Assortative Fertilization in the Elegans-Group of *Caenorhabditis*

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ASSORTATIVE FERTILIZATION IN THE ELEGANS-GROUP OF CAENORHABDITIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

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B.S., The Ohio State University, 2010

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ASSORTATIVE FERTILIZATION IN THE ELEGANS-GROUP OF CAENORHABDITIS

ASSORTATIVE FERTILIZATION

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Assortative Fertilization in the Elegans-Group of Caenorhabditis.

Assortative fertilization refers to the species-specific interactions between sperm and oocytes that affect the success of fertilization. One type of interaction is chemotaxis of sperm to oocytes. In Caenorhabditis elegans, amoeboid sperm must crawl along the uterine lining towards the spermathecae in response to oocyte-derived prostaglandin signals for fertilization. This chemotactic signaling system likely operates in other species of the Elegans-Group of Caenorhabditis as sperm of C. briggsae and C. remanei do localize to the C. elegans spermathecae. In this project the impacts of species-specific chemotaxis on fertilization and female fecundity were assessed. To accomplish this, the localization of fluorescently-labeled C. remanei sperm to the spermathecae was determined in C. nigoni, C. briggsae and various C. nigoni: C. briggsae hybrid ‘females’. Each of these crosses was also scored for cross-fertility and cross-fecundity. These data were used to study correlations between sperm chemotaxis, cross-fertility and cross-fecundity. Variation in sperm chemotaxis explained only 1% of the variation in cross-fertility and only 8% of the variation in cross-fecundity. Additionally, sperm-derived chemotactic signaling for oocyte maturation and ovulation do not appear to be species-specific. Therefore, other mechanisms, such as specific-specific receptor-ligand
interactions and/or insemination reactions, must also contribute to assortative fertilization in *Caenorhabditis*. 
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**INTRODUCTION**

**Speciation and Reproductive Isolation**

A biological species is a population of interbreeding individuals that are reproductively isolated from other species (Mayr, 1942; Dobzhansky, 1970). Reproductive isolation encompasses all genetic mechanisms that inhibit or restrict gene flow between co-existing populations (Dobzhansky, 1970; Mayr 1963). Evolutionary biologists have focused on genes coding for characteristics integral in reproduction to determine when, why and how species diverge. A comparison of two closely related species genomes’ could identify important factors leading to reproductive isolation between two populations. Reproductive isolation between two species can be caused by many factors. Three major categories of reproductive isolation are pre-mating mechanisms, post-zygotic mechanisms and assortative fertilization. Pre-mating mechanisms include differences in mating season, genitalia, behavior, or habitat preference between species (Dobzhansky, 1970). These mechanisms temporally, physically, or behaviorally isolate individuals of different species from each other. Post-zygotic mechanisms include hybrid inviability and hybrid sterility (Mayr, 1959). Even though hybridization can occur through this mechanism gene flow is restricted. Assortative fertilization mechanisms prevent or limit sperm-oocyte interactions between species (Dobzhansky, 1970). Assortative fertilization mechanisms include species-specific chemotaxis of sperm towards oocyte, receptor-ligand interactions between gametes and acrosome reactions.
Over time evolutionary forces change a population’s allele frequencies to increase fitness for its environment. Fitness can be defined as the reproductive output in lifetime. A population’s fitness can be represented by the peak heights on an adaptive landscape of genotypes [Figure 1]. Wright’s shifting-balance model attempts to explain multiple evolutionary forces influence on adaptive genotypes (Coyne et al., 1997) [Figure 1]. Each peak in Wright’s model represents an independent genotype. All individuals within can interbreed. Populations can occupy peaks and valleys. In some cases gene flow can occur between peaks. Reproductive isolation between two peaks is represented by a valley. If hybrids between two species are fit then the valley slope between the two parental peaks is shallow. When a hybrid is less fit than its parental species a steep valley will form between the two peaks (Ayala et al., 1974; Ayala, 1975). Speciation could result in the splitting of one peak into two peaks. The peaks in Wright’s theory are a result of multiple evolutionary forces constructing allele-specific gene complexes. An adaptive gene complex consists of linkage or interaction among several alleles conferring an increase in fitness for a population in the environment. It is an allele-specific network of genes that is responsible for the interactions of genotypes, proteins, and phenotypes (Goodnight, 1995). These complexes arise by molding maladaptive allelic combinations until a peak of fitness is attained (Goodnight, 1995). Adaptive gene complexes will increase in frequency if they confer a higher fitness in the current environment.
**Figure 1**: Wright’s Shifting Balance theory. Adapted version of Sewall Wright’s theory on adaptive gene complexes. Each peak represents a genotype with the highest fitness in the adaptive landscape. Each valley represents a genotype with the lowest fitness in the adaptive landscape. Selection will drive populations towards a peak or valley in response to environmental conditions. Adapted from Kauffman and Levin, 1987.
Adaptive gene complexes are resistant to genetic recombination and are often inherited as an entire unit. Linkage disequilibrium is the non-random association of two or more alleles (Falconer and Mackay, 1996). Linkage disequilibrium can decrease recombination along chromosomes, thus producing heritable blocks of the genome (Viard et al., 1997). If linkage disequilibrium between an allele-specific complex increases fitness, then this complex will be passed onto future generations. One example of adaptive gene complexes is observed in five populations of Hawaiian Drosophila (Ohta, 1980). Two different gene complexes have evolved due to ecological differences in their mating sites. The D. grimshawi-Maui complex populations have a polymorphic inversion sequence on chromosome 4 and lay their eggs on a variety of plants. Other D. grimshawi populations retain the primary ancestral sequence on chromosome 4 and lay their eggs specifically on rotting bark of a Wikstroemia tree. Fertile F1 hybrids are produced between the D. grimshawi-Maui complex and other D. grimshawi populations (Ohta, 1980). Dysgenic interactions between adaptive gene complexes result in hybrid infertility in F2 generations (Ohta, 1980). The degree of reproductive isolation between the D. grimshawi-Maui complex and other D. grimshawi populations only becomes apparent in the low fitness values observed in hybrids (Ohta, 1980).

The evolutionary forces acting upon a population’s genome are mutation, genetic drift, gene flow and natural selection (Johnson, 2008). In every generation new mutations arise and go extinct. These mutations introduce new allelic variants that may be fixed or lost in a population (Halliburton, 2004). The persistence of a mutation in a population is
based on its impact on the phenotype and ultimately fitness. If a new allelic variant in the gene complex confers an increase in fitness in the current environment then it increases in frequency throughout the population (Kim and Rieseberg, 2001). New selectively advantageous allele-specific complexes will increase the height of its population’s peak in Wright’s model [Figure 1].

Mutations not changing the amino acid sequence or, located in non-coding regions are often referred to as ‘neutral’. Neutral mutations do not change phenotypic expression. Evolutionary biologists calculate divergence times between closely-related species by quantifying the accumulation of neutral mutations (Halliburton, 2004). Some mutations in non-coding regions are not neutral. Mutations in the non-coding region of the genome could inhibit or prevent gene expression. Mutations are an evolutionary force that continually introduces novel allelic variants thereby increasing a population’s genetic diversity.

In contrast, genetic drift decreases the amount of genetic diversity within a population (Halliburton, 2004). Genetic drift is the random fluctuation of allele frequencies due to sampling error of gametes and chance events (Halliburton, 2004). Two disadvantages of genetic drift for populations are the increase in frequency of deleterious alleles and loss of beneficial alleles from a population. Genetic drift can cause a population to move from one peak to the base of another peak (Johnson, 2008; Coyne et al., 1997). In Wright’s model, genetic drift allows populations to traverse the adaptive landscape to attain a higher fitness in the current environment. This evolutionary force is
stochastic and its effect is inversely proportional to population size. During the 20th century, evolutionary biologists like Ernst Mayr purported new species formation could only have resulted from genetic drift in allopatric populations (Mayr, 1942).

The accumulation of novel allelic variants between two populations since isolation often results in genomic incompatibilities. These incompatibilities are observed in hybrids with a lower fitness than either parental population (Halliburton, 2004). In addition to genetic drift, two populations separated by physical barriers also experience different environmental pressures like competition and predation. These environmental pressures change the population’s genome by increasing the frequency of allelic variants conferring a higher rate of survival and fitness of an individual. Populations separated by geographic barriers are often referred to as allopatric populations. Allopatric populations experiencing secondary contact produce hybrids but these hybrids may not be advantageous in the environment in comparison to parental populations (Wallace, 1912) [Figure 2]. Hybrid disadvantages include decreased reproductive output, competitiveness and survival in either parental environment. The production of hybrid progeny varies from embryonic lethality to F1 hybrid progeny reaching reproductive maturity (Dobzhansky, 1970). In succeeding generations of F2 and F3 hybrids, hybrid breakdown can occur. Hybrid breakdown is lower if not variable rate of fitness in comparison to parental and F1 generations. Decreases in fitness experienced by hybrids are due to incompatibilities from genetic variants accrued since isolation. Each population’s genome will drift independently in isolation. The impact of genetic drift upon two populations
that were previously isolated is not realized until secondary contact. Dysgenic incompatibilities between populations with different adaptive gene complexes will decrease gene flow.

Dysgenic incompatibilities can also arise within populations with gene flow. These populations are referred to as sympatric populations. Sympatric populations are not separated by physical barriers [Figure 2]. The ‘Speciation with Gene Flow’ model proposes a population genomic landscape with gene flow at certain loci and the prevention of gene flow at other loci (Wu, 2001) [Figure 3]. This model highlights the reduction of gene flow at certain loci between populations due to dysgenic hybrid incompatibilities. In Wright’s model, migrants from highly fit populations will increase the fitness of surrounding populations by introducing new adaptive complexes (Coyne et al., 1997; Halliburton, 2004). Increases in the fitness of surrounding populations will increase the frequency of the adaptive complex in Wright’s model.
Figure 2: Allopatric vs Sympatric speciation. Allopatric populations will diverge when geographic barriers prevent gene flow. Each population will be subject to differing evolutionary forces and environmental constraints. New species are formed due to accumulation of mutations during the time since contact. In contrast, under sympatric models of speciation new species can form within a population. Selection for different phenotypic variants gives rise to new species even when the entire population is in contact. Adapted from http://kullee.myblog.it/2012/01/09/sympatric/
Figure 3: Speciation with gene flow model. This speciation model theorizes selection for environmentally adapted characteristics will gradually lead to an increase in reproductive isolation between sympatric populations. From Wu, 2001.
Mechanisms that can reduce gene flow are gene linkage and selection. If advantageous allelic variants are linked to adjacent loci then these loci may hitchhike their way to fixation through low rates of recombination and natural selection. If novel allelic variants in loci coding for reproductive processes hitchhike their way to fixation then barriers to reproduction can form despite gene flow (Wu, 2001).

