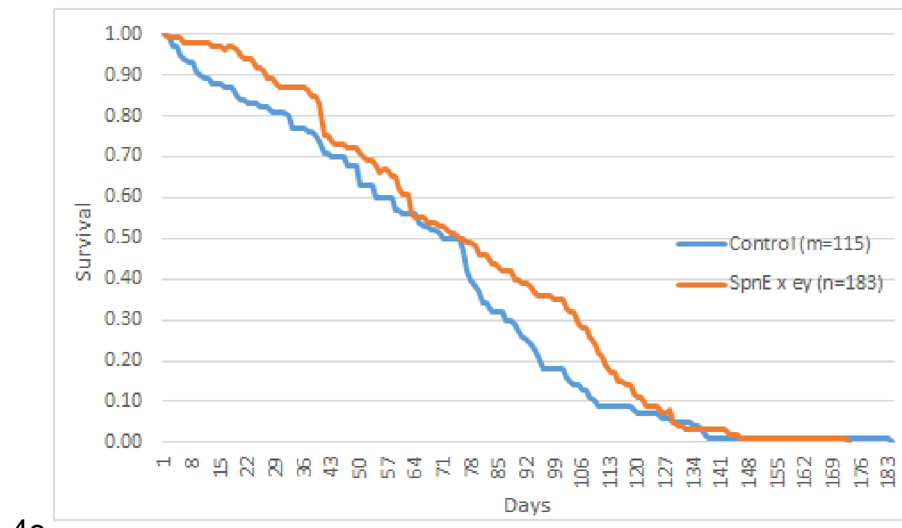

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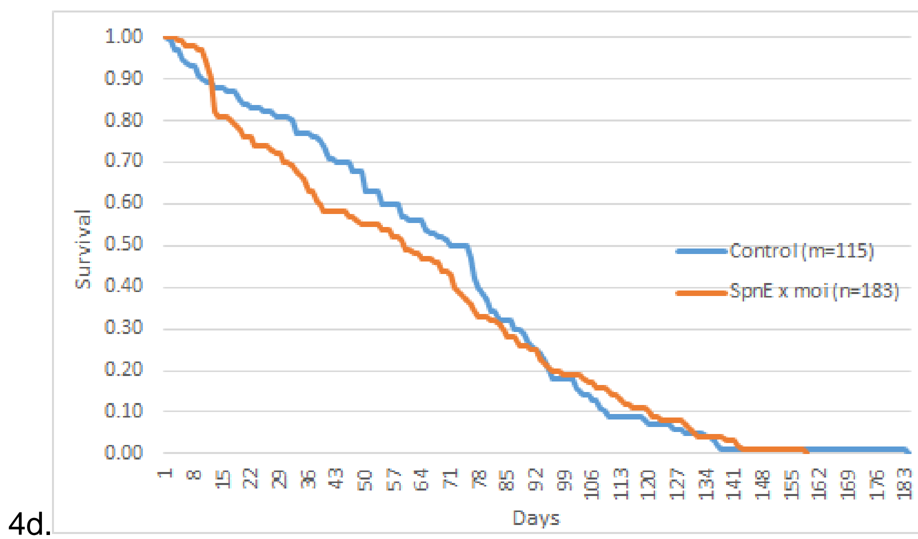


4d.

4e.

