The Effect of RNAi Knockdown of *atfs-1*, *hsp-6*, *dpy-30*, *set-25*, and C47E12.2 on *C. elegans* Early Brood Count

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Abstract

Caenorhabditis elegans is a species of nematode that is often used as a model species in genetic research. In order for a model species to be useful in research, it is crucial that as much information as possible is known about that species. This experiment seeks to answer the question of how five specific genes, namely *C47E12.2*, *atfs-1*, *hsp-6*, *set-25*, and *dpy-30*, influence the early reproduction of *C. elegans*. In order to answer this question, RNA interference through feeding was performed on *C. elegans* nematodes in order to knock down the expression of each gene individually. The results of this experiment showed a significant decrease in early brood count for all five experimental genes with *hsp-6* being highly significant. In addition, it was found that high embryonic and developmental lethality was part of the reason for the low early brood count for *hsp-6*. Lastly, it was found that there is no significant difference between the early brood counts of early L4 stage and late L4 stage nematodes treated with *hsp-6* RNAi when compared to a control condition. These findings set the stage for further research into the role of *hsp-6* in *C. elegans* reproduction.

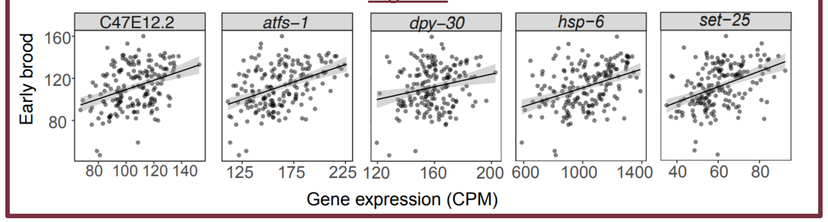
Background

Caenorhabditis elegans is a nematode that has long been used as a model in research due to its set number of cells and large number of human orthologs. While the entire genome of *C. elegans* has been mapped, the exact roles and functions of many of its genes are still being elucidated. The purpose of this research is to gather information on the possible roles of some of these genes in *C. elegans* reproduction.

There are a total of five genes being tested in this research: *atfs-1*, *hsp-6*, *set-25*, *dpy-30*, and C47E12.2. Both *atfs-1* and *hsp-6* are involved in the mitochondrial unfolded protein response (UPRmt) of C. elegans. The UPRmt, a biological process that is meant to respond to mitochondrial distress, is activated when the *atfs-1* protein, ATFS-1, is translocated to the nucleus, where it acts as a transcription factor for many genes involved in the UPRmt (Anderson & Pukkila-Worley, 2020). The gene *hsp-6* is one of *atfs-1*'s target genes and it encodes the chaperone and heat shock protein HSP-6 (Yoneda et al., 2004). While *hsp-6* expression is significantly up-regulated during UPRmt activation, it is also expressed normally throughout the nematode's lifespan (Heschl & Baillie, 1990). The gene *set-25* encodes the H3K9 methyltransferase SET-25 which is involved in heterochromatin formation and negative regulation of gene expression (Gonzalez-Aguilera et al., 2014; Kishore et al., 2024). The gene *dpy-30* encodes the protein DPY-30 and is implicated in several processes including dosage compensation and transdifferentiation (Ercan & Lieb, 2009; Kishore et al., 2024). Lastly, the gene C47E12.2 is not very well studied, but it is predicted to be involved in mitochondrial membrane permeability and mitochondrial DNA protection (Addo et al., 2010; Kishore et al., 2024).

While each of these genes are involved in vastly different processes, in a previous experiment done by Dr. Webster (Figure 1), they were all implicated in early brood count (Webster et al., 2025). Thus, this experiment aims to test if the relationship between these genes and early brood count is causal through RNA interference.

Figure 1



Methods

- ❖ RNAi Prep and Nematode strains
  - ➢ Two strains of *C. elegans* nematodes were used in this experiment
    - ➢ N2 strain
    - ➢ DG4215 strain (*puf-5*::GFP reporter)
  - ➢ All *C. elegans* nematodes were kept at 20 degrees Celsius on NGM plates and were fed OP50 *E. coli*
  - ➢ RNAi for this experiment was administered through feeding
- ❖ Experimental Procedure
  - ➢ Pick at least 5 hermaphrodite nematodes at the L4 stage of development onto a starting RNAi plate
    - ▪ Repeat for each gene in the experiment: EV, *atfs-1*, *hsp-6*, *dpy-30*, *set-25*, and C47E12.2
  - ➢ Wait 16-17 hours then single each nematode onto their own new RNAi plate
    - ▪ Will result in five plates for each gene- discard the starting plate
  - ➢ Wait 24 hours then remove the adult nematodes from each plate
    - ▪ Count the number of eggs laid on each plate for brood assay
  - ➢ Wait 2-3 days, then count the number of nematodes on each plate for adult assay
- ❖ Early vs Late Experimental Procedure
  - ➢ Pick at least 10 hermaphrodite nematodes at the early L4 stage of development onto a starting RNAi plate
    - ▪ Repeat for EV and *hsp-6*
  - ➢ Pick at least 10 hermaphrodite nematodes at the late L4 stage of development onto a starting RNAi plate
    - ▪ Repeat for EV and *hsp-6*
  - ➢ Wait 16-17 hours then single each nematode onto their own new RNAi plate
    - ▪ Will result in 20 plates for each gene, 10 for each condition- discard the starting plate
  - ➢ Wait 24 hours then remove the adult nematodes from each plate
  - ➢ Wait 2-3 days, then count the number of nematodes on each plate and record any noticeable phenotypes

