student_id: 100354070

5023Y-Summative-2

Studying the effect of the gene raga-1 and how it interacts with stress on reproduction in the nematode worm Caenorhabditis elegans

Introduction : Caenorhabditis elegans, also known as C. elegans is a nematode worm commonly used in biology as a model organism for various experiments ranging from genetics to neuroscience and ageing research (Markaki & Tavernarakis, 2020). The nematode C. elegans has a simple, small anatomy (about 1 mm in length and a transparent body), a short lifespan and reproduces fast.

The life cycle of C. elegans is short, meaning that the average nematode lives around 20 days and it goes through four larval stages (L1-L4) before becoming a fully developed adult (Ambros, 2000; Byerly et al., 1976). This developmental period lasts 3-4 days at 20°C and starts off as a single body cavity during the first larval stage, following further development of distinct features such as the reproductive system and repetitive episodes of moults, shedding its outer cuticle multiple times (Hirsh et al., 1976).

The focus of this paper is the interplay between genetic and environmental factors on the reproductive abilities of C. elegans. The reproductive system of this species is hermaphroditic, meaning that it is made up of both male and female reproductive organs. Thus, self-fertilisation is very common and the germ cells in the hermaphroditic gonads undergo mitosis and meiosis to produce oocytes, which are then fertilised by sperm in the uterus. Finally, the zygotes are laid through the vulva (Han et al., 2009; Kimble & White, 1981).

Under optimal conditions, C. elegans can lay up to 300 eggs during their lifetime (Ward et al., 1981). However, this reproductive lifespan can vary greatly and is influenced by various genetic and environmental factors such as: diet, temperature, and exposure to light/dark conditions. For example, the gene raga-1 is present in other organisms, including humans and its role is to encode a protein that is part of the mTORC1 signalling pathway involved in reproduction, metabolism and longevity (Bar-Peled & Sabatini, 2014; Blackwell et al., 2019). The gene has been conserved across species and studying it at a deeper level, would allow us to learn more about potential therapies for a variety of diseases including cancer, diabetes, or reproductive diseases (Condon & Sabatini, 2019).

Hypothesis: Previous scientific literature published has linked reduced fertility in nematodes with a reduced expression of the raga-1 gene. This was found to be caused by defects in sperm and oocytes since the mTORC1 pathway cannot support meiotic progression and gamete production in such instances (Fukuyama et al., 2012). Therefore, I hypothesised that there would be a statistically significant drop in offspring count in the groups of nematodes (both f0 and f1) offered RNAi treatment to lose the expression of raga-1 gene compared to the groups of nematodes offered RNAi treatment in empty vectors.

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Null Hypothesis: No statistically significant difference in the count of offspring count between the two groups of nematodes.

Data and model : Differential Raga-1 Gene Expression and its Impact on Fertility. To test the hypothesis mentioned in the previous paragraph, I compared the offspring count of 4 different groups of C. elegans nematodes.

```
library(tidyverse)
```

```
## ── Attaching core tidyverse packages ──────────────────────── tidyverse
2.0.0 ──
## ✓ dplyr     1.1.2     ✓ readr     2.1.4
## ✓ forcats   1.0.0     ✓ stringr   1.5.0
## ✓ ggplot2   3.4.2     ✓ tibble    3.2.1
## ✓ lubridate 1.9.2     ✓ tidyr     1.3.0
## ✓ purrr     1.0.1
## ── Conflicts ────────────────────────────────────────
tidyverse_conflicts() ──
## ✖ dplyr::filter() masks stats::filter()
## ✖ dplyr::lag()    masks stats::lag()
## ℹ Use the conflicted package (<http://conflicted.r-lib.org/>) to force all
conflicts to become errors
```

```
library(readxl)
library(ggpubr)
library(GGally)
```

```
## Registered S3 method overwritten by 'GGally':
##   method from
##   +.gg   ggplot2
```

```
library(performance)
library(patchwork)
library(rstatix)
```

```
##
## Attaching package: 'rstatix'
##
## The following object is masked from 'package:stats':
##
##     filter
```

```
f0_data <- read_excel(path = "C:/Users/Denisa/Documents/5023Y-Summative-
2/Data/elegans_offspring.xlsx", sheet = 1, col_names = TRUE, col_types =
NULL)
f1_data <- read_excel(path = "C:/Users/Denisa/Documents/5023Y-Summative-
```

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```
2/Data/elegans_offspring.xlsx", sheet = 2, col_names = TRUE, col_types =
NULL)

f0_data$offspring <- as.numeric(f0_data$offspring)

## Warning: NAs introduced by coercion

f1_data$offsprings <- as.numeric(f1_data$offsprings)