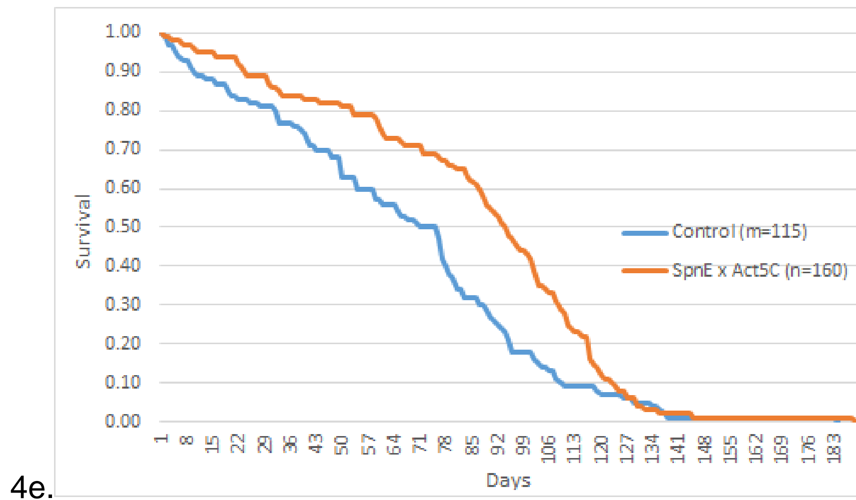
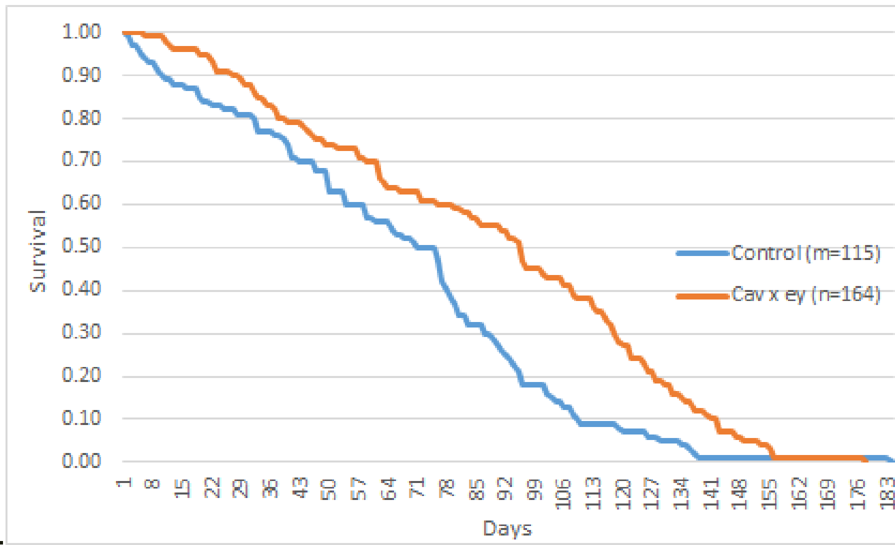
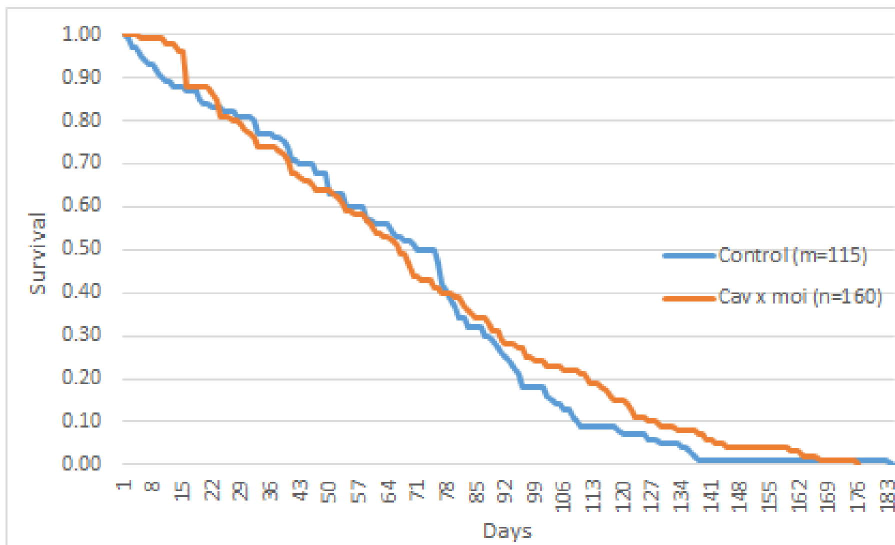


Figure 4: Comparison of the survival of the control and UAS-SpnE.RNAi F2 crosses over multiple days

