The Effect of Nutritional State on Social Behaviors in *C. elegans*

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Abstract

In order to determine if nutrition has an effect on social behaviors, this project looks at *C. elegans* and their responses to endogenously produced small-molecules called ascarosides. This project involves the classification of behavior using behavior assays and different nutritional states including well-fed, starved and a high glucose diet. In particular, the project looks at sex-specific behaviors. It was found that both males and hermaphrodites are attracted to ascr#3 and ascr#8 when well-fed. When starved, males are still attracted to ascr#8 whereas hermaphrodites lose their attraction. Growing males and hermaphrodites on glucose plates do not change their response to ascr#8 in either the fed or starved condition. Using the *daf-2* mutant (an insulin signaling mutant), growing hermaphrodites on glucose plates or agar plates did not elicit a response to ascr#8 in the fed condition. It is important to research this topic as it can provide insight into how the physiology and biochemical pathways of nutrition may be influenced in order to cause favorable social behaviors.

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1. Introduction

Proper nutrition is vital for growth and development in living organisms and plays a significant role in health and wellness. Malnutrition poses risks for disease, deficiencies in the immune system, and obesity (World Health Organization). In the United States, about 97 million adults are considered to be overweight or obese which are conditions that may lead to diseases such as diabetes, stroke, and respiratory problems, along with numerous other dangers (NHLBI Obesity Education Initiative). Undernutrition, which is classified as a shortage in vitamins and minerals, leads to a disruption in development and productivity and is the cause of approximately 1/3 of all child deaths. One indication of malnutrition is low growth rate when compared to the average growth among age groups. Globally, 165 million children have stunted growth which can lead to lack of brain development (World Health Organization). Along with impacting growth and development, nutritional status can also influence social behaviors. Research has found that malnutrition, including iron, zinc, protein and Vitamin B deficiencies can be linked to antisocial and aggressive behavior. In a University of Southern California study, researchers saw that deficiencies in nutrients associated with brain development correlated to significant increases in antisocial and aggressive behavior compared to a well-nourished control group (Sutliff, 2004). This shows that particular nutritional states may be detrimental to social behaviors. By studying the cause of social behaviors, a greater understanding of the species can be developed.

Not only is nutrition important to understand in a species as a whole, but there is also a curiosity in the differences among genders within a species. For example, in women, calcium is an essential nutrient that contributes to bone health and a lower risk of osteoporosis, but in men it actually may be harmful in large quantities. It may even increase the risk of prostate cancer

(Harvard Medical School Family Health Guide, 2006). Particularly, the physiology of eating in men and women has shown to be distinct. Several factors such as fluctuating hormones, genetic variation, and different levels of ghrelin, glucagon, insulin and leptin in the body along with many other aspects can lead to these differences (Asarian & Geary, 2012). A study conducted by Smeets et al. showed that there are distinctions in men and women in regards to the brain's response to food. In this study, twelve men and twelve women were asked to taste chocolate milk before and after they were satiated with chocolate. They were then tested using a functional magnetic resonance. Increased and decreased taste activation was seen in different parts of the brain among men and women. In particular, areas where sex differences were found in accordance with chocolate satiation were the medial prefrontal cortex, the ventral striatum and the hypothalamus (Smeets, Graaf, et al, 2006). These physiological differences may lead to behavioral differences among men and women. This may coincide with the distinct social behaviors that are varying among opposite sexes of the same species.

It is important to understand exactly how nutrition affects behavior so that detrimental behaviors due to poor nutrition can be improved upon efficiently. By testing many nutritional states on *C. elegans* and by keeping all variables constant besides the ones being tested, it can be determined if nutritional state, does, in fact, cause changes in social behaviors. A direct effect between nutrition and behaviors can be observed. This experimentation can then be further studied by looking specifically at different sexes of *C. elegans* to see if behaviors significantly vary between sexes. Overall, better nutrition may lead to a better quality of life and it is important to gain an understanding of the effects of nutritional state. In this project, both the nutritional state and the sexes of *C. elegans* were taken into consideration when looking at behavioral response to endogenous small molecules. The three nutritional states consisted of fed,

starved, and high glucose. The two sexes tested were hermaphrodites and males. This experiment allowed for determination of whether different nutritional statuses would affect the natural behavior of the animals in response to small molecule signals. It also delved into sex-specific behaviors and how these behaviors can be manipulated by nutrition.

2. Background

2.1 What are *C. elegans*?

C. elegans are small round worm nematodes that are generally found in dirt, fruit or compost. *C. elegans* are a superb model of scientific study for several reasons. They are an abundant species that are easy to cultivate leading to quick experimental results. *C. elegans* are good for experimentation as they develop into adults in 3.5 days and produce many offspring quickly (Corsi, 2006). They have about 1,000 somatic cells and are transparent allowing for direct observation of many biological aspects and behaviors. They are composed of a simple anatomy but with a variety of tissue including nerve, muscle and intestinal. *C. elegans* can also be frozen and recovered allowing for long-term storage. Although they are very different species, 40% of genes found in human disease are found as homologs in the *C. elegans* genome. This allows for *C. elegans* to be a good comparison to some elements of humans including disease research (Corsi, 2006). Among other topics, *C. elegans* are used in research involving genetics, neuroscience and social behavior.

The life cycle of *C. elegans* is important in experimentation. The life cycle of *C. elegans* depends on the temperature at which they are stored. At higher temperatures, *C. elegans* have a shorter life cycle than those kept at a lower temperature, but the average life cycle is 3 days at 20° C. After embryogenesis, the egg hatches into the first larval stage, the L1 stage, and continues with development. They then go through three more larval stages (L2-L4) until they develop into young adults and then into adults. The time between the L4 stage and the young adult stage is about 10 hours (Corsi, 2006). For the purposes of this project, the behavior of young adults was observed.

2.2 Hermaphrodites and Males

C. elegans exist as two different sexes, either male or hermaphrodite. Males have about 100 more somatic cells than hermaphrodites and have a slightly different appearance. They have broader tails yet are thinner and smaller than hermaphrodites. The tails of hermaphrodites appear to taper off rather than remaining broad. Males contain structures in their tales used for mating while hermaphrodites can self-fertilize since they store sperm before creating oocytes. The two sexes are generally distinguishable from one another at the L4 stage of life (Corsi, 2006). Due to these differences in sexes, males and hermaphrodites have different social behaviors involving mating. Images of hermaphrodites are shown in Figure 1A and males in Figure 1B shown in appendix A (Corsi, 2006). Males are infrequent in the natural *C. elegans* population as they make up only about 0.2 % of the entire population. However, mutations have been discovered which can increase the male population by up to 150 fold. This frequency increase is made possible by an escalation of X-chromosome nondisjunction. These *him* mutants are named due to the "high incidence of males" that occurs in the offspring of the mutated hermaphrodites. This is convenient when males are the main observation of study (Hodgkin, Horvitz & Brenner, 1979).

2.3 How are *C. elegans* grown and what are the different types of strains?

C. elegans may be cultivated on nematode growth medium (NGM) agar plates with bacteria or can be grown in liquid culture. The animals can also be grown in a chemically altered medium without bacteria. The benefit of this is so that the effects of multiple chemicals and nutrients can be tested on the model organisms. After the food supply is depleted, the starved animals will begin to bury themselves into the agar. In order to prevent starvation, *C. elegans* can be restored by chunking the agar and placing it onto a new supply of bacteria or individually picking animals and passing them to a new agar plate (Corsi, 2006). The most common strain of

C. elegans is the N2 Bristol strain, which is easily found in laboratories (Corsi, 2006). This strain was the main focus for this project when observing hermaphrodites The *Cel-him-5* strain was used to study males. This strain has a high incidence of males and is particularly useful for biochemical study as their fertility rates are high. These mutants also have a particular effect of the X chromosome as compared to the autosomes (Hodgkin, Horvitz & Brenner, 1979). A strain of *C. elegans* defective in insulin signaling called *daf-2 (**da**uer **f**ormation defective)* was used to study a genetic mutation involving glucose. The *Cel-daf-2* gene encodes for an insulin-like receptor and a deficiency in the function of this receptor leads to longevity of life (Boccitto, Lamitina, Kalb, 2012) This is caused by "signaling cascades mediated by inhibition of the phosphoinositide 3-kinase (*age-1*) and activation of the forkhead transcription factor DAF-16 via its nuclear localization" (Boccitto, Lamitina, Kalb, 2012). The *daf-2* gene facilitates endocrine signaling and a decrease in *daf-2* signaling generates developmental and metabolic variations. (Kimura, Tissenbaum, et. al, 1997).

