# DELAYED OUTCROSSING IN CAENORHABDITIS ELEGANS BY MATING AVOIDANCE BEHAVIOR

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A Thesis

Presented to

the Faculty of the Department of Biology and Biochemistry

University of Houston

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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By

Sneha Latha Koneru

May 2013

**DELAYED OUTCROSSING IN CAENORHABDITIS ELEGANS**

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# ACKNOWLEDGEMENTS

I wish to thank, first and foremost, my advisor, Prof. Ricardo Azevedo. I consider it a privilege to have worked with him. This project and thesis would not be possible without the support, encouragement, and endless patience of my advisor. He has been an inspirational mentor, and I hope to approach research with the same enthusiasm and motivation as he does. There are many valuable lessons, apart from the many academic skills, I learnt through my interactions with him that will no doubt help me throughout my life.

My deepest gratitude to my committee members: Profs. Blaine Cole, Meng Wang, and Timothy Cooper. Their guidance and constructive criticism helped immensely through various stages of the project and thesis. I am grateful to Prof. Weiwei Zhong for her time, and valuable discussions. I am indebted to her for letting me use the automatic microscopy system. I greatly appreciate the assistance provided by Aleman Meza-Boanerges. I would like to thank Profs. Elizabeth Ostrowski and Diane Wiernasz, for their advice and encouragement. My sincere thanks to the faculty of Ecology and Evolution for making the department a great place for research.

I express my heartfelt appreciation to my former and present research group– Maithili Gupte, Bingjun Zhang, Ata Kalirad, Jo West, Tiago Paixão, and Ilyas Patanam–for stimulating discussions, both academic and non-academic. I learnt more than I could offer to them. I am also grateful for their help, which they offered enthusiastically. I am particularly grateful to the assistance provided by Karla Melendez in the egg-counting assays and setting up mating assays. I thank her for her company during experiments that ran into the night. I am indebted to many colleagues

who helped me improve my presentation skills and scientific communication by their feedback. Thanks to Hongan Long, Kelly Phillips, Huy Vu, Yichen Zheng, Nicholas Price, Eric Bakota, Drew Russey, Lara Appleby, Kristen Dimond, Rebecca Satterwhite, Yinhua Wang, Trang Huyen, Fen Peng, Heather Votaw, Claire Manson-Bishop, Nancy Vanaphan, and Deepika Kumar.

I thank WormBase for maintaining an extensive bioinformatics database, which was a valuable resource for my project. I am grateful to the staff members of Biology and Biochemistry, especially Amanda Paul, Yonia Pulido, Rhonda James, and Rosezelia Jackson for helping me navigate graduate school smoothly. I would like to thank the Department of Biology and Biochemistry for supporting me as a graduate assistant during the course of my study.

Special thanks to Anjana Narayananan for bringing much joy and being a wonderful roommate in the short time I have known her. I am indebted to friends across the world for their help and support. I would like to thank Keertee Taneja, Andrea Colaco, Vikram Bodavula, Swetha Chaganti, Lahiri Ganti, Avinash Patlolla, Rajya Lakshmi Maram, Samarth Mohanty, Sindhura Ponnala, Shalini Gonnabathula, Shantan Deshpande, Kaushalya Murthy, Achaleshwar Sahai, Shravya Reddy, Naren Sudheendran, Meiling Wang, Halley Tej, Lekha Chowdary, Divya Parvataneni, and Ade Ajibade.

I am grateful to my parents who, in spite of their societal constraints, chose to educate me and supported me emotionally and financially. I cannot find words to express gratitude to my sister who has been the most gentle and kind person who only knows to love. Finally, and most importantly, I would like to thank my husband,

Vamsi Ganti. His encouragement to aim high and to be persistent at achieving those goals has allowed me to explore my potential. His companionship, unwavering support, and patience over the years have been instrumental in my academic pursuits. No words can do justice to his role in my personal and professional life.

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# Abstract

The species *Caenorhabditis elegans* has a mating system of androdioecy, which consists of hermaphrodites and males. The evolutionary pressures on the two sexes are different. *C. elegans* hermaphrodites make self-sperm during larval development and therefore are self-fertile, whereas the males have to mate with hermaphrodites to reproduce. Behaviors that increase the reproductive success of each sex may evolve to maximize fitness. Prior studies indicate that self-sperm exhausted hermaphrodites are more receptive to mating while a recent study suggests that males have a preference for sperm-depleted hermaphrodites. These observed behaviors are confounded with receptivity of hermaphrodites, male preference, and the effects of age. In this study, I present mating assays that attempt to disentangle the effects of age, receptivity of hermaphrodite, and male preference on mating success. In the mating assays, a higher proportion of sperm-depleted hermaphrodites mate compared to hermaphrodites that have sperm. During their self-fertile period, hermaphrodites actively avoid mating with males by sprinting away, thus, delaying outcrossing by mating avoidance. Hermaphrodites that are paralyzed due to mutations in their genes do not show mating avoidance behavior. Therefore, mating avoidance is an active behavior of hermaphrodites, which requires locomotion. The velocities of older hermaphrodites that are sperm-depleted are significantly higher than velocities of young hermaphrodites that have sperm. Therefore, older hermaphrodites are capable of mating avoidance but do not avoid mating because they are sperm-depleted. I conclude that sperm-status of the hermaphrodite is a strong predictor of mating

avoidance behavior. The sperm-sensing pathway of the hermaphrodites mediates the mating avoidance behavior by dynamically changing the behavior of hermaphrodites.

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# Glossary of Abbreviations and Acronyms

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **CGC** | Caenorhabditis Genetics Center |
| **df** | Degrees of freedom |
| **GFP** | Green fluorescent protein |
| **NGM** | Nematode growth medium |
| **CI** | Confidence Interval |
| **N2** | *C. elegans* wild type isolate |
| ***him*** | high incidence of males |
| ***fog*** | feminization of germline |
| ***lov*** | location of vulva defective |
| ***pkd*** | polycystic kidney disease |
| ***ceh*** | *C. elegans* homeobox |
| ***unc*** | Uncoordinated animals exhibit deviations in self-propelled movement on a solid medium compared to control animals |
| ***dpy*** | Dumpy animals are shorter and stouter than control animals at the same developmental stage |
| ***sma*** | Small animals are smaller than control animals at the same stage |
| ***spe*** | defective spermatogenesis |
| ***dbl*** | DPP/BMP-like |
| **PM** | Proportion of hermaphrodites mated |
| **Day 3** | Usually refers to a young hermaphrodite with sperm |
| **Day 5** | Usually refers to a sperm-depleted hermaphrodite |

# Chapter 1

# Introduction

## 1.1 Sexual conflict over mating in androdioecious mating systems

Androdioecious populations are rare in nature; to date only 50 species of plants and 36 species of animals have been found that have functional androdioecy (Charlesworth and Charlesworth, 1978; Charlesworth, 1984; Pannell, 1997, 2002; Weeks et al., 2006). Most animal androdioecious species are derived from gonochoristic ancestors (Cutter, 2008; Kiontke et al., 2004; Nayak et al., 2005; Sudhaus and Kiontke, 1996; Weeks et al., 2006). In such cases, males exist as a consequence of the genetics of sex determination inherited from the gonochoristic ancestral past. Hermaphrodites are self-fertile and do not have to mate to reproduce, whereas males need to mate with hermaphrodites to reproduce. This asymmetry in reproduction sets up a conflict over mating in androdioecious species and could be a determinant of the sex ratios in the population. This conflict is expected to drive the evolution of mating behaviors and morphological features that maximize the fitness of each sex.

The androdioecious species *Caenorhabditis elegans* and *Eulimnadia texana* are two models that can be used to study sexual conflict. These species consist of mostly self-fertilizing hermaphrodites and rare males. The presence of males at a low frequency in *C. elegans* populations remains an enigma (Anderson et al., 2010; Chasnov and Chow,

2002; Cutter and Payseur, 2003; Cutter and Ward, 2005; Stewart and Philips, 2002). The study of the mating conflict between hermaphrodites and males may help explain the proximate causes of the sex ratios in androdioecious species. I chose the *C. elegans* system to study the sexual conflict over mating due to the availability of genetic tools to manipulate hermaphrodite and male mating characteristics.

The central hypothesis of my study is that hermaphrodites show mating avoidance when they have the option to self fertilize. A recent theoretical study has concluded that selection is expected to favor hermaphrodites that avoid mating so long as the fitness of the self-progeny exceeds one-half of the fitness of the cross progeny (Chasnov, 2010). As far as we know from lab studies, this condition is met. For example, Dolgin et al. (2007) found that self-progeny had *higher* fitness than cross progeny from crosses among wild isolates of *C. elegans*. Mating avoidance could have evolved during the transition from gonochorism to androdioecy and the sexual conflict may be ongoing.

Consistent with the central hypothesis of this thesis, one study has reported that hermaphrodites in *C. elegans* actively avoid mating with males by sprinting away during their self-fertile period (Kleemann and Basolo, 2007). They also found that hermaphrodites that have run out of self-sperm do not avoid mating. The time to copulation is reported to be shorter in hermaphrodites with no sperm (Kleemann and Basolo, 2007). Another study found that *C. elegans* males have higher mating success with sperm-depleted hermaphrodites, albeit studied with a small sample size (Garcia et al., 2007). However, their experiments were designed to study male mating behavior and

were not suitable to study hermaphrodite mating behavior. The sperm depletion occurs on day 5 and thus, observed behavior of sperm-depleted hermaphrodites may be confounded with the effects of age and body size (Figure 1.1). Hermaphrodites approximately double in total body volume from day 3 (sexual maturity) to day 5 (the time they run out of self-sperm) (Knight et al., 2002, Lozano et al., 2006). The mechanism by which older hermaphrodites experience higher mating success is not understood. They could be secreting a pheromone that attract males or be receptive to mating following sperm depletion like their gonochoristic ancestor (Garcia et al., 2007; Lipton et al., 2004).

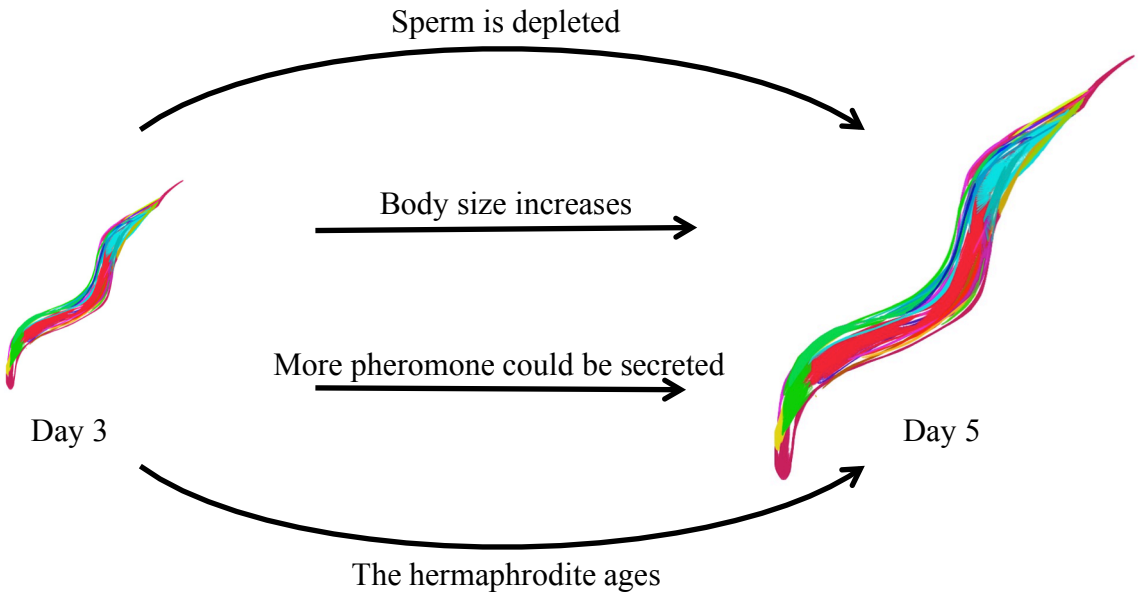



**Figure 1.1:** The changes in the hermaphrodite from sexual maturity (day 3) to the day they run out of sperm (day 5) that could affect mating avoidance behavior.

Mating avoidance in hermaphrodites is expected, however; it appears to be dependent on the sperm-status of hermaphrodites. The hermaphrodite's reproductive success is limited by its self-sperm. Therefore, the hermaphrodite may maximize its reproductive success

by mating avoidance during the self-fertile period and outcrossing later in life. However, whether outcrossing later in life is adaptive remains an open question. The mechanism by which the hermaphrodites switch to inhibition of mating avoidance could be a vestige of the mechanism in the gonochoristic ancestor and not an adaptation. There is evidence of coupling of absence of sperm and receptivity to mating in females of related gonochoristic species *C. remanei* (Garcia et al., 2007).

Recently, Morsci et al. (2011) have proposed that hermaphrodites with sperm do not actively avoid mating; instead, males prefer to mate with sperm-depleted hermaphrodites. They tested mutant males that are defective in their response to hermaphrodites, in the presence of hermaphrodites that are paralyzed due to a mutation. In this way Morsci et al. (2010) hoped to eliminate any effect of hermaphrodite locomotion. They found that males contacted sperm-depleted hermaphrodites at a higher rate than hermaphrodites that had sperm. They surmised that an, as yet unknown hermaphrodite-derived cue, released by sperm-depleted hermaphrodites stimulated male mating response.

Sperm-depleted hermaphrodites are older than hermaphrodites with self-sperm. A male preference for older hermaphrodites makes little evolutionary sense, opposed to mating avoidance by hermaphrodites with sperm. Males should evolve to prefer to mate with young hermaphrodites because the quality of the oocytes decreases with age in *C. elegans* (Andux and Ellis, 2008). Indeed, one would expect evolution of male behaviors that would allow them to identify younger hermaphrodites or evolution of their mating abilities to mate with a reluctant partner. Interestingly, a male preference for older

hermaphrodites could coevolve with mating avoidance shown by younger hermaphrodites. Inasmuch as males have a higher fitness if they mate with younger hermaphrodites, mating avoidance by those hermaphrodites may result in an advantage for the males that prefer to mate with their older counterparts.

The purpose of my thesis research was to disentangle the effects of size, age, male preference, and hermaphrodite receptivity on mating success in *C. elegans* by manipulating each of these factors individually (Figure 1.2). The hermaphrodite was viewed until recently as a passive partner during mating (Emmons 2006), and the male mating behavior has been described as overt and stereotyped. Since, male mating is considered the most complex behavior exhibited by *C. elegans,* it has been extensively studied from a neuroscience viewpoint (Barr and Garcia, 2006; Emmons 2006; Garcia et al., 2001, 2007; Lipton et al., 2004; Liu and Sternberg, 1995). However, relatively little is known about the hermaphroditic mating behavior. The goal of my thesis research is to investigate the hermaphroditic mating behavior and contribute to a better understanding of it.

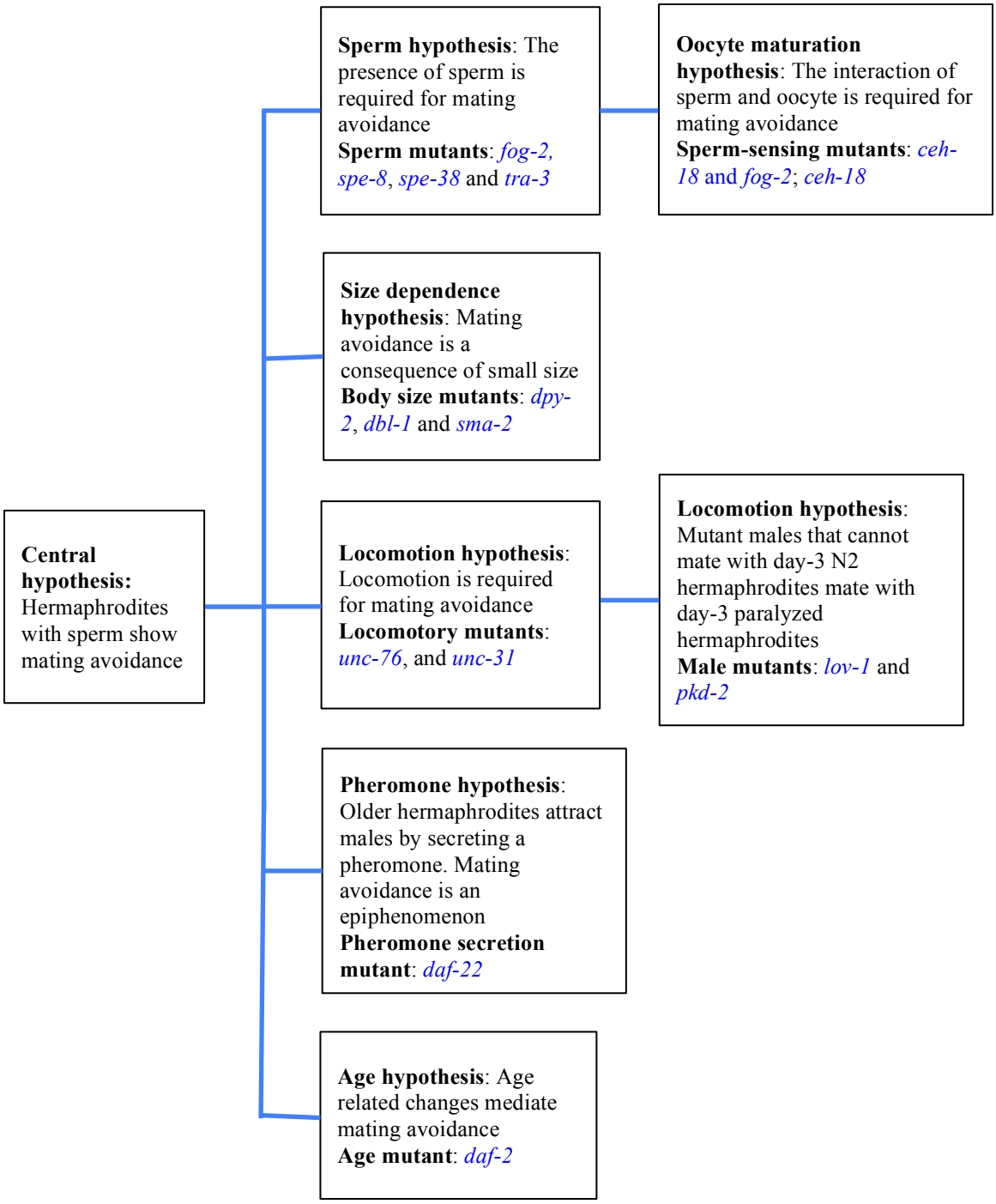



**Figure 1.2:** Experimental Design: A flowchart of the possible mechanisms that could mediate mating avoidance behavior. The experiments were conducted to test these individual hypotheses. The results presented in Section 3 are organized based on this flowchart.

