INVESTIGATING KNOCKDOWN OF MITOCHONDRIAL GENES ON REPRODUCTION IN *C. ELEGANS*

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Diana Chirila[1,2] and Amy Webster[1]

1. Department of Biological Sciences, Florida State University 2. Undergraduate Research Opportunity Program

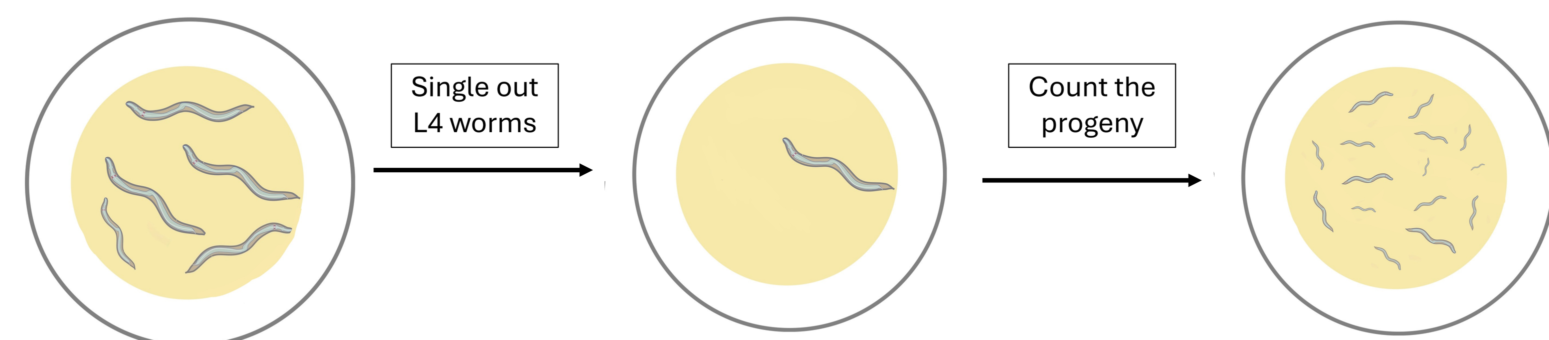
INTRODUCTION

Genetic research is essential for understanding the biological mechanisms underlying individual differences in traits and diseases. *Caenorhabditis elegans*, a widely used model organism, provides valuable insights into how genes regulate various biological processes. *C. elegans,* a species of microscopic nematodes, are highly valued as a model organism due to their short life cycle and genetic trackability (Corsi et al, 2015). With a lifespan of two weeks and the ability to reach sexual maturity in just 3 days, they offer a rare opportunity to study rapid biological processes, including reproductive output. This short life cycle makes them an ideal candidate for studying reproduction traits in a relatively short time frame. In this project, I will utilize C. elegans to investigate the effects of knocking down the expression of four specific mitochondrial elongation factor genes: *gfm-1, tsfm-1, tufm-2, and tufm-1*. These genes are all involved in coding for proteins that facilitate the movement of tRNA molecules along the mitochondrial ribosome, allowing for the assembly of amino acids into a growing polypeptide chain during protein synthesis. Mutations in these genes have been shown to cause severe growth defects and can be lethal. (Trivino & Haerry, 2011). By examining how gene expression alterations influence the reproductive capacity of C. elegans, the focus will be on the number of progenies, or offspring, they produce. Knocking down these genes will significantly decrease progeny output, providing a deeper understanding of their role in reproductive biology.

METHODS

RNAi knockdown and early brood assay:

1. Fill a test tube with 5 mL of LB for each RNAi bacteria and add carbenicillin. Using a pipette tip, select a single bacterial colony from the streaked LB plate and eject the pipette tip into the LB broth. Place all the tubes in the shaker at 37C and all leave overnight.
2. The LB + RNAi culture grown overnight should appear cloudy. Seed 10 RNAi plates with 2-3 drops of bacteria and let dry/grow overnight.
3. Transfer L4 larvae from stock plates to RNAi plates. Pick 10 L4s per RNAi, all 10 L4's will be on one plate.
4. About 16 hours later, single the 10 worms to new RNAi plates .
5. 24 hours later remove the parent worm off the plate, leaving only embryos.
6. Count the number of larvae on the plate that were laid by the parent worm by placing plate with worms on a grid and quickly count the number of worms in each square of the grid. Use a clicker counter that you press every time you see a worm in the grid you are counting.
7. Perform data analysis using R.