2.4 The insulin signaling pathway

The insulin signaling pathway plays a large role in glucose storage and uptake. Insulin is a hormone that responds to excess nutrients in the blood stream and is released by pancreatic cells. Insulin helps support the storage of nutrients into lipids, glycogen and proteins. The insulin receptors consist of two subunits and a conformational change is induced when insulin binds to a subunit. Defects in the insulin signaling pathway can lead to diseases like diabetes (Gami, 2006).

2.5 What is the role of ascarosides?

Ascarosides, or dideoxysugar ascaryloses, are small molecule signals that act as pheromones. Pheromones are chemicals secreted by organisms that may manipulate the social

behavior of other organisms, particularly in mating behaviors. The chemical structure of ascarosides include a sugar ascarylose and a side chain that is fatty-acid like. This fatty acid chain may contain a number of functional groups (Srinivasan, Kaplan, et. al, 2008). Ascarosides are identified by many chemosensory head neurons in *C.elegans* and perceptions are deliberated by G- protein coupled receptors (Ludewig & Schroeder, 2013). In *C. elegans,* ascarosides were first discovered as chemical signals that regulated entrance into the dauer stage. The dauer stage is one in which *C. elegans* cease development and become highly resilient and do not feed. This happens in the L2 larval stage. *C. elegans* can survive in the dauer stage for several months. This stage is caused by harsh environmental conditions such as lack of food, high population density, or high temperatures (Kaplan, Srinivasan, et. al, 2011). When the conditions improve, development continues as usual (Ludewig & Schroeder, 2013). Along with the regulation of dauer formation, ascarosides also play a key role in other signaling pathways.

In particular, ascarosides can influence social behaviors like male attraction and hermaphrodite repulsion. They have also been shown to have an influence in aggregation and olfactory plasticity. Even small alterations in chemical structure can lead to dramatically different behaviors. It was found that many signaling pheromones were not made up of just one compound but of a blend of several, which synergize together. Precise combinations and concentrations of specific ascarosides can have several different effects on behavior. Because of this, it is thought that ascarosides have several different receptors and each receptor displays a different affinity to individual ascarosides. To differentiate among the ascarosides, liquid culture extracts from *C. elegans* were analyzed using NMR-based comparative metabolomics (Ludewig & Schroeder, 2013).

The two ascarosides that are the focus of this this project are ascr#3 and ascr#8. The structure of ascr#3 includes a "nine-carbon α,β-unsaturated carboxylic acid side chain" (Ludewig & Schroeder, 2013). Ascr # 8 has a unique structure compared to the other ascarosides. The ascr # 8 structure consists of a "paminobenzoic acid moiety, forming an amide with a seven-carbon α,β-unsaturated fatty acid" (Ludewig & Schroeder, 2013). The structure of ascr#3 (Figure 3) and ascr#8 (Figure 4) are shown in the appendix (Kaplan, Srinivasan, et. al, 2011). In *C. elegans*, hermaphrodites secrete these molecules which attract males. This project focuses on attraction or repulsion of males and hermaphrodites to ascr#3 and ascr#8.

Research previously conducted has shown that ascr#3 is the most attractive ascaroside to males. It has also been shown to repel hermaphrodites. These results show that the reaction to ascarosides is sex-specific. (Srinivasan, Kaplan, Ajredini, Zachariah, Alborn, Teal, Malik, Edison, Sternberg, Schroeder, 2008) Other experiments have also shown that ascr#3 attracts males from a distance whereas ascr#8 holds males in an area upon them entering it. (Choe, 2012). Ascr#3 and ascr#8 both have been discovered to be attractants and the greatest amount of each molecule is released during the young adult and adult stages (Kaplan, Srinivasan, et. al, 2011).

In order to determine if nutrition has an effect on social behaviors, this project looks at *C. elegans* and their behaviors in reaction to ascarosides. The social behaviors induced by ascarosides have been studied, and testing different nutritional states along with this will add a new layer to the research. In particular, looking at different sexes will specify the behavior further. It is important to research this topic as it can provide insight into how nutrition may be improved to cause favorable social behaviors.

3. Methods and Materials

3.1 *C. elegans* strains

Three strains of *C.elegans* were used including the N2 strain, the *him-5* strain and the DAF-2 strain. The N2 strain, which supplied the hermaphrodites, and the *him-5* strain, which supplied the males, were both obtained from the Caenorhabditis Genetics Center (CGC) which is funded by the National Institutes of Health National Center for Research Resources (NIH NCRR). The *daf-2* strain, which was used to study mutated hermaphrodites, was acquired from Dr. Michelle A. Mondoux from the Cellular and Molecular Biology department of the College of the Holy Cross.

3.2 Agar plates and worm picking

C. elegans were grown on NGM agar plates which were seeded with OP50 strain *E. coli* as the provided food source. OP50 is used because of its slow growth on NGM agar plates which allows for better observation and superior mating for worms. A 1 liter quantity of the NGM agar was made by mixing 3 g NaCl, 2.5 g Bacto-Peptone, 17 g DIFCO-agar and 975 ml of distilled water. These ingredients were mixed on a stir plate and autoclaved. After the autoclave process was completed and the mixture had cooled, 1 mL of 1 M $CaCl_2$, 1 mL of 1 M $MgSO_4$ and 25 mL of 1 M KPO_4 (pH 6.0) was added in that same order. Using the created agar solution, 60 mm plates were poured. (Stiernagle, 2006).

In order to maintain the *C. elegans,* it is important to transfer them to new petri dishes consistently. This was carried out through individual worm picking which is done using a piece of platinum wire attached to the tip of a Pasture pipet (Stiernagle, 2006).

3.3 Behavior assays

In order to observe *C.elegans* behavior in response to ascarosides, behavior assay experiments were conducted.

3.3.1 Preparation for behavior assays

Approximately 14 hours prior to the start of the behavior assay experiment, 50-60 *C. elegans* in the L4 stage were passed to a new agar plate that was seeded with OP50. L4 worms were picked so that they would develop into young adults for the time of the experiment. Depending on whether the experiment was using hermaphrodites or males to study, the respective strain and gender would be picked and passed. Hermaphrodites were picked from the N2 strain while males were picked from the *him-5* strain. Hermaphrodites were also picked from the *daf-2* strain when the mutation was being considered in experiments.

For the behavior assays, assay plates were prepared one day in advance of the experiment. These plates were prepared by spreading 2-3 drops of OP50 distributed equally throughout an unseeded agar plate. This was done by using a bent Pasteur pipette as a spreader and a rotating platform to spread the bacteria evenly. Between the spreading of each plate, the Pasteur pipette was dipped in ethanol and flamed to sterilize. The bottle of OP50 was also brought to the flame to eliminate any contaminants. For this project, plates were made five at a time for the following experiments.

3.3.2 Behavior assay template

For the behavior assay, the assay plates were attached to a template (Figure 5). There are two circles which indicate where the chemical and control were placed. The placement of the chemical and control switched every other test. With a micropipette, 0.750 µl of both the chemical and control were placed into their respective circles. The solutions were allowed to dry. For the experiments in which the well-fed worms were being used, five worms, from the plate

with 50-60 worms which was prepared 14 hours prior, were picked and placed on one of the Xs on the template. Another five worms were also taken from the prepared plate and were placed on the other X on the template. After the assay plate was set, it was placed under a Leica KL 300 LED microscope which was attached to a Unibrain camera (Model No: fire- I980b) all of which was connected to a computer. Twenty minute videos were taken of each assay plate. Five assay plates were tested in each experimental session.

3.3.3 Data collection for behavior assays

Data was collected based on the interaction and behavior of the worms on the assay plates. The time in which a worm entered one of the circles, either the chemical or control, and the time in which it left were recorded. The difference of these two times was calculated in order to collect the total time that the worm spent in that area. A worm was considered to have stayed in the chemical or control if it remained there for more than four seconds. Each interaction was considered to be a new worm. If one worm left the circle for less than five seconds and returned to the circle, it would not be considered another worm, however if a worm left the circle for more than five seconds and then entered again, it was considered as a different worm.

3.4 Starved behavior assays

For the starved experiments, the protocol differed. The worms were picked fourteen hours in advance as before. During the experiment, instead of taking the worms from that plate and putting them directly onto the assay plate, ten worms were placed onto an agar plate with no food to starve them. After an hour, the original protocol for the experiment began again with the chemical and control being placed in the circles and five worms being placed on each X. Data was measured in the same manner as for the well-fed worms. Five assay plates were tested in each experimental session.