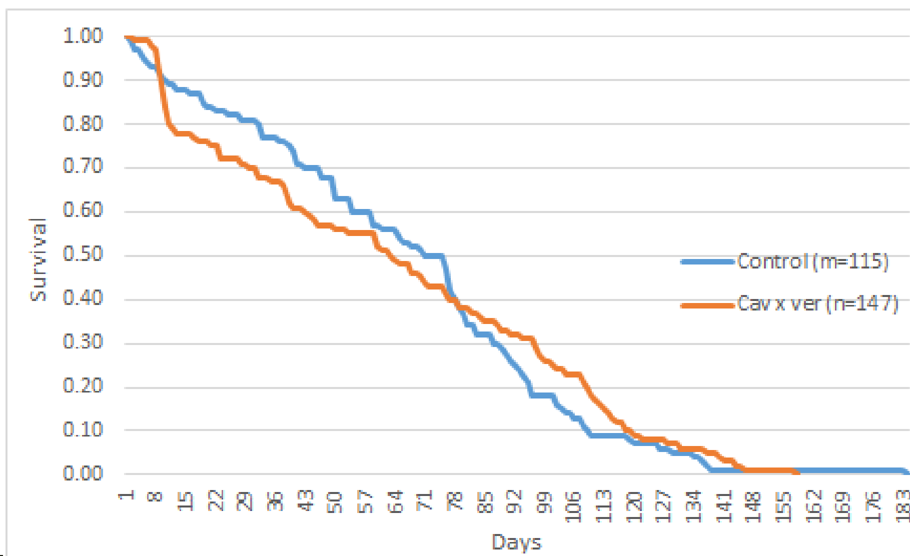
Figure 4a shows that UAS-SpnE.RNAi x nos-GAL4 had a decreased rate of survival than the control due to the curve being below the control curve and the population died off before the control population. Figure 4b shows that UAS-SpnE.RNAi x ver-GAL4 had a decreased rate of survival earlier in the experiment compared to the control, but later in the experiment it had a similar survival curve to control. Figure 4c shows that UAS-SpnE.RNAi x ey-GAL4 had a similar survival curve to the control at the start of the experiment, but the cross did not live as long as the control and had a larger rate of survival between day 78 and day 120 in the experiment. Figure 4d shows that UAS-SpnE.RNAi x moi-GAL4 had a similar survival curve to UAS-SpnE.RNAi x ver-GAL4, which included a decreased survival rate compared to the control at the start and then was more similar to the control later in the experiment. Figure 4e shows that UAS-SpnE.RNAi x Act5C-GAL4 had an increased rate of survival compared to the control since the survival curve is above the control curve and the population lived longer than the control.



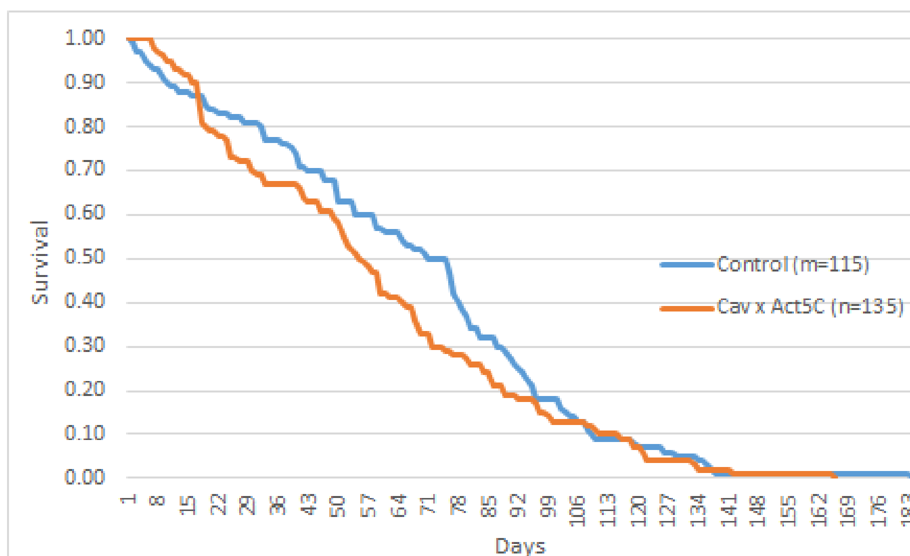
5a.



5b.



5c.



5d.

5e.