## 1.2 Proximate and ultimate causes of low frequency of *C. elegans* males

Hermaphrodites avoid mating with males during their self-fertile period. However, mating avoidance cannot evolve when there are no males to avoid. Males are rare and have been found only a few times in nature (Barriere and Felix, 2005). They appear at a frequency of between 0.1% and 0.2% in lab conditions (Hodgkin and Doniach, 1997; Ward and Carrel, 1979). There are three possible explanations for the low frequency of *C. elegans* males. First, males may be maintained at the spontaneous nondisjunction rate of the sex chromosomes. Second, the males may be outcrossing occasionally, and there may be selection against outcrossed progeny. Third, the poor mating abilities of *C. elegans* males.

*C. elegans* has a chromosomal sex-determination system where, XX eggs develop as hermaphrodites and XO eggs develop as males. In the absence of mating, hermaphrodites produce males rarely by spontaneous nondisjunction of the X chromosome. The rate of appearance of males is thought to be due to the nonadaptive spontaneous nondisjunction of the sex chromosomes that was inherited from the gonochoristic ancestor (Chasnov and Chow, 2002; Stewart and Philips, 2002). If the males are maintained at the rate of spontaneous nondisjunction without any outcrossing in nature, then the genes responsible for male development, morphology and behavior should accumulate mutations and produce nonfunctional males (Jiang et al., 2001). There is at least one instance of nonfunctional males occurring in a population. The KR314 isolate from Vancouver,

Canada contained a mutation in *mab-23* gene, which is required for normal development of the male's tail, its copulatory organ (Hodgkin and Doniach, 1997). However, this solitary example is not strong evidence for the decay of the *C. elegans* male function.

Surprisingly, there is evidence in the *C. elegans* genome that the genes specific to male soma are conserved. There has been rapid evolution of genes involved in spermatogenesis compared to genes of other phenotypic traits, which is consistent with the operation of sexual selection (Cutter and Ward, 2005). Although, it can be argued that the *C. elegans* has not evolved as an androdioecious species for long enough to observe nonfunctional males (Barriere and Felix, 2005; Loewe and Cutter, 2008). In the evolution from gonochorism to androdioecy, males could have shaped hermaphroditic behaviors that avoid mating in order to self. If this is true, the mating avoidance behavior should decay along with the appearance of nonfunctional males.

Outcrossing rates estimated from patterns of genetic variation are highly variable ranging from 0 to 22% in populations sampled across the globe (Barriere and Felix, 2005; Cutter, 2006; Sivasundar and Hey, 2005). It should be noted, however, that only one study estimated a high outcrossing rate of 22% from sampling in California, USA (Sivasundar and Hey 2005). In evolution experiments, outcrossing was favored in populations under a high mutational load and when adapting to a novel environment suggesting a role for males in adaptation to transient stressful conditions in nature (Manoel et al., 2007; Morran et al., 2009a, 2009b). This mechanism could offset the accumulation of mildly

deleterious mutations that are expected to occur in strictly selfing species called Muller's Ratchet (Loewe and Cutter, 2008; Muller, 1964).

Based on the variable rates of outcrossing across the world, there should be more males sampled in nature. This paradox can be resolved by taking into account the potential disadvantages of outcrossing. For example, Seidel et al. (2008) found widespread genetic incompatibility in *C. elegans* populations maintained by balancing selection. Genetic incompatibilities reduce the fitness of the cross-progeny. The fitness of the inbred progeny might be higher than outbred progeny owing to outbreeding depression (Dolgin et al., 2007). Therefore, the potential disadvantages of outcrossing could maintain the mating avoidance behavior of the *C. elegans* hermaphrodites.

The low frequency of males in the N2 strain, in the lab, has been attributed to the poor mating ability of N2 males (Chasnov and Chow, 2002; Stewart and Philips, 2002). N2 is the wild-type reference strain for all work on *C. elegans*. The strain was isolated from a mushroom compost near Bristol, England and was maintained in the lab for approximately a decade before being cryopreserved (Hodgkin and Doniach, 1997). The strain could have evolved to lab conditions. Thus, the male frequency of N2 may not be representative of wild populations. A study of hermaphrodites sampled from across the world shows that the male frequencies maintained in populations propagated in lab conditions could vary by at least two orders of magnitude (Anderson et al., 2010; Teotonio et al., 2006). Therefore, males may be present in higher frequencies, in certain natural populations that drive the mating avoidance behavior of *C. elegans*. If this is the

case, variation in the mating avoidance behavior is expected. Mating avoidance behavior of hermaphrodites may be directly proportional to the number of males in the population.

The presence of males at low frequency and their role in facilitating adaptation, in contemporary *C. elegans* populations, remains an unresolved issue. During the transition from gonochorism to androdioecy, males may have been in mating conflict with hermaphrodites that consequently shaped their behavior. Hermaphrodites have an advantage of selfing, therefore, may continue to avoid mating with males evolved to outcross. There is just one study that presents evidence for the mating avoidance behavior shown by *C. elegans* (Kleemann and Basolo, 2007). Currently, there is a debate on whether hermaphrodites play an active or passive role in mating behavior. Kleemann and Basolo (2011) present a case that hermaphrodites actively avoid mating by sprinting away and Morsci et al. (2011) present the case that hermaphrodites are passive in that they secrete a mating cue to modify male behavior. Further investigation is required to resolve the nature of hermaphroditic mating behavior in order to get a comprehensive view of the mating behavior of *C. elegans* species. In my thesis, I attempt to understand the nature of hermaphroditic mating behavior.

## 1.3 General biology of *C. elegans*

In the following section, I provide an overview of the *C. elegans* life cycle and its mating behavior to provide context for the work presented in this thesis.

### 1.3.1 Life cycle

*C. elegans* is used extensively as a model system in cell biology, development, genetics, genomics, neurobiology, and evolutionary biology. As a model system, it has many salient features: it is transparent, hermaphrodites have an invariant 959 somatic cells, a simple nervous system consisting of 302 neurons, short generation time of 3.5 days at 20°C, hermaphroditic mode of reproduction, and ability to survive cryo preservation.

*C. elegans* has a rare mating system of androdioecy composed of hermaphrodites and males. The population is predominantly hermaphroditic; males appear at a frequency between 0.1% and 0.2% in lab conditions (Hodgkin and Doniach, 1997; Ward and Carrel, 1979). *C. elegans* hermaphrodites cannot mate with other hermaphrodites. Hermaphrodites are protandrous; their gonads make ≈ 300 self-sperm during larval development and then irreversibly switch to oocyte production. They are limited by their self-sperm and produce only ≈ 300 self-progeny. The progeny are exclusively hermaphrodites except for the rare male produced by spontaneous nondisjunction of the X chromosome. However, hermaphrodites being female in soma can mate with male and produce additional progeny, up to 1400, half of which are males (Kimble and Ward, 1988; Mendenhall, 2011; Wu et al., 2012). Oocytes of *C. elegans* are surrounded by

ovarian sheath cells, and in the absence of sperm, are arrested in meiotic prophase (Ward and Carrel, 1979). Major Sperm Protein (MSP) is a paracrine hormone released by sperm to stimulate oocyte maturation and the rate of gonadal sheath cell contractions (Miller, 2001). In the presence of sperm, maturation, and ovulation occurs continuously until the sperm are exhausted (Ward and Carrel, 1979). Self-sperm are used to fertilize about 300 oocytes that are laid over a period of three days (Mendenhall et al., 2009).

*C. elegans* is robust; it can survive months of starvation in the lab by switching into an alternative developmental pathway called the dauer. In the absence of a food source, the larvae switch into dauer pathway allowing them to survive after desiccation and a host of environmental insults for days to months. When food becomes available, the dauer larvae quickly switch to regular development and reproduce.

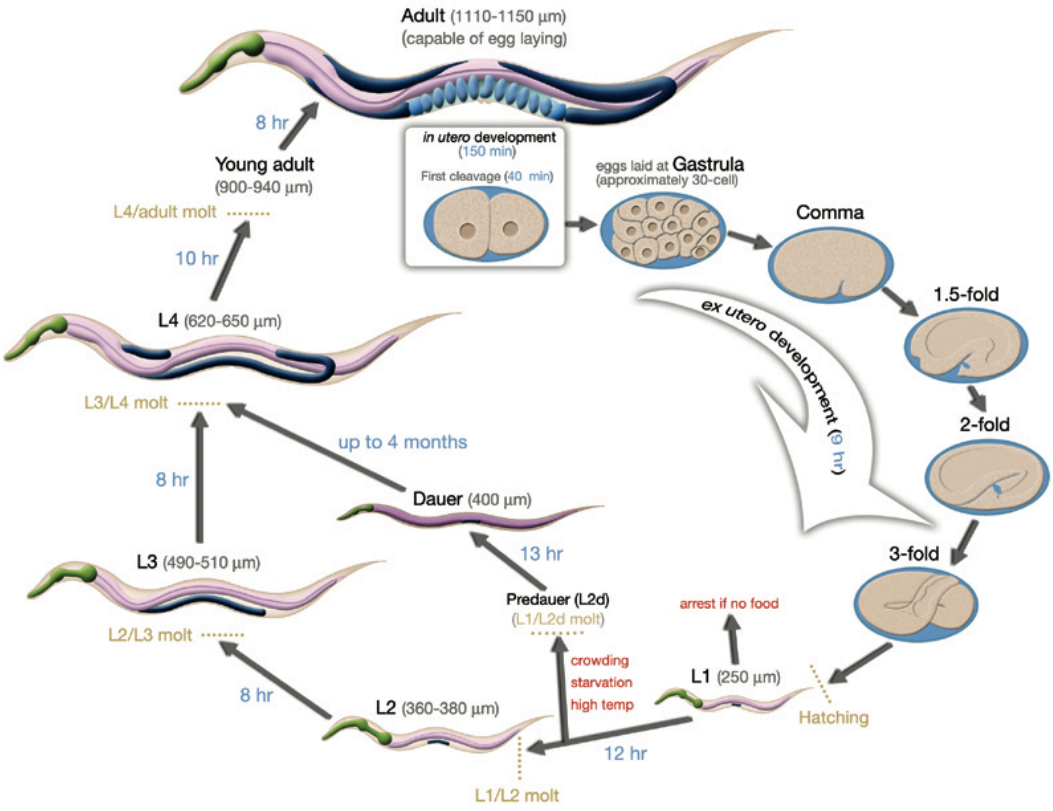



**Figure 1.3:** The life cycle of *C. elegans* at 22° C that takes about 45 hours from the time of eggs are laid, the numbers along the arrows indicate the time spent at every stage (Reprinted from wormatlas.org).

### 1.3.2 Mating behavior

Males use a combination of chemosensory and mechanosensory signals for locating and copulating with the hermaphrodite. It has been observed that hermaphrodites produce a pheromone that attracts males from a distance and retains males on the bacterial lawn (Simon and Sternberg, 2002; Srinivasan et al., 2008, 2012; White et al., 2007). In the absence of hermaphrodites, males leave the bacterial lawn and explore (Lipton et al., 2004).

The mechanosensory cues include the male's ability to sense the end of the hermaphrodite worm, which tapers at the head and the tail, to execute a sharp ventral coil to continue to be in contact with the hermaphrodite's body (Liu and Sternberg, 1995). The vulva is located on the ventral side of the hermaphrodite's body. The mechanosensory or chemosensory characteristics of the vulva of the hermaphrodite, are also required for the male's ability to copulate (Liu and Sternberg, 1995; Barr and Sternberg 1999; Barr and Garcia, 2006).

Like the mating sequence of the three-spine stickleback, the mating behavior of *C. elegans* male mating behavior can be studied as a series of stereotypical events as shown in Figure 1.4 (Tinbergen, 1951). Once the male has successfully located a hermaphrodite, the male initiates mating by placing his tail against the hermaphrodite's body to scan for the vulva by moving backwards. If the male reaches either the head or the tail of the hermaphrodite, the male makes a turn by a ventral coil and continues to scan for the vulva moving backwards. The male stops scanning when the male's tail locates the vulva, he coordinates his movements with the hermaphrodite's and inserts his spicules and ejaculates into the hermaphrodite's uterus (Liu and Sternberg, 1995; Garcia et al., 2001; Barr and Sternberg 1999; Emmons, 2006). The hermaphrodite's two gonadal arms share a single uterus. The sperm from the ejaculate migrate to the spermathecae of the hermaphrodite, and exclusively fertilize the oocytes displacing the self-sperm of the hermaphrodite (LaMunyon and Ward, 1995). The success of the mating sequence is dependent on how stationary the hermaphrodite is during mating (Garcia et al, 2007).

Males will often attempt to mate with other males, L4 stage hermaphrodites and even dead worms in the bacterial lawn.

My goal is to study mating avoidance in hermaphrodites that have self-sperm. I confirmed mating by the presence of cross-progeny because any behavior that is important in mating should cause an increase in the animal's fitness. One advantage of my approach is that I assayed mating success itself, not just behaviors associated with mating.

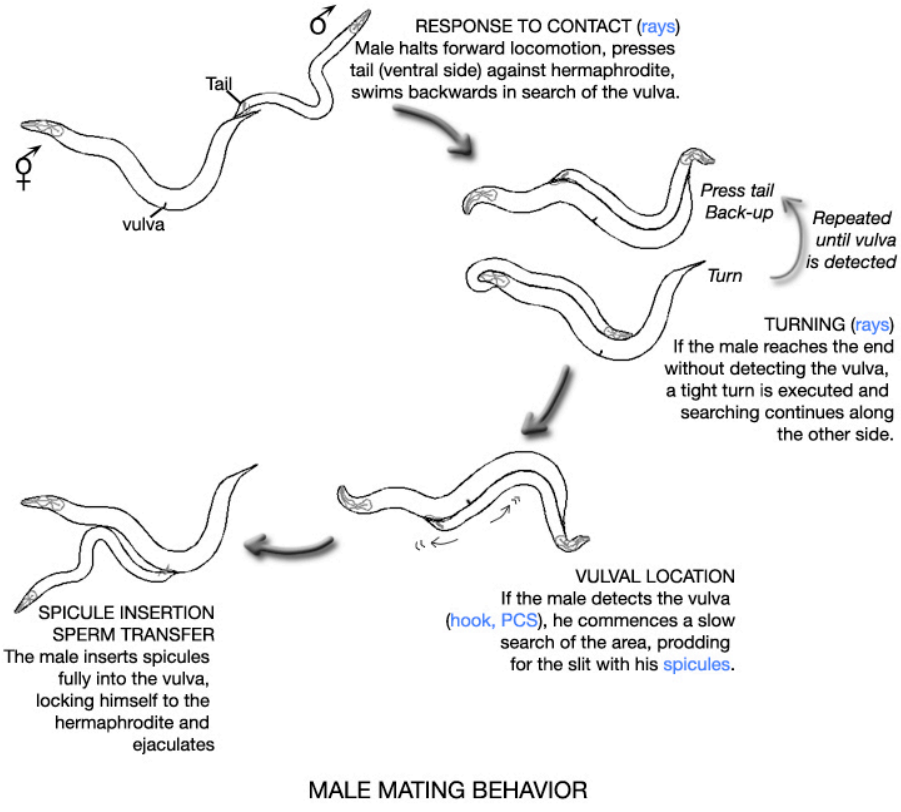



**Figure 1.4:** A schematic illustrating the male mating sequence in *C. elegans* (Reprinted from wormatlas.org).

# Chapter 2

# Methods

## 2.1 Maintenance of bacterial food source

*C. elegans* is typically maintained monoxenically using *Escherichia coli* strain OP50 (Brenner, 1974). OP50 is a uracil auxotroph whose growth is limited on the growth medium, which allows better mating of worms and easier observation. A starter culture of OP50 was obtained from the Caenorhabditis Genetics Center (CGC), St. Paul, MN. A nutritionally rich lysogeny broth (LB) was aseptically inoculated with a single colony of OP50 from the starter culture and allowed to grow overnight at room temperature (22° C). The liquid culture was stored at 4° C as back up. For mating assays and choice assays, liquid OP50 culture was incubated for no more than 12 hours at room temperature to ensure that the optical density of the culture at $OD_{600} \approx 1$.

## 2.2 Maintenance of *C. elegans*

Worms were cultured on 6 cm Petri plates (VWR® or BD Falcon™) containing Nematode Growth Medium (NGM-Lite, US Biological®) seeded with liquid OP50 culture (Brenner, 1974). The NGM Petri plates seeded with the OP50 culture were left on the bench for two days at room temperature for the bacteria to grow into a "lawn". These seeded NGM plates were stored at 4° C and were used within a month of their preparation. Worms were typically transferred every three days to new seeded NGM plates. Most of the strains were stored at 20° C with the exception of some temperature sensitive strains.

Worms have a high internal hydrostatic pressure and burst often when placed in water. M9 buffer is used to wash worms as it maintains an osmotic pressure of 300 mOsm (Starck and Brun, 1977). Additional buffers like M9, freezing and bleach solutions used were prepared according to the protocols from Stiernagle (2006).

## 2.3 Decontamination of *C. elegans* strains

From time to time, worm cultures would get contaminated with bacteria other than *E. coli* or fungi. This poses a problem for worm survival and thwarts observation. A mixture of 1N NaOH and 5% solution of sodium hypochlorite (bleach) in the ratio of 1:1 was used to decontaminate stocks. A drop of bleach solution was placed on the agar of an uncontaminated seeded NGM Petri plate; gravid hermaphrodites from the contaminated culture are dropped into the bleach solution. Eggs can be used directly if gravid hermaphrodites are not available. Hatched larvae clean from the contamination would crawl into the OP50 lawn. The larvae were picked up and transferred to another clean seeded NGM plate.

## 2.4 Cryopreservation and recovery of *C. elegans* strains

Worms can be frozen and stored at –80° C or liquid nitrogen (–196° C) for several years (Brenner, 1974). Three to five seeded 9 cm NGM plates should be started with gravid hermaphrodites and checked over a period of the following week until all the bacteria have been consumed and the plate is crowded with worms at L1 and L2 stages. Each plate was washed with 2 ml of M9 buffer to transfer the worms into the solution; the M9

from all the washed plates was collected into a 10 ml BD Falcon™ conical tube. An equal volume of freezing solution was added and mixed. This solution was divided into five aliquot parts and dispensed into NUNC® cryo tubes. The cryo tubes were placed in a styrofoam container and placed immediately in –80° C freezer. The styrofoam container facilities a slow drop in temperature as opposed to flash freezing the worms, which is desirable during freezing. The cryo tubes containing the strain to be recovered were put in a beaker with tap water until the ice thawed. The thawed solution was transferred into two seeded 9 cm NGM plates and left at room temperate to dry. The viable worms can be picked after the liquid medium dries out in the plates.