3.5 Re-fed behavior assays

Re-feeding experiments were carried out to determine if starvation was reversible. For this experiment, 60-70 worms were picked fourteen hours in advance as they were before. During this experiment, ten worms were placed onto an agar plate with no food for one hour. After an hour, they were placed onto plates that had food for fifty minutes. After being placed on the plate with no food and then the plate with food, the original protocol for the experiment began again with the chemical and control being placed in the circles and five worms being placed on each X. Data was measured in the same manner as for the well-fed worms. As a comparison, half of the worms were placed on the assay plates just after being starved for one hour. This was to observe the behavior of this set of worms in the starved condition to see if re-feeding would affect it.

3.6 Glucose plates

For the high-glucose diet experiments, worms of each strain were passed from normal NGM agar plates to glucose plates. They were allowed to grow through two generations before using for testing so that the progeny being tested would have been fully developed on the glucose plates. This was done by transferring eggs from the original plate to the glucose plate and then passing eggs of the original generation to a new glucose plate. To prepare 1 liter of solution for 250 mM glucose plates, the ingredients were as follows: 3 g of NaCl, 2.5 g peptone, 17 g agar and 775 mL of DiH2O were mixed together and autoclaved. After, this mixture was allowed to cool to approximately 68° C. Then, 1 mL cholesterol (5mg/mL), 1 mL 1 M $CaCl_2$, 1 mL 1 M $MgSO_4$, 25 mL 1 M KPO_4 (pH 6.0) and 200 mL 1.25 M filter sterile glucose solution were added. This was all mixed on a stir plate and then 10 mL per plate were poured.

4. Results

4.1 Response of hermaphrodites and males to different small molecules

In order to have a baseline comparison, the response to small molecule ascarosides of both males and hermaphrodites grown on regular agar plates was observed using a fed behavior assay. The averages were derived from multiple days of testing which consisted of 5 assays each day. The average time of each interaction with the chemical and the control were calculated. Both ascr#3 and ascr#8 were tested.

4.1.1 Ascr#3

Ascr#3 was tested first to observe the attraction of males and hermaphrodites to the chemical and t-tests were performed to check for significance. The mean time spent in the chemical (ascr#3) for well-fed males (M=121.45 SD=197.37) was significantly different than the time spent in the control (M=48.79, SD=48.35), t(88)=-2.25, p=0.027, two-tailed t-test. The mean time spent in the chemical (ascr#3) for well-fed hermaphrodites (M=52.15, SD=38.79) was significantly different than the time spent in the control (M=39.11, SD=28.14), t(135)=-2.27, p=0.025, two-tailed t-test.

Another t-test was performed to compare the response to ascr#3 of males and hermaphrodites. The mean time spent in the chemical for males (M=121.45 SD=197.37) was significantly different than the time spent in the chemical for hermaphrodites (M=52.15, SD=38.79), t(114)=2.77, p=0.007, two-tailed T-test. It appears that ascr#3 causes attraction in both males and hermaphrodites. The average time spent in ascr#3 was higher for males than for hermaphrodites indicating that ascr#3 is more attractive to males (Figure 6 and Table 1).

4.1.2 Ascr#8

Ascr#8 was also tested to observe the attraction of males and hermaphrodites to the chemical and t-tests were again performed to check for significance. Both chemicals were tested

in order to compare *C. elegans* response across ascarosides. The mean time spent in the chemical (ascr#8) for well-fed males (M=94.88, SD=73.77) was significantly different than the time spent in the control (M=52.75, SD=53.39), t(94)=-2.99, p=0.004, two-tailed t-test. The mean time spent in the chemical (ascr#8) for well-fed hermaphrodites (M=129.29, SD=141.31) was significantly different than the time spent in the control (M=49.04, SD=27.95), t(67)=-3.70, p=0.004, two-tailed t-test.

Another t-test was performed to compare the response to ascr#8 of males and hermaphrodites. The mean time spent in the chemical for males (M=94.88, SD=73.77) was not significantly different than the time spent in the chemical for hermaphrodites (M=129.29, SD=141.31), t(82)=0-1.46, p=0.15, two-tailed t-test. It appears that ascr#8 causes attraction in both males and hermaphrodites. The average time spent in ascr#8 was not significantly different between the males and the hermaphrodites, unlike ascr#3 (Figure 7 and Table 2).

4.2 Response to small molecules as an effect of nutritional state

Starvation assays were conducted to see if starvation had an effect on the hermaphrodite and male response to the chemical. The response to small molecule ascarosides as a result of nutritional state of both males and hermaphrodites grown on regular agar plates was observed using a starved behavior assay. The averages were derived from multiple days of testing which consisted of 5 assays each day. The average time of each interaction with the chemical was calculated. Only ascr#8 was observed in these assays based on the results of the previous experiments that there was a significant difference between hermaphrodites and males in the response to ascr#3 but no significant difference in response to ascr#8 between hermaphrodites and males. Ascr#8 was focused on for the rest of the project in order to determine if nutritional state could cause sex-specific behaviors since there was no original difference in response.

4.2.1 Males on agar plates

In order to see if starvation had an effect on male response to ascr#8, t-tests were used to check for significance. The mean time spent in the chemical (ascr#8) for starved males (M=29.73, SD=344.39) was significantly different than the time spent in the control (M=96.39, SD=123.36), t(36)=-2.56, *p*=0.015, two-tailed t-test. The mean time spent in the chemical for well-fed males (M=94.88, SD=73.77) was significantly different than the time spent in the chemical for starved males (M=296.73, SD=344.388), t(73)=-4.24, *p*=0.001, two-tailed t-test .It appears that the starved worms still show an attraction to the chemical ascr#8. The average time spent in the chemical for starved males was higher than that of fed males (Figure 8 and Table 3).

4.2.2 Hermaphrodites on agar plates

To compare different sexes, hermaphrodites were also used on starvation assays. In order to see if starvation had an effect on hermaphrodite response to ascr#8, t-tests were used to check for significance. The mean time spent in the chemical for starved hermaphrodites (M=86.57, SD=56.33) was not significantly different than the time spent in the control (M=144.3, SD=193.94), t(73)=-4.24, *p*=0.001, two-tailed t-test. The mean time spent in the chemical for well-fed hermaphrodites (M=129.29, SD=141.31) was not significantly different than the time spent in the chemical for starved hermaphrodites (M=86.57, SD=56.33), t(29)=0.77, *p*=0.45, two-tailed t-test. It appears that the hermaphrodites are no longer attracted to the chemical after being starved. This is the opposite response of the males. While the males were attracted to the chemical more after starvation, it appears that the hermaphrodites have lost attraction (Figure 9 and Table 4).

4.3 Effects of carbohydrates on ascr#8 responses

Carbohydrates were investigated to determine if they played a significant role in the social response to ascr # 8. To do this, males and hermaphrodites were grown on glucose plates.

To determine the effect of growing worms on glucose plates, the response to small molecule ascarosides of both males and hermaphrodites grown on glucose plates was observed with a fed and starved behavior assays. *C.elegans* were grown on glucose plates for two generations before being used for testing. The averages were derived from multiple days of testing which consisted of 5 assays each day. The average time of each interaction with the chemical was calculated.

4.3.1 Males on glucose plates

Males were grown on glucose plates and t-tests were conducted to see if their response to ascr#8 was significant. The mean time spent in the chemical for well-fed males grown on glucose plates (M=80.36, SD=69.52) was significantly different than the time spent in the control (M=38.03, SD=23.45), t(81)=-3.62, p=0.001, two-tailed t-test. The mean time spent in the chemical for starved males grown on glucose plates (M=207.06, SD=287.77) was marginally significantly different than the time spent in the control (M=76.58, SD=51.71), t(34)=-1.95, p=0.060, two-tailed t-test. The mean time spent in the chemical for well-fed males grown on glucose plates (M=80.36, SD=69.52) was significantly different than the time spent in the chemical for starved males grown on glucose plates (M=207.06, SD=287.77), t(59)=-2.75, p=0.008, two-tailed t-test. It appears that the starved worms still show an attraction to the chemical ascr#8. The average time spent in the chemical for starved males was higher than that of fed worms. These results are the same as the fed and starved males grown on normal plates indicating that growing males on glucose plates does not seem to make a difference in the response to ascr#8. It still lends to the question of why starved males are more attracted to the chemical than fed worms (Figure 10 and Table 5).