Natural selection is an evolutionary force that can inhibit the flow of genes between populations. Natural selection is a process in which alleles become more or less frequent to increase a population’s adaptation to an environment. A classic example of adaptive evolution is Charles Darwin’s finches. Increased competition for food resources within ancestral finch populations increased genetic diversity (Grant, 2003). Selection can either increase or decrease allelic diversity in response to the demands of the current environment in which the population resides. In the case of Darwin’s finches, an increase in beak diversity decreases competition for resources by increasing the variety of food the finches could consume. Selection manipulates Wright’s shifting balance theory by favoring which genotypic peaks are occupied or vacated by a population. The slope of each genotypic peak is dependent upon the strength of selection (Halliburton, 2004).

Another type of selection that may drive sympatric speciation is selection for sexual characteristics. Sexual conflict is an antagonistic relationship in sexually reproducing species with variable fitness strategies between genders leading to a coevolution of self-serving mechanisms (Parker, 2006). Selection results in the evolution of mating behavior, reproductive organ morphologies, and secretory inhibitors of
additional matings all of which can lead to rapid diversification between species (LaMunyon and Ward, 1998; Ting et al., 2014).

   Rapid diversification between sympatric species experiencing post-zygotic isolating mechanisms may be driven by reinforcement selection (Wallace, 1912). Reinforcement selection seeks to prevent the waste of reproductive efforts by increasing the frequency of pre-mating isolating mechanisms. An investment of resources into the production of inviable or sterile hybrids does not benefit either population. Moreover, the production of gametes coupled with an unsuccessful mating occurrence is a wasteful interaction. Reinforcement selection affects loci coding for reproductive characteristics. Assortative fertilization mechanisms do not prevent the waste of gametes. Reinforcement selection will drive two species towards pre-mating isolation to prevent further waste of reproductive resources on the production of hybrids (Wallace, 1912; Ridley, 2003). Variation in the degree of gamete waste depends on parental care investment. Broadcast spawners like sea urchins employ the ‘r’ strategy for reproduction by investing little into parental care and releasing copious amounts of gametes. The prevention of inter-specific sea urchin hybrids is due to inability of hetero-specific sperm to traverse the jelly layer surrounding oocytes (Suzuki and Garbers, 1984). This example of sea urchin reproduction highlights the waste of both sperm and oocytes and the prevention of hybridization due to assortative fertilization mechanisms.

   In contrast, some populations employ a ‘k’ reproductive strategy where they invest a great amount of energy into the survival of their progeny. This parental
investment is represented by infrequent production of large offspring and prolonged parental care (Reznick et al., 2002). One organism that relies on parental care to ensure offspring survivability is the American robin *Turdus migratorius*. A small clutch of 3-5 offspring are incubated by the mother alone (Alderfer, 2006). After two weeks of incubation the chicks hatch. In the following weeks the mother will provide food to maturing chicks and rid the nest of waste (Alderfer, 2006). A large amount of maternal investment into offspring survival would drive selection to guarantee that each offspring is viable and fertile (Reznick et al., 2002). The degree of selection against hybrid production is directly related to the fitness of hybrid progeny and parental care investment. An increase in maternal investment will increase how choosy a female is for potential mates (Turner et al., 2011). In the context of Wright’s model, low fitness hybrids with maladaptive genotypes are located in the valleys of the adaptive landscape (Johnson, 2008). Reinforcement selection can increase the earlier barriers to reproduction to prevent hybridization between two adaptive gene complexes (Ridley, 2003; Johnson, 2008).

Another model for speciation focuses on polyploidy preventing the production of viable and fertile hybrids. Polyploidy is a heritable condition in which an organism contains more than two complete sets of chromosomes (Weiss-Schneeweiss et al., 2013). Large-scale gene duplication events like polyploidy induces speciation by decreasing gene flow between novel and extant genomes. The disproportionate division of chromosomes during meiosis in polyploid organisms prevents hybridization with
Figure 4: Polyploid speciation in angiosperms. Rapid species divergence is evident in flowering plants. In crosses of Melampodium glabribracteatum (2x) and M. americanum (2x), hybrid progeny (M. strigosum) retain 4 sets of chromosomes. When M. strigosum (4x) is mated to M. linearilabum (2x), two new polyploid variant species are produced. Polyploid hybrid species are viable and can successfully reproduce with species with different number of chromosome sets than their own. From Weiss-Schneeweiss et al., 2013.
ancestral populations. Polyploid genomes have the potential to proliferate deleterious alleles and mask beneficial alleles to the detriment of the organism. More recent paleological evidence reveals advantages of polyploidy: genomic diversity, masking deleterious alleles and species richness (Weiss-Schneeweiss et al., 2013). For example, polyploid plant organisms have an advantage in environments with increased competition for niche availability (Weiss-Schneeweiss et al., 2013). All angiosperms show evidence of at least one whole genome duplication event and there is a positive correlation between species richness and polyploidy (Weiss-Schneeweiss et al., 2013) [Figure 4]. Despite differences in the ‘how’ and ‘why’ of species divergence, all speciation models attempt to explain how new species evolve.

Many models have been proposed since Darwin’s extensive investigation into natural selection and species formation (Orr, 1996). Despite this, evolutionary biologists still struggle with the concept of hybrid male sterility and lethality. How can homozygous species (AA) arise from a homozygous ancestral population (aa), if there is lethality in heterozygous individuals (Aa)? Bateson (1909) first proposed the complementary model. In this model, two complementary factors (presumably loci) gained by species after isolation would explain hybrid male sterility upon secondary contact. A similar model was proposed by Dobzhansky (1936), emphasizing the need for at least two complementary genes necessary to explain male hybrid incompatibilities. Both models focused on a multi-gene dysgenic interaction between allopatric species. Muller (1940; 1942) integrated epistasis into the model focusing on interactions between these genes.
and subsequent changes to its function. The changes in function Muller referred to are sterility and fertility. In its entirety the Bateson-Dobzhansky-Muller (BDM) model gives evolutionary biologist a genetic foundation in which to build models to explain reproductive isolation. Despite the simplicity of each speciation model, variable environmental constraints add complexity to new species formation.
**Sperm Chemotaxis/Prostaglandin signaling**

During fertilization, one of the earliest stages of gametic interaction involves the movement of sperm to the oocyte in response to a chemical stimulus. Females maximize the probability of producing viable progeny by producing specific chemical signals to attract the ‘right’ sperm or sperm from a male of the same species. Species-specific chemoattractants deter sperm from other species from directing themselves towards the unfertilized oocyte (Evans, 2012). In the absence of egg chemoattractants, sperm locating female gametes left to random chance.

Species-specific chemotaxis is of particular importance for non-motile marine invertebrates like blue mussels that use broadcast spawning to reproduce. Precedence for compatible gamete interactions by chemotactic signaling prevents undesirable combinations. The degree of selection on genes involved in the variation in chemotactic signaling would be strong in organisms that reproduce using broadcast spawning (Evans, 2012). The reduction of gametic interactions in sessile marine invertebrates using broadcast reproduction reinforces the selection for species-specific gametes (Evans, 2012). This reinforcement selection is a result of gamete waste during sexual interactions that do not result in fertile progeny. The type of natural selection involved in female mate choice and evolution of reproductive characteristics is sexual selection. Sexual selection increases the probability of mating and producing viable progeny (Darwin, 1859). In marine invertebrates, sexual selection of species-specific chemical attractants differentiates potential mates and ensures successful fertilization events.
Evidence of intra-specific gamete chemoattractants is observed in blue mussels, *Mytilus galloprovincialis* (Evans, 2012). The blue mussel example highlights two mechanisms that inhibit gamete interactions: receptor-ligand interactions on the surface of gametes and chemical attractants localizing the sperm toward the oocyte. In dichotomous mate choice experiments of blue mussels, variation in the chemosensory response of male sperm is dependent on individual female egg chemoattractants (Evans, 2012) [Figure 5]. Male preference for female-specific chemical stimuli in broadcast reproducers suggests a possible motive for strong selection on loci involved in egg chemoattractants in *Mytilus galloprovincialis*. In sessile marine invertebrates, chemical attractants would differentiate potential mates and reduce energy expenditures on failed attempts at reproduction (Evans et al., 2012). In the context of reinforcement selection, the sexual selection observed in broadcast spawning species like the blue mussels should drive reproductive characteristics towards pre-zygotic barriers. The cost of reproduction and importance of mate choice will drive selection to act upon earlier stages of gametic interactions to increase a population’s fitness. Chemotaxis of sperm towards the oocyte precludes cell-surface interactions between gametes.

**Receptor-ligand Interactions**

Every cell exhibits proteins on its extra-cellular membrane (ECM) for communication. Gametic cell ECM proteins are highly polymorphic and allow for specificity in signaling between sperm and oocytes. Important functions of gamete ECM proteins are communication between sperm and oocytes and fusion of sperm cells to the
**Figure 5:** Female-specific sperm chemotaxis. Experimental design testing the role of female-specific sperm chemotaxis in *Mytilus galloprovincialis*. The diagram tests the preference of Male A and Male B sperm for either Female A or Female B eggs. In this study, sperm chemotaxis was found to be female-specific. Adapted from Evans et al., 2012.
“oolemma”, the plasma membrane of the oocyte. Strain-specificity of ECM protein interaction is observed in sea urchins, sessile marine invertebrates that reproduce using broadcast spawning. In sea urchins, inter-specific sperm-oocyte interactions are prevented due to a disjunction between protein receptors and corresponding ligands (Lopez et al., 1993). Sea urchin eggs are surrounded by a jelly layer that inhibits spermatozoa from penetrating the extracellular matrix to induce fertilization (Suzuki and Garbers, 1984). In *Strongylocentrotus purpuratus*, a sperm-activating peptide, speract, has been isolated from the jelly layer. Speract increases sperm respiration and motility through the jelly layer (LaMunyon et al., 1985). *Arbacia punctulata* produce a sperm-activating protein called resact. Species-specific sperm activating peptides like speract and resact allow sperm to reach the plasma membrane of the egg and fuse for fertilization [Figure 6]. The species-specificity observed in the sperm activating peptide amino acid sequence forms a barrier to hybridization between species of sea urchins (Suzuki and Garbers, 1984). Elucidating the forces inducing variation in the amino acid sequence of gametic cell proteins could provide researchers with a key to understanding the evolution of reproductively isolation.

