How Do Genes Work Together to Regulate Aging in *C. elegans*?

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Abstract

Although a variety of genes effect aging in the nematode *Caenorhabditis elegans*, there are few studies that systematically combine and test mutant combinations of lifespan-regulating genes. This project aims to make *C. elegans* mutants with multiple selected transcription factor deficits and record changes in aging-related phenotypes.

Introduction

Why C. elegans?

C. elegans is a widely used model organism and a simple animal with which we can study aging. Pioneering studies have identified genes that regulate aging in *C. elegans* which are conserved in flies, mice, and possibly humans.

Why Transcription Factors?

Transcription factors are interesting because they coordinate the function of many other genes. Testing a variety of transcription factors will help to identify the genes that normally regulate aging. This project focuses on *daf-16*, a well-characterized aging-related transcription factor.
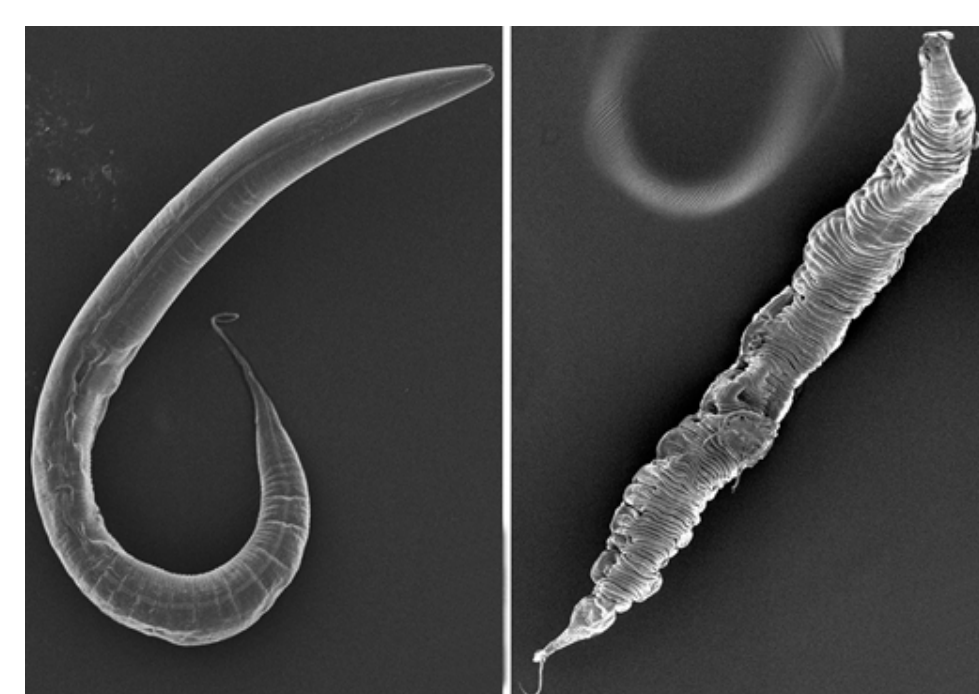
1 Day Old   13 Days Old