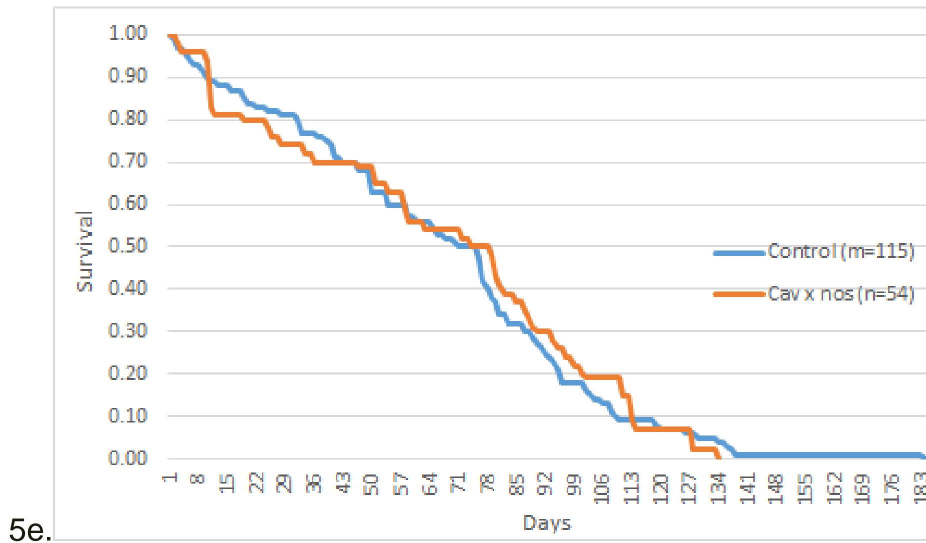


Figure 5: Comparison of the survival of the control and UAS-Cav.RNAi F2 crosses over multiple days.

Figure 5a shows that UAS-Cav.RNAi x ey-GAL4 had a larger rate of survival than the control due to the survival curve being above the curve of the control. Figure 5b shows that UAS-Cav.RNAi x moi-GAL4 had a similar rate of survival to the control. Figure 5c shows that UAS-Cav.RNAi x ver-GAL4 had a similar survival curve compared to the control, but the population dies off before the control. Figure 5d shows that UAS-Cav.RNAi x Act5C-GAL4 had a decreased survival curve to the control at the start of the experiment and becomes similar to the control later in the experiment, but the cross did not live longer. Figure 5e shows that UAS-Cav.RNAi x nos-GAL4 had a survival curve similar to the control, but the population died off before the control.

Cross	D-max	Critical value
UAS-SpnE.RNAi x Act5C	0.2960*	0.256816
UAS-SpnE.RNAi x ver-GAL4	0.2166	0.232218
UAS-SpnE.RNAi x moi-GAL4	0.1532	0.237715
UAS-SpnE.RNAi x ey-GAL4	0.3143*	0.227577
UAS-SpnE.RNAi x nos-GAL4	0.3542	0.438324

Table 4: UAS-SpnE.RNAi-GAL4 F1 crosses max difference compared to the critical value (=significant).*

Table 4 shows that after comparing the F1 generation UAS-SpnE.RNAi-GAL4 crosses to the control and using the KS Test that UAS-SpnE.RNAi x Act5C and UAS-SpnE.RNAi x ey-GAL4 have a significant difference in survival compared to the control.

Cross	D-max	Critical value
UAS-Cav.RNAi x moi-GAL4	0.1673	0.234611
UAS-Cav.RNAi x ver-GAL4	0.2295	0.233677
UAS-Cav.RNAi x Act5C-GAL4	0.4200*	0.290752
UAS-Cav.RNAi x ey-GAL4	0.1799*	0.161609
UAS-Cav.RNAi x nos-GAL4	0.3500*	0.312927

Table 5: UAS-Cav.RNAi-GAL4 F1 crosses max difference compared to the critical value (=significant).*

Table 5 shows that after comparing the UAS-Cav.RNAi-GAL4 crosses to the control and using the KS Test that UAS-Cav.RNAi x Act5C, UAS-SpnE.RNAi x nos-GAL4 and UAS-Cav.RNAi x ey-GAL4 have a significant difference in survival compared to the control.