Recently a proteomic analysis of vertebrate sperm proteins and other genes involved in sperm traits revealed evidence of adaptive evolution and positive selection on sperm traits (Meslin, 2012; Vicens, 2014) [Table 1]. In particular, more proteins involved in sperm motility and sperm-egg fusion exhibited positive selection than genes involved in other sperm-egg interactions (Vicens, 2014). A substitution or change in amino acids
coding for sperm-oocyte ECM proteins initiates specificity between gametes (Meslin, 2012). Species-specificity is borne from differential positive selection across all gamete recognition proteins further initiating a coevolution between sperm and oocytes (Vicens, 2014).
Figure 6: Species-specificity in receptor-ligand interactions in sea urchins. *Arbacia punctulata* sperm in response to different sperm chemotactrant a) resact and b) speract. Resact increases the chemotaxis of *A. punctulata* sperm towards *A. punctulata* eggs and allows for agglutination of eggs. Speract peptide produced by *Strongylocentrotus purpuratus* does not attract *A. punctulata* sperm and eggs are randomly dispersed. Bar, 300μm x 41. From LaMunyon et al., 1985.
**Table 1:** Proteomic analysis of vertebrate sperm proteins. P-values above the diagonal represent the average number of non-synonymous substitutions. In pairwise comparisons of non-synonymous substitutions of coding regions for sperm proteins, sperm-egg interactions were significantly higher than all other processes except sperm motility. The highest percentages of genes with positive selection were involved in sperm-egg interactions. Adapted from Vicens et al., 2014.

<table>
<thead>
<tr>
<th>Reproductive process</th>
<th>Spermatogenesis</th>
<th>Sperm metabolism</th>
<th>Sperm motility</th>
<th>Capacitation</th>
<th>Acrosome reaction</th>
<th>Sperm-egg interaction</th>
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<tbody>
<tr>
<td>Spermatogenesis</td>
<td>-</td>
<td>0.88</td>
<td>0.06</td>
<td>0.30</td>
<td>0.86</td>
<td>0.0021 (***)</td>
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<tr>
<td>Sperm metabolism</td>
<td>0.05</td>
<td>-</td>
<td>0.06</td>
<td>0.64</td>
<td>0.54</td>
<td>0.0005 (**)</td>
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<tr>
<td>Sperm motility</td>
<td>0.002 (**)</td>
<td>0.15</td>
<td>-</td>
<td>0.028 (*)</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>Capacitation</td>
<td>0.75</td>
<td>0.25</td>
<td>0.025 (*)</td>
<td>-</td>
<td>0.96</td>
<td>0.0003 (**)</td>
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<tr>
<td>Acrosome reaction</td>
<td>0.05</td>
<td>0.93</td>
<td>0.18</td>
<td>0.21</td>
<td>-</td>
<td>0.024 (*)</td>
</tr>
<tr>
<td>Sperm-egg interaction</td>
<td>0.09</td>
<td>0.92</td>
<td>0.14</td>
<td>0.09</td>
<td>0.94</td>
<td>-</td>
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</table>
Oocytes are larger and more complex than sperm. Females must produce a gamete containing resources necessary for directing oocyte growth and function after fertilization. Due to complexity and maternal investment into oocyte production females should prevent gamete waste (Parker, 1979; 1984). Ovulation and maturation of oocytes in response to proximity of male gametes is a conservative mechanism to prevent wasted energy expenditures on gamete production. Female gametes of most animal species arrest during meiotic prophase I. These primary oocytes arrest in earlier stages of meiosis until intracellular signaling initiates oocyte maturation (Kosinkski et al., 2005; Govindan et al., 2009; Kobayashi et al., 2002; Miller et al., 2002) [Figure 7]. Oocytes arrest at this stage of development to limit female energy expenditures in sexual reproduction but also to prepare for the presence of potential mates (Miller et al., 2002). In some cases the presence of sperm does not induce oocyte ovulation. Typically a reproductively mature human female will ovulate oocyte(s) for a short period each month from puberty till menopause (Young, 2006). This ovulation strategy of the human female prevents the expense of multiple oocytes maturing at once. The average female 28-day cycle habituates the ovulation process and removes oestrus variability for potential mates. This is one example of selection affecting ovulation and maturation of human oocytes (Szalay and Costello, 1990). The evolutionary arms race of sexual characteristics could result in new species formation.
Figure 7: Oogenesis in a diploid animal. Primary oocytes arrest during prophase I of meiosis. In response to hormone signaling, primary oocytes will continue through meiosis.

From Young, 2006.
*Caenorhabditis*

*Caenorhabditis* is a genus of non-parasitic nematodes with a world-wide distribution in both temperate and tropical environments (Kiontke et al., 2004; Kiontke et al., 2011; Felix et al., 2014) [Figure 8]. These microscopic organisms live in bacteria-enriched environments, compost heaps and rotting fruit (Kiontke and Sudhaus, 2006; Kiontke et al., 2011). In *Caenorhabditis*, females have two X chromosomes and males have one X chromosome [Figure 9]. Geographically some species are isolated from one another while others live in sympatry. Within *Caenorhabditis*, species most closely related to *C. elegans* are collectively referred to as the Elegans-group. Within the Elegans-group of *Caenorhabditis*, all pairwise combinations of species will mate with each other [Figure 8; Table 2]. Among these, many combinations are cross-fertile and in many cases, variants within species affects cross fertility between species.

Closely related species of *Caenorhabditis* employ different modes of reproduction: gonochorism and hermaphroditism. Gonochorism is a form of sexual reproduction with both male and female genders. Hermaphroditic species have a males and hermaphrodite. Hermaphrodites produce and store self-sperm in the last larval stage of their development. Once the hermaphrodites are reproductively mature they produce oocytes and self-fertilize producing self-progeny. Hermaphrodites are modified females; males also exist and may mate with hermaphrodites. Hermaphrodites can be depleted of their sperm. Sperm-depleted hermaphrodites can be mated a male. Three of the species in the Elegans-Group are hermaphroditic: *C. briggsae*, *C. elegans* and *C. tropicalis*. The
A hermaphroditic form of reproduction arose three independent times within this Elegans-Group (Kiontke et al., 2004). Other species within the Elegans-Group employ facultative sexual reproduction. Live cultures are available for 26 species of *Caenorhabditis* (Kiontke et al., 2011; Felix et al., 2014). Of the 26 species available, *C. elegans* is the most widely studied. *C. elegans* is an ideal model for research in genetics due to their short life cycles, large numbers of offspring, low cost maintenance, simple anatomy, and small but well-documented genome (Strange, 2006). The discovery and subsequent characterization of additional species of *Caenorhabditis* has revealed some of its evolutionary history and appropriateness as a model organism for evolutionary biology (Kiontke et al., 2004; Kiontke et al., 2011; Felix et al., 2014; Brenner, 1974).
Figure 8: Caenohabditis phylogeny based on genetic and morphologic characteristics. Isolates that were previously identified by species number are immediately adjacent to their corresponding species name. Hermaphroditic species are indicated in red and gonochoristic species are indicated in blue. From Felix et al., 2014.
Figure 9: Anatomy of (XX) hermaphrodite/ female and (XO) male from Caenorhabditis.Wormbook.org
Table 2: Table of pairwise comparisons of cross fertility in the Elegans-Group of *Caenorhabditis*. Cross-fertile combinations are indicated in green, cross-infertile in red, strain-dependent cross-fertility in yellow, combinations not tested in white and intra-specific crosses in black. All species within the Elegans-Group will mate with each other. Despite this some crosses remain infertile. Assortative fertilization is observed within the Elegans-Group of *Caenorhabditis*. Adapted from Baird and Seibert, 2013.
**Assortative fertilization in *Caenorhabditis***

Assortative fertilization includes all genetic mechanisms that differentiate between con- and inter-specific sperm. Discriminatory mechanisms include species-specific receptor-ligand interactions involved in sperm-ovum fusion, chemotaxis of sperm toward ova and acrosome reactions. In the Elegans-Group of *Caenorhabditis* males and females (hermaphrodites) will mate with each other in all pairwise combinations (Baird et al., 1992; Baird et al., 2000; Baird, 2002; Woodruff et al., 2010; Kiontke et al., 2011). Assortative fertilization acts in any of these crosses within the Elegans-Group in which fertilization is limited or absent (Hill and L’Hernault, 2001; Baird and Seibert, 2013). In inter-specific mating crosses between three closely-related species of *Caenorhabditis*: *C. remanei*, *C. elegans* and *C. briggsae*, scientists have observed the localization of sperm near unfertilized oocytes (Hill and L’Hernault, 2001) [Figure 10]. Despite the proximity of *C. remanei* sperm to *C. elegans* unfertilized oocytes, the cross remains infertile [Table 2]. *C. elegans* oocytes successfully localized *C. remanei* sperm but defects in other assortative fertilization mechanisms like receptor-ligand interactions prevent fertilization (Hill and L’Hernault, 2001).