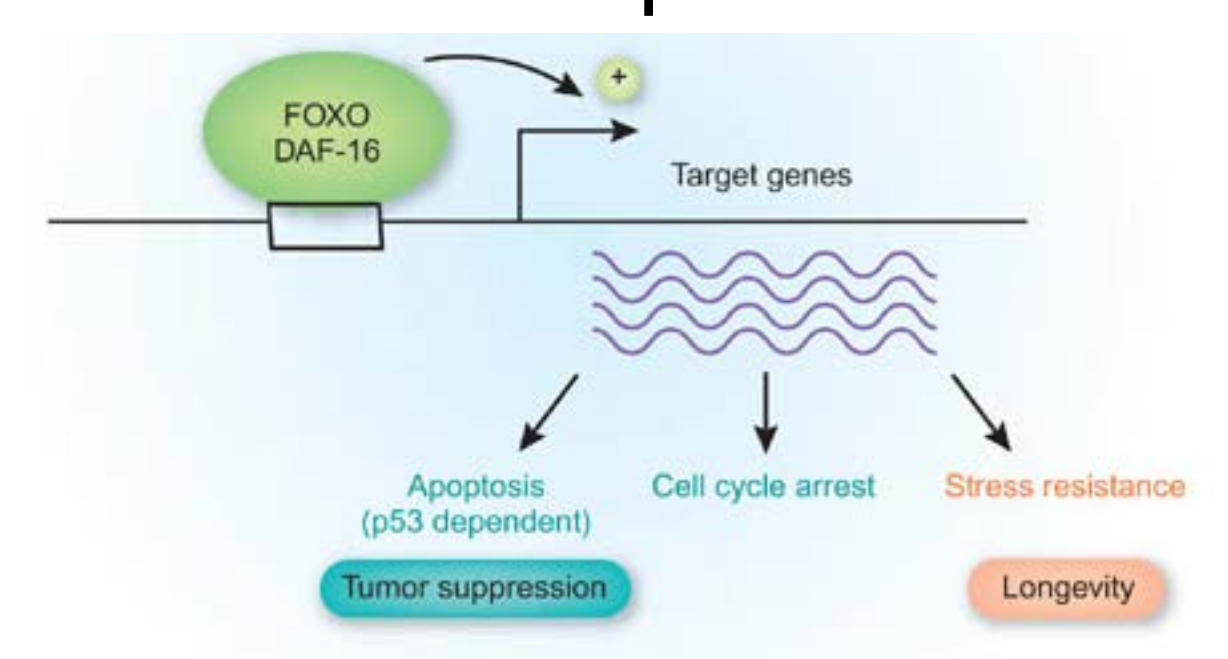Figure 1: Even in worms, aging changes appearance. Photos taken by the Ghazi Lab at Children's Hospital of Pittsburgh.

Figure 2: Cropped to simplify and emphasize importance of *daf-16*. From Brunet A. 2007. doi:10.1038/ng1107-1306