## 2.5 *C. elegans* strains

Worm strains were procured primarily from CGC, St. Paul, MN and some were from Silencing Genomes, a worm repository at Cold Spring Harbor Laboratory, NY. The strains used in the study are presented in the Table 2.1.

| STRAIN | GENOTYPE | PHENOTYPE |
|---|---|---|
| AD271 | *spe-38 (eb44) I, him-5 (e1490) V, asEx78* | Hermaphrodites produce sperm that fail to fertilize oocytes. |
| BA785 | *spe-8 (hc40) I* | Hermaphrodites produce nonfunctional, non-motile sperm that fail to fertilize oocytes. |
| BE13 | *sqt-1 (sc1) II* | Left-handed roller. |
| BE93 | *dpy-2 (e8) II* | Dumpy, encodes an unusual cuticular collagen, left-handed roller. |
| CB4108 | *fog-2 (q71) V* | Feminization of the germ line, hermaphrodites are converted to functional females. The strain was backcrossed ten times with N2. |
| CB1370 | *daf-2 (e1370) III* | Long-lived. |
| CB169 | *unc-31 (e169) IV* | Slow moving uncoordinated locomotion. |
| CB4419 | *tra-3 (e2333) IV* | Produce 500 rather than 330 self-progeny. Egg laying variant. |
| CB502 | *sma-2 (e502) III* | Small, endoreduplication mutant, extended reproductive life span. |
| DG1604 | *fog-2 (q71) V, ceh-18 (mg57) X* | Hermaphrodites make no sperm making them females, defects in oocyte cell cycle arrest, gonad migration and hypodermal differentiation. |
| DR476 | *daf-22 (m130) II* | Hermaphrodites are defective in short-ascaroside synthesis. |
| DR96 | *unc-76 (e911) V* | Uncoordinated locomotion. The body of worm coils when it attempts to move. |
| GR1034 | *ceh-18 (mg57) X* | Defects in oocyte cell cycle arrest, gonad migration, and hypodermal differentiation. |
| GR1373 | *eri-1(mg366) IV* | Temperature sensitive, sterile at 25° C. |
| CX10 | *osm-9 (ky10) IV* | Diacetyl chemotaxis defective. |
| MT6160 | *unc-76 (n2397) V* | Uncoordinated locomotion. |
| N2 | *Wild-type (Bristol)* | Reference strain. |
| NU3 | *dbl-1(nk3) V* | Short, endoreduplication mutant, extended reproductive life span. |
| PS3401 | *lov-1(sy582) II; him-5 (e1490) V* | Males are defective in response and vulva location mating behaviors. |
| PT8 | *pkd-2 (sy606) IV; him-5 (e1490) V* | Males are defective in response and vulva location mating behaviors. |
| SP346 | *4n* | Tetraploid, larger than wild-type. |

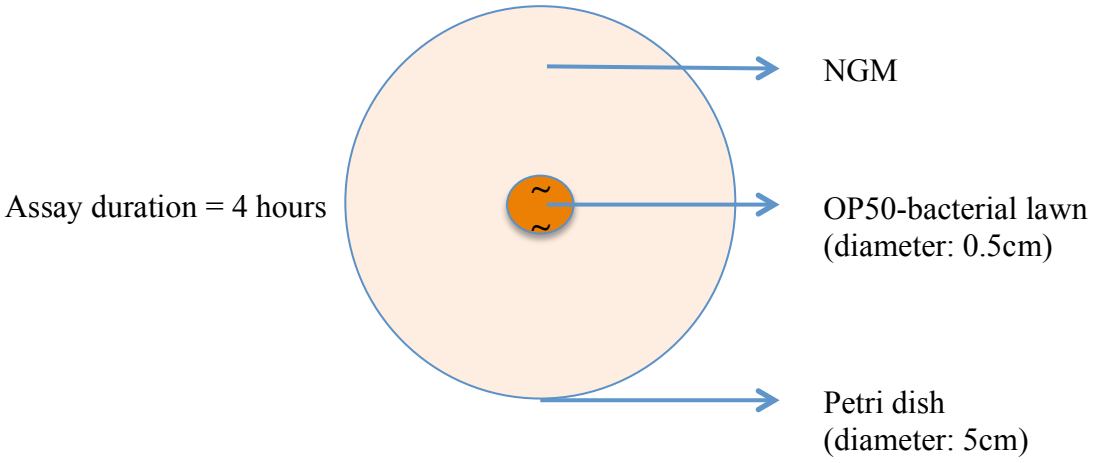
**Table 2.1:** A brief description of *C. elegans* strains used in the experiments (wormbase.org)

## 2.6 Mating assays

The aim of this assay is to evaluate the willingness to mate by hermaphrodites of a certain genotype and age. The age of a worm is counted from the time of egg laying (day 0; Herndon et al., 2002). Worms for the mating assays are collected as late L4s, that is, on day 2 at 20 °C (Plasterk, 1995). The mating assays are done 24-28 hours after being collected as L4s—day 3. The L4s worms picked up three days before the mating assay are day-5 adults.

Six cm Petri plates were used for this assay predominately, with the exception of one replicate for which 6-well cell culture plates were used. For each replicate, three days before the mating assay, NGM was autoclaved and poured into the plates and set on the bench to allow the medium to solidify. On the same day, at least thirty L4 stage hermaphrodites of the strains in a cohort be tested were picked and placed in seeded NGM plates. These became the day-5 animals (newly laid eggs were counted as day 0) for the purpose of the mating assay. These were transferred a day before the mating assay to a new set of seeded NGM plates to avoid overcrowding and starvation. A day before the mating assay, another thirty L4 stage hermaphrodites of the strains to be tested were picked up and these became the day-3 animals. Additionally, at least two hundred L4 stage N2 (wild-type) males or mutant males were picked and placed in seeded NGM plates.

The mating assay plates were seeded with 20µl of OP50 liquid culture at the center of the plate as shown in the Figure 3.1. A 20µl pipette fitted with 10µl tips was used to make a repeatable, uniform sized lawn. The seeded NGM plates are left on the bench overnight. On the day of the mating assay, a single hermaphrodite or female was placed in the lawn with a male for a period of four hours. After four hours, the males are killed. The experiment is done in a staggered fashion to allow time for manipulation.




$$\text{Proportion mated} = \frac{\text{Number of hermaphrodites mated in 4 hrs}}{\text{Total number of hermaphrodites assayed}}$$

**Figure 2.1:** Mating assay.

The plates were checked two days after the males were killed and scored for the presence of cross-progeny, i.e., the presence of >0.01% males. If mating has occurred, males have to be present at a frequency higher than the spontaneous nondisjunction frequency of 0.01%. In day-3 N2 worms, the presence of 3 males in ≈ 300 progeny is statistically significant and can only be a product of outcrossing. However, I used a more stringent measure of at least 10 males in the progeny to be scored as positive. I would like to

measure the likelihood of a hermaphrodite to mate and presence of 3 males is not convincing of prolonged mating.

Day-5 N2 worms produce about ≈ 4 progeny via self-fertilization on the day of the scoring if no mating occurred (Figure 3.7). Therefore, presence of 10-15 progeny is highly significant could be a result of mating. I would however, not score the plate as positive if 100% of the 10-15 progeny were hermaphrodites. If mating occurred, males have to be present. Thus, the presence of at least 3 males in 10 total progeny was counted as positive. All mutations used in the assays were recessive. Heterozygotes produced by mating with wild-type males would be wild-type for the trait. Therefore, the presence of wild-type hermaphrodites and males in an uncoordinated mutant plate was scored as positive. A few ambiguous cases arose and were carefully examined and scored based on the above criteria.

I tested mating of hermaphrodites mainly with N2 males. N2 is the reference strain for all *C. elegans* research and all the mutations were first isolated in this background. N2 males are considered inferior in their mating abilities compared to males from other natural isolates of *C. elegans* (Hodgkin and Doniach, 1997, LaMunyon and Ward, 1998). Ultimately, males from other natural isolates would have to be tested to understand the variability of mating avoidance with male mating ability.

## 2.7 Egg-counting assays

Some of the mutants did not lay all their eggs by day 3, posing a problem for testing the sperm hypothesis, which was contingent on worms having exhausted all their sperm on day 5. The egg-counting assay was done to estimate the amount of sperm present in day-5 hermaphrodites on the day of the mating assay.

In hermaphrodites, each oocyte is fertilized by only one sperm (Singson et al., 2001; Ward and Carrel, 1979). Therefore, we reasoned that the number of eggs laid after day 5 could be used as a proxy for the number of self-sperm in the hermaphrodite. This technique is valid only if the strain does not have egg laying defective phenotype. In egg laying defective mutants, fertilized eggs are retained in the gonad and laid much later in adult life. In extreme cases, the eggs hatch inside the hermaphrodite causing a phenotype called bag of worms.

For this assay, three days before the start of a mating assay, an additional twenty L4 worms were picked and allowed to age. On the day of the mating assay, ten day-5 worms were placed individually on seeded NGM plates. The worms would lay the eggs that would hatch into L1/L2 larvae by the next day, the number of larvae serves as proxy for the sperm that was present in day-5 hermaphrodites for similar aged hermaphrodites used in the mating assay. The L1/L2 larvae were counted until day 7.

## 2.8 Measuring velocities of hermaphrodites in the presence of males

The protocol for this experiment was adapted from (Morsci et al., 2011). Day-3 and day-5 hermaphrodites and males were obtained as described in the mating assays. The assay plates were seeded with 13 μl of OP50 liquid culture at the center of the plate as shown in the Figure 3.1 to make a smaller bacterial lawn compared to the mating assay. At the magnification used for recording such that the worms can be seen, a 20 μl bacterial lawn was too big and extended beyond the field of view. The worms would wander away from the field of view. Therefore, a 13μl bacterial lawn was used to restrict the movement of the worms.

Twenty hermaphrodites of the same age and genotype were placed in the lawn and allowed to acclimatize for one minute. Four adult males were placed at the center of lawn and the video was recorded for four minutes. I manually tracked each of the twenty worms in each video using mTrackJ© plugin of ImageJ (Meijering et al., 2012). However, tracking each worm using this software is a tedious process. Hence, I was restricted to a smaller data set of 60 worms per genotype per day. The plugin computes various parameters such as velocity, distance from the center of the bacterial lawn and distance traveled based on the coordinates.

## 2.9 Statistical analyses

All statistical analyses were conducted using R 2.15.3 (R Core Team, 2013). For the mating assays, I performed analysis of variance (ANOVA) on the proportion mated (PM) data obtained for each genotype / age / cohort combination using the 'lm' or 'aov' commands in R. Genotype and age were treated as fixed effects; cohort was treated as a blocking variable. The sample sizes for each cohort were added as weights in the linear model. If age or genotype were found to have a significant effect on PM, then I performed Tukey's Honest Significant Difference (HSD) post-hoc tests to find the differences between the means of all pairwise combinations of the main effects.

In the egg-counting assays, ANOVA was used to detect significant differences in the number of eggs laid by each genotype. Since the sample sizes for each estimate were the same, no weights were added. In a two-way ANOVA, post-hoc Tukey's HSD test was used to find the differences between the means of all pairwise combination of the main effects.

Fligner-Killeen test for homogeneity of variances was used to test if the variance of the distributions of velocities of day-3 and day-5 hermaphrodites is the same. To correct for heterogeneity of variances, pairwise Welch's t-tests were used to test for significance for the mean velocities and mean distances from the center of the bacterial lawn of the hermaphrodites that differed in age and genotype.

# Chapter 3

# Results

I conducted mating assays in multiple independent cohorts to test a series of individual hypotheses as described in Figure 1.2. There were at least three independent cohorts for every group of mutants tested. Every cohort has N2 (day-3 and day-5) hermaphrodites and *fog-2 (q71)* (day-3 and day-5) hermaphrodites as controls. In the results presented below, PM refers to proportion of hermaphrodites mated. Young (day-3) N2 males were used in all mating assays unless stated otherwise. N2 is a wild-type strain of *C. elegans* and is used as a reference. All other strains were derived from N2.

## 3.1 Hermaphrodites prefer selfing over outcrossing

My central hypothesis is that, if given a choice, hermaphrodites prefer selfing over outcrossing (see Section 1.1). A prediction from the central hypothesis is that hermaphrodites with sperm should outcross less compared to hermaphrodites without sperm. Outcrossing is measured by the presence of cross-progeny in the mating assay as described in Section 2.6.

To test the central hypothesis, I use *fog*-2 hermaphrodites that do not have sperm on day 3. *fog-2* hermaphrodites are wild-type except for their inability to produce sperm. The gene *fog-2* triggers a temporary masculinization of the germ line of hermaphrodites, allowing them to produce sperm transiently. Therefore, loss-of-function mutants in *fog-2* are unable to produce sperm making them functionally females (Clifford et al., 2000;

Nayak et al., 2005). By comparing *fog-2* mutants that lack sperm on day 3 with N2 on day 3, I can control for the effects of age of the hermaphrodite on PM. They should fail to show mating avoidance on day 3 when N2 hermaphrodites show mating avoidance. Therefore, day-3 *fog-2* hermaphrodites will have a higher PM in the mating assay compared to day-3 N2 hermaphrodites.

N2 hermaphrodites have self-sperm on day 3 and exhaust them by day 5 (Mendenhall, 2009). Thus, another prediction from the central hypothesis is that day-5 N2 hermaphrodites that have depleted their sperm would have higher PM compared to day-3 hermaphrodites. I refer to this as the "N2 trend". I perform mating assays as described in the methods using day-3 and day-5 hermaphrodites of N2 and *fog-2 (q71)* strains.

The outcomes of 19 independent mating assays are shown in Figure 3.1. N2 males were used in 16 cohorts, and *fog-2* males were used for three cohorts. I performed three-way analysis of variance (ANOVA) of the PM data obtained for each hermaphrodite genotype / age / male genotype /cohort combination. There was no significant effect of male genotype (N2 and *fog-2,* $p=0.12$) or significant interaction between the hermaphrodite and male genotype ($p=0.80$). Hence, I simplified the analysis by not taking male genotype into account.

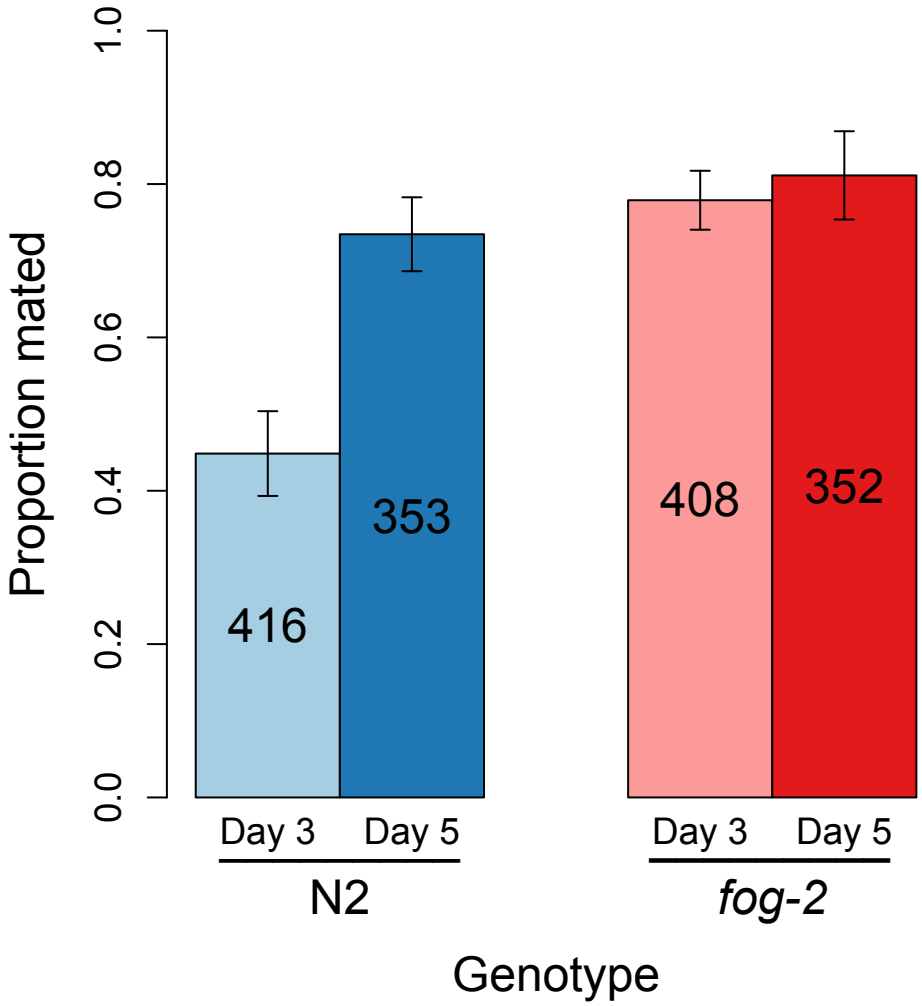



**Figure 3.1:** The proportion of hermaphrodites mated by wild-type males in 4 hours. The number inside each bar represents the total number of hermaphrodites assayed over all the independent cohorts. The genotype of hermaphrodite is shown on x-axis. The error bars are 95% CIs.

Excluding the male genotype, to test the effect of hermaphrodite genotype (N2 and *fog-2*) and age (day 3 and day 5) on PM, I performed two-way ANOVA. The results are summarized in Table 3.1. The analysis shows that there is a significant interaction between genotype and age. I performed Tukey's HSD post-hoc tests on the differences between the means of all pairwise combinations of the main effects (Table 3.2). The PM

of N2 hermaphrodites is significantly lower on day 3 compared to day 5 (Tukey's HSD, $p<0.00001$, Figure 3.1), which I will call the "N2 trend". There is no corresponding trend for *fog-2* females ($p=0.61$). There is also a significant difference in PM between N2 hermaphrodites and *fog-2* females on day 3 (Tukey's HSD, $p<0.00001$). The responses of other genotypes to age were compared separately to N2 and *fog-2* because of the interaction discussed above. The presence of sperm does indeed decrease the PM of day-3 hermaphrodites. I call this phenomenon mating avoidance of hermaphrodite because males are kept constant in the mating assays.

| **Factor** | **df** | **Sum of squares** | **F** | **p** |
|---|---|---|---|---|
| Genotype | 1 | 19.3952 | 77.071 | <0.00001 |
| Age | 1 | 11.0048 | 43.730 | <0.00001 |
| Cohort | 17 | 6.4898 | 1.517 | 0.1263 |
| Genotype × Age | 1 | 5.8960 | 23.429 | <0.00001 |
| Error | 51 | 12.8343 | | |

**Table 3.1:** Analysis of Variance (ANOVA) for mating assay of N2 and *fog-2* hermaphrodites. Genotype and age (day 3 and day 5) were treated as fixed effects

| Genotype: Age - Genotype:Age | Difference | Lower CI | Upper CI | p adjusted |
|---|---|---|---|---|
| N2:3 - *fog-2*:3 | -0.3395 | -0.3591 | -0.3198 | 0.0000 |
| *fog-2*:5 - *fog-2*:3 | 0.0450 | 0.0253 | 0.0646 | 0.6145 |
| N2:5 - *fog-2*:3 | -0.0453 | -0.0650 | -0.0257 | 0.6080 |
| *fog-2*:5 - N2:3 | 0.3844 | 0.3648 | 0.4041 | 0.0000 |
| N2:5 - N2:3 | 0.2941 | 0.2745 | 0.3138 | 0.0000 |
| N2:5 - *fog-2*:5 | -0.0903 | -0.1100 | -0.0706 | 0.0789 |

**Table 3.2:** Pairwise comparisons of main effects using Tukey's Honest Significant Difference (HSD) test

## 3.2 The presence of sperm in the hermaphrodite is required for mating avoidance

Presence of sperm seems to be a reliable predictor of mating avoidance. I refer to this as the sperm hypothesis. This result is consistent with the conclusions of Kleemann and Basolo (2007) that self-sperm status affects the likelihood of mating. The study by Morsci et al. (2011) concluded that males prefer sperm-depleted hermaphrodites. To confirm that just the presence of sperm and not fertilization in the gonad causes mating avoidance I use *spe-8* and *spe-38* hermaphrodites that have defective sperm mutant that are incapable of fertilizing the oocytes.