**Sperm chemotaxis/Prostaglandin signaling in *C. elegans***

*Caenorhabditis* females have two U-shaped gonadal arms with germ cells progressing from distal tip of each arm to the proximal uterus of the female (Lee et al., 2007; Singson, 2001) [Figure 11]. Oocytes arrest during meiosis I prophase 1 within the proximal gonad (Govindan et al., 2009; Han et al., 2010; Singson, 2001). Adjacent to the
proximal gonad is the spermathecae, site of fertilization. A centrally located uterus and vulvae connect both gonadal arms. *Caenorhabditis* males have a binary U-shaped reproductive tract ending in a distal fan-like cloaca tail (Maggenti, 1981). During mating the male will attach its tail to the female’s vulvae.
Figure 10: Sperm chemotaxis in inter-specific crosses of *Caenorhabditis*. (a) Differential interference contrast (DIC) image of *C. remanei* sperm near the spermathecae of a *C. briggsae* hermaphrodite. (b) SYTO17 fluorescent image of fluorescently labeled *C. remanei* sperm near the spermathecae of a *C. briggsae* hermaphrodite. (c) Table summarizing sperm localization between inter-specific crosses of *C. briggsae*, *C. elegans*, and *C. remanei*. Positive sperm localization near the spermathecae is indicated by a (+) and negative sperm localization is indicated by a (-). A score of (+a) indicates the positive localization of sperm near the spermathecae is inferred based on observations of cross-fertility. Combinations with a (+a) were not scored for sperm localization. Both reciprocal crosses of *C. remanei* and *C. elegans* are cross-infertile despite the localization of *C. remanei* sperm near *C. elegans* spermathecae. From Hill and L’Hernault, 2001.
Figure 11: Magnified view of *Caenorhabditis* female reproductive tract. The female reproductive tract includes two proximal gonads with a centrally located uterus. Immediately adjacent to the uterus is the spermathecae site of fertilization and sperm storage. Mature oocytes are squeezed out of the proximal gonad into the spermathecae in response to the presence of sperm. Oocytes are fertilized in the spermathecae and then pushed into the uterus. Eventually the fertilized embryo is ejected from the uterus through the vulvae. From Burrows et al., 2006.
The male will then insert its spicule through the vulvae and into the uterus of the female (Maggenti, 1981; Baird et al., 1992). Amoeboid sperm are then ejaculated into the uterus of the female. Males produce a gelatinous mating plug to prevent sperm from leaking out (Maggenti, 1981). The amoeboid sperm must then crawl along the uterine lining towards the spermathecae in response to chemical signaling from oocytes, i.e. sperm chemotaxis (Kubagawa et al., 2006; Edmonds et al., 2010; Han et al., 2010) [Figure 12]. Due to the constrictive nature of the valve between the spermathecae and proximal gonad, sperm remain in the spermathecae and wait for a mature oocyte to ovulate (Maggenti, 1981; Kosinski et al., 2005; Burrows et al., 2006; Singson, 2001; Ward and Carrel, 1979). The oocyte immediately adjacent to the spermathecae is ovulated into the spermathecae (Maggenti, 1981; Han et al., 2010). Sperm fuse to the plasma membrane of the oocyte to induce a fertilization reaction. The fertilized oocyte passes into the uterus where it will eventually exit the female through the vulvae (Burrows et al., 2006). In *C. elegans*, the oocyte releases a chemoattractant that guides the sperm towards the unfertilized oocyte in the spermathecae (Kubagawa et al., 2006; Edmonds et al., 2010; Han et al., 2010). Many species use diffusible secreted signaling molecules to help facilitate sperm-oocyte interactions (Han et al., 2010; Evans et al., 2012; Kosinski et al., 2005; Govindan et al., 2009; Kobayashi et al., 2002; Pate et al., 2008; Yeates et al., 2013).

Previous studies have highlighted a relationship between irregularities in insulin signaling and defects in fertility in *C. elegans* (Fielenbach and Antebi, 2008; Han et al., 2010; Edmonds et al., 2010; Kubagawa et al., 2006). Through a cascade of events insulin
Figure 12: Chemotactic signaling in *Caenorhabditis* female reproductive tract. Oocytes produce chemical signals from poly-unsaturated fatty acids. These signals are released to chemoattract amoeboid sperm towards the unfertilized oocyte. Hermaphrodite-derived sperm are produced in the last larval stage of development and stored in the spermathecae. Spermatozoa exocytose major sperm protein (MSP) for oocyte maturation and gonadal sheath contraction. From Han et al., 2010.
receptors regulate the synthesis of prostaglandins important in sperm motility and reproduction (Edmonds et al., 2010; Han et al., 2010). Lipid or prostaglandin signaling is the movement of a lipid messenger that binds to a protein receptor, kinase or phosphatase (Kubagawa et al, 2006; Edmonds et al., 2010).

Lipid messengers are manufactured in the intestine and distributed throughout an organism (Kubagawa et al., 2006). Fat-2 is a gene that codes for the production of poly-unsaturated fatty acids, necessary for prostaglandin production [Figure 13 a,b]. In C. elegans fat-2 female mutants, the absence of poly-unsaturated fatty acids (PUFAs) in oocytes resulted in a reduction of sperm velocity, reduced directional sperm velocity and increased reversal frequency of sperm (Kubagawa et al., 2006; Edmonds et al., 2010) [Figure 13c]. Kubagawa et al. (2006) found that poly-unsaturated fatty acids (PUFAs) are necessary precursors of signals that attract sperm to the spermathecae and eventually the oocyte for fertilization.

**Receptor-ligand interactions in C. elegans**

Fertilization between gametes cannot occur without successful interactions between transmembrane proteins (Putiri et al., 2004). Several transmembrane proteins, receptors and ligands, have been observed affecting the efficacy of sperm motility and respiration in C. elegans. One such transmembrane protein identified in C. elegans male gametes is spe-9 (Singson et al., 1998). Mutations in the extra-cellular domain (ECD) amino acid coding sequence may truncate or change the function of the protein.
**Figure 13:** *C. elegans* fat mutant female. (a) Fluorescently-labeled wild type sperm was observed localizing near the wild type female spermathecae (outlined in yellow). Fluorescently-labeled wild type sperm were unable to localize near the spermathecae of fat-2 mutant females. Fat-2 mutants are unable to produce prostaglandin signal essential for sperm localization. (b) Fat-2 mutant females experience decreases in wild-type sperm velocity and directional velocity towards the spermathecae. Additionally, fat-2 mutant females lack of chemotactic signaling increases the reversal frequency of wild-type sperm. From Han et al., 2010.
In several *spe-9* mutants with truncated ECD protein structure impacts on sterility and temperature-sensitivity of the male gamete were observed (Putiri et al., 2004).

Additional transmembrane proteins have been identified on female gametes in *C. elegans*. The EGG-1 gene codes for a transmembrane protein with extracellular receptors for male-derived ligands on the oocyte surface (Kandandale et al., 2005). EGG-1 is required for hermaphrodite/female fertility. EGG-1 and EGG-2 are paralogs of each other that arose from a gene duplication in *C. elegans* after it split from its last common ancestor with *C. briggsae* and *C. remanei* (Kandandale et al., 2005; Johnston et al., 2010; Edmonds et al., 2010). *C. elegans egg-1* mutants contain defects in meiotic maturation, ovulation, sperm migration, and sperm retention in the reproductive tract [Figure 14]. Reduction of the sperm retention in the *C. elegans egg-1* mutant reproductive tract is due to the inability of the sperm to retain position within the uterus after fertilized oocytes pass through the uterus (Kandandale et al., 2005). In *C. elegans egg-1* mutants, residual fertility is likely due to the preservation of *egg-2* function. Based on these results, researchers concluded that fertility is a highly process function in *C. elegans*. EGG-1 and EGG-2 transmembrane receptors are displayed on the oocyte surface where they can bind to a sperm ligand (Kandandale et al., 2005; Johnston et al., 2010). Species-specificity of receptors and ligands is implied from failure of sperm to initiate a fertilization reaction despite their close proximity to mature oocytes (Hill and L’Hernault, 2001; Baird and Seibert, 2013). Additionally, *C. elegans* sperm fuse to the plasma membrane of the oocyte instead of being engulfed by the oocyte (Kandandale et al., 2005). Failure to fuse
Figure 14: *Caenorhabditis elegans* mock, *egg-1* mutant and *egg-2* mutant. (A) *C. elegans* mock trial reveals typical ovulation (i.e. positive control). (B) *C. elegans egg-1* RNAi mutants are defective in oocyte maturation, ovulation, sperm migration and sperm retention. (C) *C. elegans egg-2* RNAi mutants showed the same results as observed in *egg-1* mutants. Kandandale et al., 2005
and induce an acrosome reaction with the oocyte membrane may also be due to species-specific protein interactions within *Caenorhabditis*. Several other transmembrane proteins important for fertilization have been identified on both the male and female gametes of *C. elegans* (Liau et al., 2013; Edmonds et al., 2010; Wilson et al., 2011; Singson, 2001; Singson et al., 2008). The interaction between transmembrane proteins from both male and female gametes is still largely a mystery. Researchers do not know specifically which proteins from the male gametes are interacting with proteins on the egg surface.

**Oocyte maturation and ovulation in *C. elegans***

Unfertilized oocytes arrest in prophase I of meiosis I and remain in the proximal gonad of the female reproductive tract until sperm is present. The proximal gonad is lined with gonadal sheath cells, which are integral in the movement of germ cells through the female reproductive tract and the formation of gap junctions with developing oocytes (Miller et al., 2002; Govindan et al., 2009). Oocyte maturation and ovulation is accomplished through the detection of the major sperm protein (MSP) [Figure 12]. MSP is a cytoplasmic protein hormone signal released by exocytosis from sperm to promote oocyte nuclear envelope breakdown, cytoskeletal rearrangement, spindle assembly and gonadal sheath contraction (Kosinski et al., 2005) [Figure 15]. MSP binds to VAB-1 Eph receptor protein-tyrosine kinase and other unidentified receptors on the oocyte and sheath cells [Figure 14]. Signaling from VAB-1 Eph receptors negatively regulate oocyte maturation and ovulation (Miller et al., 2002). Sperm release MSP to counteract the
negative impact VAB-1 and other receptors (Miller et al., 2002). This antagonistic behavior of MSP allows sheath contraction and oocyte maturation. Gonadal sheath cells in the female reproductive tract control the response of oocyte to presence of MSP. cAMP signaling in gonadal sheath cells is required for oocyte response to sperm presence. Goαs–coupled receptors on the surface of sheath cells receive MSP signals and then inhibit or prohibit the maturation of oocytes through cAMP signaling and gap junctions (Govindan et al., 2009). Selectively storing or ovulating oocytes in the absence or presence of sperm, respectively, is a cost effective way of reducing the amount of female energy expended reproduction. This is an energetically favorable strategy that is conserved across many animal taxa (Palumbi et al., 2008; Yeates et al., 2013; Govindan et al., 2009).

For example, human females are born with a limited amount of oocytes arrested in meiotic prophase 1. Luteinizing hormone (LH) has a similar function to MSP. LH signals Goαs–coupled receptors female oocytes to ovulate (Govindan et al., 2009). Luteinizing hormone (LH) is required for ovulation and meiotic maturation of human oocytes.
Inter-specific crosses in the Elegans-Group of *Caenorhabditis*

All species within the Elegans-Group of *Caenorhabditis* will mate with each other. In some crosses, F1 progeny are obtained. The viability of inter-specific hybrids ranges from embryonic lethality to viable F2s (Baird et al., 1992; Baird et al., 2000; Baird, 2002; Woodruff et al., 2010; Kiontke et al., 2011). In Baird et al., 1992, an exploration into the inter-specific fertility within *Caenorhabditis* demonstrated the incompleteness of the reproductive isolation between species within this genus. Further investigation, into the fertility between combinations revealed a greater degree of variation among species combinations. Cross-fertility is measured by the presence of one fertilized embryo from a post-mated female. 44% are cross-fertile, 33% of the pairwise mating crosses are cross-infertile, 8.8% cross-fertility is dependent on which natural isolate was used (Baird and Seibert, 2013) [Table 2].