Cross	D-max	Critical value
UAS-SpnE.RNAi x Act5C	0.3283*	0.166031
UAS-SpnE.RNAi x ver-GAL4	0.1665*	0.152928
UAS-SpnE.RNAi x moi-GAL4	0.1544	0.161609
UAS-SpnE.RNAi x ey-GAL4	0.1799*	0.161609
UAS-SpnE.RNAi x nos-GAL4	0.2836*	0.191134

Table 6: UAS-SpnE.RNAi-GAL4 F2 crosses max difference compared to the critical value (=significant).*

Table 6 shows that after comparing the UAS-SpnE.RNAi-GAL4 F2 crosses to the control and using the KS Test that UAS-SpnE.RNAi x ver-GAL4, UAS-SpnE.RNAi x ey-GAL4, UAS-SpnE.RNAi x Act5C and UAS-SpnE.RNAi x nos-GAL4 have a significant difference in survival compared to the control.

Cross	D-max	Critical value
UAS-Cav.RNAi x moi-GAL4	0.1255	0.166031
UAS-Cav.RNAi x ver-GAL4	0.1329	0.169073
UAS-Cav.RNAi x Act5C-GAL4	0.2081*	0.17234
UAS-Cav.RNAi x nos-GAL4	0.1000	0.224042
UAS-Cav.RNAi x ey-GAL4	0.2983*	0.165182

Table 7: UAS-Cav.RNAi-GAL4 F2 crosses max difference compared to the critical value (=significant).*

Table 7 shows that after comparing the UAS-Cav.RNAi-GAL4 F2 crosses to the control and using the KS Test that UAS-Cav.RNAi x Act5C-GAL4 and UAS-Cav.RNAi x ey-GAL4 have a significant difference in survival compared to the control.

Discussion:

The use of RNAi in knocking down the expression of the telomere specific genes *spindle-E* and *cav* appeared to have an effect on the life cycle and the life span of *D. melanogaster*. When looking at table 2 there is an increase in the average life cycle of the UAS.Spn-E.RNAi.-GAL4 and the UAS.Cav.RNAi.-GAL4 since all of their average life cycles are above 11.6 days, which is the average life cycle of the control. The lack of *spindle-E* and *cav* in *D. melanogaster* appears to affect their ability to develop into adults since the metamorphosis process requires an increased time span. Table 3 shows that the average life cycle of the F2 generation was more varied since there were crosses with a shorter average life cycle than the control, which had an average life cycle of 12.8 days. There were crosses that had an average life cycle longer than 12.8 days, for example UAS-SpnE.RNAi x Act5C-GAL4 had the longest average life cycle of 15 days.

The lack of *cav* being expressed in the eye region of the UAS.Cav.RNAi. x ey-GAL4 also appears to affect the development of the eyes and the mouth. This result possibly supports the idea that the loss of the Cav protein has a possible effect on genes that have a role in cell cycle control and cellular senescence (Bai *et al.* 2014). By knocking down *cav* the formation of cells in the eye could have been affected causing a decrease in the amount of cells in the eye. There could be increased cellular senescence, which would be an increase in the number of cells that have stopped proliferating (Campisi, 2013). If cellular senescence was increased in the eyes, then multiple eye cells would have stopped growing and limited the number of cells in the eye causing the size of the eye to be decreased.

When looking at Figures 2 and 3 there appears to be a decrease in lifespan of UAS.Spn-E.RNAi. x nos-GAL4, which had a survival curve that was below the control curve thus having a decreased rate of survival. This may be due to the lack of *spindle-E* present in the germline and *spindle-E* is necessary for telomere maintenance within the germline (Savitsky *et al.* 2006). Without *spindle-E* the telomeres are not being maintained in the germline and it has a negative effect on the survival of that population.

Figure 2 and 3 show that there is an increase in lifespan of the F1 generations: UAS.Spn-E.RNAi. x Act5C-GAL4, UAS.Cav.RNAi. x Act5C-GAL4, UAS.Cav.RNAi. x nos-GAL4, which all have survival curves above the control curve. The F1 generation of UAS.Spn-E.RNAi. x nos-GAL4 had a decreased lifespan. However, based on the Kolmogorov-Smirnov test the F1 generations of UAS.Spn-E.RNAi. x Act5C-GAL4, UAS.Spn-E.RNAi. x ey-GAL4, UAS.Cav.RNAi. x Act5C-GAL4, UAS-Cav.RNAi. x ey-GAL4, UAS.Cav.RNAi. x nos-GAL4 all had a significant difference in survival compared to the control. These crosses resulted in a significant increase in lifespan when compared to the wildtype control.