Methods & Results

Step 1: Select & Outcross Single Mutants

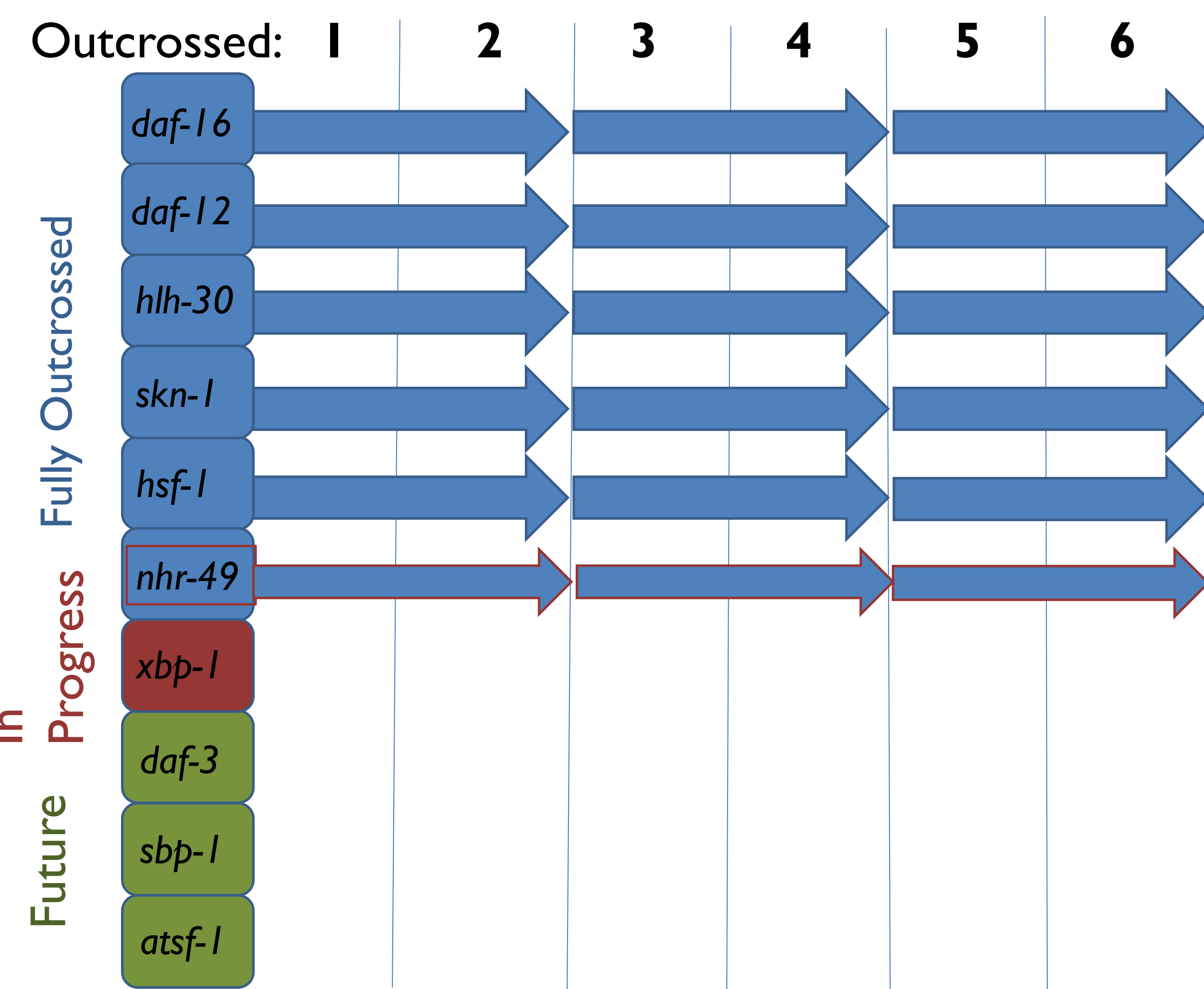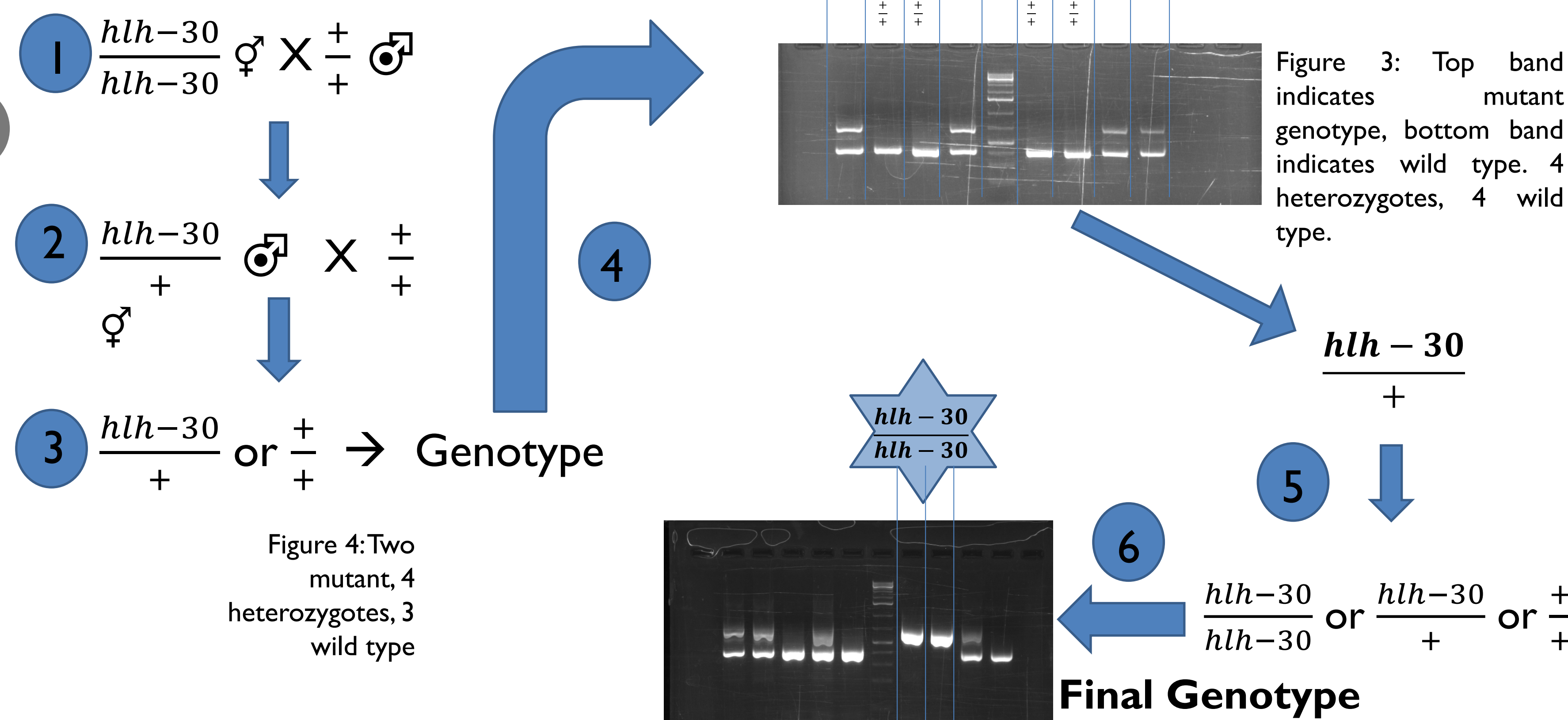Outcrossed: 1   2   3   4   5   6

