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Epigenetic effects of stress on survival in *Caenorhabditis elegans*

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ABSTRACT

Caenorhabditis elegans are members of the nematode family and have been model organisms for epigenetic studies for many years due to their small size, transparent body, short life cycle, and easy maintenance. For this study, *C. elegans* were used to explore the epigenetic effects of stress on the survival of the worms. The animals in this study were subjected to the environmental stressor starvation and showed an increase in overall lifespan based on the statistical analysis of a Kaplan-Meier Survivorship test. This increased survival rate was also found to be passed to at least three subsequent generations who were grown under unstressed conditions. These results showcase the hormetic epigenetic effects of starvation in *Caenorhabditis elegans.*

Keywords: *Epigenetics, Stress, Caenorhabditis elegans*

INTRODUCTION

Epigenetic changes within the environment, whether initiated by DNA methylation, histone modification, or non-coding RNA-associated gene silencing, can lead to modifications in gene expression by switching certain genes on or off, controlling which proteins will be transcribed (Simmons, 2008). These changes in gene expression may cause little to no effect on an organism, however, it is also possible that these changes may cause more damaging effects that could lead to the acquisition of diseases such as cancer. Understanding the epigenetic effects of certain environmental factors could help show the role that epigenetics plays in the health field and could even allow for the early diagnoses and better medical treatment of many disorders.

There are many environmental factors that may be potential modifiers of gene expression including a factor of recent interest, stress caused by a restriction of diet. For instance, an article published by Rechavi *et al.* in July of 2014 titled "Starvation-Induced Transgenerational Inheritance of Small RNAs in *C. elegans*" found that the experience of a specific environmental factor (in this case starvation) by an ancestor is enough to cause genetic modifications in the descendants of that ancestor for up to three consecutive generations. Other recent studies have also concluded that these gene modifications, caused by the environmental factors, can create what is known as an epigenetic memory, which explains why the effects of an event experienced by an ancestor are present in the subsequent generations (Gaydos *et. al.,* 2014).

Another topic of recent interest is hormesis. Hormesis effects are thought to be possible beneficial effects that are brought on by low-dose exposure to specific conditions and stressors. This low-dose exposure to stressors early in an organism's life has even been found to go as far as extending the organism's overall lifespan (Yanase, S. *et al*., 1999).

For this research project, I will attempt to address the research question "Does stress have a transgenerational effect on survival in *Caenorhabditis elegans*?"

MATERIALS AND METHODS

For this experiment, I will be using the methods laid out in the paper "Environmental stresses induce transgenerationally inheritable survival advantages via germline-to-soma communication in *Caenorhabditis elegans*" (Kishimoto, 2017).

These methods are as follows: All nematodes will be cultured using standard *C. elegans* methods. The experimental and the control group studies were performed at 25 °C. The N2 wild type strain of *C. elegans* will be used in this experiment.

Starvation:

For this experiment, I obtained one NGM plate to be used for my control groups. Then, I seeded the plate with 0.05 mL *E. coli* OP50 liquid culture and spread the *E. coli* to create a lawn. Next, I transferred L1 stage N2 worms onto the *E. coli* seeded NGM plate, allowing the worms on the plate to grow under normal conditions for three days. Then, I transferred fifteen of the L4 worms onto new *E. coli* containing NGM plates, without inducing starvation, and allowed the animals to lay eggs for twenty-four hours. After the worms reproduced, the parents were removed from the plates to obtain the F1 generation which I kept incubated for four days. I then repeated this process to obtain F2 and F3 generations.

For the experimental groups, I began by obtaining one NGM plate. Then, I seeded the plate with 0.05 mL *E. coli* OP50 liquid culture and spread the *E. coli* to create a lawn. Next, I transferred L1 stage N2 worms onto the *E. coli* seeded NGM plate allowed the worms to grow under normal conditions for three days. Then, I transferred fifteen of the L4 stage animals onto a new NGM plate without *E. coli* OP50 to induce starvation. I then incubated the animals for twenty-four hours. Next, I transferred the L4 animals onto new plates that

once again contained *E. coli* OP50 and allowed the worms on each plate to grow under normal conditions for three days. Next, I transferred fifteen of the adults onto new *E. coli* containing NGM plates without inducing starvation and allowed the animals to lay eggs for twenty-four hours. After the worms reproduced, the parents were removed from the plates to obtain the F1 generation which I kept incubated for four days. This process was then repeated to obtain F2 and F3 generations.

The F1-3 generations of both the control and experimental groups were not subjected to any type of environmental stressors during their lifespan.

Assessing epigenetic effects:

In order to determine the epigenetic effects caused by the starvation environmental stressor I assessed the lifespan of the F1-3 generations.