RESULTS

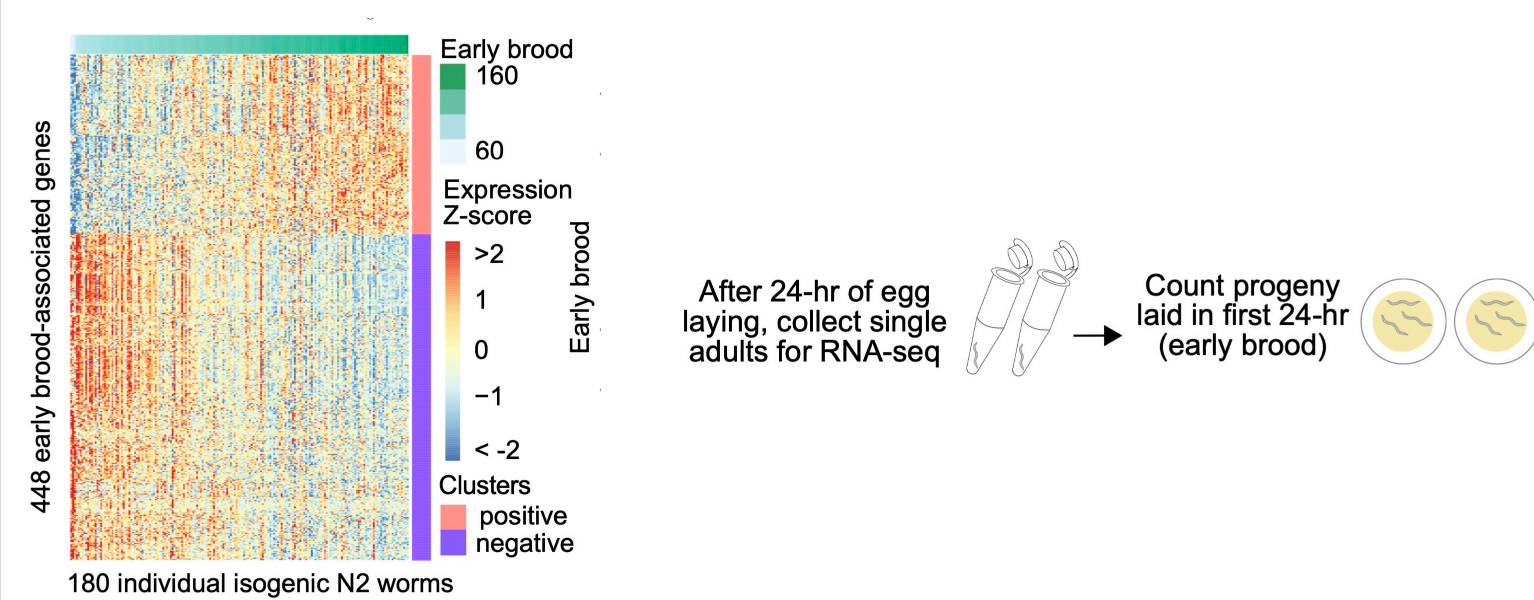


Figure 1: Preliminary study done, shows schematic of heatmap and genes that are associated with early brood.