The prediction of the sperm hypothesis is that both day-3 animals of *spe-8* and *spe-38* should show the N2 trend in spite of being sterile because they have sperm. *spe-8* is required for hermaphrodite spermatogenesis or sperm activation but not for the activation

of male-derived sperm. *spe-38* is required for both male and hermaphrodite sperm for fertilization of oocytes. *spe-8 (hc40)* hermaphrodites are maintained by mating with males of the same strain. *spe-38 (eb44*) hermaphrodites are fertile on account of a extra chromosomal array with cDNA of *spe-38* linked to myosin GFP, GFP+ animals are fertile, non-GFP animals are sterile.

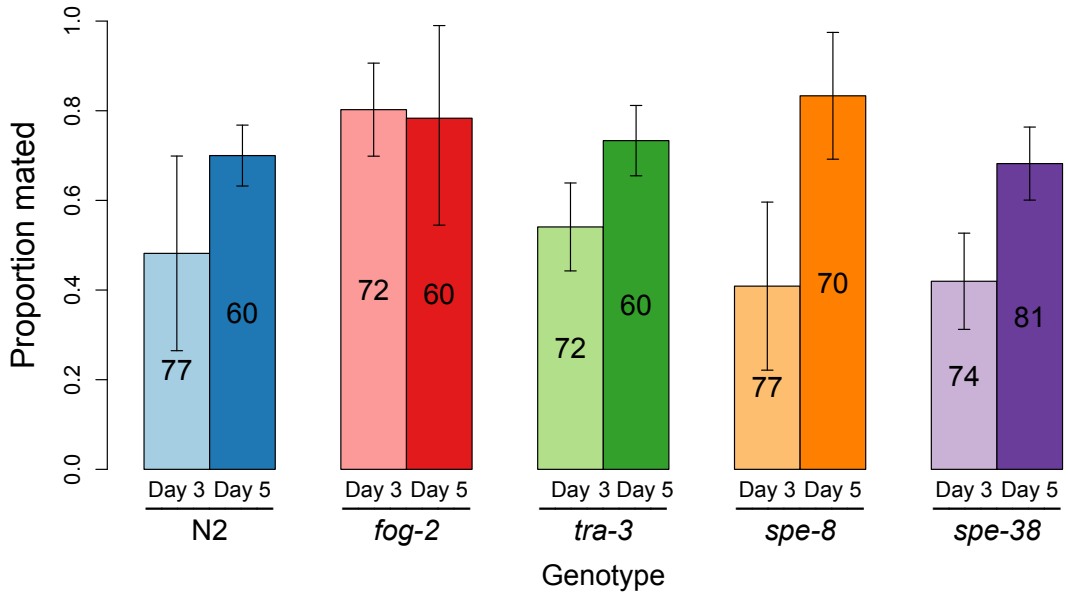



**Figure 3.2:** The proportion of hermaphrodites mated by wild-type males during 4 hours. The number inside each bar represents the total number of hermaphrodites assayed over all the independent cohorts. The genotype of hermaphrodite is shown on x-axis. The error bars are 95%

The outcomes of 3 independent mating assays are presented in Figure 3.2. To test sperm hypothesis, I compared the PM of the mutants that have defective sperm with N2 using two-way ANOVA. The results are summarized in Table 3.3. The analysis shows that there is a significant effect of age on PM ($p<0.00001$). N2, *spe-8* and *spe-38* show the N2 trend, i.e., the PM of day-3 hermaphrodites is lower than day-5 hermaphrodites (Tukey's

HSD, P<0.047). Therefore the sperm mutants also behave like N2, suggesting that the presence of even defective sperm is enough to elicit the mating avoidance behavior.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 2 | 0.243 | 0.926 | 0.42763 |
| Age | 1 | 11.257 | 85.928 | <0.00001 |
| Cohort | 2 | 2.079 | 7.934 | 0.00864 |
| Age × Genotype | 2 | 0.849 | 3.241 | 0.08223 |
| Error | 10 | 1.310 | | |

**Table 3.3:** ANOVA for the mating assay data of *spe-8*, *spe-38* and N2 hermaphrodites. Hermaphrodite genotype and age were treated as fixed effects.

To test the hypothesis that N2 day-5 hermaphrodites would also show sperm avoidance behavior if they have sperm. I used *tra-3,* which make more sperm and therefore on day 5 should behave like day-3 hermaphrodites with sperm. *tra-3* is thought to promote female development in XX hermaphrodites. *tra-3 (e2333)* hermaphrodites lay 499 rather than 327 self-progeny due to extended sperm synthesis during L4 stage (Hodgkin and Barnes, 1991). A prediction that follows from the sperm hypothesis is that *tra-3* will not show the N2 trend as they would not have run out of sperm by day 5. The results of the mating assays are presented in Figure 3.2.

I tested the sperm hypothesis by comparing PM of *tra-3* and N2 using two-way ANOVA, the results are summarized in Table 3.4. There is a significant difference in PM between day-3 and day-5 hermaphrodites (p=0.01). Both N2 and *tra-3* show the N2 trend. This was surprising because *tra-3* hermaphrodites make more sperm than N2 hermaphrodites,

yet it behaves likes sperm-depleted day-5 N2 hermaphrodites. *tra-3* makes more sperm, but the sperm may or may not be exhausted by day 5. I use the egg-counting assay to estimate the number of sperm present in day-5 *tra-3* hermaphrodites.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 1 | 0.2376 | 1.006 | 0.3546 |
| Age | 1 | 2.997 | 12.691 | 0.0119 |
| Cohort | 2 | 0.4876 | 1.032 | 0.4118 |
| Age × Genotype | 1 | 0.026 | 0.11 | 0.7513 |
| Error | 6 | 1.4169 | 1.006 | |

**Table 3.4:** ANOVA for the mating assay data of *tra-3* and N2 hermaphrodites. Hermaphrodite genotype and age were treated as fixed effects.

### 3.2.1 *tra-3* mutants have an extended egg-laying schedule

The data from 2 independent egg-counting assays are presented in Figure 3.3. I performed one-way ANOVA on the mean total progeny between day 5 to day 7 obtained for each genotype. The results are presented in Table 3.5. There is a significant difference between the number of eggs laid by *tra-3* and N2 hermaphrodites between day 5 and day 7 (ANOVA, $F_{(3,6)}=10.24$, p= 0.0015). *tra-3* retain more fertilized eggs in the uterus than N2 animals (McMullen et al., 2012). Therefore, the egg-counting assay is not an appropriate method to estimate the amounts of sperm *tra-3* hermaphrodites have. Thus, the results from mating assays of *tra-3* assays are inconclusive and further experiments are required to understand the behavior of day-5 hermaphrodites with sperm.

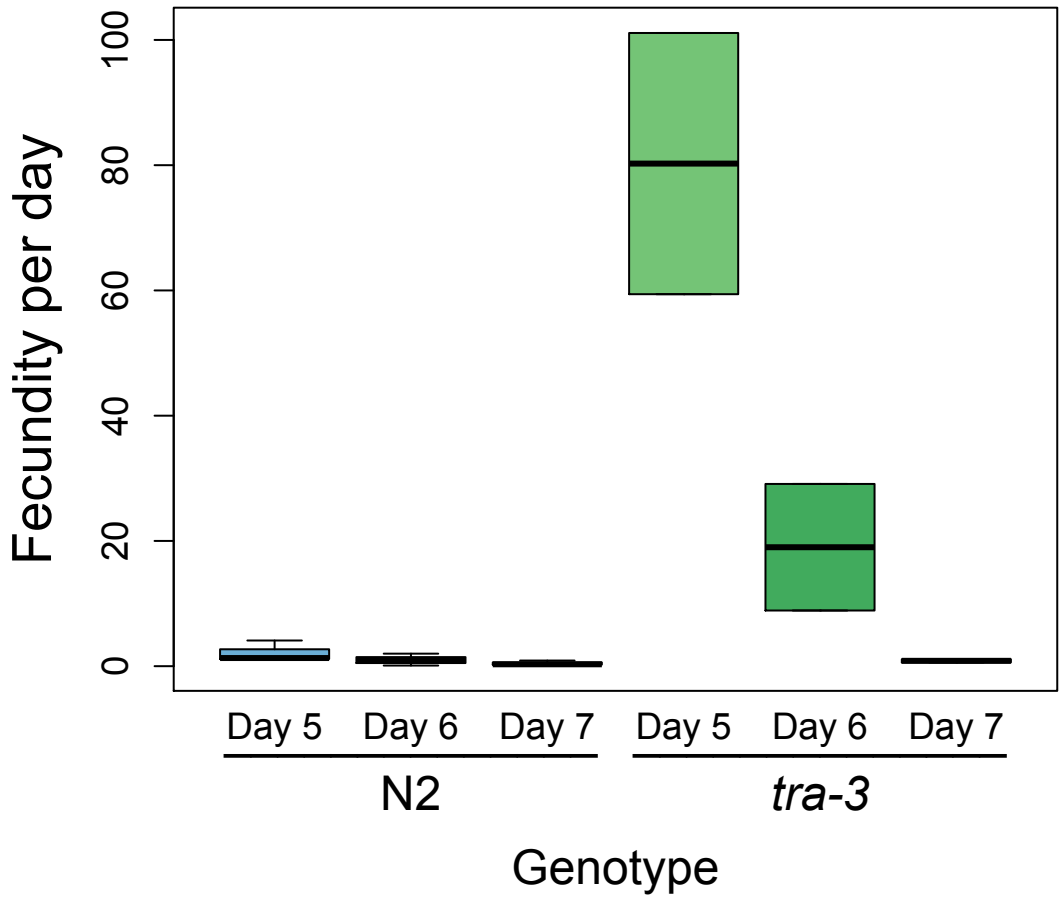



**Figure 3.3:** The number of eggs laid per day per genotype between days 5 and 7. *tra-3* lays most of its eggs on day 5 and it retains more eggs in its gonad compared to N2. Therefore the egg-counting assay is not an accurate estimate of the amount of sperm in *tra-3*

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 1 | 11176 | 128.9 | 0.00147 |
| Error | 3 | 260 | | |

**Table 3.5:** ANOVA for the mean total progeny between day 5 to day 7 obtained for *tra-3* and N2. Hermaphrodite genotype is treated as a fixed effect.

### 3.2.2 Interaction between sperm and oocytes in the gonad of the hermaphrodite is required for mating avoidance

If the presence of sperm causes mating avoidance in hermaphrodites, there should be a mechanism for sensing the sperm-status. Since the *ceh-18* gene is involved in a sperm-sensing checkpoint mechanism that prevents oocyte maturation in the absence of sperm, a *ceh-18* mutant has constitutive oocyte maturation and therefore behaves like a hermaphrodite with sperm. The gene *ceh-18* is required in hermaphrodites for specific aspects of gonadal sheath cell differentiation, such as normal cell shape and position, that are essential for negative regulation of oocyte meiotic maturation by sheath cells (Greenstein, 1994; Miller, 2003; Rose, 1997).

Morsci et al. (2011) showed that day-5 hermaphrodites of *ceh-18* fail to elicit an increase in mutant male response (RE) shown by day-5 N2 hermaphrodites. They found that day-3 hermaphrodites of *fog-2* lose their attractiveness if they carry the mutation *ceh-18*. Morsci et al. (2011) concluded that the gene *ceh-18* mediates the increase in RE in the absence of sperm. I refer to this as the oocyte maturation hypothesis. The prediction from this hypothesis is that *ceh-18* mutants should not show the N2 trend and double mutant (*fog-2;ceh-18*) should show mating avoidance unlike *fog-2* hermaphrodites as described in Section 3.1.

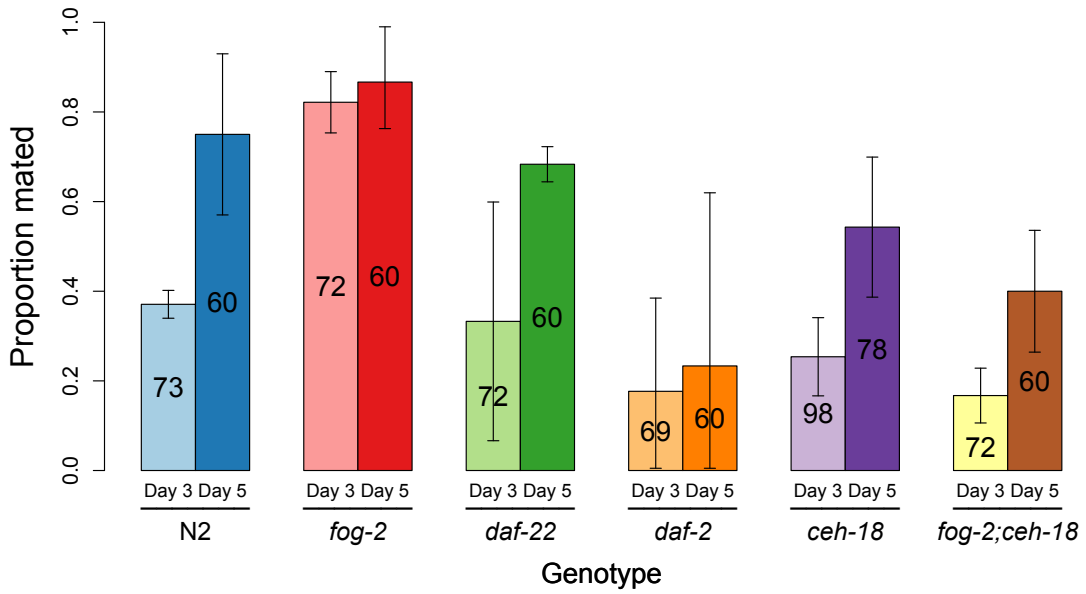



**Figure 3.4:** The proportion of hermaphrodites mated by wild-type males during 4 hours. The number inside each bar represents the total number of hermaphrodites assayed over all the independent cohorts. The genotype of hermaphrodite is shown on x-axis. The error bars are 95% CIs.

The outcomes from 3 independent mating assays are presented in Figure 3.4. To test the effect of constitutive oocyte maturation on mating avoidance, I compare the PM of *ceh-18* mutants and *fog2;ceh-18* double mutants with that of N2 hermaphrodites using two-way ANOVA. The results are summarized in Table 3.6. This analysis shows that there are significant effects of genotype and age on PM. The overall PM of *ceh-18* is lower than N2 (Tukey's HSD, p=0.032) even though *ceh-18* shows the N2 trend (Tukey's HSD, p=0.019). The PM of day-5 *ceh-18* hermaphrodites is not significantly different for day-5 N2 (Tukey's HSD p=0.14). This was unexpected, as *ceh-18* should cause day-5 hermaphrodites to behave as if they have sperm. The gene *ceh-18* has pleiotropic effects on the phenotype. Hermaphrodites of this genotype are heterogeneous in their phenotype especially on day 5. The overall PM of *fog-2;ceh-18* is significantly lower than that of N2

(Tukey's HSD, p=0.0019) which is surprising. It suggests that constitutive oocyte maturation causes more mating avoidance than even the presence of sperm. Thus, the presence of sperm and it interaction with oocytes is the mechanism for mating avoidance. Both day-3 and day-5 *fog-2;ceh-18* hermaphrodites should behave like N2 on day 3 due to constitutive oocyte maturation. PM of *fog-2*;*ceh-18* on day 3 and day 5 is not significantly different from PM of N2 on day 3 (p>0.21).

| **Factor** | **df** | **Sum of squares** | **F** | **p** |
|---|---|---|---|---|
| Genotype | 2 | 4.857 | 11.247 | 0.00219 |
| Age | 1 | 9.66 | 44.737 | < 0.00001 |
| Cohort | 3 | 0.576 | 0.89 | 0.4767 |
| Age × Genotype | 2 | 0.366 | 0.848 | 0.45427 |

**Table 3.6:** ANOVA for mating assay data of N2, *ceh-18* and *fog2; ceh-18* hermaphrodites. Hermaphrodite genotype and age (day 3 and day 5) were treated as fixed effects.

To test the effect of constitutive oocyte maturation on mating avoidance, I compare the PM of *ceh-18* mutants and *fog2;ceh-18* double mutants with that of *fog2* hermaphrodites using two-way ANOVA. The results are summarized in Table 3.7. The analysis shows that there are significant effects of genotype and age on PM. There overall PM of *fog-2;ceh-18* genotypes is significantly lower than *fog-2* (p<0.00001), the PM of *fog-2;ceh-18* (day-3) hermaphrodites is significantly lower than of *fog-2* (day-3) hermaphrodites (Tukey's HSD, P=0.000046). The PM of *fog-2;ceh-18* (day-5) hermaphrodites is significantly lower than of *fog-2* (day-5) hermaphrodites (Tukey's HSD, p=0.00094). Therefore, the introduction of *ceh-18* into *fog-2* background reduces the PM of *fog-2*.

Thus, presence of a *ceh-18* wild-type allele is required for the decrease in mating avoidance in the absence of sperm.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 2 | 24.583 | 63.060 | < 0.00001 |
| Age | 1 | 4.241 | 21.757 | 0.00069 |
| Cohort | 3 | 0.426 | 0.729 | 0.55597 |
| Genotype × Age | 2 | 1.112 | 2.853 | 0.10040 |
| Error | 11 | 2.144 | | |

**Table 3.7:** ANOVA for mating assay data of *fog-2*, *ceh-18* and *fog2; ceh-18* hermaphrodites. Hermaphrodite genotype and age (day 3 and day 5) were treated as fixed effects.