4.3.2 Hermaphrodites on glucose plates

Hermaphrodites were also grown on glucose plates to test for any sex-specific differences and to compare to agar plates. The mean time spent in the chemical for well-fed hermaphrodites

grown on glucose plates (M=132.21, SD=216.49) was marginally significantly different than the time spent in the control (M=57.43, SD=80.32), t(68)=-1.20, *p*=0.05, two-tailed T-test. The mean time spent in the chemical for starved hermaphrodites grown on glucose plates (M=78.06, SD=71.10) was not significantly different than the time spent in the control (M=69.88, SD=58.64), t(31)=-0.36, *p*=0.72, two-tailed T-test. The mean time spent in the chemical for well-fed hermaphrodites grown on glucose plates (M=132.21, SD=216.49) was not significantly different than the time spent in the chemical for starved hermaphrodites grown on glucose plates (M=78.06, SD=71.10), t(47)=0.97, *p*=0.34, two-tailed T-test. It appears that the hermaphrodites are no longer attracted to the chemical after being starved. This is the opposite response of the males. While the males were attracted to the chemical more after starvation, it appears that the hermaphrodites have lost attraction. This is the same result for regular plates indicating that growing the hermaphrodites on glucose did not make a difference. It seems that's growing the hermaphrodites and males on glucose plates did not change their response to the chemical (Figure 11 and Table 6).

4.4 Effect of glucose plates on *Cel-daf-2* strain

A mutation of *C.elegans* was observed in order to try to reveal the biochemical pathway that mediates ascaroside response. T-tests were performed to look for significant differences. In order to determine if glucose plates had an effect on the *daf-2* mutant strain of *C. elegans*, a fed behavior assay was carried out on agar plates and glucose plates. The mean time spent in the chemical for well-fed *daf-2* hermaphrodites grown on agar plates (M=238.0, SD=362.48) was not significantly different than the time spent in the control (M=69.12, SD=109.16), t(26)=-1.81, *p*=0.08, two-tailed test. The mean time spent in the chemical for well-fed *daf-2* hermaphrodites grown on glucose plates (M=111.17, SD=172.14) was not significantly different than the time

spent in the control (M=145.11, SD=12.18), t(28)=0.62, *p*=0.54, two-tailed t-test. The mean time spent in the chemical for well-fed *daf-2* hermaphrodites grown on agar plates (M=238, SD=362.48) was not significantly different than the time spent in the chemical for well-fed *daf-2* hermaphrodites grown on glucose plates (M=111.17, SD=172.14), t(21)=1.09, *p*=0.29, two-tailed t-test. On both agar and glucose plates, there was no significant response to the chemical. It appears that growing *daf-2 C. elegans* on glucose plates did not make a significant difference in their response to the chemical (Figure 12 and Table 7).

4.5 The effect of re-feeding on the response to ascr#8 of hermaphrodites

Hermaphrodites in the fed state had an attraction to the chemical while those in the starved state did not. This led to the experiment of starving and re-feeding the hermaphrodites to see if the *C. elegans* would regain their response after being fed again. In order to determine if starvation is reversible, experiments in which *C. elegans* were starved for 1 hour and re-fed for 50 minutes were conducted. It appears that there is more activity from the hermaphrodites after being re-fed but more experiments need to be conducted (Figure 13 and Table 8).

5. Discussion and Conclusion

The aim of this project was to discover if nutritional state had an effect on social behavior. This was conducted using *C. elegans* and their behavioral response to small endogenously produced molecules called ascarosides. In particular, sex-specific behaviors were investigated. A further point of inquiry was the biochemical pathway that mediates the ascaroside response. It was discovered through this project that both males and hermaphrodites were attracted to ascr#3 and ascr#8 in the fed condition. After starvation, hermaphrodites were no longer attracted to ascr#8 while males still were, indicating that nutritional state affects social behavior and is sex-specific. Growing hermaphrodites and males on glucose did not affect their response to ascr#8, indicating that carbohydrates do not play a role in social signaling response. Finally, in both conditions of growing the *daf-2* mutants on glucose and agar plates, there was no response to ascr#8 demonstrating that the *daf-2* gene is essential in the physiology of nutrition in *C. elegans*.

It is known that ascarosides play a factor in sexual attraction and that males are attracted to hermaphrodites by these small molecule signals. Research has also shown that ascr#3 is more attractive to males than it is to hermaphrodites (Srinivasan, 2008). The data for this project also supports this claim as the mean time spent in the chemical was significant for both males and hermaphrodites but higher for males. Males spent about 2.3 times longer in the ascr#3 spot than the hermaphrodites. It makes sense that males would be more attracted to the chemical since they cannot reproduce on their own and therefore would be attracted to chemical signals.

Both fed hermaphrodites and males were significantly attracted to ascr#8, although unlike ascr#3, males were not anymore attracted to ascr#8 than hermaphrodites were. The structure of ascr#8 is unique from several other ascarosides that have been identified in that it forms an amide with a seven-carbon α,β-unsaturated fatty acid. Ascr#8 was focused on in this project to

look at the effect of nutritional state since it was not significantly more attractive to males or females.

After starvation, males displayed the same favoring response to ascr#8 as they had when they were fed. In fact, starved males spent about 3.12 times longer in the ascr#8 than the fed males. On the other hand, after starvation hermaphrodites were no longer attracted to ascr#8. This indicates that there is a sex-specific difference in males and hermaphrodites involving starvation. Whereas males were more heavily attracted to the chemical, hermaphrodites lost attraction altogether. A hypothesis that could explain this behavior is that males know that they need to find a mate quickly in order to pass along their genes before they are completely starved. In the case of hermaphrodites, they may no longer be attracted to the chemical in order to conserve energy.

In order to determine the effects of carbohydrates on the ascr#8 response, hermaphrodite and male *C. elegans* were grown for two generations on glucose plates. The fed and starved behavioral assays were then carried out again. The results were similar to those of the *C. elegans* grown on regular plates. The males were still attracted to the chemical, even more so while starved, while the hermaphrodites lost attraction after starvation. This indicates that carbohydrates did not play a significant role in the social response to ascr#8.

The results for *daf-2* showed that there was not a significant change in the response to ascr#8 for *daf-2* hermaphrodites grown on agar plates as compared to grown on glucose plates. Neither the *daf-2* hermaphrodites grown on the glucose plates nor the *daf-2* hermaphrodites grown on the agar plates spent significantly more time in the chemical than the control. *Cel-daf-2* hermaphrodites acted as starved hermaphrodites did, as they did not show a response to the

chemical. Therefore, the *daf-2* gene and insulin signaling pathway is essential to the fed physiology of *C.elegans*.

There are many factors that can be considered when thinking about future experimentation. Some aspects that should be considered is changing the concentration of the ascaroside or using a different ascaroside to elicit responses. There are several other ascarosides to be tested. In addition, it has been shown that mating signals are made up of a synergistic blend of ascarosides and therefore testing them together may provide different results (Srinivasan, 2008). Continuation with *daf-2 C. elegans* would be of interest to study especially when looking at starvation assays. This would provide more information on the insulin signaling pathway and its potential role in social signaling. Other mutations may also be of interest to elucidate the biochemical pathways that mediate ascaroside response. Notably, more experiments involving re-feeding are important for future research. It was shown that hermaphrodites are attracted to the chemical when fed but lose attraction when starved. It would be interesting to conduct more experiments in which the *C. elegans* are starved and re-fed to see if the behavior is restored indicating that starvation is reversible. There are several directions in which this research project can be taken.

This project has laid down the foundation of social behaviors as an effect of nutritional state in *C.elegans.* It has been shown that C.elegans are attracted to small molecule signals and that nutritional state can influence their responses. This establishment can lead to several more directions including further research about biochemical pathways that affect the physiology of nutrition and social behaviors. With these results as a basis, additional testing and analysis about nutrition and behavior can proceed.