Figure 3: Ten selected genes and progress outcrossing.

Methods & Results Continued

Step 1 involves the identification of mutations in transcription factor genes that effect lifespan. Those mutations must be outcrossed using classical and molecular genetic techniques to remove background mutations.

Procedure for Two Outcrosses:

$$ \textbf{1} \quad \frac{hlh-30}{hlh-30} \,♀\, \times\, \frac{+}{+} \,♂ $$

$$ \textbf{2} \quad \frac{hlh-30}{+} \,♂\, \times\, \frac{+}{+} \,♀ $$

$$ \textbf{3} \quad \frac{hlh-30}{+} \text{ or } \frac{+}{+} \rightarrow \text{Genotype} $$

Figure 4: Two mutant, 4 heterozygotes, 3 wild type

Figure 3: Top band indicates mutant genotype, bottom band indicates wild type. 4 heterozygotes, 4 wild type.

$$ \frac{hlh-30}{+} $$

$$ \frac{hlh-30}{hlh-30} $$

$$ \textbf{6} \quad \frac{hlh-30}{hlh-30} \text{ or } \frac{hlh-30}{+} \text{ or } \frac{+}{+} $$

Final Genotype

### Step 2: Build Double Mutant Collection

Once strains are outcrossed, they can be systematically combined with *daf-16* using similar classical and molecular genetic techniques.

Figure 5: I have created two double mutants: *daf-16; daf-12* and *daf-16; hlh-30*. I am in the process of creating two more double mutants: the *daf-16; skn-1* mutant is challenging because *skn-1* is maternal effect lethal, while the *daf-16 hsf-1* mutant is challenging because *daf-16* and *hsf-1* are near each other on the 1st chromosome, and therefore the isolation of a double mutant requires a recombination event.

daf-16	
daf-12	daf-16; daf-12
hlh-30	daf-16; hlh-30
skn-1	daf-16 hsf-1
hsf-1	daf-16; skn-1
nhr-49	daf-16 nhr-49
xbp-1	daf-16; xbp-1
daf-3	daf-16; daf-3
sbp-1	daf-16; sbp-1
atsf-1	daf-16; atsf-1