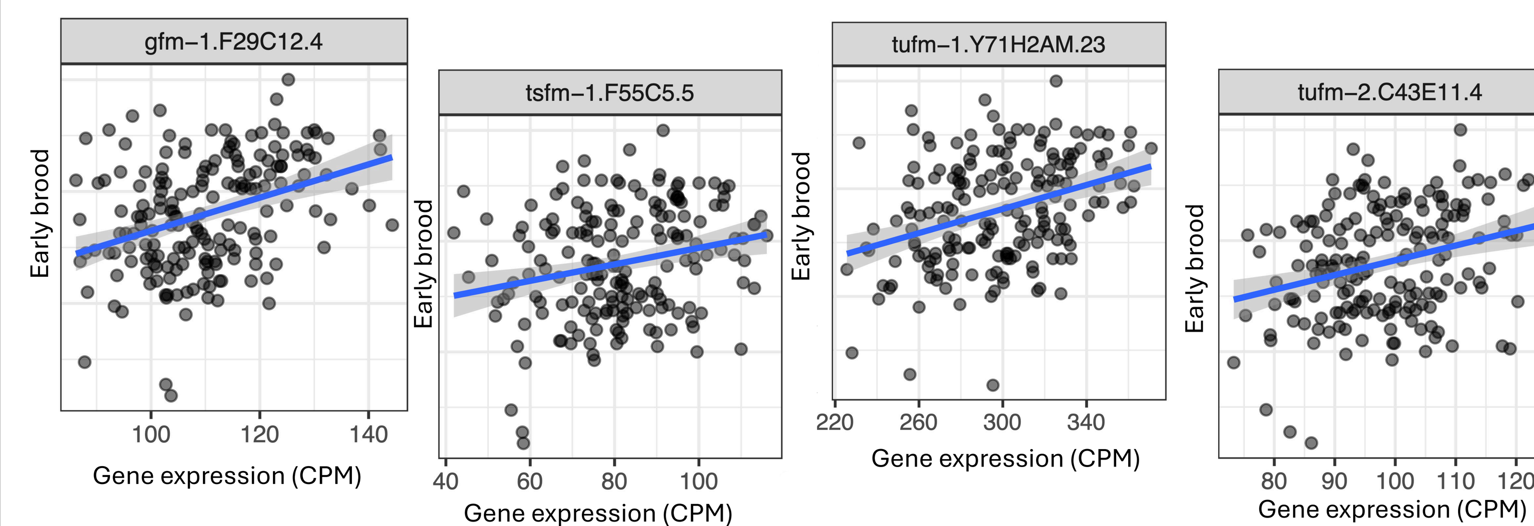


Figure 2: The relationship between gene expression (CPM) and amount of early brood for my genes of interest.