Assortative fertilization is observed in all infertile crosses. In rare cases cross-fertile combinations results in hybrid progeny. One combination producing fertile hybrid progeny is *C. nigoni* and *C. briggsae* [Figure 15]. Despite the production of fertile F1 female hybrids between *C. nigoni* and *C. briggsae*, F1 hybrid males are embryonically lethal or sterile. *C. nigoni: C. briggsae* F1 hybrid female are fertile but only produce viable offspring when backcrossed to *C. nigoni* males (Woodruff et al., 2010). The asymmetry in hemi-zygous male hybrids (XO) fitness compared to homozygous female hybrids (XX) follows Haldane’s Rule. Haldane’s Rule is hemizygous hybrids will have a
Figure 15: Asymmetrical hybrid construction between *C. briggsae* and *C. nigoni* (formerly *C. species 9*). Each block represents a different cross: parents listed first in black/white and observed progeny in grey. Block (A) and (B) are reciprocal mating crosses between *C. briggsae* and *C. nigoni*. Assymetry is observed in F1 hybrid progeny. F1 males are either sterile (A) or inviable (B) depending on the direction of the mating cross. Mating crosses between F1 female progeny and *C. briggsae* males produces no viable progeny (Block E and C). Viable backcrossed progeny are produced in crosses between F1 female progeny and *C. nigoni* males (Block D and F). Adapted from Woodruff et al., 2010.
lower fitness than homozygous. The best explanation of the Haldane’s rule between
_C. nigoni_ and _C. briggsae_ is the dominance theory (Kozlowska et al., 2012). The
dominance theory is the phenotypic expression of the one particular allele over another.
In 1908, Charles Davenport proposed a genetic model where hybrids were advantageous
due to the suppression of deleterious alleles inherited by either parental population.

Furthermore, any hybrid incompatibilities, specifically those observed in hemizygous
individuals, were largely due to inability to suppress deleterious alleles with a
compensatory non-deleterious allele (Davenport, 1908). Between _C. nigoni_ and _C.
briggsae_ asymmetries in reciprocal crosses occur in hybrids are due to the effects of
inherited incompatibilities on the X-chromosome (Kozlowska et al., 2012). This implies
that in the earliest stages of speciation, genetic incompatibilities may initiate hybrid male
sterility or lethality. Nuclear and mitochondrial incompatibilities explain the low
occurrence and fitness of hybrid between _C. briggsae_ and _C. nigoni_ (Kozlowska et al.,
2012).

_C. briggsae_ and _C. nigoni_ employ different modes of reproduction: _C. briggsae_ self-
fertilizes with sperm produced before reproductive maturity is attained. _C. nigoni_ is an
obligate outcrossing species with male and females genders (Kiontke et al., 2004). Mate
availability does not impact _C. briggsae_ due their production of self-progeny. _C. nigoni_
females are dependent upon the presence of _C. nigoni_ males to ovulate mature oocytes
and produce progeny. Positive selection has been observed in male sperm competition
and female defenses against heterospecific progeny (Ting et al., 2014; LaMunyon, 1999; 2002; 2007; LaMunyon and Ward, 1998).

Females have evolved sophisticated mechanisms to prevent heterospecific sperm from reaching mature oocyte for cross-fertilization (Edmonds et al., 2010; Kubagawa et al., 2006; Markert and Garcia, 2013). One such mechanism is observed in *C. briggsae* hermaphrodite 48 hours after mating with *C. nigoni* males. The sterility observed in *C. briggsae* hermaphrodites induced due to heterospecific sperm presence in their reproductive tract is called an insemination reaction (Kozlowska et al., 2012). *C. briggsae* hermaphrodites have lesser degree of this insemination reaction in comparison to gonochoristic species like *C. nigoni*. Therefore, in inter-specific crosses between *C. briggsae* and *C. nigoni*, we would expect *C. briggsae* hermaphrodites to have more heterospecific sperm fertilize oocytes and larger brood sizes when mated to *C. nigoni* males than the reciprocal cross (Kozlowska et al., 2012). This is due in part to greater degree of selection for ovum defensive mechanisms and sperm fitness in sexually reproducing species. *C. nigoni* populations have selected for defenses against heterospecific sperm to prevent the waste of gametes on less fit hybrids (Kozlowska et al., 2012). Moreover, *C. nigoni* males are constantly competing for females and the production of progeny. Sperm competition between *C. nigoni* males would increase fitness by selecting for traits that would increase the success of their sperm fertilizing an oocyte. These traits could include pheromone signaling between potential mates, sperm motility, sperm size, viscosity of mating plugs, and etc (LaMunyon and Ward, 1998;
LaMunyon, 1999; 2002; 2007; Markert and Garcia, 2013; Edmonds et al., 2010; Han et al., 2010). Markert and Garcia (2013), found that coital signals released by males during mating are species-specific. Furthermore, these coital signals were also observed to attract additional females to the copulating pair. These coital signals, soporific factors, allow a male from a gonochoristic population to immobilize hermaphrodites for copulation. Males from hermaphroditic populations do not produce soporific factors to the same efficacy of gonochoristic males. Hermaphrodites do not always respond to these aired signals due in part to the non-productive copulations resulting from hetero-specific crosses. If this signal was a broadcast to all females in general, increases in hybrid breakdown and non-productive mating occurrences (Markert and Garcia, 2013).

Greater degree of selection on reproductive characteristics in gonochoristic species like C. nigoni would give them an advantage over hermaphroditic species like C. briggsae in cross-fertilization. In the laboratory, the probability of a male present in a hermaphroditic population is 0.1% (Chasnov and Chow, 2002). This male is a result of non-disjunction of the X chromosome during meiosis (Hodgkins, 1989). Selection has little effect on C. briggsae male populations due to its small size and unapparent differences in fitness. C. briggsae male sperm will not be as successful in a C. nigoni female reproductive tract because of C. nigoni’s selection for ovum defenses and no selection for increases in fitness for C. briggsae sperm (Kozlowska et al., 2012).

Researchers believe that deleterious polymorphisms from C. nigoni cause the hybrid incompatibilities and initiated reproductive isolation between C. nigoni and C. briggsae
(Kozlowska et al., 2012). This theory is supported by the inviability of the progeny from F1 female hybrids backcrossed to *C. briggsae* males (Woodruff et al., 2010). Dominant or deleterious variants of *C. nigoni* prevent further hybridization with *C. briggsae* (Kozlowska et al., 2012). In contrast, F1 female hybrids have a higher rate of producing viable offspring when crossed to *C. nigoni* males (Woodruff et al., 2010). Genetic explanations for the increased F1 hybrid viability and F1 female fertility relative to parental strains may be due to nuclear-mitochondrial interactions, paternal effect or dominant factors causing hybrid incompatibilities (Kozlowska et al., 2012). Future work should focus on these elucidating the dysgenic interaction between polymorphic loci involved in reproductive isolation to build a model for speciation. Additionally, investigations into other evolutionary forces besides selection that may be affecting this interaction will further help in our understanding of hybrid incompatibilities (Kozlowska et al., 2012).

Recent laboratory breeding experiments have revealed another pair of sister species within the Elegans-Group of *Caenorhabditis* that can produce viable F1 hybrids: *C. remanei* and *C. latens*. Before 2012, *C. latens* was categorized as an Asian subspecies of *C. remanei* until crosses between F1 hybrids revealed the extent of their reproductive isolation (Dey et al., 2012). When F1 male hybrids are mated to F1 female hybrids they experience F2 hybrid breakdown (Dey et al., 2012). Further experimentation will determine if asymmetrical inheritance of genes from either *C. latens* or *C. remanei* are responsible for this F2 hybrid breakdown. There are many interesting differences in this
pair of sister species, *C. remanei* and *C. latens*, from *C. briggsae* and *C. nigoni*. Both *C. remanei* and *C. latens* are gonochoristic species. This introduces selection on both species for sexual characteristics, along with greater population diversity. Greater population diversity would increase rates of recombination this potentially complicating the identification of reproductively isolating genes. Genetic diversity of gonochoristic populations is 20 x greater than in closely related cosmopolitan hermaphroditic populations (Cutter, 2008). In addition, *C. remanei* and *C. latens* have a more recent time of divergence relative to *C. briggsae* and *C. nigoni* (Felix et al., 2014). *C. briggsae* and *C. nigoni* diverged approximately $10^7$ generations ago while *C. latens* diverged from *C. remanei* $10^6$ generations ago. The differences in divergence times could drastically reduce the amount of substitutions that have accumulated in each species since divergence (Felix et al., 2014). A decrease in the amount of mutations would narrow the genomic spectrum responsible for the reproductive isolation observed between the two species. *C. remanei* is a cosmopolitan temperate species of *Caenorhabditis* with an overlapping range with *C. latens* (Dey et al., 2012). Further investigation, will determine if there is gene flow between Asian populations of *C. remanei* and *C. latens* and which evolutionary forces initiated reproductive isolation between these two species (Dey et al., 2012).
**Assortative fertilization in inter-specific crosses**

Previous research has demonstrated the presence of assortative fertilization in hermaphroditic species of *Caenorhabditis*. Evidence of inter-specific mating can be achieved by using mitochondria-tracker dyes that reveal the position of inter-specific male sperm within the hermaphrodite’s spermathecae (Hill and L’Hernault, 2001; Edmonds et al., 2010; Han et al., 2010; Singson). Several inter-specific combinations of *Caenorhabditis* have previously confirmed copulation and sperm transfer but oocytes remain unfertilized [Table 2]. In inter-specific mating of *C. briggsae* and *C. remanei* males with *C. elegans* hermaphrodites, the probability of cross-fertilization is small. The hermaphrodite produces spermatozoa that out competes other male-derived sperm resulting in self-progeny (Hill and L’Hernault, 2001). Additionally, despite the localization of *C. remanei* sperm in *C. elegans* hermaphrodites at the spermathecae this mating cross is infertile. In the reciprocal cross there is no localization of *C. elegans* male sperm in *C. remanei* females and the cross is infertile. Hill and L’Hernault’s (2001) inter-specific mating cross results highlight two other assortative fertilization mechanisms that may also be impacting cross-fertilization: receptor-ligand interactions and an insemination reaction. The inability of *C. remanei* sperm to induce a fertilization reaction despite their presence near the *C. elegans* mature oocyte points to protein-protein interactions on the surface of the gametic cells. Previous work has found evidence of inter-specific female infertility 48 hours after mating. For example, *C. briggsae*
hermaphrodites will mate and produce viable offspring when mated to *C. nigoni* males (Woodruff et al., 2011). After 48 hours, the hermaphrodite will experience a delayed insemination reaction. This implies the hermaphrodite is inducing additional isolating mechanisms to prevent hetero-sperm fertilization (Kozlowska et al., 2012).