Created / Future

Step 3: Characterize Genetic Interactions for Lifespan

Figure 6: Lifespan analysis of the *daf-16; daf-12* double mutant against controls. If *daf-16* and *daf-12* were working independently, then the double mutant would live much shorter than either single mutant. Instead, this data suggests that *daf-16* and *daf-12* regulate aging in very similar ways.
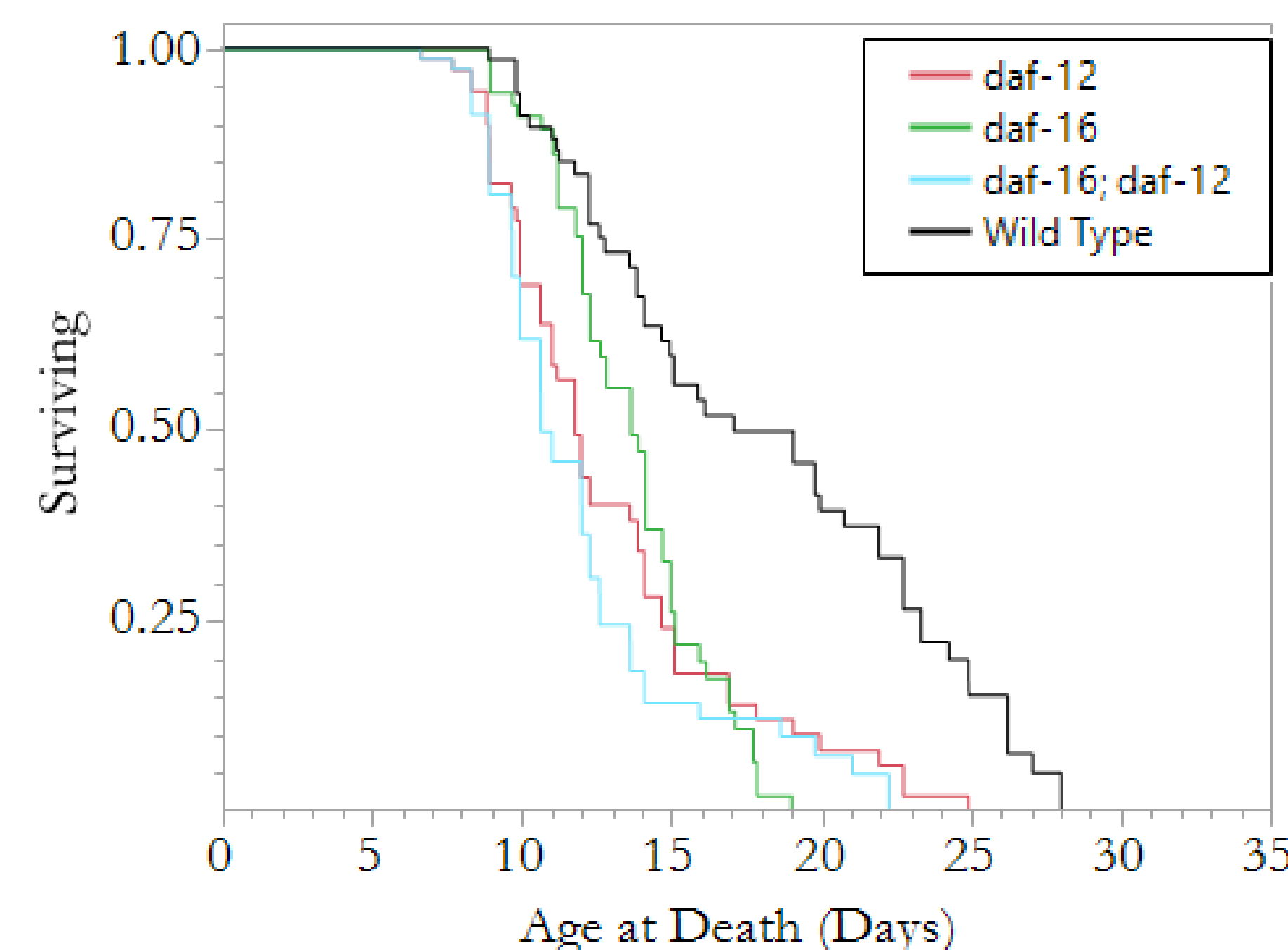
Methods & Results Continued

Step 4: Characterize Additional Phenotypes

Once a novel double mutant is created, phenotypes that are related to aging can give insight into the health of the new mutant. Further, additional phenotypes can also uncover effects that interactions between transcription factors have on different pathways, such as those that regulate the immune response and reproductive success.