To assess lifespan, the P0 (parental) generation animals were raised under the indicated environmental stress condition for four days while the F1-F3 generation animals were raised without environmental stressors for four days. Fifteen animals from each generation were then transferred to separate 5' flurodeoxyuridine-containing NGM plates seeded with ultraviolet-treated *E. coli* to inhibit progeny growth. The day the worms were transferred to the FUdR-containing NGM plates was defined as *t*=1 day. Deaths were recorded every four days. Animals were scored as dead if they fail to respond to touch by a platinum wire picker.

Microsoft Excel was used to organize the collected data and SigmaPlot was used to conduct a Kaplan-Meier Log Rank Test on the data. The Log Rank Test was chosen due to its ability to compare the survival distributions of the two groups (experimental and control) for each of the four generations (P0, F1, F2, & F3). For this test, statistical significance was defined as a P-value < 0.05 and the data was censored to ensure the removal of worms from the remaining data once they were defined as dead.

RESULTS

For this experiment, I attempted to recreate or replicate certain aspects of a study published by Saya Kishimoto, Masaharu, Emiko Okabe, Masanori Nono, & Eisuke Nishida published in January of 2017 titled "Environmental Stresses Induce Transgenerationally Inheritable Survival Advantages via Germline-to-Soma Communication in *Caenorhabditis elegans.*" In their experiment Kishimoto *et al.* looked at hormesis effects caused by environmental stressors as well as the resulting germ-to-soma communication.

For my experiment, I focused solely on the epigenetic effects of the starvation stressor on survival in the *C. elegans.* I grew hermaphroditic L4 stage worms under fasting conditions (for this experiment we shall define fasting/starvation as a highly limited supply of E. coli food) for a total of four days. After the four days, the worms were transferred onto plates that contained an unlimited supply of E. coli and remained on plates such as these for the remainder of their lifespan. Subsequent generations were produced and transferred onto new plates without being subjected to the stressor to determine if there was a transgenerational effect taking place. To compare the experimental and control groups I used the data collected from the experiment to conduct a Kaplan-Meier Survival Analysis.

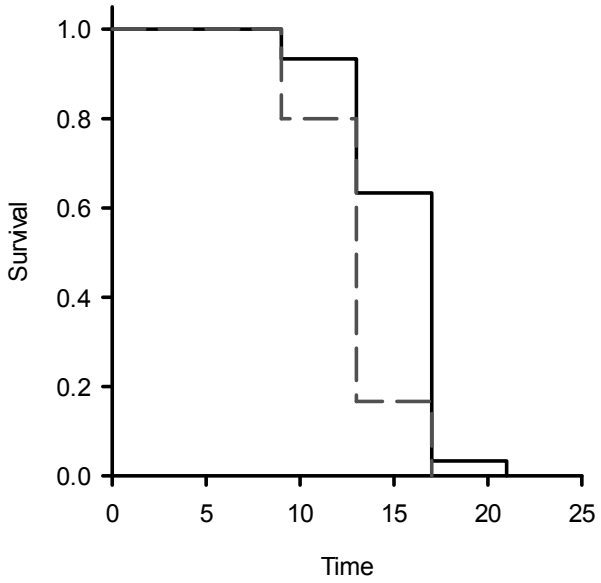

Figure 1. Comparison between control (---) and experimental (—) groups of parental generation.

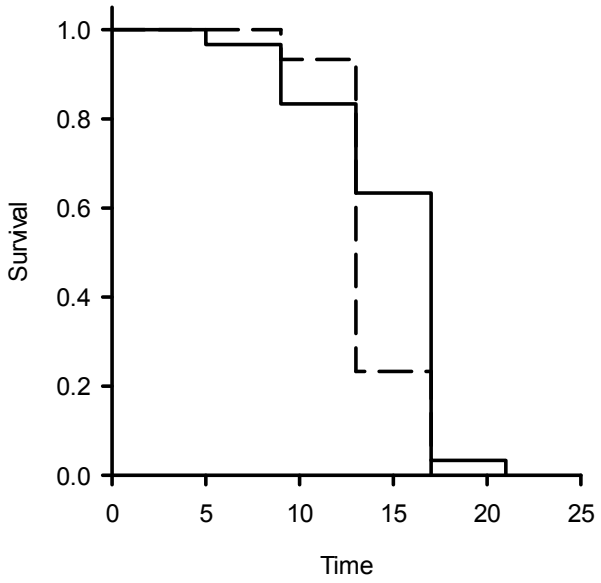

Figure 2. Comparison between control (---) and experimental (—) groups of F1 generation.