Hill and L’Hernault (2001) inferred the localization of *C. briggsae* sperm in *C. remanei* females based cross-fertility data. When *C. briggsae* males are mated to *C. remanei* females, arrested embryos are produced. Recent analysis of fluorescently-labeled *C. briggsae* sperm in *C. remanei* females reveals defects in sperm chemotaxis (Seibert and Baird, unpublished data) [Figure 16]. Further exploration into the strength of correlation between sperm chemotaxis and cross-fertility is needed to discriminate the impact different sperm-oocyte interactions have on fertilization in *Caenorhabditis*. 
Figure 16: Chemotactic defects in mitotracker-labeled *C. briggsae* (AF16) sperm in *C. remanei* (EM464) females. White arrow: vulvae; yellow arrow: spermathecae.

unpublished, Seibert and Baird.
Specific Aims

Aim 1: To characterize the segregation of assortative fertilization mechanisms in *Caenorhabditis*.

Aim 2: To determine the association between sperm chemotaxis, cross-fertility, and fecundity.

Aim 3: To determine if species-specificity of major sperm proteins affects ovulation.
Materials and Methods

Nematode strains and maintenance

All nematode strains were maintained on 60mm agar plates seeded with *E. coli* strain DA857 and incubated at 20°C (Brenner, 1974). *C. briggsae* strains AF16 originated from Gujarat, India and RE771 is a gonochoristic, *she-1(v51)* mutant derivative of AF16. RE771 was obtained from Ron Ellis. *C. nigoni* strain EG5268 originated from Congo, Africa. *C. nigoni* strain JU1422 is an inbred lab derivative of JU1325 which is a natural isolate from Kerala, India. *C. remanei* strains EM464 and PB4641 originated from Brooklyn, New York, USA and EM464 lab derivative, respectively. These strains are available from the *Caenorhabditis* Genetics Center (CGC), from Marie-Anne Félix or Ron Ellis.

Hybrid construction

*C. briggsae* males were obtained by heat shocking *C. briggsae* L4 hermaphrodites at 30°C for 2 hours to stimulate nondisjunction of the X chromosome during meiosis, producing XO males (Hodgkins, 1983). Once males were obtained via heat-shock, populations of males were maintained by mating males to hermaphrodites. *C. briggsae* hermaphrodites were depleted of their sperm for 5-6 days prior to mating with male. Hermaphrodites were isolated and transferred each day to distinguish between adults and self-progeny. In *C. briggsae* she-1(v51) sperm-less hermaphrodites, sperm depletion was
not required due to their disruption of spermatogenesis in the L4 larval stage of development.

Inter-specific hybrids were constructed by mating 5 males and 3 females of different strain designations of *C. briggsae* and *C. nigoni* on a 60 mm agarose plate with an approximate 1 cm circle of *E. coli* strain DA857 for a period of 24 hours. After 24 hours each individual female was transferred to an agarose plate where they laid F1 hybrid progeny for 72 hours. In backcrossed hybrid construction, *C. briggsae: C. nigoni* F1 females were mated to *C. nigoni* males. All other combinations of *C. briggsae: C. nigoni* F1 progeny mated to their *C. briggsae* parental strain are infertile (Woodruff et al., 2010). F1 and backcrossed hybrid females were mated to *C. remanei* males to assess the inheritance of strong chemotaxis from *C. briggsae* and *C. nigoni*.

**Sperm chemotaxis**

Sperm chemotaxis was measured by staining *C. remanei* male sperm and observing its localization in parental, F1 hybrid and backcrossed hybrid females. This was accomplished by suspending *C. remanei* L4 males in a glass depression slide containing a fluorescent stain, Mitotracker CMXROs (Invitrogen) (Hill and L’Hernault, 2001). This fluorescent dye, 24 µL M9 buffer (5.8g Na₂HPO₄, 3.0g KH₂PO₄, 0.5g NaCl, 1.0 g NH₄Cl, 1L of dH₂O), 1 µL of Mitotracker CMXROs Stock solution (50 µg Mitotracker CMXROs and 37.6 µL of Dimethyl sulfoxide)) preferentially stains nematode sperm mitochondria. This fluorescent stain does not negatively affect sperm mobility and is used to visualize its localization within the female reproductive tract in
vivo. The glass slide was placed in a dark incubator at 25 °C for 2-3 hours. Fluorescently-labeled *C. remanei* males were transferred, rinsed with M9 buffer and allowed to recover on an agarose plate with L4 females overnight. Fluorescently-labeled *C. remanei* males (5) were mated to L4 females (3) overnight in a darkened incubator at 25 °C (adapted from Hill and L’Hernault, 2001). The next morning, females were then isolated for 4 hours to ensure sperm from last mating occurrence would have reached the spermathecae. Females were then anaesthetized in 0.25% sodium azide NaN₃ and mounted on 2% agar pads under coverslips. Females were then examined by differential interference contrast (DIC) and fluorescence microscopy in the rhodamine channel for the localization of *C. remanei* sperm (Adapted from Hill and L’Hernault, 2001). Females were scored strong or defective based on the localization of *C. remanei* male sperm near the spermathecae. A chemotactic score of strong means most if not all *C. remanei* sperm localized near the spermathecae. A chemotactic score of defective means most of the *C. remanei* sperm did not localize near the spermathecae.

**Scoring cross-fertilization and fecundity**

Mating crosses consisted of 5 males and 3 females placed on a 60 mm agar plate with an approximate 1 cm circle of *E. coli* strain DA857 for a period of 24 hours. After the 24 hours each individual female was transferred to an agar plate for 72 hours. After 72 hours, each female was scored for the presence or absence of fertilized oocytes (Maggenti, 1981). Fertilized oocytes have a keratinized shell and characteristically oval
shape. Additionally, the number of fertilized oocytes laid by each female after 72 hours was recorded to measure fecundity.

**Ovulation**

To determine if *C. remanei* major sperm protein can initiate oocyte maturation and ovulation in *C. briggsae* and *C. nigoni* virgin females, ovulation assays were performed on virgin and mated females. L4 females from *C. remanei*, *C. briggsae*, and both strains of *C. nigoni* were isolated for 24 and 48 hours. After 24 and 48 hour virgin females were either mated to *C. remanei* males overnight or anaesthetized in 0.25% sodium azide NaN₃ and mounted on 2% agar pads under coverslips. Females were then examined by differential interference contrast (DIC). Females were scored based on the presence of stacked oocytes in their proximal gonad.

**Chi-squared Statistical Analysis**

To determine if our observed indices for sperm chemotaxis and cross-fertility for each subset of females were significantly different than one another I used chi-squared statistical analyses. A contingency table from Vassar Stats was used to measure significant differences among all females scored. [http://vassarstats.net/newcs.html](http://vassarstats.net/newcs.html)

**Student’s T-test Statistical Analysis**

To determine if our observed fecundity indices for each subset of females were significantly different than one another I used a student’s T test. [http://www.graphpad.com/quickcalcs/ttest1/](http://www.graphpad.com/quickcalcs/ttest1/)
Results

I. **Species-specific chemotaxis**

To observe the localization of sperm, males are soaked in fluorescent dye that preferentially stains sperm mitochondria. Males are then allowed to mate with females overnight. The next day each female was scored for localization to the spermathecae. Strong or positive sperm chemotaxis is characterized by a large portion of the sperm localizing at the female spermathecae [Figure 17]. Weak or absent sperm chemotaxis is characterized by random localization of sperm in the female uterus. Chemotaxis index is defined as the total percent females with strong sperm localization of sperm near the female spermathecae.

When *C. briggsae* males are mated to *C. remanei* females arrested embryos are laid. Based on these results, Hill and L’Hernault (2001) assumed the positive localization of *C. briggsae* sperm in *C. remanei* females. To confirm Hill and L’Hernault’s results, *C. remanei* females were mated to fluorescently stained *C. briggsae* males [Figure 18]. Only 25% of the total *C. remanei* females scored had strong sperm chemotaxis of *C. briggsae* male sperm (Figure 18). Upon further investigation, reciprocal crosses of *C. briggsae* females had a 65% chemotactic index when mated to *C. remanei* males. In *C. remanei* sperm chemotaxis assays, *C. nigoni* strain EG5268 females had a higher chemotaxis index (0.46) than JU1422 females (0.25, Chi-squared p value < 0.3771). *C. briggsae* and *C. nigoni* diverged $10^7$ generations ago (Cutter et al., 2010). To examine the segregation
of assortative fertilization mechanisms *C. nigoni* and *C. briggsae*, sperm chemotaxis was assessed in hybrids.
Figure 17: Chemotaxis of mitotracker-labeled *Caenorhabditis remanei* strain EM464 sperm in *Caenorhabditis nigoni* strain JU1422 females. Variation in sperm chemotaxis is observed within this cross and other hybrid crosses in the Elegans-Group. A) Weak or absent sperm chemotaxis and B) Strong sperm chemotaxis. Yellow arrow = spermathecae; White arrow = vulvae.
Variation in species-specific chemotaxis

Figure 18: Variation in species-specific chemotaxis observed between several species of *Caenorhabditis*. Chemotaxis index: total percent females with strong sperm chemotaxis of *C. remanei* sperm. Error bars: 2x SEM.