Appendix A: Figures

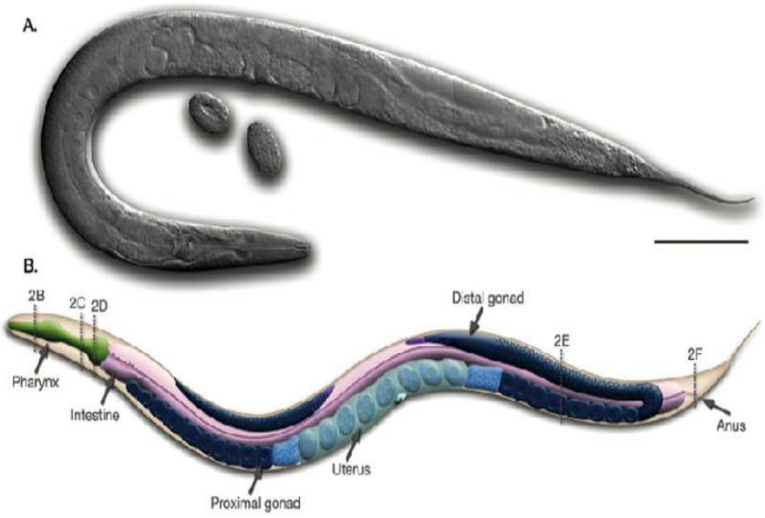


Figure 1: *C.elegans* hermaphrodite lateral view
(A) Differential Interference Contrast Micrograph of a hermaphrodite. Two embryos are shown. (B) Anatomy of the hermaphrodite. Major anatomical features are shown including the tapered off tail of the hermaphrodite. Source: (Corsi, 2006)

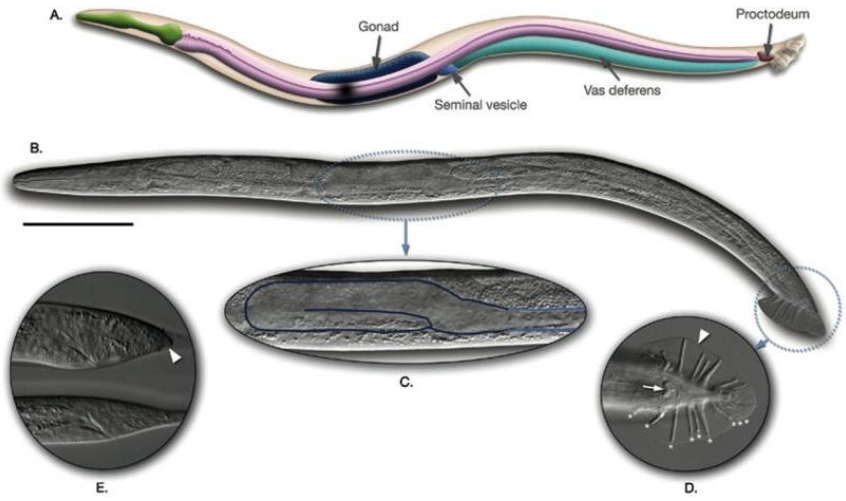


Figure 2: *C. elegans* male lateral view
(A) Anatomy of the male. Major anatomical features are shown including the broad tail of the male. (B)Micrograph of male. Source: (Corsi, 2006)

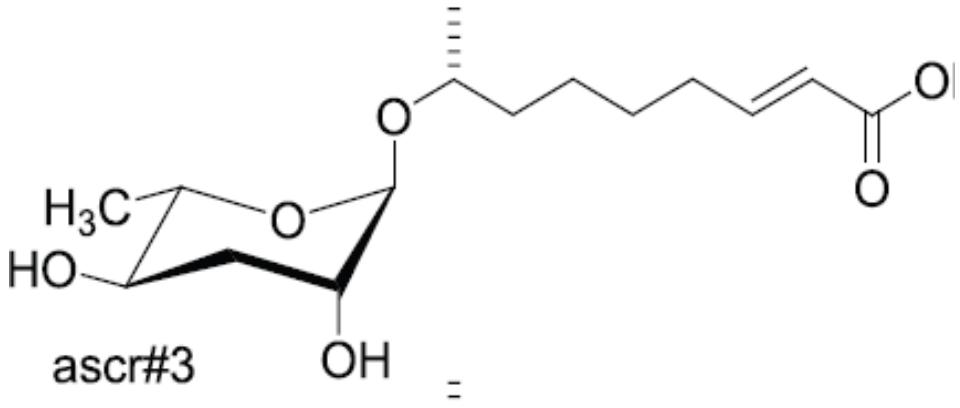


Figure 3: The Structure of Ascr#3

The structure of ascr#3 showing the ascarylose sugar and fatty-acid like chain. The structure consists of nine-carbon α,β-unsaturated carboxylic acid side chain.

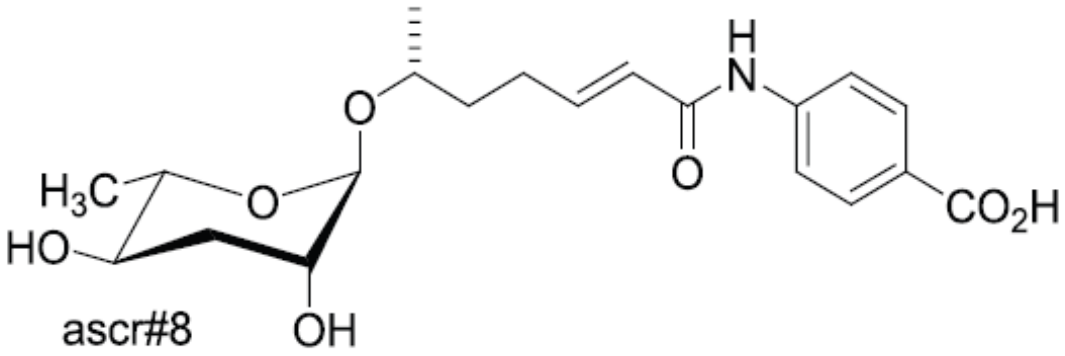


Figure 4: The Structure of Ascr#8

The structure of ascr#8 showing the ascarylose sugar and fatty-acid like chain. The structure consists of a paminobenzoic acid moiety, forming an amide with a seven-carbon α,β-unsaturated fatty acid.

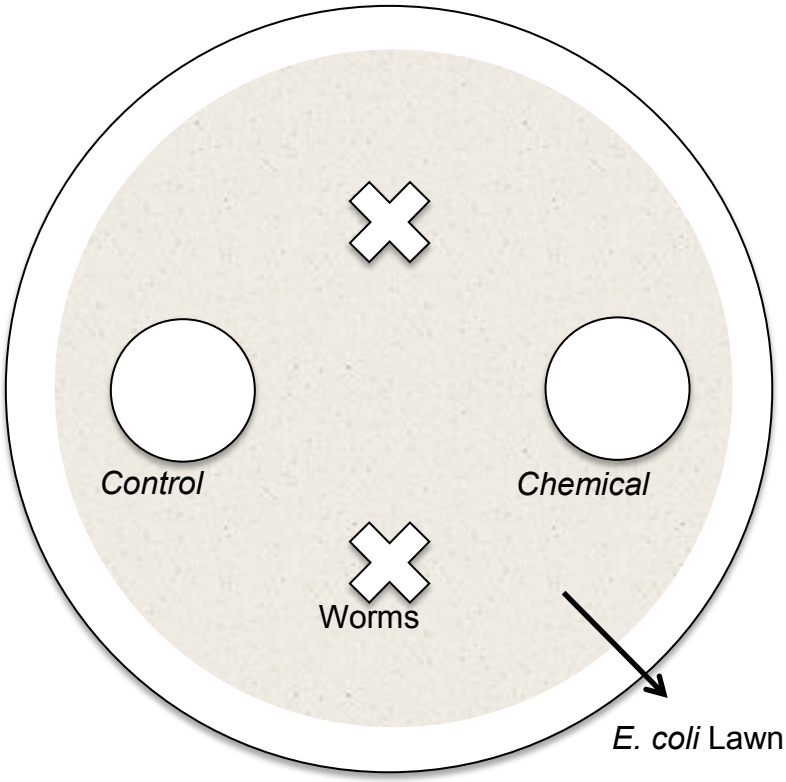


Figure 5: Template for Behavior Assay
The control was placed on the circle labeled C and the ascaroside was placed on the circle labeled Ch. Five *C. elegans* were placed on each X and a twenty minute video was recorded for each assay