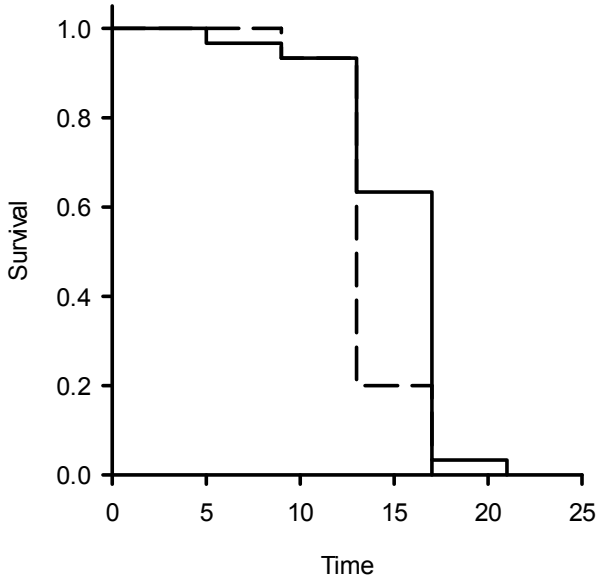

Figure 3. Comparison between control (---) and experimental (—) groups of F2 generation.

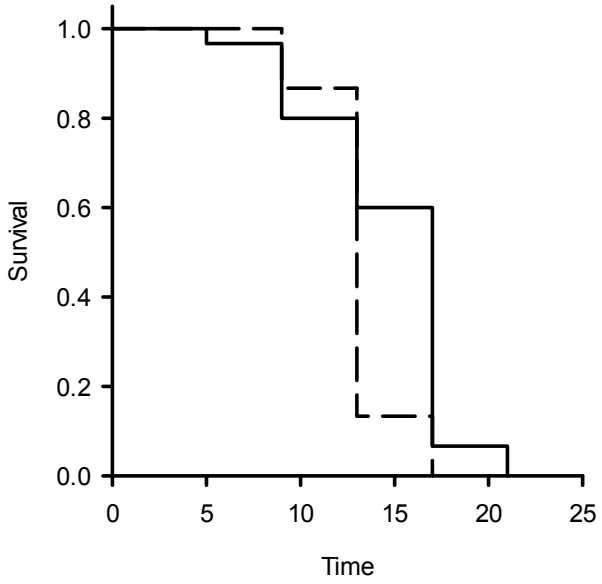

Figure 4. Comparison between control (---) and experimental (—) groups of F3 generation.

Table 1. LogRank test statistics for the comparisons between the experimental and control generations.

	Statistic	DF	P-Value
P0	13.195	1	<0.001
F1	5.742	1	0.017
F2	9.636	1	0.002
F3	8.067	1	0.005

DISCUSSION

The main focus of this experiment was to determine whether exposure to an environmental stressor during developmental stages of *C. elegan* worms would induce a transgenerational effect through three subsequent generations in the form of an increased lifespan. To determine the presence of an epigenetic effect such as this, a graphical and statistical comparison between each experimental and control group was done.

The comparisons between the four experimental and control generations resulted in P-values of <0.001, 0.017, 0.002, and 0.005 respectively. The cutoff for significance in this experiment was defined as a P-value of 0.05 therefore, these P-values allow for the rejection of the null hypothesis and the conclusion that a significant difference does exist between the experimental and control groups of each generation. The LogRank test statistics for the comparisons were found to be 13.195, 5.742, 9.636, and 8.067. Each LogRank statistic is greater than what would be expected by chance again proving that there is a statistically significant difference between the survival curves.

Based on these calculated statistical results from the Kaplan-Meier survivorship LogRank test, I can conclude that stress exposure during the developmental period of the parental generation of *C. elegan* worms can produce beneficial effects, such as increased longevity in the subsequent generations. These findings agree with the results published by Saya Kishimoto, Masaharu, Emiko Okabe, Masanori Nono, & Eisuke Nishida in which they found that subjecting the worms to various stressors provides beneficial effects as well as phenotypic effects in the unstressed generations that follow. This study revealed that not only do the three subsequent generations experience a greater longevity, but also that the experimental parental generation, which was the only generation subjected to the environmental stressor, also experienced an increase in overall lifespan when compared to the control parental generation.

It is thought that the production of small-RNAs are behind these epigenetic changes within the worms and that the mechanism by which they function is a method the parents use to help prepare their future generations to face hardships like the ones they themselves experienced (Rechavi *et. al.*, 2014). This theory reverts to Darwin's original thinking of survival of the fittest where only individuals who are equipped with certain traits will be able to survive and, more importantly, reproduce.

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