A. *C. remanei* EM464 wild-isolate from Brooklyn, New York.
B. *C. remanei* EM464 wild-isolated from Brooklyn, New York.
C. *C. briggsae* AF16 wild-isolate from Kerala, India and *she-1*(v51) AF16 lab derivative.
D. *C. nigoni* strain JU1422 wild-isolate from Kerala, India.
E. *C. nigoni* strain EG5268 wild-isolate from Rep. of Congo.
II. Segregation of *C. remanei* sperm chemoattraction

II.  *C. nigoni* intra-specific hybrid females

In *C. remanei* sperm chemotaxis assays, *C. nigoni* strain EG5268 females had a higher chemotaxis index (0.46) than JU1422 females (0.25, p < 0.3771). To understand the segregation of allelic variants associated with sperm chemotaxis, intra-specific *C. nigoni* hybrid females were scored for *C. remanei* sperm chemotaxis. F1 females constructed from JU1422 males crossed to EG5268 females had a higher chemotactic index (0.4) than reciprocal F1 females (0.25). The chemotactic indexes for F1 females were not significantly different than one another and their JU1422 and EG5268 females (Chi-squared values: p < 0.92 and p < 0.8415, respectively) [Figure 19]. This F1 data suggests that a maternal effect is responsible for the higher chemotactic index for F1 females with EG5268 mitochondria. Maternal effects occur when mRNA or proteins supplied by the mother during oogenesis affects the phenotype of the progeny regardless of genotype and mito-nuclear effect. To further eliminate the possibility of mito-nuclear interactions or a maternal effect explaining the variation observed in F1 females, F2 females were constructed.

F2 females were constructed from F1 males crossed to F1 females to distinguish between maternal effect or an effect of the mitochondria responsible for the variation in chemotactic indices. F2 females will have the same mitochondria as those F1 females from which they were constructed. Any maternal effect in F2 females is a result of both JU1422 and EG5268 nuclear genome in their F1 mothers. Phenotype of the progeny
reflects the genotype of the mother. F2 females with JU1422 mitochondria (0.66) had a higher chemotactic index than both F1 and C. nigoni females but the difference is not significant. This F2 data eliminates differences in mito-nuclear interactions and maternal effect as an explanation for higher chemotactic index in F1 females. This F2 data implies that transgressive segregation of allelic variants associated with higher chemotaxis is cryptic.

Backcross females were constructed to examine patterns of segregation of chemotactic indices and if allelic variants with either C. nigoni strain were responsible for the chemotactic indices observed in F1 and F2 females. All 4 B2 females constructed from F1 females backcrossed to either C. nigoni strain JU1422 did not have significantly different chemotaxis indices from one another (p < 0.8231). Similar results were observed for all 4 EG5268 B2 females (p < 0.5657). In general, all backcross females were not significantly different than parental and other hybrid females.

Based on these results for C. nigoni female chemotactic indices, no maternal or mito-nuclear effect was observed. Hybrid incompatibilities between C. nigoni strain genomes results in cryptic variation of chemotactic indices of females scored for C. remanei sperm localization. One possible explanation for the variation observed in the chemotactic indices of P0, F1, and B2 C. nigoni females is transgressive segregation. The transgressive segregation of allelic variants observed in these females could be caused by recombination between additive alleles or disruption of an epistatic complex involved in the production of sperm chemoattractant.
Chemotaxis Index of *C. remanei* sperm in *C. nigoni* females

**Figure 19:** Strain-specific variation of *C. remanei* sperm chemotaxis is observed between *C. nigoni* strains EG5268 and JU1422. Chemotaxis index: total percent females with strong sperm chemotaxis of *C. remanei* sperm. F1 and B2 females constructed from various crosses between *C. nigoni* strains JU1422 and EG5268 were also scored for *C. remanei* sperm chemotaxis. Error bars: 2x SEM.
III. *C. briggsae* and *C. nigoni* inter-specific hybrid females

*C briggsae* females had the highest chemotaxis index (0.6525; JU1422, p < 0.010; EG5268, p < 0.38). Comparatively, the *C. nigoni* strain EG5268 (0.46) had a higher chemotaxis index than *C. nigoni* strain JU1422 (0.25, p < 0.377). To understand the segregation of allelic variants associated with sperm chemotaxis, inter-specific F1 hybrid females were scored for *C. remanei* sperm chemotaxis. *C. briggsae* and *C. nigoni* can produce F1 and B2 inter-specific hybrids. Backcross females (B2) are only produced when F1 females are backcrossed to *C. nigoni* males (Woodruff et al., 2010). Reciprocal crosses of *C. briggsae* males mated to *C. briggsae*: *C. nigoni* F1 females result in arrest hybrid embryos (Woodruff et al., 2010). *C. briggsae*: *C. nigoni* F1 males are either sterile or inviable (Woodruff et al., 2010).

*C. briggsae* and *C. nigoni* strain JU1422 inter-specific hybrid females

0.80 F1 females constructed from *C. nigoni* strain JU1422 mated to *C. briggsae* did not have a significantly different chemotactic index than reciprocal F1 females (0.57, p < 0.1492) [Figure 20]. Both F1 females from *C. nigoni* strain JU1422 mated to *C. briggsae* were combined for our analysis. Similar results were observed between *C. nigoni* strain EG5268: *C. briggsae* F1 females (0.86) and reciprocal F1 females (0.70, p < 0.4708). JU1422 x *C. briggsae* F1 females (0.6875) also had significantly higher chemotaxis index than females from their *C. nigoni* parental strain (0.25, p < 0.002). B2 females derived from *C. nigoni* strain JU1422: *C. briggsae* F1 females mated to JU1422 males had different chemotactic indices (0.5) than *C. nigoni* strain JU1422: *C. briggsae*
F1 females (0.6875, p < 0.0979) and *C. nigoni* strain JU1422 females (0.25, p < 0.0812). This dominant effect is observed in *C. nigoni* JU1422:*C. briggsae* F1 females (0.62) with a chemotaxis index similar to *C. briggsae* females (0.68, p < 0.60). If *C. briggsae* allelic variants are responsible for the high rate of chemotaxis in F1 hybrid females then the B2 hybrid females should have a relatively lower rate of chemotaxis. Another possible explanation of high chemotaxis indices of F1 hybrid females is the expression of a dominant phenotype inherited by either parent. If hybrid female results correspond to a rate of chemotactic signaling observed in parental generations, it is assumed the corresponding allelic variants are dominant. For example, if F1 female’s chemotactic index is not significantly different than that of *C. briggsae* it can be assumed the *C. briggsae* allelic variant is dominantly expressed. The lower chemotaxis index of B2 hybrid females is result of hybrid construction.

B2 hybrid females are constructed from F1 hybrid females crossed to *C. nigoni* males. B2 females have a higher percentage of *C. nigoni* genome than *C. briggsae*. If *C. briggsae* allelic variants are dominant then we should observe a reduction in the percentage of B2 females with high chemotactic indices. A reduction of chemotactic index of B2 females with respect to F1 females is observed between F1 and B2 females derived from *C. briggsae* and *C. nigoni* females. B2 females derived from *C. nigoni* strain JU1422: *C. briggsae* F1 females mated to JU1422 males had different chemotactic indices (0.5) than *C. nigoni* strain JU1422: *C. briggsae* F1 females (0.6875, p < 0.0979) and *C. nigoni* strain JU1422 females (0.25, p < 0.0812). Additionally, backcross females
constructed from *C. briggsae* and *C. nigoni* strain EG5268 had a significantly higher chemotactic index than JU1422 B2 females (0.033). It appears that *C. briggsae* allelic variants are responsible for the variation of observed in chemotactic indices of *C. briggsae* and *C. nigoni* JU1422 females.
Chemotaxis Index of *C. remanei* sperm in *C. nigoni* JU1422 inter-specific hybrid females

**A**

F1 female; A, JU1422 x *C. briggsae* derived from JU1422 and *C. briggsae* mothers, combined.

**B**

B2 female; B, JU1422 x F1, females derived from JU1422 males crossed to F1 females, combined

Figure 20: Strain-specific variation of *C. remanei* sperm chemotaxis is observed between *C. nigoni* strains JU1422. Chemotaxis index: total percent females with strong sperm chemotaxis of *C. remanei* sperm. F1 and B2 females constructed from various crosses between *C. nigoni* strains JU1422 were also scored for *C. remanei* sperm chemotaxis. Error bars: 2x SEM.
**C. briggsae and C. nigoni EG5268 inter-specific hybrid females**

*C. briggsae: C. nigoni* EG5268 F1 females (0.79) had a significantly higher chemotaxis index than *C. nigoni* strain EG5268 females (0.46, p < 0.05) [Figure 21]. The higher chemotaxis index in F1 females in comparison to either parental female suggests overdominance in F1 *C. briggsae: C. nigoni* females. Overdominance is observed in heterozygous F1 hybrid females with an increase in fitness compared to their parents. Moreover, the high rate of sperm chemotaxis in F1 females is likely due to the additive impact of multiple prostaglandin signals from their *C. briggsae* and *C. nigoni* parents for sperm chemotaxis. F1 females will employ a suite of prostaglandins from both *C. briggsae* and *C. nigoni*. EG5268 B2 females do not have a significant decrease in chemotactic index in comparison to EG5268 F1 females. This B2 data supports my theory of overdominance because F1 and B2 hybrid females have a higher chemotactic index than either parent.

*C. briggsae: C. nigoni* EG5268 F1 females did not have a higher chemotaxis index (0.79) in comparison to F1 hybrid females constructed from the *C. nigoni* JU1422 strain (0.69, p < 0.4131). Both F1 females had significantly higher chemotaxis indices than females of their *C. nigoni* parental strain. These data suggest that maternal effects cannot explain the variation observed between F1 chemotactic indices for both strains of *C. nigoni*. In general, the transgressive segregation of allelic variants associated with chemotaxis is cryptic. Moreover, it appears that *C. briggsae* allelic variants coding for
chemotactic signaling are dominantly expressed but no consistent pattern of segregation is discernible.
Chemotaxis Index of *C. remanei* sperm in *C. nigoni* EG5268 interspecific hybrid females

![Chart showing the chemotaxis index of *C. remanei* sperm in *C. nigoni* EG5268 interspecific hybrid females.]

**C. briggsae**  *C. briggsae* wild-isolate from Kerala, India and lab derivative she-1 (v51)

**EG5268**  *C. nigoni* strain EG5268 wild-isolate from Kerala, India

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<tr>
<td>A</td>
<td>F1 female; A. EG5268 x <em>C. briggsae</em></td>
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<td>B</td>
<td>B2 female; B. EG5268 x F1, females derived from EG5268 males crossed to F1 females, combined</td>
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**Figure 21:** Strain-specific variation of *C. remanei* sperm chemotaxis is observed between *C. nigoni* strains EG5268. Chemotaxis index: total percent females with strong sperm chemotaxis of *C. remanei* sperm. F1 and B2 females constructed from various crosses between *C. nigoni* strains EG5268 were also scored for *C. remanei* sperm chemotaxis.