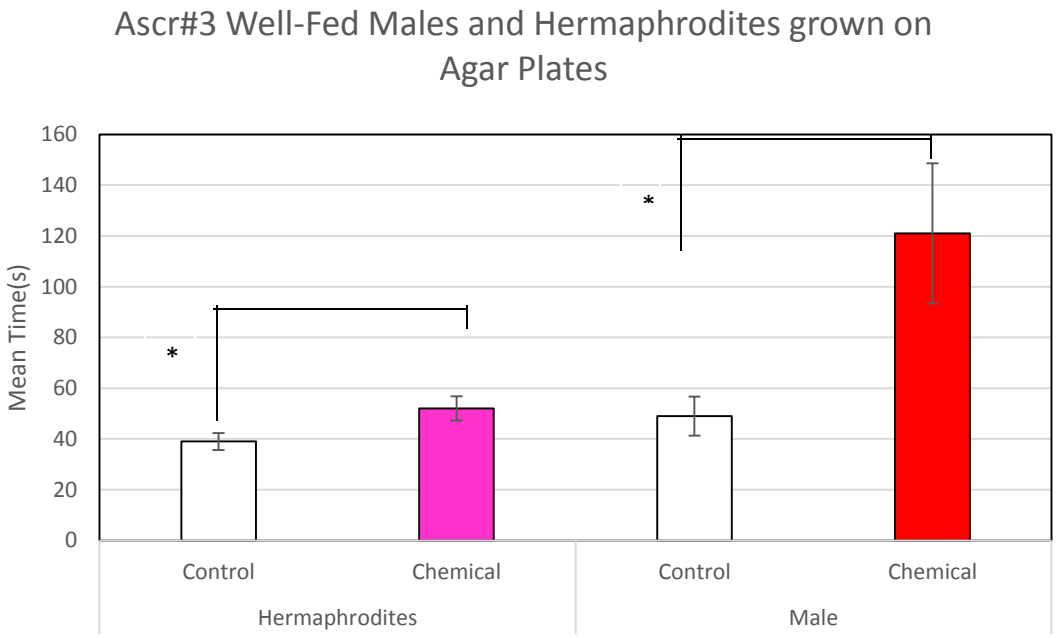


Figure 6: Mean time spent in the chemical (ascr#3) and the control for well-fed males and hermaphrodites fed on agar plates

Well-fed males ($p=0.03$) and hermaphrodites ($p=0.03$) grown on agar plates are both significantly attracted to ascr#3. The mean time spent in the chemical for males is higher than the mean time spent in the chemical for hermaphrodites indicating that males are more attracted to ascr#3 than hermaphrodites.

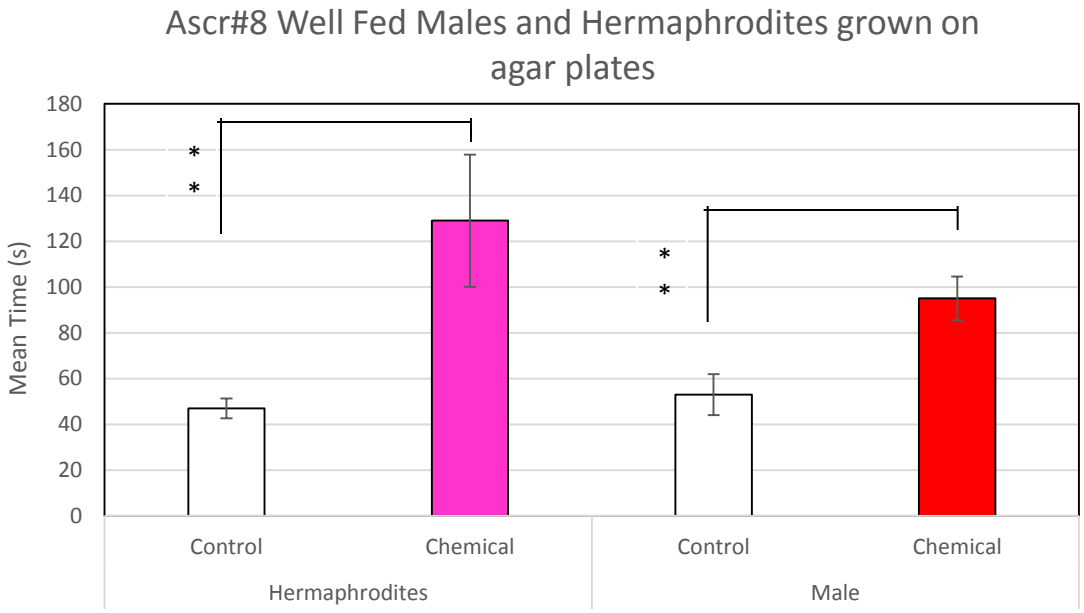


Figure 7: Mean time spent in the chemical (ascr#8) and the control for well-fed males and hermaphrodites fed on agar plates

Well-fed males (p=0.004) and hermaphrodites (p=0.004) grown on agar plates are both significantly attracted to ascr#8. The mean time spent in the chemical for the males was not significantly different than the mean time spent in the chemical for hermaphrodites.

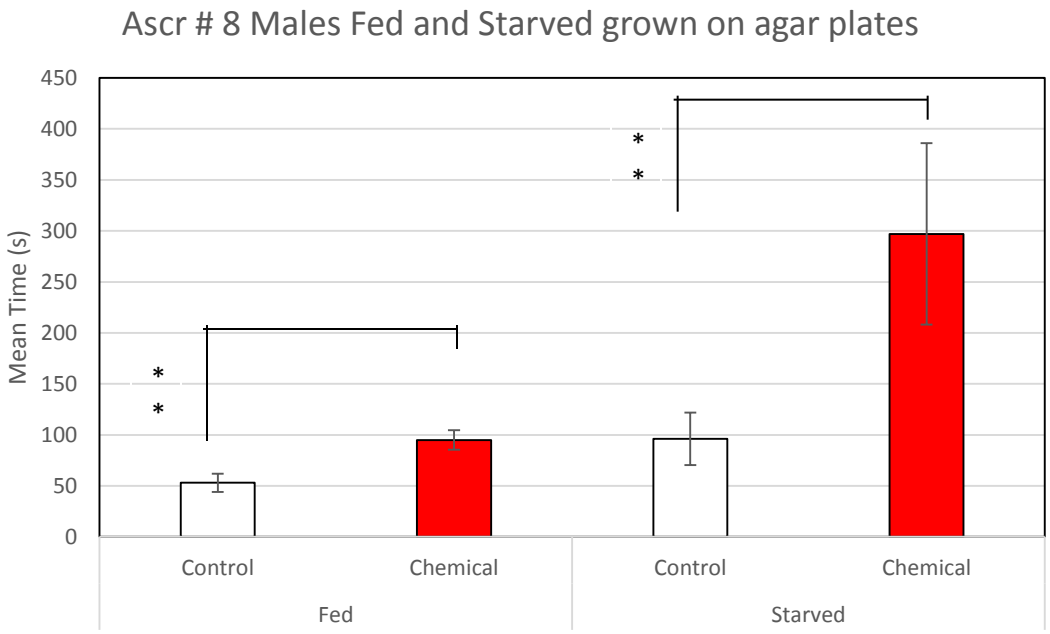


Figure 8: Mean time spent in the chemical (ascr#8) and the control for starved and fed males on agar plates

Starved males grown on agar plates ($p=0.01$) retained their response to ascr#8. The mean time spent in the chemical for starved worms was higher than that for fed worms.

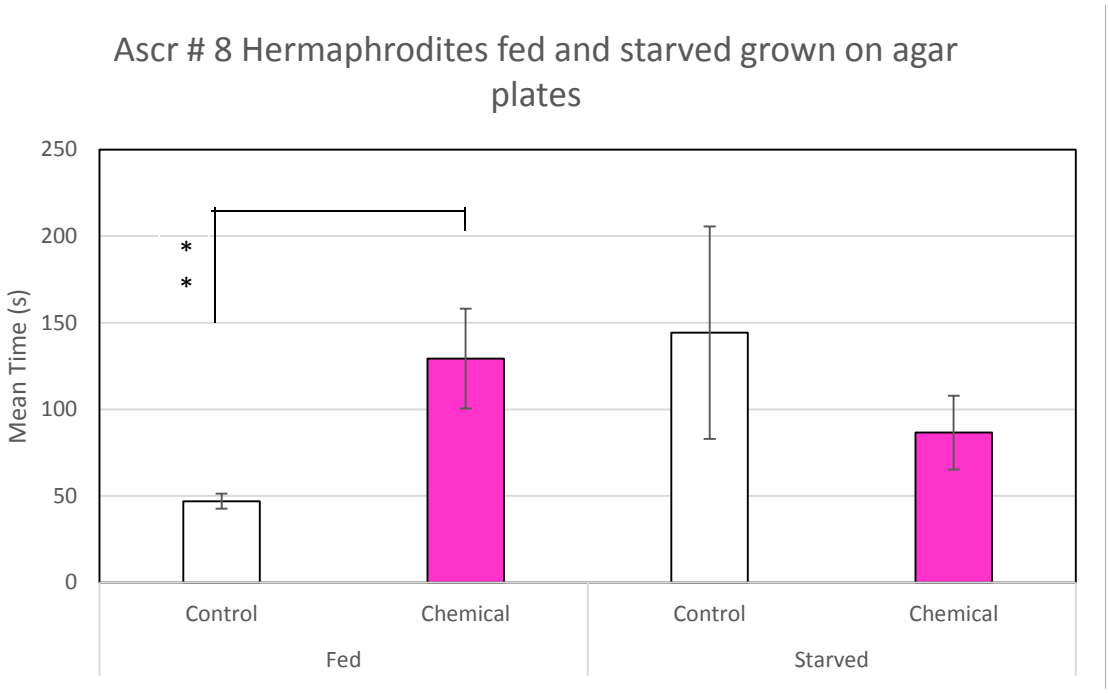


Figure 9: Mean time spent in the chemical (ascr#8) and the control for starved and fed hermaphrodites on agar plates

Starved hermaphrodites lost their attraction to ascr#8. There was a significant difference between the time spent in the control and the chemical for the fed condition (p= 0.004) but not for the starved condition.