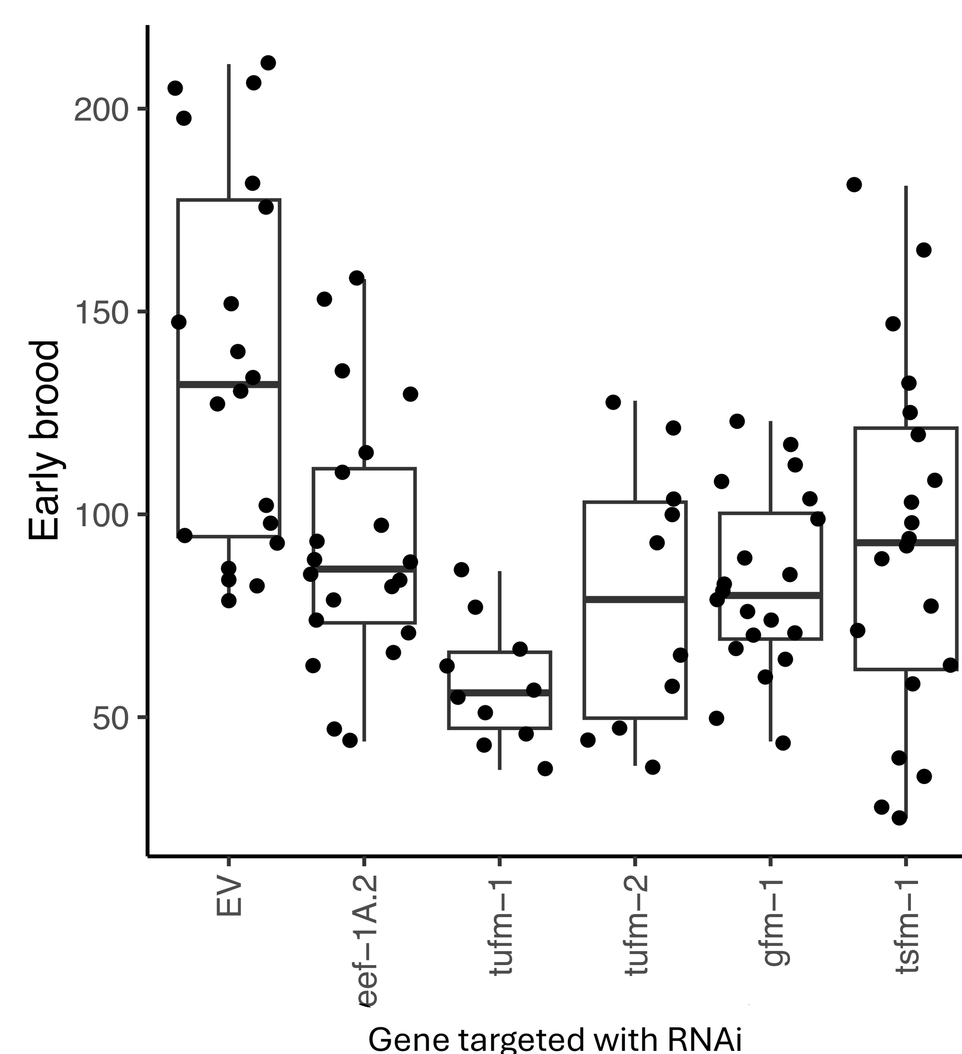


Figure 3: Graph illustrating the quantity of early brood produced from knockdown genes targeted using RNAi.

DISCUSSION

When comparing the RNAi knockdown effects of *eef-1A.2* and *tufm-1* in figure 3, the data indicate that knockdown was more effective in *tufm-1* than in *eef-1A.2*. Additionally, all tested genes showed a significant decrease in progeny count compared to the empty vector (EV) control (figure 3). Since *tufm-1* impacts progeny production more than *eef-1A.2*, it likely exerts a direct effect on brood size. *tufm-1* plays a role in mitochondrial translational elongation, a crucial process for protein synthesis and gamete production. Meanwhile, *eef-1A.2* is essential for binding and activating GTPase proteins, which are key regulators of protein synthesis and signal transduction, both of which are vital for gamete formation. The knockdown of *gfm-1* resulted in progeny counts comparable to those of *eef-1A.2*, while *tsfm-1 and tufm-2* exhibited a significant reduction in progeny output, though not as pronounced as in the *eef-1A.2* control (figure 3). These findings suggest that mitochondrial translation factors differentially impact brood size, potentially through their roles in energy production and cellular homeostasis.

CONCLUSION

The RNAi knockdown of *gfm-1*, *tsfm-1*, *tufm-2*, and *tufm-1* had varying effects on progeny production in *C. elegans*. Notably, the knockdown of *tufm-1* led to developmental arrest, with offspring failing to progress beyond the L1 and L2 stages, preventing them from reaching adulthood. These results demonstrate that mitochondrial translation elongation factors play distinct roles in reproduction. The knockdowns of *gfm-1, tsfm-1, tufm-1, and tufm-2* resulted in a significant reduction in progeny compared to the EV. These findings underscore the functional differences among mitochondrial translation elongation factors and suggest that *tufm-1* is more critical for reproductive viability. Further studies are needed to investigate the specific mechanisms through which these genes influence mitochondrial function and development.

FUTURE DIRECTIONS

- More replicates with *gfm-1, tsfm-1, tufm-2, and tufm-1*
- A replicate that has an RNAi knockdown of both *tufm-1* and *tufm-2*
- Replicates with knockdowns of other mitochondrial genes

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Trivigno, C., & Haerry, T. E. (2011). The Drosophila mitochondrial translation elongation factor G1 contains a nuclear localization signal and inhibits growth and DPP signaling. PloS one, 6(2), e16799. https://doi.org/10.1371/journal.pone.0016799

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