## 3.3 Mating avoidance is independent of body size

The body size of *C. elegans* increases during post-larval stages and through adult life. *C. elegans* approximately doubles in size from sexual maturity to the time they run out of self-sperm (Knight et al., 2002, Lozano et al., 2006). Therefore, day-5 hermaphrodites have larger body sizes than day-3 hermaphrodites. The study by Kleemann and Basolo (2007) found no significant difference in the time it took the males to make first contact with day-3 and day-5 hermaphrodites in their assay spanning over an hour. However, the mating assay in this study spans over 4 hours in which the rate of encounter with a larger worm (in a limited area of the bacterial lawn) could be higher and cause higher PM.

By using mutants that were defective for body size to varying degrees and due to different mutations, I test the size-dependence hypothesis. The null expectation, if mating

avoidance is independent of body size, is that the PM should be the same for day-3 N2 and day-3 “small body size mutants”. An additional prediction is that all the mutants should show the N2 trend, which is the increase in proportion of mated day-5 hermaphrodites.

*sma-2* and *dbl-1* genes function in the TGF-beta signaling pathway to regulate body size and reproductive aging (Shijing et al., 2009). *dpy-2* encodes a unusual cuticular collagen that is required for maintaining the normal body length of the animal in later larval stages. The body size of *sma-2 (e502)* hermaphrodites is 13% of N2, *dbl-1 (nk3)* hermaphrodites is 38% of N2 and *dpy-2 (e8)* hermaphrodites is 40% or 54% of N2 depending on the study (Flemming, 2000; Lozano, 2006). *sma-2 (e502)* and *dbl-1 (nk3)* are endoreduplication mutants, they do not grow after maturity and therefore day-3 and day-5 hermaphrodites have the same body size. *dpy-2 (e8)* on the other hand grows like N2 after maturity via endoreduplication.

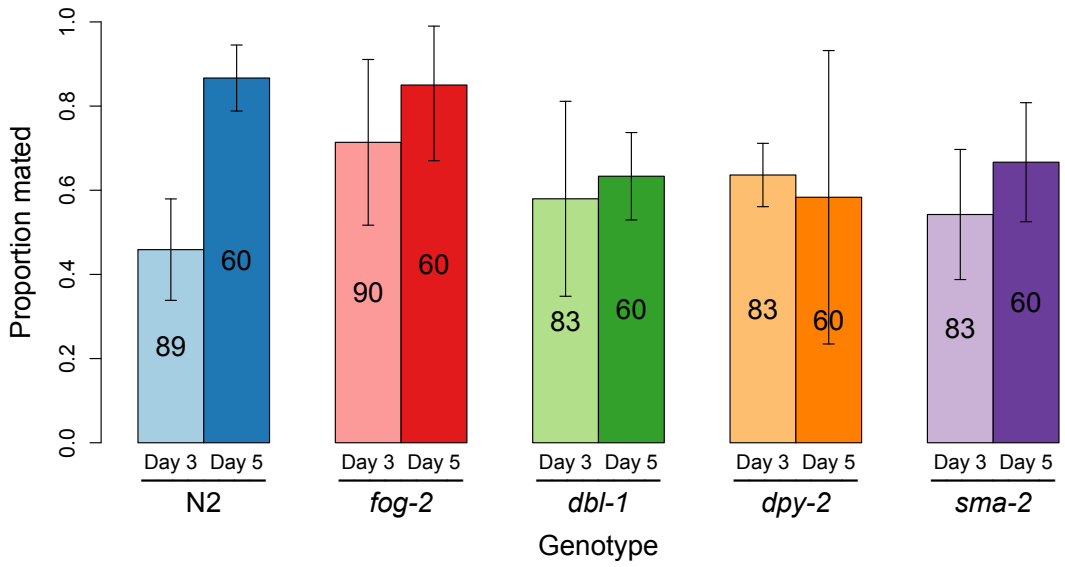



**Figure 3.5:** The proportion of hermaphrodites mated by wild-type males during 4 hours. The number inside each bar represents the total number of hermaphrodites assayed over all the independent cohorts. The genotype of hermaphrodite is shown on x-axis. The error bars are 95% CIs.

The outcomes of 3 independent mating assays for the cohort of mutants differing in body size are presented in Figure 3.5. To test the effect of small body size on mating avoidance, I compare PM of the small mutants (*dbl-1*, *dpy-2* and *sma-2*) with N2 hermaphrodites using two-way ANOVA. The results are summarized in Table 3.8. The analysis shows there is a significant interaction between genotype and age. There is no significant difference in PM between day-3 hermaphrodites of the mutants (*dbl-1*, *dpy-2* and *sma-2*) and N2 (Tukey's HSD, $p>0.39$), although there is a trend towards increasing PM, suggesting that a decrease in size may decrease mating avoidance, perhaps by interfering with locomotory performance (Brenner, 1974). Surprisingly, hermaphrodites of the small mutants (*dbl-1*, *dpy-2* and *sma-2*) did not show the N2 trend on day 5, showing a marked reduction in PM. These results are consistent with increased size

causing higher PM. An alternative possibility is that day-5 hermaphrodites of the size mutants were not sperm depleted. Consistent with this possibility, I noticed that in the day-5 mating assays, many small mutant hermaphrodites produced larger numbers of self-progeny than N2. Therefore I measured the fecundity of the different strains after day 5 as a way to estimate the number of sperm they had on day 5.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 3 | 0.151 | 0.182 | 0.90706 |
| Age | 1 | 2.65 | 9.542 | 0.00801 |
| Cohort | 2 | 2.169 | 3.905 | 0.04489 |
| Age × Genotype | 3 | 4.689 | 5.628 | 0.00959 |
| Error | 14 | 3.888 | | |

**Table 3.8:** ANOVA for mating assay data of *dbl-1*, *dpy-2*, *sma-2,* and N2 hermaphrodites. Hermaphrodite genotype and age (day 5 and day 7) were treated as a fixed effect.

### 3.3.1 Small body mutants show mating avoidance on day 5 because they have not exhausted self-sperm

The data from 3 independent egg-counting assays are displayed in Figure 3.6. I performed one-way ANOVA on the mean total progeny between day 5 to day 7 obtained for each genotype. The results are presented in Table 3.9. There is a significant effect of the genotype on the eggs laid between day 5 and day 7 (ANOVA, $F_{(3,2)}$ =10.24, p=0.0089). *sma-2* hermaphrodites lay a significantly larger number of eggs than N2 (Tukey's HSD, p=0.0063). *dbl-1* and *dpy-2* show similar trends although the patterns are

marginally significant (p≈0.05). These results are consistent with the central hypothesis that presence of sperm causes mating avoidance.

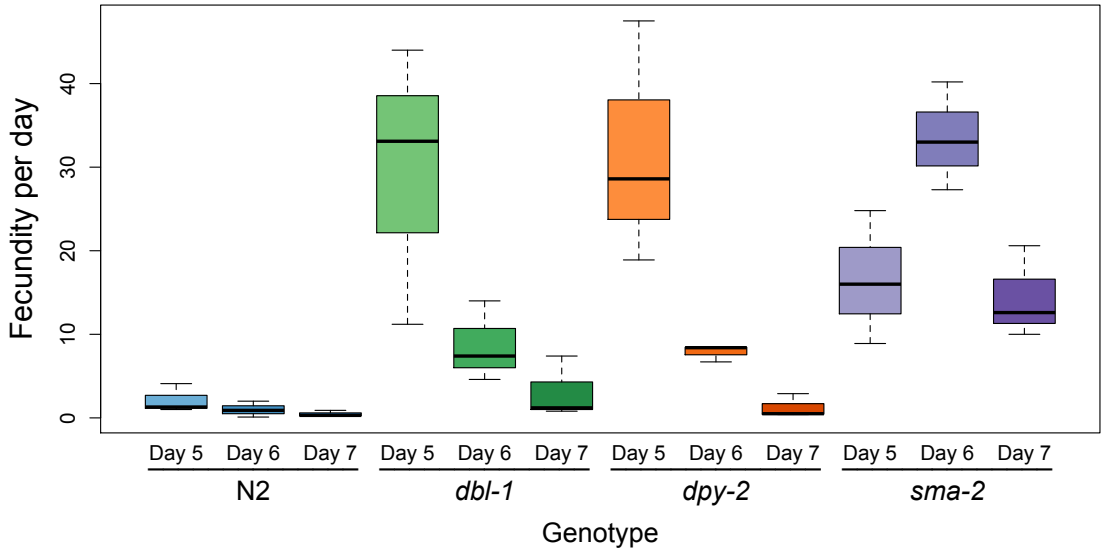



**Figure 3.6:** The number of eggs laid per day per genotype between the days 5 and 7. *dbl-1* and *dpy-2* show a similar trend, they lay most of their eggs on day 5, on the day of the mating assay. *sma-2* hermaphrodites lay most of their eggs on day 6, a day after the mating assay.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 3 | 5704 | 10.24 | 0.00894 |
| Cohort | 2 | 290 | 0.78 | 0.49978 |
| Error | 6 | 1114 | | |

**Table 3.9:** Analysis of Variance (ANOVA) for fecundity data of *dbl-1*, *dpy-2*, *sma-2,* and N2 hermaphrodites. Hermaphrodite genotype was treated as a fixed effect.

## 3.4 Locomotion is required for hermaphrodite's mating avoidance

In the genus *Caenorhabditis*, mating success of a male depends on the hermaphrodite/female being stationary. *C. remanei* males secrete a soporific factor during copulation to immobilize their conspecific females. The factor also widens the vulval slit facilitating copulation (Garcia et al., 2007). Since, *C. elegans* males do not secrete any factor to paralyze the hermaphrodites or any known factor to modify hermaphrodite behavior, successful mating is dependent on hermaphrodites being stationary during copulation. Kleemann and Basolo (2007) showed that hermaphrodites that have sperm sprint away at a higher rate than sperm-depleted hermaphrodites. This indicates that mating avoidance behavior shown by hermaphrodites that have sperm may require locomotion. I refer to this as the locomotion hypothesis.

Many *C. elegans* researchers know this by the observation that it is not easy to generate and maintain high frequency of males in N2 strain but it is easy to generate males from paralyzed hermaphrodites. Small mutants have locomotion defects, for example, *dpy-2* has a mild "roller" phenotype—worms rotate around their long axis and move in a corkscrew fashion. They do not move in the sinusoidal wave motion of N2 hermaphrodites. There is even hint of this trend in Figure 3.2 that the small mutants, including *dpy-2*, have higher PM than the N2 hermaphrodites on day 3.

I tested the importance of locomotion in mating avoidance by using uncoordinated locomotion mutants *unc-76 (e911/n2397)* and *unc-31 (e169)* that are paralyzed due to mutations in different genetic pathways. These hermaphrodites are reproductively equivalent to wild-type worms; they are capable of self-fertilization and have sperm at day 3. *unc-76* is required for normal axonal outgrowth and fasciculation and hence, normal locomotion. *unc-31* is required for egg laying, locomotion, pharyngeal pumping and recovery from dauer larval stage. *unc-76* has a severe locomotion defect compared to *unc-31*. *unc-31* hermaphrodites are characterized as sluggish whereas *unc-76* hermaphrodites coil as they move. The prediction from locomotion hypothesis is that day-3 uncoordinated hermaphrodites would have higher proportion of mated hermaphrodites compared to day-3 N2 worms because they are not capable of the mating avoidance.

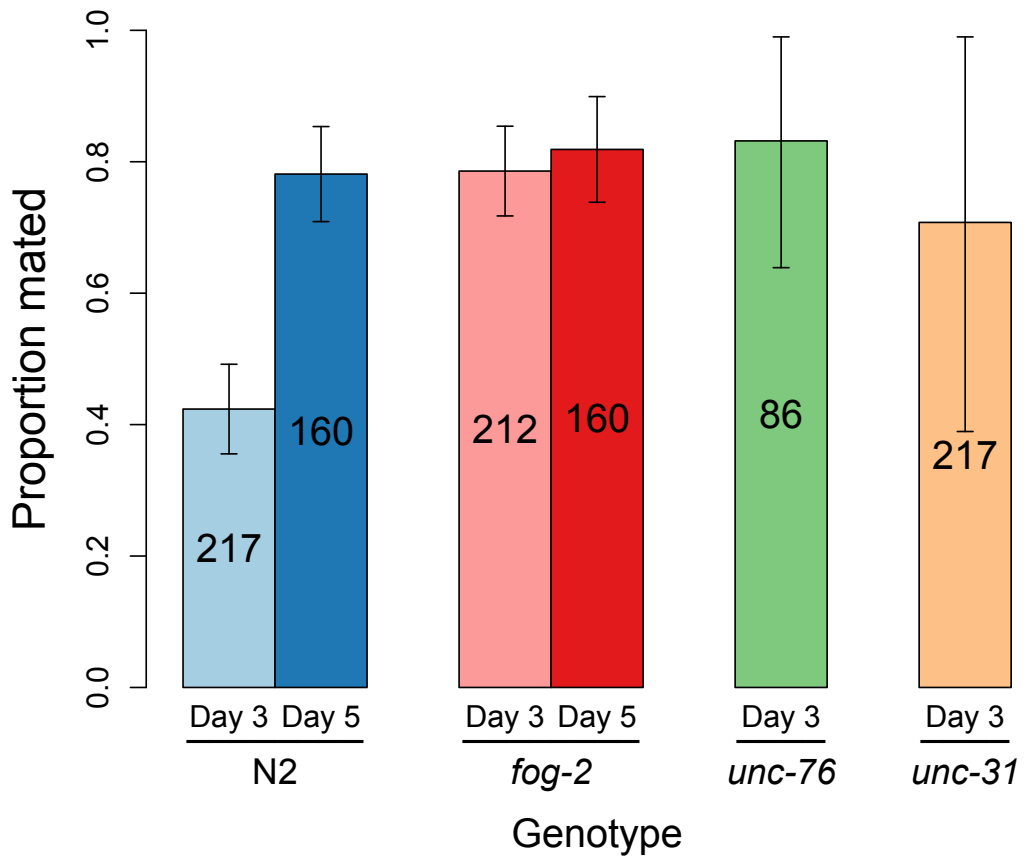



**Figure 3.7:** The proportion of hermaphrodites mated by wild-type males during 4 hours. The number inside each bar represents the total number of hermaphrodites assayed over all the independent cohorts. The genotype of hermaphrodite is shown on x-axis. The error bars are 95% CIs.

The outcomes from mating assays for cohort of uncoordinated locomotion mutants are presented in Figure 3.7. I conducted 3 independent cohorts with *unc-76* and 5 independent cohorts with *unc-31*. Mating assays were performed separately for *unc-76* and *unc-31* and therefore, not directly comparable. Hence, I performed statistical analysis for *unc-76* and *unc-31,* separately. I performed a one-way ANOVA on the PM data obtained for day-3 *unc-76* hermaphrodites to compare it to N2 hermaphrodites. The results are summarized in Table 3.10. There is a significant difference between PM of

day-3 hermaphrodites of N2 and *unc-76* genotype (ANOVA, $F_{(1,2)} = 23.081$, p=0.01) indicating that locomotion is necessary for mating avoidance of day-3 hermaphrodites.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 1 | 5.811 | 23.082 | 0.0172 |
| Cohort | 2 | 1.048 | 4.164 | 0.1363 |
| Error | 3 | 0.252 | | |

**Table 3.10:** ANOVA for the mating assay data of N2 and *unc-76* hermaphrodites. Hermaphrodite genotype was treated as a fixed effect.

To test if uncoordinated mutants have PMs equivalent to day-3 *fog-2* hermaphrodites, I compared the PM of *unc-76* with *fog-2* using one-way ANOVA (Table 3.11). There is no significant difference in PM between the two genotypes (ANOVA, $F_{(1,2)} = 0.2028$, p=0.68). Therefore, PM of *unc-76* on day 3 is equivalent to *fog-2* hermaphrodites due to its inability to avoid mating.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 1 | 0.21248 | 0.2028 | 0.6830 |
| Cohort | 2 | 0.21426 | 0.1022 | 0.9058 |
| Error | 3 | 3.14340 | | |

**Table 3.11:** Analysis of Variance (ANOVA) for the mating assay data of *fog-2* and *unc-76* hermaphrodites. Hermaphrodite genotype was treated as a fixed effect.

To test if *unc-31* supports the locomotion hypothesis like *unc-76,* I compared PM data for day-3 *unc-31* hermaphrodites to day-3 N2 hermaphrodites using one-way ANOVA. The results are summarized in Table 3.12. There is no significant difference between the

proportion mated in day-3 hermaphrodites of N2 and *unc-31* (ANOVA, $F_{(1,4)} = 4.0825$, p=0.11). However, there was an outlier for *unc-31* genotype in cohort 3 data. I performed one-way ANOVA excluding data from cohort 3, the data is presented in Table 3.13. I found significant difference in PM of day-3 hermaphrodites of N2 and *unc-31* (ANOVA, $F_{(1,4)} = 12.1932$, p=0.04). PM of both the locomotory mutants is higher than of day-3 N2 hermaphrodites confirming that locomotion is a necessary condition for mating avoidance.

| **Factor** | **df** | **Sum of squares** | **F** | **p** |
|---|---|---|---|---|
| Genotype | 1 | 7.2227 | 4.0825 | 0.1134 |
| Cohort | 4 | 5.8878 | 0.8320 | 0.5686 |
| Error | 4 | 7.0767 | | |

**Table 3.12:** ANOVA for the mating assay data of N2 and *unc-31* hermaphrodites. Hermaphrodite genotype was treated as a fixed effect.

| **Factor** | **df** | **Sum of squares** | **F** | **p** |
|---|---|---|---|---|
| Genotype | 1 | 6.4493 | 12.1932 | 0.03971 |
| Cohort | 4 | 11.3763 | 5.3771 | 0.09920 |
| Error | 3 | 1.5868 | | |

**Table 3.13:** ANOVA for the mating assay data of N2 and *unc-31* hermaphrodites excluding cohort 3 data. Hermaphrodite genotype was treated as a fixed effect.

To test if uncoordinated mutants have PMs equivalent to day-3 *fog-2* hermaphrodites, I performed one-way ANOVA on the PM data of day-3 *unc-31* and day-3 *fog-2* hermaphrodites (Table 3.14). There is no significant difference in PM between the two

genotypes (ANOVA, $F_{(1,4)}$ = 0.3688, p=0.58). Therefore, PM of *unc-31* on day 3 is equivalent to *fog-2* hermaphrodites due to its inability to avoid mating.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Genotype | 1 | 0.5460 | 0.3688 | 0.5765 |
| Cohort | 4 | 5.8672 | 0.9907 | 0.5035 |
| Error | 4 | 5.9223 | | |

**Table 3.14:** ANOVA for the mating assay data of *fog-2* and *unc-31* hermaphrodites. Hermaphrodite genotype was treated as a fixed effect.