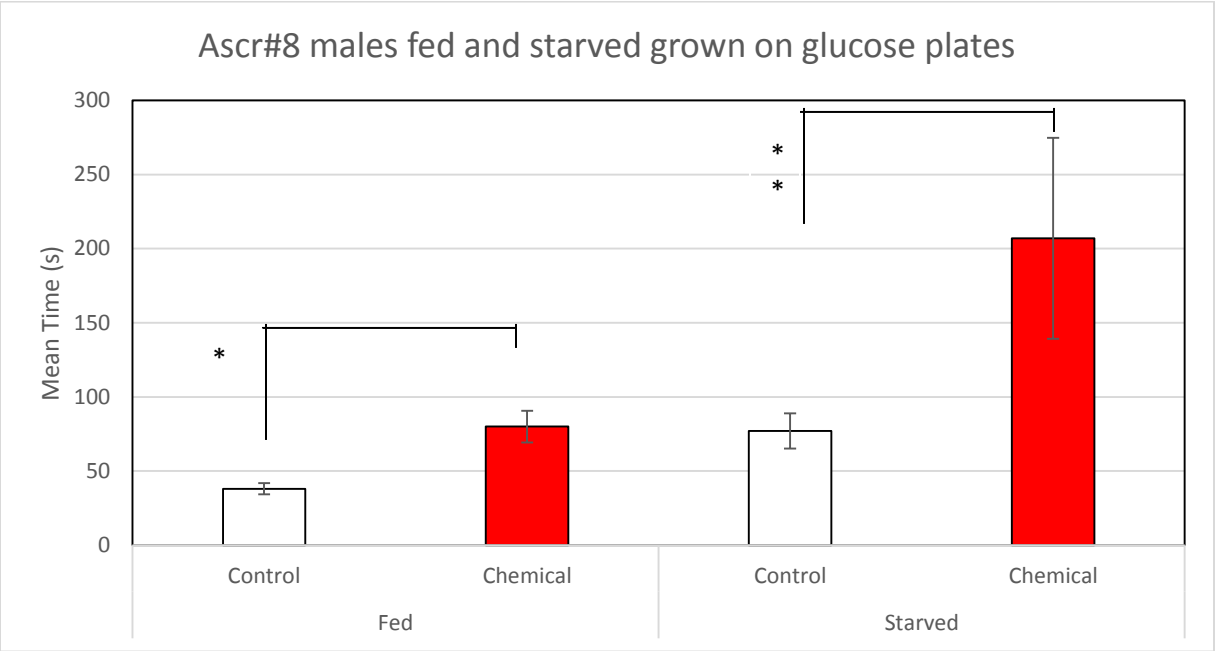


Figure 10: Mean time spent in the control and chemical (ascr#8) for fed and starved males grown on glucose plates

Starved males grown on glucose plates retained their response to ascr#8. The mean time spent in the chemical for starved worms was higher than that for fed worms. Both fed (p=0.001) and starved males (p=0.06) responded similarly when grown on glucose plates and when grown on agar plates.

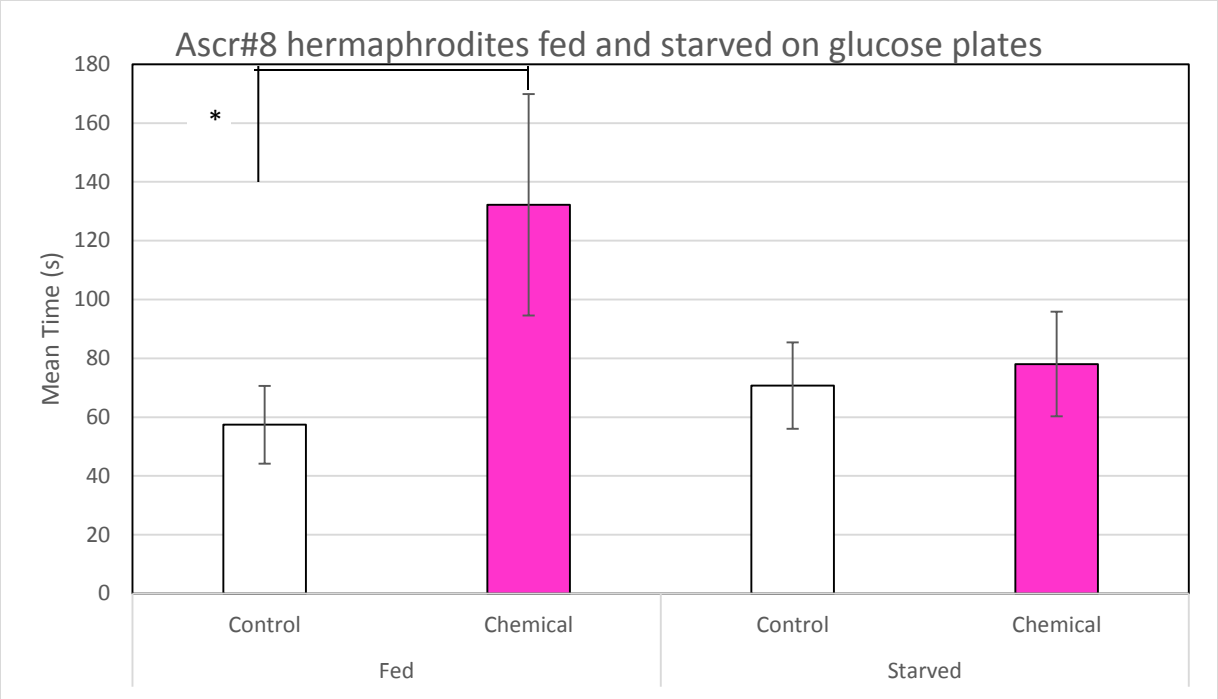


Figure 11: Mean time spent in the control and chemical (ascr#8) for fed and starved hermaphrodites grown on glucose plates

Starved hermaphrodites lost their attraction to ascr#8. There was a significant difference between the time spent in the control and the chemical for the fed condition ($p=0.05$) but not for the starved condition. ($p=0.72$) Both fed and starved hermaphrodites responded similarly when grown on glucose plates and when grown on agar plates.

