Investigating the effect of RNAi knockdown of *pgl-1* and *ppw-2* in *C. elegans*

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Introduction

Genetically identical individuals can exhibit differences in their traits; however, the mechanisms underlying this phenomenon remain obscure. Using *C. elegans*, a roundworm commonly used as a model organism for biological research, we can create genetically identical individuals and use these individuals to test the influence of gene expression differences on traits of interest. Previous research has shown that variation in gene expression across individuals is associated with differences in reproductive traits. To test whether these genes are causal to affect reproduction in *C. elegans*, I am using RNAi to reduce mRNA expression and score reproductive traits. I am studying two genes of interest, *pgl-1* and *ppw-2*. Preliminary results indicate that *pgl-1* knockdown reduces progeny production, suggesting that *pgl-1* function is typically required for normal progeny production. In contrast, *ppw-2* does not appear to have an effect on progeny production in this experiment. Continued experimentation will further elucidate the role of these genes and others in *C. elegans* reproduction.

Background

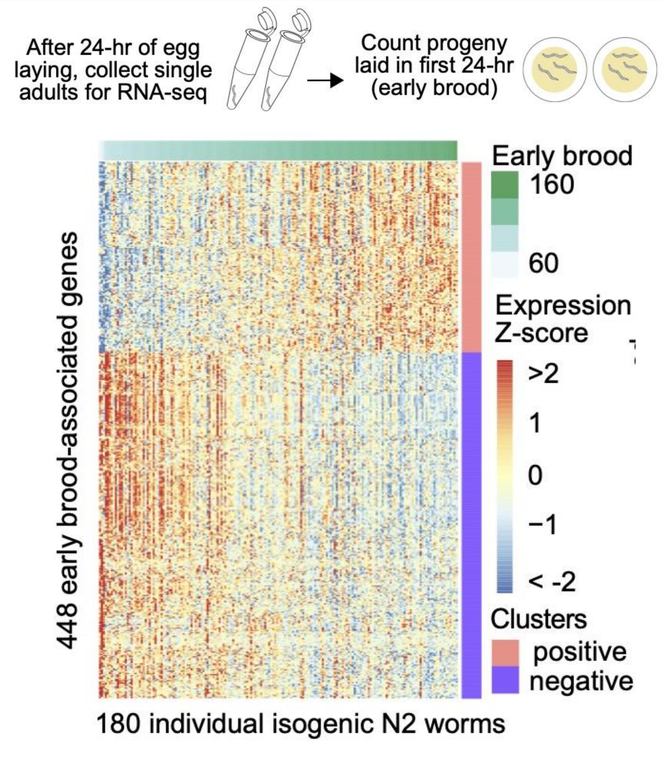

Figure 1: Our previous study used mRNA-seq to identify 448 genes with differences in levels of mRNA that are associated with early brood across genetically identical *C. elegans*.

Results

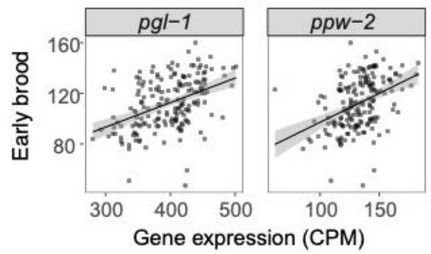

Figure 2: Gene expression levels of *pgl-1* and *ppw-2* vary among genetically identical individuals and are positively associated with early brood.

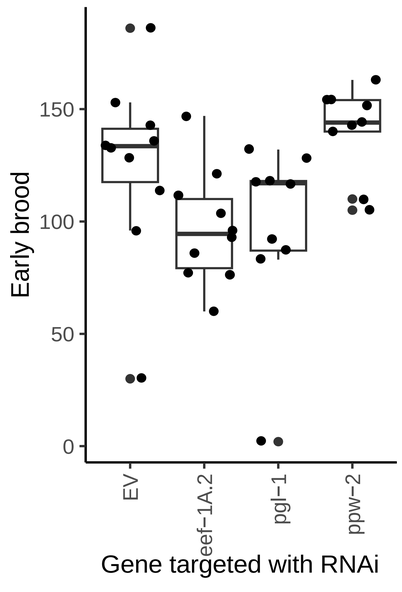

Figure 3: RNAi knockdown of *pgl-1* reduces early brood relative to empty vector (EV). *ppw-2* does not significantly impact early brood. *eef-1A.2* knockdown serves as a positive control for RNAi.

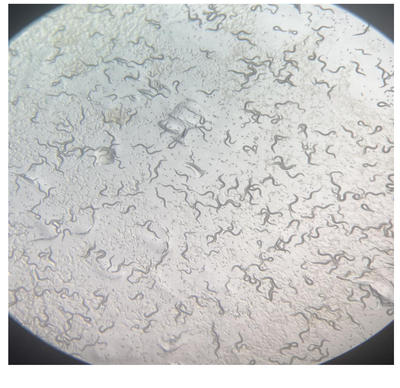

Figure 4: A plate of wild-type *C. elegans* nematodes is shown, primarily consisting of adults and embryos.

Methods

•**RNAi bacterial growth**: First, test tubes were labeled for each RNAi that would be used. Each tube was filled with 5 mL of Lysogeny Broth (LB), a medium used to grow bacteria. Subsequently, 1.25 microliters of the antibiotic carbenicillin were added to each tube. The addition of carbenicillin made it possible for RNAi clones to grow in the presence of the antibiotic, while other bacteria (not containing RNAi clones) could not, due to the resistance of RNAi to carbenicillin. The last step was to include the bacteria containing my genes of interest. To do this a pipette tip was used to select a single bacterial colony from LB plates and ejected into the test tube. All of the tubes containing LB+ RNAi cultures were then placed in the shaker at 37 C and 180 rpm and left to incubate overnight.

•**Preparation of RNAI plates**: After a night of growth, the LB+ RNAi cultures appeared cloudy and were used to seed my RNAi plates. To seed, each plate received 2-3 drops of culture and was left overnight to dry.

•**Early brood assay:** After the RNAi plates dried, 10 L4 C. elegans were transferred from a stock plate to 1 RNAi plate. 16 hours later when these worms matured into adults, they were singled to individual plates. Exactly 24 hours later, the progeny that each adult laid on their plate was counted.

Discussion and Future Research

These figures represent the genes of interest (*pgl-1* and *ppw-2)* that were tested alongside positive and negative control RNAi. I counted the number of progeny individual adult worms produced in a 24 hour window after RNAi knockdown. While results are preliminary, they suggest that knockdown of *pgl-1* in adults reduces progeny production. In contrast, *ppm-2* does not significantly impact progeny production. Some *pgl-1* mutations have been shown to cause sterility (Kawasaki 4), so the effects of RNAi are consistent with its role in fertility. This presentation only includes one biological replicate of two genes of interest. In the future, I plan to complete additional biological replicates for *pgl-1* and *ppw-2* in addition to assaying early brood on three additional genes of interest (*his-35, his-37,* and *wago-1*) that are similarly predicted to impact early brood.

Resources

Kawasaki, I et al. “PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in C. elegans.” *Cell* vol. 94,5 (1998): 635-45. doi:10.1016/s0092-8674(00)81605-0

Liu, Lei et al. “Systematic characterization of small RNAs associated with C. elegans Argonautes.” *Science China. Life sciences* vol. 66,6 (2023): 1303-1322. doi:10.1007/s11427-022-2304-8

Amy K. Webster, John H. Willis, Erik Johnson, Peter Sarkies, Patrick C. Phillips bioRxiv 2023.10.13.562270; doi: https://doi.org/10.1101/2023.10.13.562270

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