Data

Figure 2

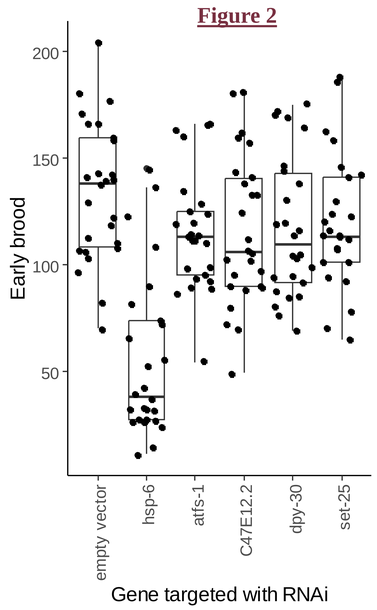


Figure 3

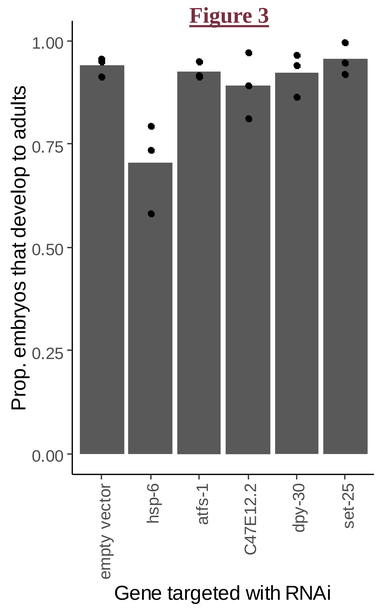
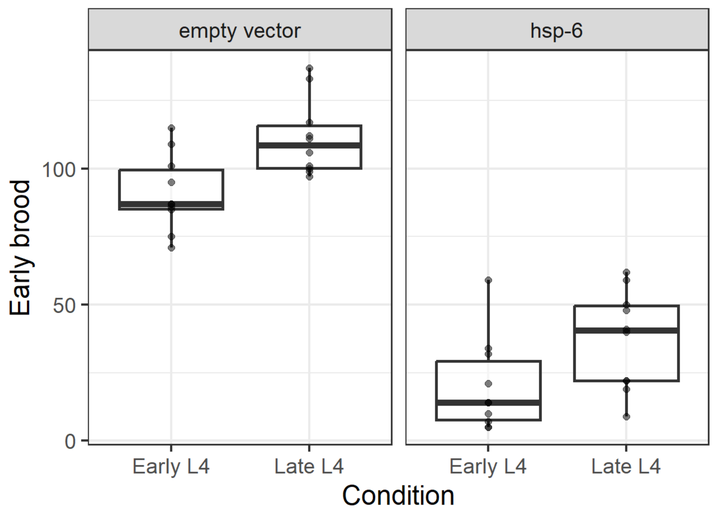


Figure 4



Results

The results of this experiment are broken up into three main portions represented by figures 2-4. The first portion of the experiment was an RNAi knockdown of all five experimental genes and a control (empty vector) group with the early brood being given 2-3 days to grow before being counted (Figure 2). The result of this was a significant decrease in early brood count for all five experimental groups ($p<0.05$) with *hsp-6* being highly significant. In addition to its high significance, the *hsp-6* knockdown experimental group displayed slowed growth and embryonic lethality as noticeable phenotypes. The second portion of the experiment sought to determine if the lower brood count of the experimental genes could be attributed to a low proportion of early brood surviving until they were counted. Thus, the early brood was counted both before and after the 2-3 days allowed for growth to determine the proportion of early brood that developed into adults (Figure 3). The result of this was a significant decrease in the proportion of brood that survived until the second count in *hsp-6*, but no significant decrease in any of the other experimental groups. The third portion of this experiment, in response to a noticeably higher standard deviation in *hsp-6* results along with heterogeneity in phenotype appears, sought to determine if the time of RNAi onset during the L4 stage of development was significant for the early brood count of *hsp-6*. Thus, early L4 and late L4 stage nematodes were compared for both the *hsp-6* experimental condition and the empty vector control condition (Figure 4). The result of this was a significant decrease in early brood count between the early and late L4 groups for both the control and experimental conditions ($p<0.05$), but an insignificant decrease in early brood count between the early and late L4 groups for the *hsp-6* experimental condition when compared to the control condition ($p=0.76$).

Future Directions

The next experiment will most likely be a tissue-specific knockdown of *hsp-6* to test if the phenotypic changes seen in the *hsp-6* experimental group (slowed development, embryonic lethality, and decreased early brood) are tied to a specific tissue type. This experiment also aims to test if these phenotypes can be de-coupled, which would suggest that the *hsp-6* expression in different tissues are responsible for part of the overall phenotype of a full *hsp-6* knockdown, rather than a single tissue being responsible for all the phenotypical changes.

References

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