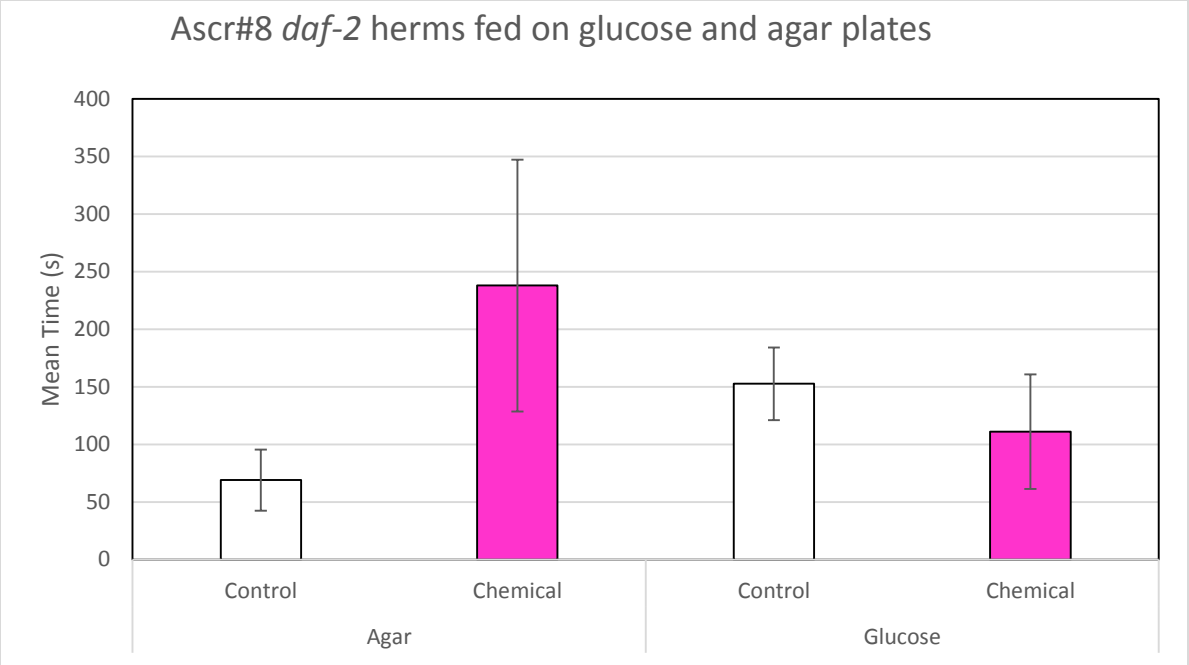
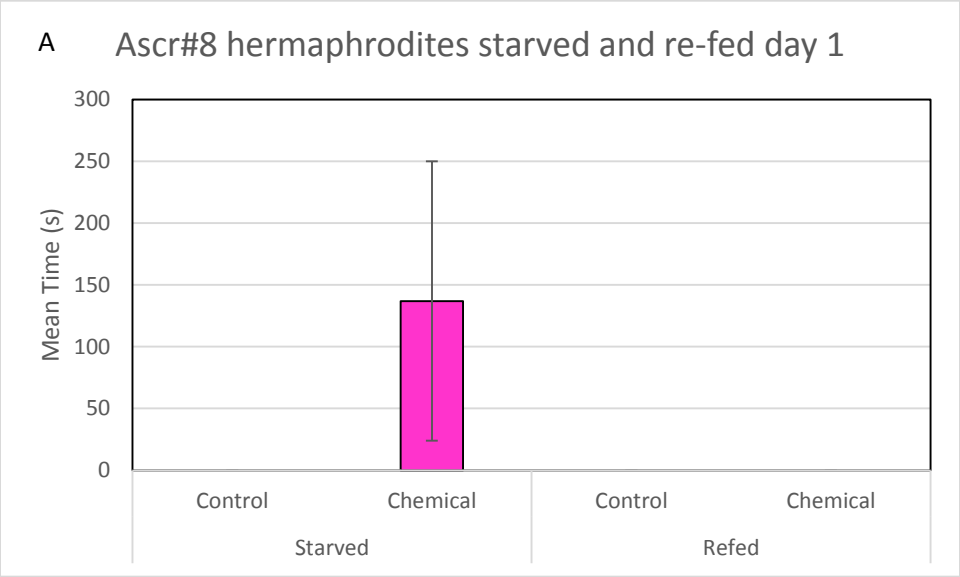


Figure 12: Mean time spent in the control and chemical (ascr#8) for *daf-2* hermaphrodites on agar and glucose plates

The *daf-2* hermaphrodites did not spend a significantly different time in the chemical as compared to the control on either the agar plates (p=0.081) or glucose plates (p=0.539).



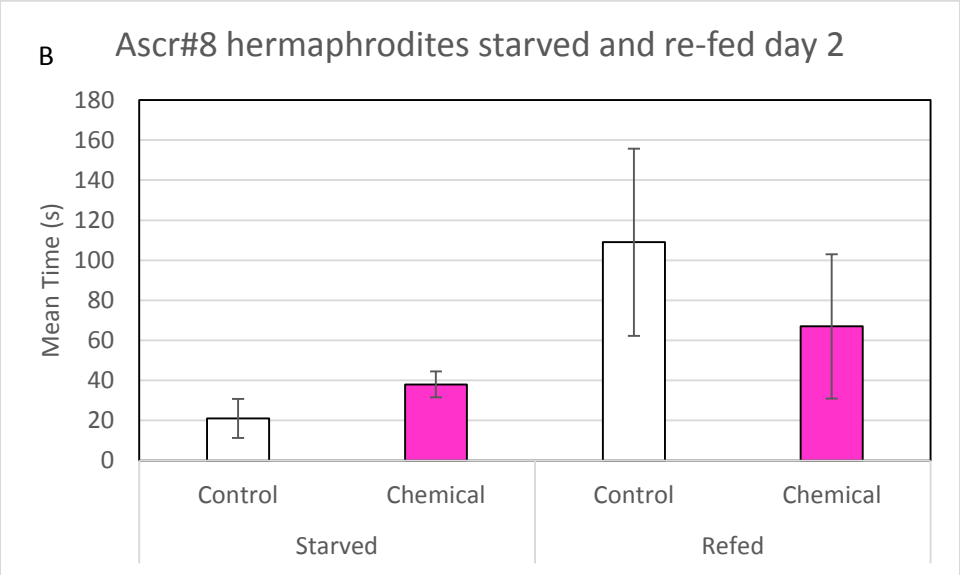


Figure 13: Mean time spent in the control and chemical (ascr#8) for starved and re-fed hermaphrodites

(A) Hermaphrodites were attracted to the chemical after being starved and showed no response after being re-fed for experiment 1. (B) There was no significant difference in time spent in the chemical as compared with the control for starved or re-fed hermaphrodites.

Appendix B: Tables

Table 1: Mean time, standard deviation, number of worm interactions and standard error for well-fed males and hermaphrodites fed on agar plates in response to ascr#3

		Mean time (s)	STDEV	n	SEM
Hermaphrodites	Control	39	28.14	72	3.32
	Chemical	52	38.79	65	4.81
Male	Control	49	48.35	39	7.74
	Chemical	121	197.37	51	27.64

Table 2: Mean time, standard deviation, number of worm interactions and standard error for well-fed males and hermaphrodites fed on agar plates in response to ascr#8

		Mean time (s)	STDEV	n	SEM
Hermaphrodites	Control	47	29.11	45	4.34
	Chemical	129	141.31	24	28.84
Male	Control	53	53.39	36	8.90
	Chemical	95	73.77	60	9.52

Table 3: Mean time, standard deviation, number of worm interactions and standard error for well-fed and starved males on agar plates in response to ascr#8

		Mean time (s)	STDEV	n	SEM
Fed	Control	53	53.39	36	8.90
	Chemical	95	73.77	60	9.52
Starved	Control	96	123.36	23	25.72
	Chemical	297	344.39	15	88.92

Table 4: Mean time, standard deviation, number of worm interactions and standard error for well-fed and starved hermaphrodites on agar plates in response to ascr#8

		Mean time (s)	STDEV	n	SEM
Fed	Control	46.96	29.11	45	4.34
	Chemical	129.29	141.31	24	28.84
Starved	Control	144.3	193.94	10	61.33
	Chemical	86.57	56.33	7	21.29

Table 5: Mean time, standard deviation, number of worm interactions and standard error for well-fed and starved males on glucose plates in response to ascr#8

		Mean time (s)	STDEV	n	SEM
Fed	Control	38	23.45	39	3.75
	Chemical	80	70.11	43	10.69
Starved	Control	77	51.71	19	11.86
	Chemical	207	287.77	18	67.83

Table 6: Mean time, standard deviation, number of worm interactions and standard error for well-fed and starved hermaphrodites on glucose plates in response to ascr#8

		Mean time (s)	STDEV	n	SEM
Fed	Control	57.43	80.32	37	13.20
	Chemical	132.21	216.49	33	37.69
Starved	Control	70.75	60.45	17	14.66
	Chemical	78.06	71.10	16	17.77

Table 7: Mean time, standard deviation, number of worm interactions and standard error for *daf-2* hermaphrodites grown on agar and glucose plates in response to ascr#8

		Mean time	STDEV	n	SEM
Agar	Control	69	109.16	17	26.48
	Chemical	238	362.48	11	109.29
Glucose	Control	152.63	132.12	18	31.42
	Chemical	111.17	172.14	12	49.69

Table 8: Mean time, standard deviation, number of worm interactions and standard error for starved and re-fed hermaphrodites day 1 and day 2

Day 1			Mean Time	STDEV	n	SEM
	Starved	Control	0	0	0	0
		Chemical	137	225.90	4	112.95
	Refed	Control	0	0	0	0
		Chemical	0	0	0	0
Day 2	Starved	Control	21	29.38	9	9.79
		Chemical	38	9.19	2	6.5
	Refed	Control	109	114.33	6	46.67
		Chemical	67	50.91	2	36

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