## 3.5 Mutant males are sensitive to locomotion of the hermaphrodite

I wanted to disentangle the male preference from the hermaphrodite preference by mating hermaphrodites of N2 (day 3 and day 5), *fog-2* (day 3 and day 5) and *unc-31* (day 3) with males of N2, *lov-1* and *pkd-2*. I predict that mutant males that are partially defective in their response to hermaphrodite contact (*lov-1* and *pkd-2)* have difficulty in finding the vulva due to the mating avoidance behavior of day-3 N2 hermaphrodites. Therefore, they should mate a higher proportion of day-3 *unc-31* compared to day-3 N2 hermaphrodites. Another prediction is that eve the mutant males will have mated a higher proportion of day-3 *fog-2* animals, which do not avoid mating. The mutant males should also have mated a higher proportion of day-5 N2/*fog-2* hermaphrodites.

*lov-1* and *pkd-2* genes are required for male response to the hermaphrodite contact and vulva location. Since mutant males of these genes are not capable of mating effectively, a strain containing a *him-5 (e1490)* mutation, which increases the frequency of X

nondisjunction events resulting in male progeny was used. *lov-1(sy582)* males show a 14% response to hermaphrodite contact (Barr and Sternberg, 1999, Barr et al., 2001). *pkd-2 (sy606)* males show 48% response to hermaphrodite contact (Barr et al., 2001).

Day-5 hermaphrodites of *unc-31* genotype were not included in the assay, as they tend to have higher mortality rates during the mating assay. A negative result of no outcrossed progeny in the mating assay is difficult to interpret as it could be due to mortality of the worm itself or mating avoidance. Uncoordinated locomotory mutant *unc-31 (e169)* was used to remove the locomotion component by which mating can be avoided.

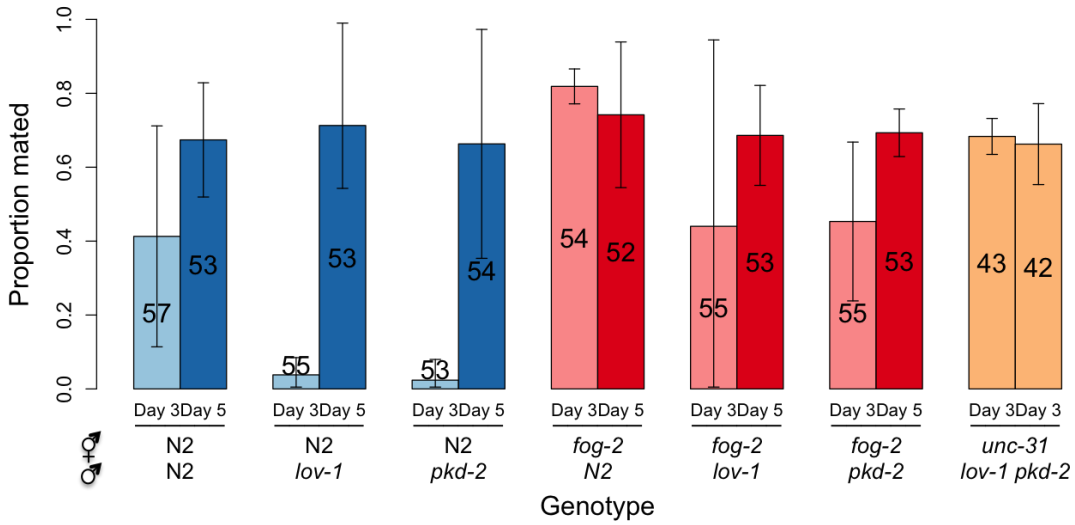



**Figure 3.8:** The proportion of hermaphrodites mated by wild-type males during 4 hours. The number inside each bar represents the total number of hermaphrodites assayed over all the independent cohorts. The genotype of both the hermaphrodite and male is shown on x-axis. The error bars are 95% CIs.

The data from 3 independent mating assays for the cohort of mutant males (*lov-1* and *pkd-2)* are presented in Figure 3.8. *unc-31* genotype was not tested in cohort 1 because of

unavailability of L4s. I performed two-way ANOVA on the PM data obtained for each male genotype / hermaphrodite genotype / cohort combination. Hermaphrodite genotype (N2 and *unc-31*) and male genotype (*lov-1* and *pkd-2)* were treated as fixed effects; cohort was treated as a blocking variable. The results are presented in Table 3.15. The PM of day-3 N2 hermaphrodites is significantly lower than day-3 of *unc-31* ($p<0.00001$). *lov-1* and *pkd-2* males are equivalent in mating with hermaphrodites of the same genotype, there is no difference of PM (Tukey's HSD, $p=0.99$ for *unc-31* hermaphrodites and $p=0.99$ for N2 hermaphrodites). There is a significant difference in the PM of N2 hermaphrodites by *lov-1* and *pkd-2* males compared to N2 males ($p=0.03$ for *lov-1,* $p=0.02$ for *pkd-2)*. *lov-1* and *pkd-2* mate significantly higher proportion of *unc-31* hermaphrodites compared to N2 hermaphrodites ($p=0.0034$ for *lov-1* and $p=0.0039$ for *pkd-2*).

| **Factor** | **df** | **Sum of squares** | **F** | **p** |
|---|---|---|---|---|
| Male | 2 | 0.4435 | 1.3141 | 0.3363 |
| Genotype | 1 | 18.9458 | 112.2855 | < 0.00001 |
| Cohort | 2 | 0.8590 | 2.5455 | 0.1583 |
| Male × Genotype | 1 | 0.0032 | 0.0188 | 0.8955 |
| Error | 6 | 1.0124 | | |

**Table 3.15:** ANOVA for the mating assay data of N2 and *unc-31* hermaphrodites. Hermaphrodite genotype, male genotype (*lov-1*, *pkd-2* and N2) and age were treated as fixed effects.

From the data in Figure 3.8, there seems to be a difference in PM of day-3 *fog-2* compared to day-5 *fog-2* hermaphrodites mated by *lov-1* and *pkd-2*. I performed two-way ANOVA on the PM data obtained for each male genotype / hermaphrodite genotype / cohort combination. Hermaphrodite genotype (*fog-2* and *unc-31*) and male genotype (*lov-1* and *pkd-2)* were treated as fixed effects; cohort was treated as a blocking variable. The results of the two-way ANOVA are summarized in Table 3.16. There is no significant difference between *fog-2* and *unc-31* genotype on PM ($p=0.24$). There is also no significant effect of males (*lov-1*, *pkd-2* and N2) on PM ($p=0.052$). Since, the p-value is close to the alpha of 0.05, I performed post-hoc Tukey's HSD test but there is no significant difference in PM due to males on *fog-2* and *unc-31* genotypes. Therefore, the mutant males (*lov-1* and *pkd-2)* are equivalent to N2 males when mating with *fog-2* and *unc-31* hermaphrodites. The results above taken together suggest that the mutant males are capable of mating with both day-3 and day-5 hermaphrodites but the hermaphrodite's locomotion poses a challenge to them.

| Factor | df | Sum of squares | F | p |
|---|---|---|---|---|
| Age | **1** | 1.4509 | 3.2755 | 0.09542 |
| Genotype | 1 | 0.6779 | 1.5304 | 0.23971 |
| Male | 2 | 3.3824 | 3.818 | 0.05209 |
| Cohort | 2 | 1.2933 | 1.4598 | 0.27073 |
| Age × Male | 2 | 2.3887 | 2.6962 | 0.10787 |
| Genotype × Male | 1 | 0.0564 | 0.1273 | 0.72744 |
| Error | 12 | 5.3156 | | |

**Table 3.16:** ANOVA for the mating assay data of *fog-2* and *unc-31* hermaphrodites. Hermaphrodite genotype, male genotype (*lov-1*, *pkd-2* and *fog-2*) and age were treated as fixed effects.

## 3.6 The decline in mating avoidance is not mediated through the pheromone

Older hermaphrodites that are sperm-depleted may attract males by secreting the pheromone and thus increase their mating success. I call this the pheromone hypothesis. If the day-5 N2 hermaphrodites increase their probability of mating by increasing the secretion of pheromone to attract males, then I predict that a mutant in the pheromone biosynthesis pathway would not show the N2 trend. To test the pheromone hypothesis, I use *daf-22* mutants, which are partially defective in producing the mating cue (pheromone) that attracts males from a distance (Simon and Sternberg, 2002; Srinivasan et al., 2012). The gene *daf-22* is required for short-chain ascaroside synthesis (Srinivasan et al., 2008, 2012).

Morsi et al. (2012) showed that *daf-22 (m130)* hermaphrodites show the N2 trend suggesting that a pathway other than pheromone biosynthesis is responsible for the N2 trend. However, the assay for testing was not done on individual worms, where the role of a pheromone that attracts males from a distance could have been overlooked. Hence, I chose to test this mutant in a different assay, which is appropriate for studying the role of a pheromone.

The data from 3 independent mating assays of *daf-22* and N2 are shown in Figure 3.4. To test the effect of pheromone on the PM, I compare the data of *daf-22* and N2 using one-way ANOVA. The results are summarized in Table 3.17. There is no effect of the genotype but there is a significant effect of the age on PM (p= 0.0020). Therefore *daf-22* shows the N2 trend. Therefore, the pheromone does not mediate the decline of mating avoidance from day 3 to day 5.

| **Factor** | **df** | **Sum of squares** | **F** | **p** |
|---|---|---|---|---|
| Genotype | 1 | 0.159 | 0.492 | 0.50911 |
| Age | 1 | 8.753 | 27.179 | 0.00199 |
| Cohort | 2 | 0.593 | 0.921 | 0.44774 |
| Age × Genotype | 1 | 0.016 | 0.049 | 0.83185 |
| Error | 6 | 1.932 | | |

**Table 3.17:** ANOVA for mating assay of N2 and *daf-22* hermaphrodites. Hermaphrodite genotype and age (day 3 and day 5) were treated as fixed effects.

## 3.7 Age-related changes in *C. elegans* do not mediate inhibition of mating avoidance

The decrease in mating avoidance in day-5 hermaphrodites by sperm-depletion is confounded with the effects of age. Age-related locomotory decline could inhibit mating avoidance in day-5 hermaphrodites. I refer to this as the age hypothesis. One of the findings of Kleemann and Basolo (2007) is that hermaphrodites that have exhausted self-sperm show a lower rate of sprinting away from the male. However, self-sperm hermaphrodites happen to be older as well. They found no effect of age of the hermaphrodite on the likelihood of mating or correlation between sprint rate (mechanism of mating avoidance) and age in their sample size. They note, that an effect of age may be found in a larger sample size. Hence, I test the effect of age, specifically on locomotion of the hermaphrodites in a large sample size.

There is evidence against the age hypothesis in Section 3.1, *fog-2* hermaphrodites that are of the same age as N2 hermaphrodites fail to show mating avoidance in spite of having wild type locomotion. Inhibition of mating avoidance in day-3 *fog-2* hermaphrodites could be due to a behavioral effect of the mutation or sperm while the inhibition of mating avoidance in day-5 N2 hermaphrodites might be due to age. Therefore, I take two different approaches to test the age hypothesis. First, I directly measure mean velocities of day-3 and day-5 hermaphrodites of N2 and *fog-2*. I recorded the locomotion of 20 hermaphrodites in the presence of 4 males for 4 minutes and measured their mean

velocities. If *C. elegans* locomotory function declines with age, then the speed of day-5 hermaphrodites should be lower than that of day-3 hermaphrodites.

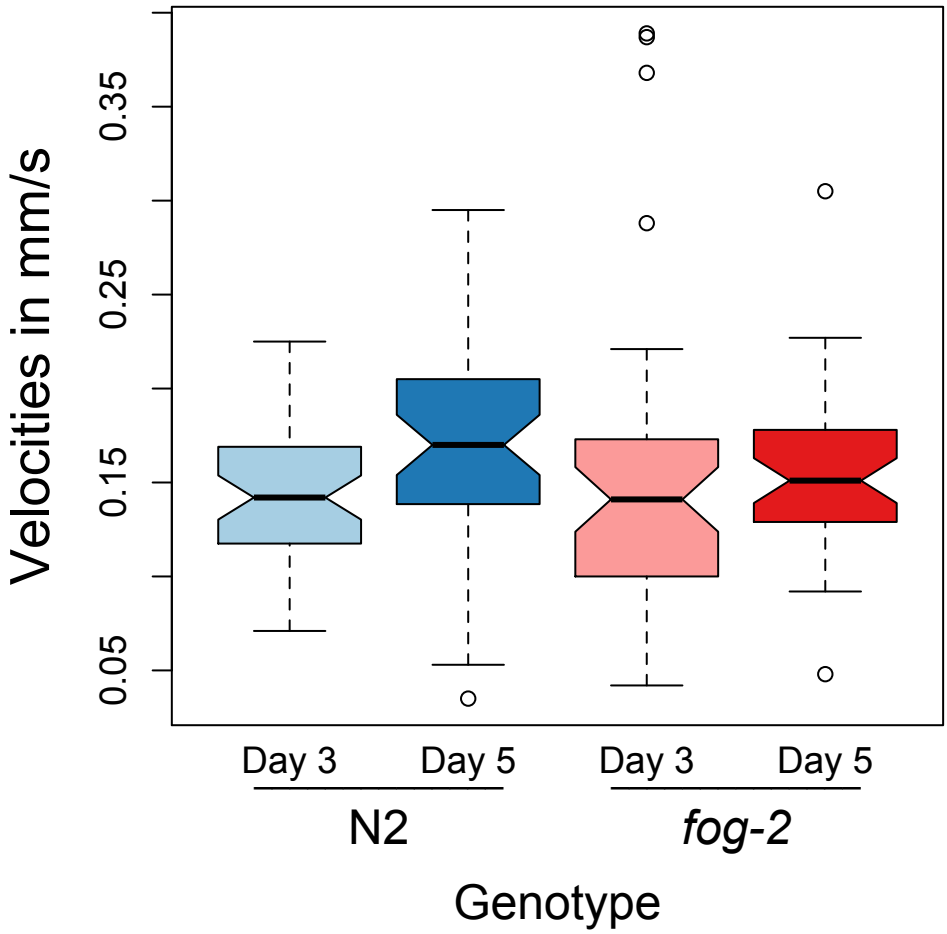



**Figure 3.9:** The distribution of velocities of hermaphrodites on days 3 and 5. The genotype of hermaphrodite is shown on x-axis.

I performed video analysis on twelve videos, each of 4-minute duration. The data from ImageJ analysis of the videos are presented in Figure 3.9. I tested the velocities of the four groups shown in Figure 3.9 with Fligner-Killeen test for homogeneity of variances and found that they are significant different (p=0.034).

I performed pairwise Welch's t-tests of N2 and *fog-2* hermaphrodites on day 3 and day 5 in homogenous condition (n=40). There is no significant difference in the mean velocities of N2 and *fog-2* (p=0.66 for day-3 and p=0.14 for day-5 hermaphrodites). There is a significant difference between the mean velocities of day-3 and day-5 N2 hermaphrodites (p=0.011). Day-5 hermaphrodites have higher velocities than day-3 hermaphrodites. Therefore, there is no evidence that day-5 hermaphrodites are slower than day-3 hermaphrodites. There is no significant difference between the mean velocities of day-3 and day-5 *fog-2* hermaphrodites (p=0.707). Thus, there is no age-related decline that could be detected in my analysis.

The spatial exploratory pattern of N2 and *fog-2* is shown in Figures 3.10 and 3.11 respectively. I attempted to quantify this behavior by measuring the mean distance of the worms from the center by analyzing the tracks using ImageJ. The data of distance from the center is displayed in Figure 3.12. The spatial exploratory pattern displayed by *fog-2* hermaphrodites is distinct from that of N2 hermaphrodites. I tested the distances of the hermaphrodites from the center of the bacterial lawn for the four groups shown in Figure 3.12, with Fligner-Killeen test for homogeneity of variances, and found that they are significant different (p=0.0012). I performed Welch's pairwise t-tests of the distances of N2 and *fog-2* hermaphrodites on day 3 and day 5 from the center of the bacterial lawn in (n=40). Day-5 N2 hermaphrodites are significantly farther away from the center of the bacterial lawn compared to day-3 N2 hermaphrodites (p=0.031). There is no significant difference in the distances of day-3 hermaphrodites of N2 and *fog-2* from the center of bacterial lawn (p=0.95). There is no significant difference in the distances of day-3 and

day-5 *fog-2* hermaphrodites from the center of bacterial lawn (p=0.056). There was no age-related locomotory pattern that could explain the PM of N2 and *fog-2* at day 3 and day 5.

The second approach I took to test the effects of aging on mating avoidance is to assay a long-lived mutant in the mating assays. I used *daf-2* hermaphrodites to test if age-related physiological changes cause the decrease in mating avoidance. A long-lived mutant is physiologically younger than N2 hermaphrodites at the same age. The prediction is that *daf-2* would not show the N2 trend because at day 5, *daf-2* worms are young. *daf-2* is a gene that regulates lifespan, reproduction, and diapause, *daf-2 (e1370)* hermaphrodite continue to produce progeny much longer and are physiologically younger than wild-type worms.

However, *daf-2 (e1370)* mutant had a variable phenotype in the lab, with the majority of the worms showing protruding vulva and sterility. If the hermaphrodite is incapable of mating due to protruding vulva, the mating assay cannot be used to study its mating behavior. The mating assay results for *daf-2* are shown in Figure 3.4. The experiments with *daf-2* are inconclusive due to the mutant's variable phenotype and more experiments are needed to explore the effects of aging on mating avoidance.

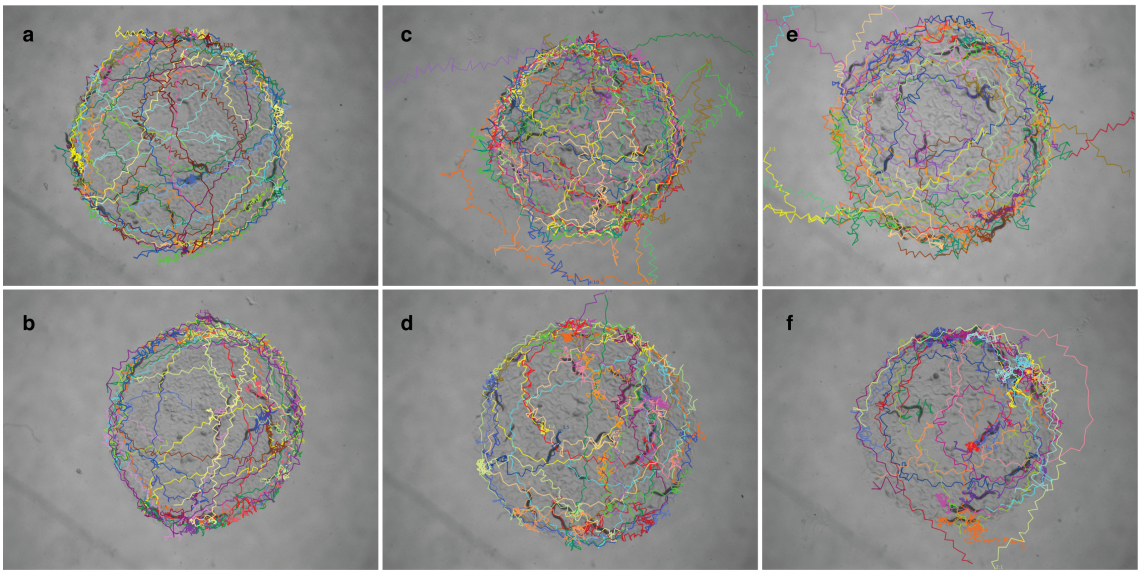



**Figure 3.10:** Tracks of N2 hermaphrodites in the presence of four males over a duration of 4 minutes. (a, b) Twenty day-3 hermaphrodites, (c, d) Ten day-3 and ten day-5 hermaphrodites, (e, f) Twenty day-5 hermaphrodites.