## Warning: NAs introduced by coercion

colnames(f1_data)[4] <- "offspring"

f0_data <- na.omit(f0_data)
f1_data <- na.omit(f1_data)

f0_data$generation <- "f0"
f1_data$generation <- "f1"

colnames(f1_data)[1] <- "rnai"
f1_data <- select(f1_data, -parental_treatment)
f0_data <- select(f0_data, -replicate)

df <- rbind(f0_data, f1_data)

df$treatment <- factor(df$treatment, levels = c("light", "dark"))
df$rnai <- factor(df$rnai, levels = c("raga", "ev"))
df$generation <- factor(df$generation, levels = c("f1", "f0"))

ggplot(df, aes(x = rnai, y = offspring, fill = treatment)) +
  geom_boxplot(color = "black") +
  facet_wrap(~ generation, ncol = 2) +
  scale_fill_manual(values = c("white", "gray")) +
  labs(x = "RNAi Treatment of f0", y = "Offspring Count") +
  theme_classic()
```

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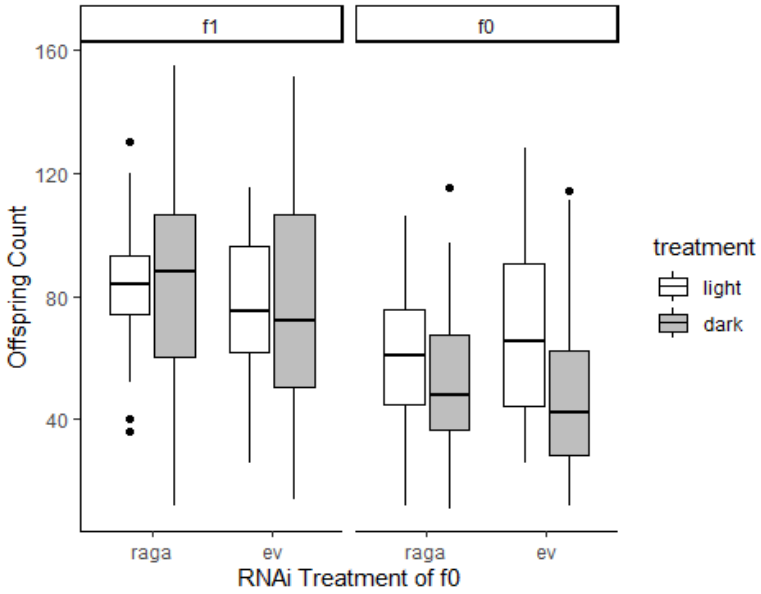

Figure 1. Boxplot comparing the offspring count of 2 different generations of C. elegans nematodes, treated with either an empty vector or a raga-1 RNAi genetic knockdown treatment. They have been kept under different light environments; grey represents the sub-groups of nematodes grown in the dark whilst the white represents the sub-groups exposed to light. F1 stands for first filial generation, f0 stands for parental generation.

This boxplot doesn't necessarily show a pattern to the naked eye, but to test whether the nematodes with reduced raga expression have a statistically significant lower offspring count than the ev subjects, I ran a t-test to compare the mean offspring counts between the raga and ev treatments. Using a general linear model with raga/eve gene treatment as a factorial predictor variable against offspring count (on separate occasions for each generation). This analysis showed that raga treated f0 nematodes reproduced only slightly more often than the f0 ev nematodes. [95% Cl; 39-56.6] (P>0.05). Similarly, in the f1 generation, the raga treated nematodes produced on average, a very similar amount of offspring to the ev mutants. [95% Cl; 8-8.9] (P>0.05)

f0 generation

Predictors	Estimates	Z-value	P	Lower 95% CI	Upper 95% CI
(Intercept)	47.82	10.75	0.00	39.01	56.63
rnairaga	4.34	0.67	0.50	-8.42	17.09
treatmentlight	22.00	3.32	0.00	8.89	35.11
rnairaga:treatmentlight	-13.24	-1.38	0.17	-32.17	5.70

f1 Generation

Predictors	Estimates	Z-value	P	Lower 95% CI	Upper 95% CI
(Intercept)	8.49	34.97	0.00	8.01	8.96
rnairaga	0.50	1.52	0.13	-0.15	1.15
treatmentlight	0.15	0.43	0.67	-0.52	0.81
rnairaga:treatmentlight	-0.04	-0.09	0.93	-0.96	0.88

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Results and discussion : My chosen significance level is 0.005 and my p-values are greater than this. As a result the null hypothesis is accepted and the alternative hypothesis is rejected. In this case, the difference in the count of offspring count between the two groups of nematodes (raga and ev mutants) in both f0 and f1 generations is not statistically significant.

Light exposure could have impacted the nematodes' behaviour to a larger degree than the gene raga-1, leading them to mate more frequently or for longer periods of time, which could result in increased offspring production rather than decreased (Mei & Singson, 2020). Possible explanations for this effect mentioned that light exposure can influence the nematodes' reproductive physiology and thus offspring count. One of the reasons this happens has been linked to the activation of mTORC1 signaling pathway and other downstream effectors linked to the pathway such as DAF-16, which are also controlled by the raga-1 gene. This means that environmental factors such as light exposure can affect gene expression despite the genetic treatment given beforehand and potentially switch genes on or off (Tissenbaum, 2018). While genetic knockdowns can significantly reduce the expression of a particular gene, confounding effects could have altered the distribution of our data. Further research of the relationship between light exposure, the raga gene, and nematode reproduction is needed for a clear, robust conclusion.

Additionally, it's worth noting that even in groups where the light exposure is dark and the RNAi treatment is raga knockdown, the median count of offspring is not significantly lower than the rest of the groups. However, further statistical modelling and analysis must be carried out to be certain. It is possible that other environmental factors such as the diet (including the quality and quantity) provided during the experiment could have also influenced the results. However, further research is be needed to confirm and expand upon these findings.

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