Figure 4 and 5 show that there is a decreased rate of survival of UAS-Spn-E.RNAi. x nos-GAL4, UAS.Cav-RNAi. x Act5C, and UAS.SpnE-RNAi. x ver-GAL4 because the curve was below the control survival curve. UAS-Cav.RNAi x ey-GAL4, UAS.Spn-E.RNAi. x ey-GAL4 and UAS-SpnE.RNAi x Act5C had an increased rate of survival because the curve was above the control survival curve. However, based on the Kolmogorov-Smirnov test UAS-Cav.RNAi x ey-GAL4, UAS-SpnE.RNAi x ey-GAL4 and UAS-SpnE.RNAi x Act5C have a significant increase in survival. UAS-Cav.RNAi x Act5C-GAL4, UAS-SpnE.RNAi x nos-GAL4, and SpnE.RNAi x ver-GAL4 have a

significant decrease in survival.

The increased lifespan of crosses (particularly the F1 generation) with decreased expression of *spindle-E* and *cav* in the somatic cells appears to show that those telomere genes are not required for the survival of the organism because *spindle-E* functions in the germline and other telomere genes may be able to function in place of *cav*. When *spindle-E* is not expressed in the eye there is no detrimental effect possibly due to *spindle-E* functioning in the telomere maintenance in germ cells and not eye cells. The lack of *cav* being expressed in the germline does not have a negative effect possibly because other telomere genes, such as *spindle-E* have a more significant role in telomere maintenance then *cav.*

Before starting the experiment, I hypothesized that knocking down expression of *spindle-E* and *cav* would result in a decreased lifespan and thus a decreased survival. I expected this because with decreased *spn-E* and *cav* I predicted that there would be a decreased flow of telomere elements to the ends of chromosomes and formation of HOAP. These aspects are important in maintaining the telomeres and will cause decreased maintenance and increased DNA damage that affects the stability of the cells and various functions within the organism. The data does not support my hypothesis because there was an increase in lifespan in some of the crosses. This may have occurred because of the cell checkpoint system. Mutations in *cav* can result in end-to-end fusion of the chromosomes. *cav* codes for HOAP and the loss of HOAP can produce uncapped or dysfunctional telomeres, which activates the DNA damage response (DDR) and spindle assembly checkpoint(SAC) (Ciappioni and Cenci, 2008; Cenci, 2009). The mutation of *cav* can affect the progression of the cell cycle resulting

in a decreased number of cells in the mitosis phase compared to the number of cells in interphase (Ciapponi and Cenci, 2008). The lack of HOAP in the telomeres can result in the cell cycle stopping between metaphase and anaphase, with less cells entering the anaphase stage of mitosis (Ciapponi and Cenci, 2008). The transition from metaphase to anaphase is also prevented by SAC, which is activated by the accumulation of the SAC kinase BubR1 at the ends of the chromosome (Cenci, 2009)

Some additional functions of *spn-E* that may have an effect on the lifespan are positive regulation of cellular process, mitotic sister chromatid segregation and that it is expressed in microtubules (Flybase Consortium 1999). With the decreased expression of *spn-E* it might have an effect on the segregation of sister chromatids during mitosis and it may affect the formation of microtubules, which are needed to separate the sister chromatids. Thus the cell cycle may stop or be delayed if the chromatids are not separated. *spn-E* is required for telomere maintenance and with decreased *spn-E* there may be telomere shrinkage that will trigger DDR and result in cells not entering mitosis. *spn-E* also is involved in controlling telomere maintenance within the germline and with decreased expression it may have affected the proliferation of the germline due to unmaintained telomeres resulting in cells that are genetically unstable.

The decreased expression of *cav* and *spn-E* resulted in increased lifespan and life cycle possibly because the cell cycle was delayed or stopped in the development of the flies resulting in a longer period of time required for the organism to develop since cell division was not occurring or was occurring at a slower rate. It has been shown in other studies that removing the germline can extend lifespan and that germline signaling has a role in the regulation of longevity (Dottermusch et al. 2016). The cell cycle

inhibition caused by decreased *spn-E* and *cav* could have resulted in a reduction of germ cell numbers, which initiated the lifespan regulating mechanisms that prolonged the life of D. melanogaster (Dottermusch et al. 2016).