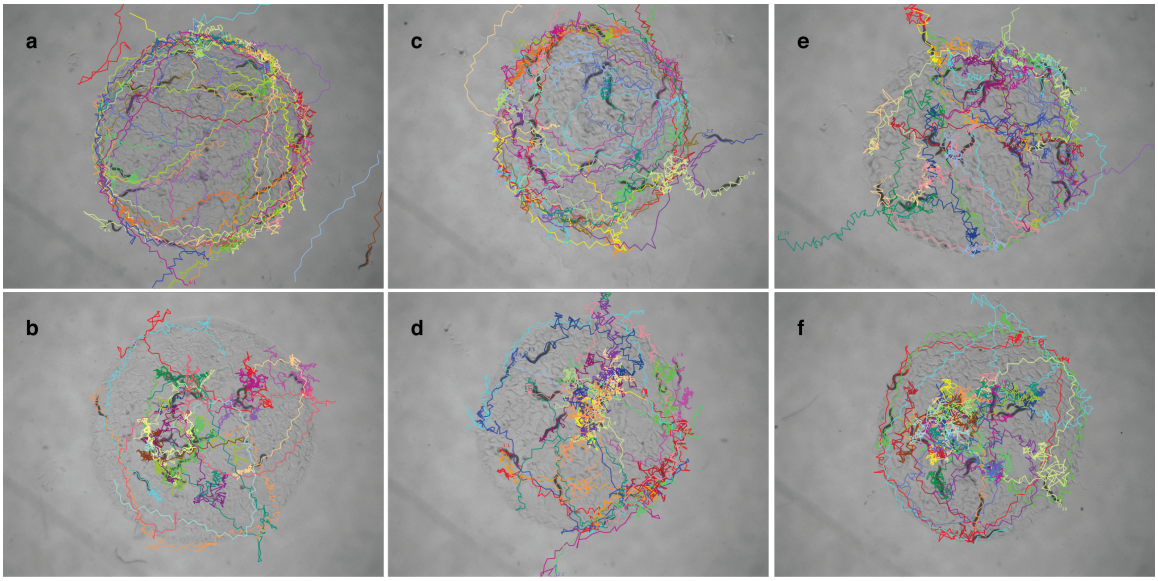



**Figure 3.11:** Tracks of *fog-2* hermaphrodites in the presence of four males over a duration of 4 minutes. (a, b) Twenty day-3 hermaphrodites, (c, d) Ten day-3 and ten day-5 hermaphrodites, (e, f) Twenty day-5 hermaphrodites.

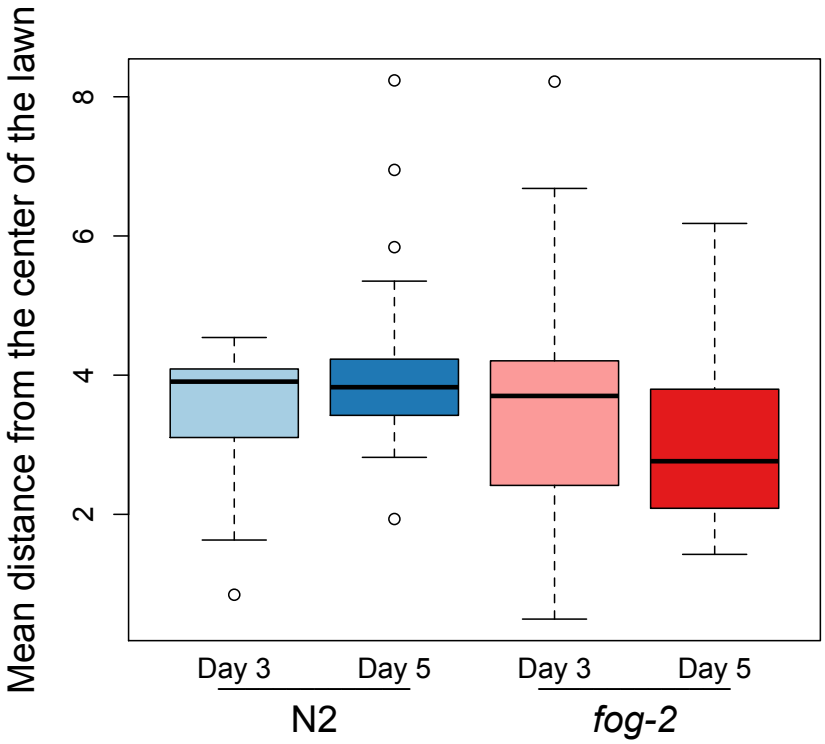



**Figure 3.12:** The mean distance of hermaphrodites from the center of the lawn over duration of 4 minutes in mm.

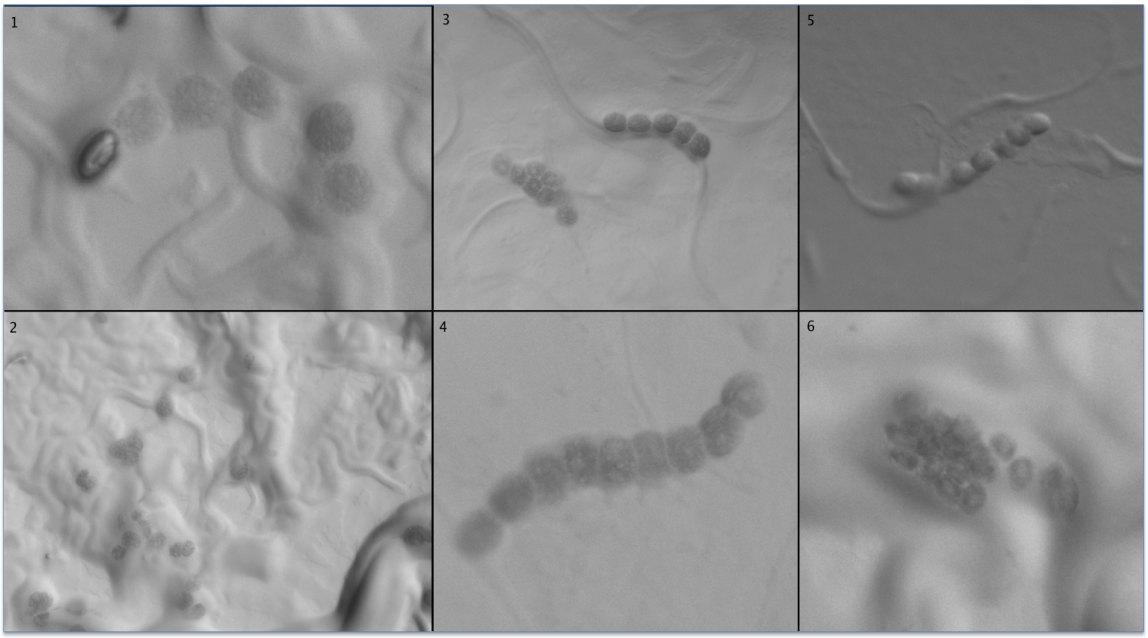



**Figure 3.13: (**1, 2) are representative images of the unfertilized eggs laid by a day-5 N2 hermaphrodite. In panel 1, there are 5 unfertilized eggs and 1 fertilized egg. (3, 4) are representative images of unfertilized eggs laid by *spe-8* mutant. (5, 6) are representative images of unfertilized eggs laid by *spe-38* mutant.


**Figure 3.14:** The arrows in all the panels point to the gonad of the hermaphrodite. 1) The gonad of a day-3 N2 hermaphrodite is filled with fertilized eggs waiting to be laid. 2) The gonad of a day-5 N2 hermaphrodite contains maturing oocytes that are compacted resembling 'piano keys'. 3) The gonad of a day-3 *fog-2* hermaphrodite contains few maturing oocytes that are compacted, it resembles gonad of a day-5 hermaphrodite. 4) The gonad of a day-5 *fog-2* hermaphrodite contains maturing oocytes that are compacted, it resembles gonad of a day-5 hermaphrodite.

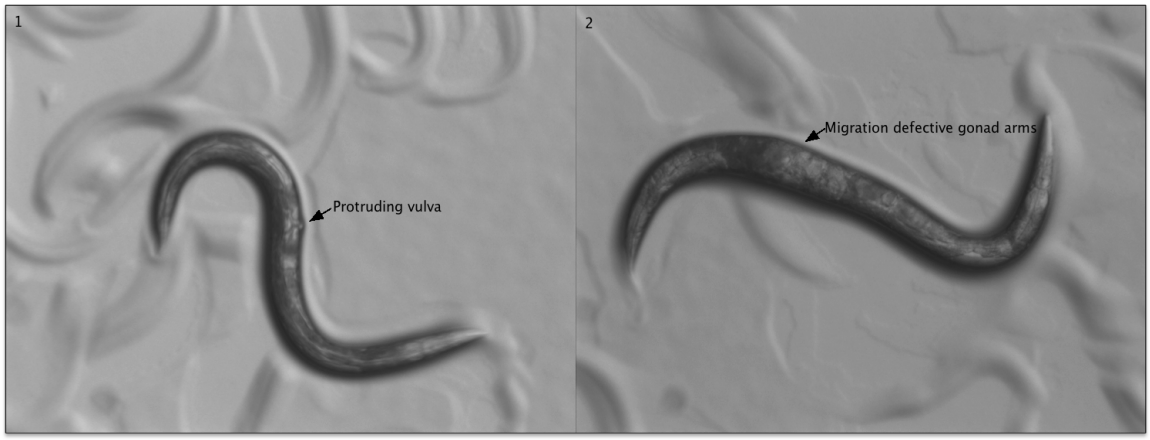



**Figure 3.15:** Panels 1 and 2 are representative images of the mutant *ceh-18*. *ceh-18* shows abnormalities of the gonad and sheath cell differentiation. The arrows in the panels point to different phenotypes exhibited by day-5 *ceh-18* hermaphrodites.

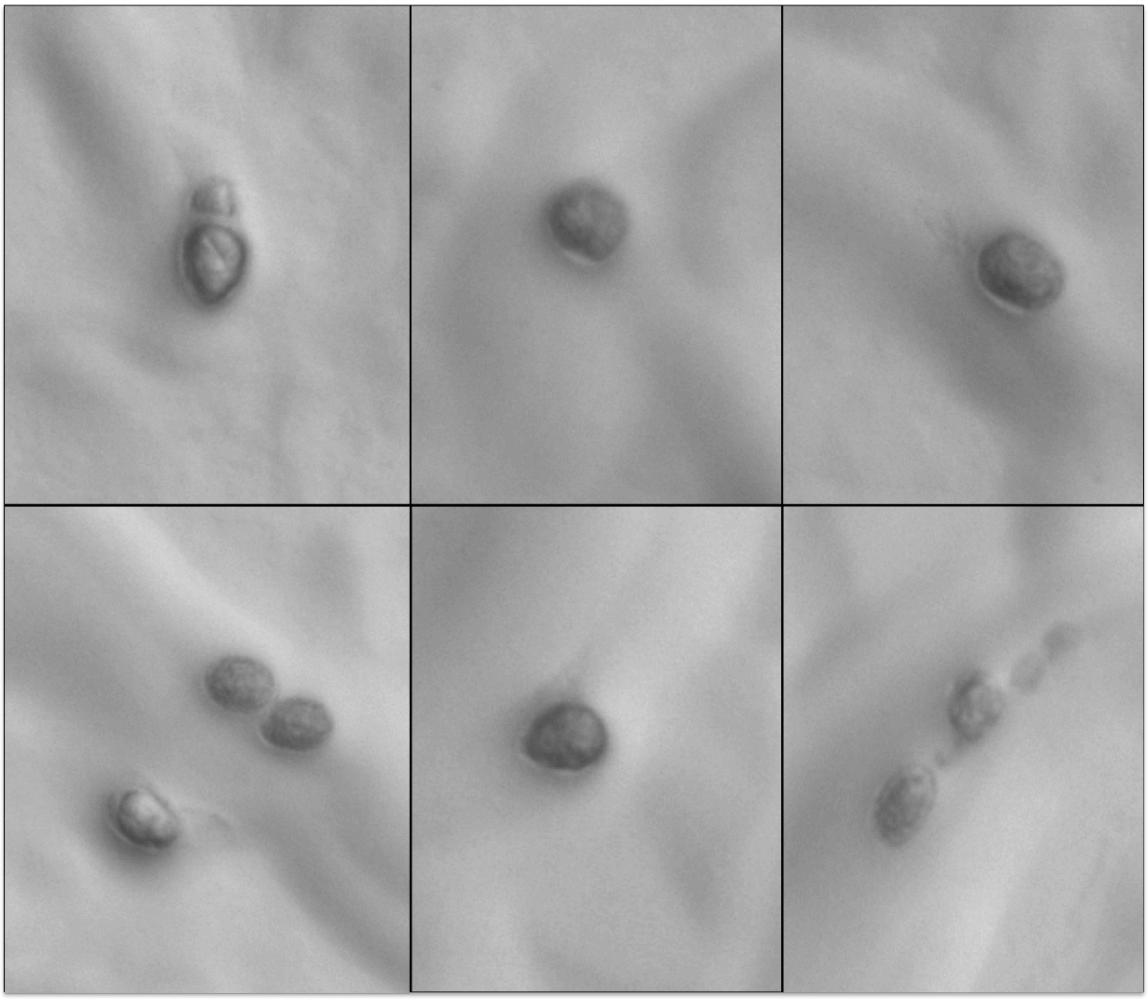


**Figure 3.16:** *ceh-18* hermaphrodites also lay endomitotic oocytes that are not viable. Eggs are abnormally shaped and the panels are representative of the phenotype. There is significant embryonic lethality in the progeny causing a reduction in brood size.

# Chapter 4

# Discussion

To understand the mating avoidance in *C. elegans*, I designed and conducted a series of experiments to disentangle the effects of hermaphrodite age, body size, sperm status, and receptivity to mate and male preference. It is not trivial to disentangle hermaphrodite receptivity to mate from male preference because they could be inextricably linked to each other.

## 4.1 Hermaphrodites with sperm show mating avoidance

*C. elegans* ecology is poorly understood, but it is inferred that their food source is ephemeral as they are often found in the dauer stage (Barriere and Felix, 2005). There is an advantage to hermaphrodites that prefer to self over outcrossing due to early reproduction in a life cycle of rapid colonization followed by extinction (Hodgkin and Barnes, 1991). During the evolution of hermaphroditism, genes required for successful copulation with males might be under relaxed selection in hermaphrodites or they may have evolved to actively avoid mating with males.

My results provide support for the hypothesis that the presence of sperm causes mating avoidance by hermaphrodites. I found that animals that contain sperm are less likely to mate. As hermaphrodites deplete their self-sperm; they mate more. N2 hermaphrodites do not mate at a higher rate on day 3 when they have sperm compared to N2 hermaphrodites

on day 5 that are sperm-depleted. N2 shows this trend consistently across many independent experiments. *fog-2* hermaphrodites are not capable of making sperm, therefore, are likely to mate even as day-3 adults. *fog-2* hermaphrodites are of the same age as and about the same body size as day-3 N2 hermaphrodites serving as a control for effects of age.

Hermaphrodites evolving from a gonochoristic female ancestor could lose receptivity to mating which is important for the female to mate but not necessary for hermaphroditic reproduction. In gonochoristic species like *C. remanei* and *C. sp. 4,* males produce a soporific inducing factor that immobilizes the female and causes the vulval slit to widen and spicule insertion occurs immediately. Consistent with the idea that hermaphrodites should lose receptivity to males, *C. elegans* and *C. briggsae* hermaphrodites, fail to respond to this factor when mated with *C. remanei* males (Garcia et al., 2007). However, there is no evidence that this mechanism is involved in the mating avoidance studied here.

*C. remanei* females show a shift in behavior after mating. Mating is associated with costs; females may suffer direct physical damage, behavioral or physiological manipulation (Fowler and Partridge, 1989; Gems and Riddle, 1996; Wu et al., 2012). It is in the interest of the female/hermaphrodite to avoid unnecessary matings. Therefore, a mechanism that allows hermaphrodites/females to optimize mating with males could be coupled with the amount of sperm in its gonad.

There is evidence for precisely such a coupling in *C. remanei*. Mated *C. remanei* females stop responding to the soporific inducing factor produced by the males. It is the presence of sperm and not ejaculate that causes them to stop responding to the soporific inducing factor. Germ-line ablated males (spermless) can induce the paralysis in virgin *C. remanei* females, but once mated by the germ-line ablated males, *C. remanei* respond to subsequent male's soporific inducing factor suggesting that *C. remanei* when mated behaves like hermaphrodites with sperm (Garcia et al., 2007). To reduce the probability of additional mating, *C. remanei* females also stop producing the potent sex pheromone once mated (Chasnov and Chow, 2002).

These observations suggest two scenarios for the evolution of mating avoidance by young *C. elegans* hermaphrodites. First, the mating avoidance behavior might be inherited from a *C. remanei*-like gonochoristic ancestor. The hermaphrodites do not respond to male contact because they have sperm and behave like mated females. Second, the mating avoidance behavior could have evolved during the transition from gonochorism to androdioecy, to actively avoid outcrossing. The behavioral analysis by Kleemann and Basolo (2007) suggests *C. elegans* hermaphrodites are not just unresponsive or passive to mating encounters; they actively avoid mating during their self-fertile period.

## 4.2 The mechanistic basis for mating avoidance

Hermaphrodites with mutant sperm that cannot fertilize oocytes also show mating avoidance. *spe-8* hermaphrodites that contain inactivated sperm show mating avoidance on day 3. The mutant sperm like wild-type sperm causes oocyte maturation and these hermaphrodites lay unfertilized eggs, curiously on the agar, as opposed to the bacterial lawn (Figure 3.12). *spe-38* hermaphrodites have sperm that is morphologically wild-type but fails to fertilize oocytes shows mating avoidance. These mutants also lay unfertilized eggs in the culture plate (Figure 3.10). Therefore, the mere presence of sperm, not fertilization or presence of eggs in the gonad is responsible for mating avoidance behavior shown by *C. elegans.*

### 4.2.1 Sperm sensing

It is possible that the mechanism for mating avoidance in the presence of sperm is inherited from the gonochoristic ancestor of *C. elegans.* It is likely that a mechanism was used by gonochoristic female to sense sperm for two reasons. Firstly, to couple oocyte maturation with the presence of sperm because oocytes are metabolically costly to produce and secondly, for the sake of optimizing mating as mating is associated with costs. In the closely related gonochoristic species *C. remanei*, oocytes arrest in meiotic prophase in the absence of sperm in *C. elegans* because oocytes are metabolically costly to produce, therefore, ovulation is coupled to the presence of sperm (Kionte et al., 2004).