Health-Related Phenotypes

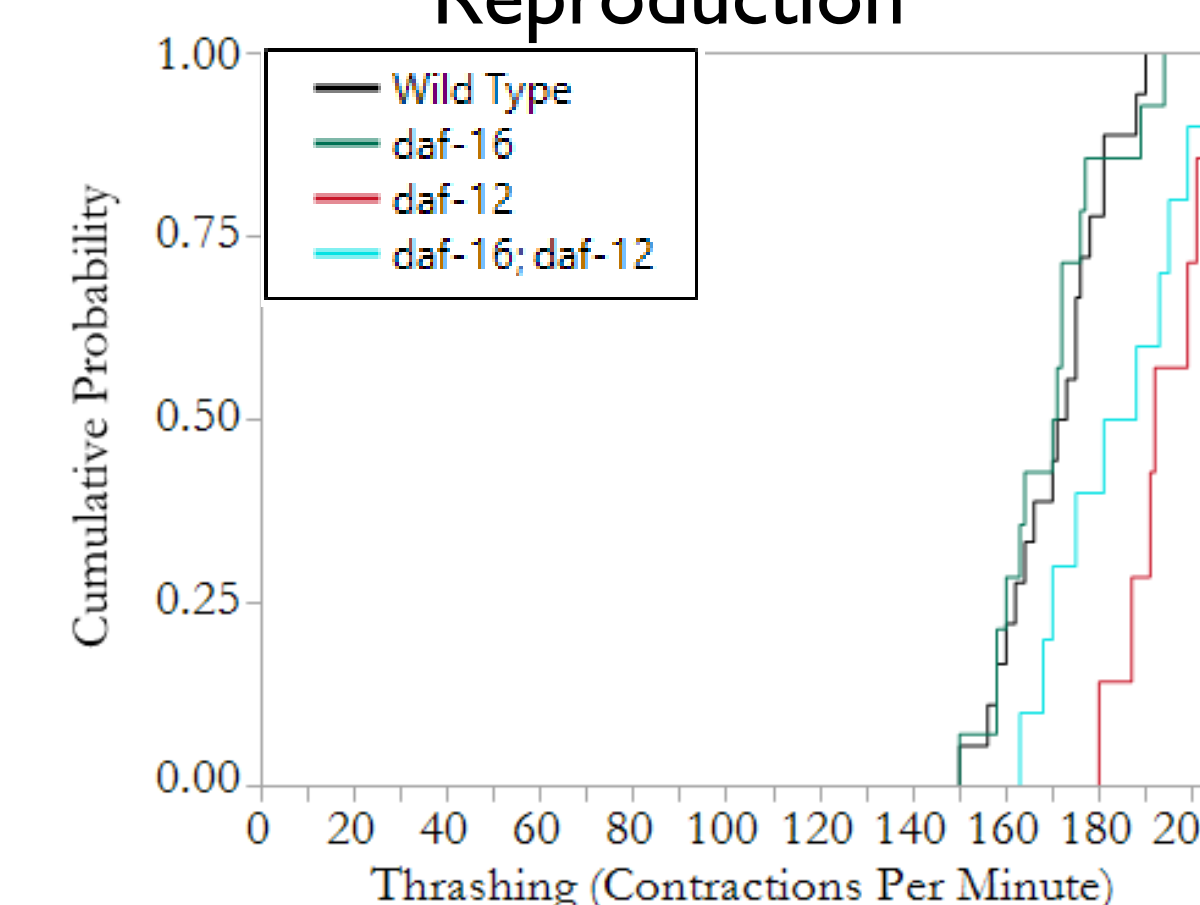Ex: Swimming (Shown Below), Feeding, Development, Reproduction

Figure 7: Swimming (rate of midline contraction in water) shows a potential effect of *daf-12* on movement.