In this project there are some limitations to the experiment. Some of the *D. melanogaster* stocks had balancer chromosomes such as CyO (curly wings) and if the offspring had curly wings that means that they are not expressing the GAL4 gene and the RNAi.UAS gene will also not be expressed. The flies in the F1 and F2 generation were not searched through to specifically select and study only the flies with wildtype wings. So the data collected included the lifespan for flies with and without the knocked down gene. Another limitation is that the lifespan was an average of male and female lifespan and did not show if there was a greater effect on male or female, especially in the crosses with nos since nos has a role in oogenesis and could have affected females more than males. Another possible factor that could have been affected by the knocked down expression besides life cycle and lifespan is fecundity. However, fecundity was not measured and it is possible that the number of offspring would have decreased to focus resources and energy on survival. This data would have also helped to support or reject the idea of decreased germ cell proliferation that resulted from the decreased expression of the two telomere genes. The number of flies per vial was not controlled, which could have affected the lifespan of flies if the vials was too crowded.

The next future steps for this project would involve overexpressing *spn-E* and *cav* to observe if it would result in an opposite effect in lifespan. For example, the overexpression of the telomere gene would result in a shortened lifespan in crosses that have shown increased lifespan in this experiment. Another possibility is to measure the

telomere lengths of the F1 and F2 generations produced from the different crosses to determine if the telomere lengths shortened, lengthened or remained the same as the control. The telomere length could be measured through the use of Q-PCR or Q-FISH. DNA microarray is another possible future study, which could be used to determine the genes that are turned on or off in the F1 and F2 generations. This could help to determine if cell cycle specific genes are being turned off with the decreased expression of *spn-E* and *cav*, resulting in the inhibition of the cell cycle and the increased lifespan that has been observed. The expression of germline specific genes could also be studied to determine if there is an effect in the F1 and F2 generations. The microarray may also show if the expression of other genes is affected by the decreased expression of *spn-E* and *cav*, possibly determining if other genes may function in place of the knocked down genes.

This project is significant because it focuses on the topic of aging, which is a process that occurs in every living organism and it is important to understand it. Aging is not a completely understood science so research focused on aging will be able to help further the understanding of that process. More research into aging is needed since the aging process is in need of improvement because as age increases it tends to come with an increased chance of developing a disease. Thus aging and the quality of life could be improved by decreasing the chance of disease or removing of those age-related diseases. Multiple telomere factors are needed to prevent end-to-end fusion of the telomeres in *D.* melanogaster and these factors have also been found in humans, thus studying telomeres in *D. melanogaster* will assist in better understanding the organization and function of human telomeres (Cenci, 2009).

Appendix

D.melanogaster strains ordered from Bloomington:

35303

y1 sc* v1; P{TRiP.GL00206}attP2

spn-EGL00206

63688

y1 v1; P{TRiP.HMJ30256}attP40/CyO

Dmel\cav

62754 w1118; PBac{IT.GAL4}ver0280-G4

Dmel\ver

64725 moi-GAL4: w[1118]; PBac{w[+mC]=IT.GAL4}moi[0489-G4] Tgs1[0489-G4]/TM6B, Tb[1]

25394 nos-GAL4: w[*]; Pen[D14]/CyO; P{w[+mC]=GAL4-nos.NGT}9/TM6B, Tb[+]

64308 nos-GAL4: y[1] w[*]; P{w[+mC]=GAL4-nos.NGT}40; P{w[+mC]=GAL4-nos.NGT}A

25374 Act5C-GAL4: y[1] w[*]; P{Act5C-GAL4-w}E1/CyO

5535 ey-GAL4: w[*]; P{w[+m*]=GAL4-ey.H}4-8/CyO

KS Test sample Mathematica equation:

```
n1 = sample size of other cross;

n2 = sample size of control;

\[Alpha] = 0.05;

pr[x_] := (Sqrt[2*Pi]/x)*
  Sum[E^((-(2*k - 1)^2)*(Pi^2/(8*x^2))), {k, 1, 100}]

crit[\[Alpha]_, n1_,
  n2_] := (x /. FindRoot[pr[x] == 1 - \[Alpha], {x, 0.5}])*
  Sqrt[(n1 + n2)/(n1*n2)]

b = crit[\[Alpha], n1, n2]

b = critical value
```

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