Comparing the molecular mechanism for the sperm-sensing in closely related gonochoristic and androdioecious *Caenorhabditis* species, is one way to investigate if the mechanism of sperm sensing is inherited from a gonochoristic ancestor. Another approach is to mate day-3 *C. elegans fog-2* hermaphrodites with *C. remanei* males, a mating event that will not result in progeny. Then a mating assay can be conducted with the mated *fog-2* hermaphrodites and N2 males. If sperm-sensing pathway is conserved, the prediction is that mated *fog-2* hermaphrodites will show mating avoidance, which can be inferred, from a low PM.

### 4.2.2 Interaction of sperm and oocytes in the gonad

Hermaphrodites in *C. elegans* also need sperm for oocyte meiotic maturation and ovulation (Greenstein et al., 1994; Rose et al., 1997; Miller et al., 2001; 2003). In the absence of the sperm, oocytes mature at a very low rate and accumulate in the proximal gonad distal to the spermatheca (Figure 3.11). In *fog-2* hermaphrodites the number of oocytes maturing per gonad arm per hour is ≈0.09, when the *fog-2* hermaphrodites are mated, oocyte maturation resumes at the rate of ≈2.27 per gonad arm per hour comparable to ≈2.52 per gonad per arm per hour in day-3 N2 hermaphrodites (Miller et al., 2003). *fog-2* lays very few unfertilized eggs, in contrast to the *spe-8* and *spe-38* mutants. *spe-8* and *spe-38* lay many unfertilized eggs on the plate due to the interaction of the oocytes and the mutant sperm, and they are indistinguishable in their mating trends from N2 indicating that the interaction between sperm and oocytes is necessary for the N2 trend.

Mutated *ceh-18* gene causes oocyte maturation in the absence of sperm in hermaphrodites. If oocyte maturation mediates the mating avoidance behavior, then *ceh-18* mutants should not show N2 trend because oocyte maturation would be constitutive and they should not be likely to mate even on day 5. Morsci et al. (2011) found that day-5 *ceh-18* mutants did not elicit higher response that day-5 N2 hermaphrodites elicit in the mutant males. I found that *ceh-18* mutants showed the N2 trend, but they mate less overall compared to N2. Since *ceh-18* shows the N2 trend, it appears as though oocyte maturation does not mediate mating avoidance. However, higher mating seen in *fog-2* hermaphrodites is inhibited by *ceh-18* mutation, *fog-2;ceh18* double mutant hermaphrodites mate significantly less overall than *fog-2*. When introducing *ceh-18* mutation into the *fog-2* background turns on the sperm-sensing pathway constitutively, *fog-2* displays mating avoidance.

In my study, *ceh-18* mutants show a highly variable phenotype and other morphological abnormalities. They have migration defective gonad arms, protruding vulva (Figure 3.15) and improper differentiation of the sheath cells often becoming severe with age (Greenstein et al., 1994). Therefore, it is possible that *ceh-18* experiences an increase in mating due to loss of locomotion, which is necessary for mating avoidance. The mutants also display sterility, embryonic lethality, larval arrest and the eggs are often chopped up; some of the phenotypes are displayed in Figure 3.16 (Greenstein et al., 1994; Rose et al., 1997). Therefore, more experiments with *ceh-18* with an allele that is not as severe as *mg57* could help understand the mechanism better.

I found that small body size mutants *dpy-2*, *sma-2* and *dbl-1* showed mating avoidance on day 5, a time when N2 hermaphrodites have become more receptive to mating. However, my data indicate that mating avoidance in these mutants is caused by the presence of sperm and not reduced body size. These mutants show an extended egg-laying schedule; they lay eggs into day 7 suggesting that these mutants still contain self-sperm, therefore, behave like day-3 N2 hermaphrodites on day 5. Since they are also older on day 5 and yet show mating avoidance, the results indicate that age is not responsible for the increase in mating as seen in N2 day-5 hermaphrodites.

To confirm the sperm dependence of mating avoidance behavior, mating assays can be conducted with the small body mutant hermaphrodites on consecutive days until they have exhausted their self-sperm. The expectation is that they would experience an increase in mating on the day they exhaust self-sperm. However, the small body mutants also die early due to internal hatching of larvae (bagging or matricide) (Shijing et al., 2009). Most body size mutants owing to their small size of the gonad seem to show either the extended egg-laying schedule or bagging phenotype.

One question that remains is whether hermaphrodites display mating avoidance, not just in response to their own sperm, but also to sperm obtained through mating. To address this question, I would use mated *fog-2* or day-5 N2 hermaphrodites (primary mating) in a mating assay and then test whether they avoid mating like N2 hermaphrodites on day 3. This would require the use of sperm labeled with GFP or another marker so that a successful secondary mating could be identified.

## 4.3 Locomotion is required for hermaphrodite's mating avoidance behavior

The proportion of hermaphrodites mated in the mating assays is affected by hermaphroditic behavior, or modification of male behavior by hermaphrodites as I have kept males constant throughout the experiments and only manipulated the hermaphrodites. All hermaphrodites that I tested that have sperm and are capable of locomotion show the mating avoidance behavior.

When young hermaphrodites carry a mutation that causes paralysis and are not sperm-depleted, they are unable to exercise their mating avoidance behavior as evidenced by the higher proportion of worms being mated. Surprisingly, N2 males do not mate 100% of young paralyzed worms in 4 hours raising the possibility that even paralyzed worms can exercise some choice. Mutant males cannot mate N2 hermaphrodites, but they mate successfully with *fog-2* hermaphrodites, which are capable of locomotion. Therefore, the increase in mating on day 5 is facilitated by a decrease in mating avoidance.

## 4.4 Avoidance behaviors

Kleemann and Basolo (2007) showed that hermaphrodites can avoid mating through the pre-copulatory mechanism of sprinting away from males attempting to mate and through the post-copulatory mechanism of expelling male ejaculate from their uteruses. Either of these mechanisms or both one of them could be at work in the mating assays presented in this study. In the mating assay, the proportion mated is measured by the presence of

outcrossed progeny. When there is no outcrossed progeny, it could be due to the hermaphrodites avoiding mating by sprinting away or by expulsion of sperm. To understand the exact mechanistic basis of the N2 trend the mating assays have to be recorded for the duration of 4 hours and analyzed for factors like rates of encounter, time to finding a mate, length of copulation, and expulsion of male ejaculate.

Successful mating in nematodes requires the hermaphrodite to be stationary (Garcia et al, 2007). The increase in mating of day-5 hermaphrodites could be due the ease of insertion of the spicules of the males. The males prod the vulval slit of their partner with their spicules and then penetrate the vulva. This takes longer in younger hermaphrodites (Garcia et al., 2001). Therefore, the increase in the mating of day-3 *fog-2* is due to locomotion because males mate them successfully.

There could be a behavioral switch in the hermaphrodites following sperm-depletion. *fog-2* has been shown to affect the behavior of hermaphrodites, they leave the bacterial lawn and explore ('mate-searching' behavior) compared to N2 hermaphrodites, which almost never leave the lawn when sperm is present (Lipton et al., 2004). I found that sperm-depleted N2 hermaphrodites displayed 'mate-searching' behavior by leaving the bacterial (Figure 3.7). Therefore, an experiment in which the exploratory behavior of *fog-2* and sperm-depleted hermaphrodites in the presence and absence of males is assayed can shed some light on the mechanism that mediates higher mating success.

Hermaphrodites could have evolved to detect males by chemotaxis to avoid them or seek them when they are sperm-depleted to increase the probability of mating. A study by Izrayelit et al. (2012) showed that *C. elegans* males also produce ascarosides and their chemical profiles are different from hermaphrodites. A component of the male ascaroside profile ascr#10 has been shown, to strongly attract and retain hermaphrodites, even at extremely low quantities.

N2 hermaphrodites produce ascr#3 which repels other hermaphrodites (Srinivasan et al., 2008). In the locomotion assays, young N2 animals seem to avoid aggregation and spread out on the plate also called the solitary behavior shown by *npr-1 (215 V)* (Gloria-Soria and Azevedo, 2008; Macosko et al., 2009). However, hermaphrodites with no sperm seem to be switching between two behaviors, exploratory behavior shown by N2 (day-5) hermaphrodites and *fog-2* (day-3 and day-5) hermaphrodites or spending more time in a clump or the center in the plates like day-3 *fog-2* hermaphrodites (Figure 3.13 and 3.14). Gregarious strains like CB4856 maintain a higher percentage of males in the population (Wegewitz, 2008; Anderson et al., 2010), indicating an alternative mechanism that mediates higher mating in sperm-depleted hermaphrodites. The mating avoidance in the day-3 hermaphrodites of CBS4856 is predicted to be higher due to a higher percentage of males in the population.

## 4.5 The decline in mating avoidance is not mediated through the pheromone

It has been observed that hermaphrodites produce a pheromone that attracts males from a distance (Simon and Sternberg, 2002; Srinivasan et al., 2008, 2012, White et al., 2007). If the decline in mating avoidance in sperm-depleted hermaphrodites is due to increased secretion of pheromone that attracts males from a distance, then a mutant in pheromone biosynthesis pathway, when sperm-depleted would not mate at a higher rate.

Like Morsci et al. (2011) I found that the N2 trend is unlikely to be through the pheromone found by Simon and Sternberg (2002) because *daf-22* mutants that are partially defective for short-chain ascaroside synthesis also show the N2 trend (Srinivasan et al., 2012). *daf-22* showed the N2 trend. Even day-3 *daf-22* hermaphrodites were mated in the same proportion as N2 hermaphrodites suggesting that the absence of pheromone does not decrease the likelihood of mating in the mating assay set up.

## 4.6 Age-related changes in *C. elegans* do not mediate inhibition of mating avoidance

If age-related changes are responsible for inhibition of mating avoidance, then mating avoidance should have decreased in day-5 hermaphrodites consistently across all assays resulting in a N2 trend. However, the results on *fog-2* and small body mutants (*sma-2*, *dbl-1* and *dpy-2*) do not support the age hypothesis. I measured the mean velocities of

both N2 and *fog-2* hermaphrodites on day 3 and day 5. There was no age-related decline in locomotion that could be detected in my analysis.

In addition, there is no evidence in the aging literature that hermaphrodites on day 5 are defective in locomotion compared to day-3 hermaphrodites (Croll et al., 1977; Herndon et al., 2002; Huang et al., 2004). I measured speeds of day-3 hermaphrodites and day-5 hermaphrodites and found that the average day-5 hermaphrodites were faster than day-3 hermaphrodites. Hermaphrodites live for up to 3 weeks (Klass, 1977). Type B animals do not move unless prodded. They leave non-sinusoidal tracks first appear only between day 6 and day 7 (Herndon et al., 2002). In another study, significant decline in fast body movement (type A) starts from day 10 (Huang et al., 2004). Therefore, age-related changes are likely not significant enough on day 5 to cause the decline in mating avoidance.

I tested a narrow age hypothesis, insofar as age causes locomotory decline. There could be other effects of age, for an increase in mating, like structural changes in the vulva that increases the ease of mating for the male. The hermaphrodites might have an ability to sense the male probing their bodies, and they might lose the ability to sense this as they age. Garcia et al. (2001) present data that males prodded the vulval slit for longer in younger hermaphrodites than older hermaphrodites. The mechanistic basis of the response of the vulval slit to prodding by the spicules could make clear, if the hermaphrodite is receptive or the vulva is worn out due to egg laying or effects of age.

To test the age hypothesis further, I used *daf-2* mutant hermaphrodites that are long-lived mutants (morphologically younger on day 5 compared to day-5 N2 hermaphrodites) to disentangle the effects of aging and presence of sperm on mating. *daf-2* presented with a protruding vulva phenotype and a sterile phenotype that did not result in self-progeny or cross-progeny making it unsuitable for the mating assays. The data are presented in Figure 3.10, but more studies need to be conducted to draw conclusions.

## 4.7 Male preference for sperm-depleted hermaphrodites

Morsci et al. (2011) presented evidence that a hermaphrodite-derived cue that is dynamically regulated by its sperm-status stimulates male mating behavior. They detected higher response efficiency (RE) towards day-5 hermaphrodites compared to day-3 hermaphrodites and concluded that males prefer older hermaphrodites. This conclusion is questionable for three reasons. First, a strict choice assay was not performed. Instead, hermaphrodites of different ages were assayed separately, and the choice was inferred from the number of times mutant males contacted hermaphrodites. Second, they did not use a strong enough locomotory mutant. *unc-31* can be characterized more accurately as sluggish, rather than paralyzed. Therefore, they were not able to totally isolate the effect of male preference and eliminate the effect of hermaphrodite locomotion. Third, they used mutant males in the assays whose response efficiency (RE) itself depends on the locomotion of the hermaphrodites. The fact that mutant males are sensitive to hermaphrodite locomotion, in that they respond more to a paralyzed worm, and the locomotory defect of *unc-31* becomes severe with age, introduces a bias towards older hermaphrodites in the study.

If there is a male preference for sperm-depleted hermaphrodites, that should be done in a choice assay with the ability of the male to distinguish between the two. They did not perform an actual choice assay; instead, day-3 and day-5 animals were assayed separately. They performed the assay on a 5mm lawn of *E. coli,* where 20 hermaphrodites are placed in the lawn and allowed to acclimatize for 10 minutes prior to the assay. Then, 5 males that are to be assayed are placed in the center of the mating lawn and observed for a 4-minute period. RE is scored as positive when a male made flush contact with the ventral side of his tail against the hermaphrodite and initiates scanning along her body. They find that males contact sperm-depleted hermaphrodites at a higher rate.

An actual choice assay with day-3 and day-5 N2 hermaphrodites was performed by Morsci et al. (2011) and they found that N2 males contacted more sperm-depleted hermaphrodites than hermaphrodites with sperm. I tried replicating a choice assay based on their assay with both *unc-31* and N2 hermaphrodites, but it was difficult to control for the relative positions of the hermaphrodites.

Morsci et al. (2011) used genetically immobilized *unc-31 (e169)* hermaphrodites to avoid the confounding factor of the hermaphrodite's locomotion, but *unc-31 (e169)* is sluggish in its locomotion. *unc-31* is not a completely paralyzed strain, and like many uncoordinated mutants in *C. elegans,* hermaphrodites' locomotion gets progressively worse with age (Hodgkin et al., 1988; Avery et al., 1993; Speece et al., 2007). Day-5 *unc-31* hermaphrodites are worse in locomotion than day-3 hermaphrodites, and would not move away in the 10 minutes given for them to acclimatize in the assay. If the

phenotype of the hermaphrodite is variable with age, that could lead to biased results. In 4-min assay, the male preference and hermaphrodite preference are difficult to disentangle because day-3 hermaphrodites might move away slightly and the mutant male being sensitive to locomotion might not be able to respond to contact with a hermaphrodite; therefore, it would be counted as no RE of the male.

In the assay by Morsci et al. (2010), RE in 4-minute time period is used as a proxy for mating preference. The RE of the male itself depends on the locomotion of the hermaphrodite. In fact, the *lov-1* mutant males exhibit high mating efficiency as measured the presence of cross-progeny with severely paralyzed *unc-52* mutant hermaphrodites (Barr and Sternberg, 1999). Using mutant males and RE, which itself is dependent on the locomotion of the hermaphrodite introduces two confounding factors. When they repeated the assay with N2 males and their response *unc-31* hermaphrodites, there was no significant difference between the response to day-3 and day-5 hermaphrodites. Thus, there is evidence within their own study that N2 males do not show the preference. Males will, in fact, attempt to mate with every individual they contact (Barrios et al., 2008; Stewart and Philips, 2002).

The data from mating assays with mutant males (*pkd-2* and *lov-1)* presented in this study show that the mutant males cannot mate effectively with N2 hermaphrodites, but are able to mate successfully with paralyzed *unc-31* hermaphrodites (Figure 3.5). The mutant males are more sensitive to the mate avoidance behavior of day-3 N2 hermaphrodites. The mutant males might fail to respond to the *unc-31* hermaphrodites as well on some

encounters, but during the assay that spans over 4 hours, they have been able to mate successfully. Thus, these observations do not support the idea that males have a preference for sperm-depleted hermaphrodites put forth by Morsci et al. (2011).

Finally, Morsci et al. (2011) did not identify that mating-cue that stimulates the male mating behavior to prefer older hermaphrodites. As a mating strategy, males should evolve to mate with younger hermaphrodites as the quality of their oocytes decreases with age (Andux and Ellis, 2008). A male preference for older hermaphrodites could coevolve with mating avoidance showed by younger hermaphrodites. In the mating assays (Figure 3.5), I did not explicitly test for the mechanism by which mutant males have higher mating success with sperm-depleted hermaphrodites. The fact that they can mate equally well with day-3 paralyzed hermaphrodites which are like day-3 N2 hermaphrodites in all other aspects except in locomotion as far as I can tell, indicates that it is likely a hermaphrodite locomotory effect on the males. Ultimately, a strict assay between the day-3 and day-5 N2 hermaphrodites paralyzed in a noninvasive method without affecting the male behavior would be required to disentangle male and hermaphrodite behavior.

# Chapter 5

# Conclusion

In this study, I set out to disentangle the effects of age, male preference, and hermaphrodite receptivity on mating success in *C. elegans*. My experiments have led to three main conclusions. First, I found that hermaphrodites that run out of sperm are more likely to mate. Hermaphrodites that have sperm, actively avoid mating by their ability to sprint away, which I call mating avoidance. When hermaphrodites are paralyzed, they cannot exercise this behavior and hence have higher PM. Second, the presence of sperm and its interaction with oocytes is likely one of the phenomenon that mediates the mating avoidance behavior. There is circumstantial evidence for the link between gonadal sheath cell contraction and locomotion of hermaphrodites, hermaphrodites that are sperm-depleted have lower gonadal sheath cell contractions and also display a different pattern of locomotion. The mutations affecting gonadal sheath cell contractions have been studied extensively at a physiological and molecular level, but the behavioral phenotypes associated with them have not been of interest. Quantifying the locomotion of mutants like *ceh-18* and *vab-1* which function in the sperm-sensing pathway will expand the knowledge on the mechanism of mating avoidance. Third, I did not find evidence that age-related locomotory changes mediate the mating avoidance behavior. There might be some structural and physiological changes caused by age, which I have not tested in this study that are responsible for the shift in the mating behavior.

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