Stress-Related Phenotypes

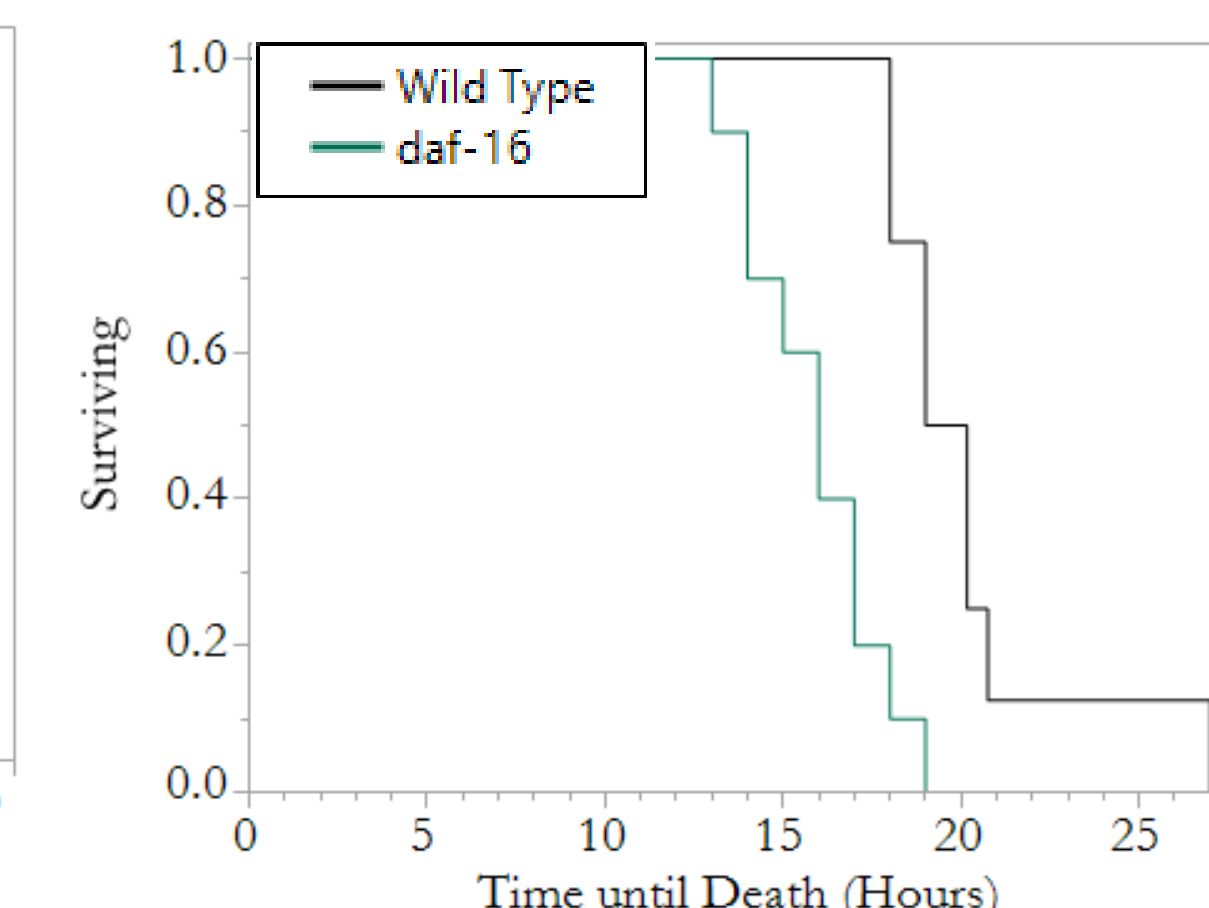Ex: Oxidative Stress (Shown Below), Heat Stress, Pathogenic Stress

Figure 8: *daf-16* mutants are less resistant to oxidative stress than wild type.

Conclusions & Further Study

I will continue to outcross new single mutants, create new double mutants, and quantify their lifespan, health, and resistance to stress.

Acknowledgments

References

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