Error bars: 2x SEM.
III. Segregation of cross-fertilization

a. Cross-fertility Index of *C. nigoni* females

In our cross-fertility assays, virgin females were mated to virgin males overnight. The next day mated females were isolated and allowed to lay eggs for 72 hours. After 72 hours, females were scored for cross-fertility based on the presence of one or more fertilized embryo. Cross-fertility index: total percent females that laid at least one fertilized egg.

Strain-specific variation of cross-fertility was observed between females of *C. nigoni* strains JU1422 and EG5268 when mated to *C. remanei* males. *C. nigoni* strain EG5268 females had a higher cross-fertility index (0.71) compared to *C. nigoni* strain JU1422 females (0.16) (p < 0.0001). F1 females constructed from *C. nigoni* EG5268 males mated to *C. nigoni* JU1422 females had a significantly higher cross-fertility index (0.67) than their *C. nigoni* JU1422 mother (p < 0.0001) [Figure 22]. F1 females constructed from *C. nigoni* JU1422 males mated to *C. nigoni* EG5268 females had an intermediate cross-fertility index between females of both parental strains (0.42, JU1422: p < 0.0005; EG5268: p < 0.0001) and lower rates than reciprocal F1 females (0.67, p < 0.0004). The difference in cross-fertility indices between F1 females suggests maternal effects. However in comparison to the JU1422 female index, both F1 female cross-fertility indices increase. This suggests the differences in hybrid nuclear genomes are a viable explanation for the increase of cross-fertility index regardless of maternal contribution. The F1 female nuclear genome is composed of both EG5268 and JU1422.
To determine if hybrid nuclear genome or maternal contributions explain the variation in cross-fertility index F2 females were constructed.

*C. nigoni* F2 females were constructed from F1 males mated to F1 females. All F2 females have the same mitochondria as the F1 females from which they were derived. F2 females will reveal if maternal effects are responsible for the variation in cross-fertility observed in F1 females. Regardless of which F1 mother was used, all F2 females were not significantly different than one another (p < 1.0). When mated to *C. remanei* males, F2 females have a markedly different cross-fertility index than *C. nigoni* strain JU1422 (p < 0.0001), EG5268 (p ≤ 0.0016), and F1 females EG5268 males mated to JU1422 females (p < 0.0001). Cross-fertility indices for both sets of F2 females derived from crosses of F1 males mated to F1 females were not significantly different (p < 1.0). The cross-fertility data for F2 females eliminates maternal effects as an explanation for the variation observed in cross-fertility.

It appears that differences in hybrid nuclear genomes cause variation in cross-fertility indices for P0, F1 and F2 females. Backcross females constructed from F1 progeny backcrossed to *C. nigoni* were also scored for cross-fertility. Backcross females allow us to determine which EG5268 or JU1422 nuclear genome allelic variant is causing the increase in cross-fertility indices observed in F1 and F2 females. All backcross *C. nigoni* females had significantly different cross-fertility rates than *C. nigoni* JU1422 females (p ≤ 0.0152). Generally, backcross females from F1 progeny mated to EG5268 had a higher cross-fertility index than reciprocal females. EG5268 B2 females containing
EG5268 mitochondria had a comparatively higher cross-fertility index than EG5268 B2 females with JU1422 mitochondria. Any maternal or cyto-nuclear effect theory is abandoned due to EG5268 B2 females with JU1422 mitochondria having a higher cross-fertility index than all JU1422 B2 females. Moreover, JU1422 B2 females with EG5268 mitochondria had a low cross-fertility index further suggesting that nuclear genome composition is the likely source of these variable cross-fertility indices.

Our claim that differences in hybrid nuclear genome as an explanation for the observed variation in cross-fertility indices is further supported by backcross female data. Furthermore, it appears allelic variants associated with the *C. nigoni* EG5268 genome are associated with high cross-fertility index with *C. remanei* males. Complications from epistatic interactions or transgressive segregation inhibit our analysis of patterns of genetic inheritance in *C. nigoni* females.
Cross-fertility Index of *C. nigoni* females

![Cross-fertility Index Graph](image)

**Figure 22**: Strain-specific variation between *C. nigoni* strains JU1422 and EG5268 cross fertilization when mated to *C. remanei* males. Cross-fertility index: total percent females that laid one fertilized egg. F1, F2 and B2 females constructed from various crosses between *C. nigoni* strains JU1422 and EG5268 and then mated to *C. remanei* males.

Error bars: 2x SEM.
b. Cross-fertility Index of *C. briggsae* and *C. nigoni* females

Variation of cross-fertility is also observed between species of *C. briggsae* and *C. nigoni* females when mated to *C. remanei* males. *C. nigoni* strain EG5268 had the highest cross-fertility index (0.71) in the parental females [Figure 23]. *C. briggsae* (0.68, p < 0.8875) and *C. nigoni* strain JU1422 (0.17, p < 0.0001) females had lower chemotaxis indices. To determine a mode of genetic inheritance of allelic variants associated with cross-fertility F1 and backcrossed inter-specific hybrids were constructed between *C. briggsae* and *C. nigoni*. Backcross females were constructed only from *C. nigoni* males mated to *C. briggsae*: *C. nigoni* F1 females.

F1 hybrid females constructed from *C. nigoni* strain EG5268 and *C. briggsae* have a higher cross-fertility index (0.86) than females from both parental species (EG5268, p < 0.0136; *C. briggsae*, p < 0.0218) [Figure 23]. All 4 B2 hybrid types constructed from F1 EG5268: *C. briggsae* F1 females mated to EG5268 were not significantly different than one another; they were combined to represent one population of B2 hybrid females. A similar result of non-significance of cross-fertility indices between B2 hybrid types was also observed in JU1422 B2 females. Females constructed from *C. nigoni* strain EG5268 and *C. briggsae* had a lower cross-fertility in comparison to F1 hybrid females (0.60, p < 0.001). F1 (0.62) and B2 hybrid (0.60) females constructed from *C. nigoni* strain JU1422 and *C. briggsae* have a significantly higher cross-fertility index than their *C. nigoni* strain JU1422 parent (p < 0.001).
It appears that *C. briggsae* allelic variants affecting cross-fertility are dominantly expressed in F1 and B2 hybrids. If overdominance was occurring we would expect both F1 females to have a higher cross-fertility index than both *C. briggsae* and *C. nigoni* females. JU1422: *C. briggsae* F1 females do not have a higher cross-fertility index than *C. briggsae* females. Therefore, overdominance cannot explain the variation of cross-fertility indices observed in *C. briggsae* and *C. nigoni* females. If *C. briggsae* allelic variants are affecting cross-fertility indices, then there should be a reduction in the cross-fertility indices of backcross females. In comparison to F1 females, there is a reduction of *C. briggsae* genome in backcross females; therefore we expect a reduction in cross-fertility. Both B2 females have lower cross-fertility indices in comparison to their corresponding F1 female. A dominantly expressed *C. briggsae* allelic variants is likely responsible for the variation observed in cross-fertility indices of *C. briggsae* and *C. nigoni* females.
Cross-fertility Index of *C. briggsae* and *C. nigoni* females

![Graph showing cross-fertility index]

**Figure 23**: Variation in cross-fertility between *C. briggsae*, *C. nigoni* and *C. briggsae*: *C. nigoni* inter-specific hybrid females when mated to *C. remanei* males. Cross-fertility index: total percent females that laid one fertilized egg. Reciprocal F1 females were not significantly different and were combined. EG5268 x F1 females includes 4 different backcrossed hybrid females that are not significantly different than one another. JU1422 x F1 female includes 4 different backcrossed hybrid females that are not significantly different than one another. Error bars: 2x SEM.

*C. briggsae*  *C. briggsae* wild-isolate from Kerala, India and lab derivative *she-1 (v51)*

JU1422  *C. nigoni* wild-isolate from Kerala, India

EG5268  *C. nigoni* wild-isolate from the Congo

A-B  F1 female; A, JU1422 x *C. briggsae*

B, EG5268 x *C. briggsae*

C-D  B2 female; C, JU1422 x F1, females derived from JU1422 males crossed to F1 females, combined

D, EG5268 x F1, females derived from EG5268 males crossed to F1 females, combined
IV. Segregation of fecundity

a. *C. briggsae* and *C. nigoni* females

The fecundity of each female was determined by measuring the brood size of each female when mated to *C. remanei* males. The average brood size of each female was assessed to determine if the presence of *C. remanei* sperm within the female reproductive tract had any impact on female fitness. After 72 hours, post-mated females were scored for the number of dead embryos laid. On average, a reproductively mature female will lay 200-300 embryos during her lifetime (Diaz et al., 2008). *C. nigoni* strain EG5268 had a higher average brood size (3.75) per cross-fertile female than females from *C. nigoni* strain JU1422 (2, p< 0.3671) when mated to *C. remanei* males. *C. briggsae* females had the higher average brood sizes than females of both *C. nigoni* strains.

Within and between species variation is observed in the average brood size of *C. nigoni* and *C. briggsae*. *C. briggsae: C. nigoni* strain JU1422 F1 females (6.27) scored significantly higher than females from parental species *C. nigoni* JU1422 (p< 0.01497), reciprocal F1s (p< 0.001245) and B2s (p< 0.00972) derived from *C. briggsae: C. nigoni* strain JU1422 F1 females [Figure 24]. *C. briggsae: C. nigoni* strain EG5268 F1 females had the highest average brood size (16.76) of any females scored and were significantly higher than females from parental species *C. briggsae* (p< 0.324) and *C. nigoni* (p< 0.0011), reciprocal F1s (p< 0.00001) and B2s (p< 0.00001) derived from *C. briggsae: C. nigoni* strain EG5268 F1 females. This F1 female data reveals a *C. nigoni* maternal effect and/or overdominance due to the combination of *C. briggsae: C. nigoni* allelic variants.
To further investigate the mode of inheritance allelic variants associated with female fecundity, backcross hybrid females were scored for average brood size. B2 hybrids constructed from *C. briggsae* and *C. nigoni* strain JU1422 deviated significantly from each other (p< 0.0137). Furthermore, B2 hybrids constructed from *C. briggsae* and *C. nigoni* strain EG5268 showed marked differences in their average brood size from one another (p< 0.005). This B2 female data eliminates maternal effects as a possible genetic explanation for the variation observed in inter-specific hybrid females. All inter-specific hybrids had greater fecundity indices than their respective *C. nigoni* mothers. Despite this increase in hybrid fecundity, no discernible pattern of segregation was observed.
**Fecundity Index of *C. briggsae*: *C. nigoni* inter-specific hybrid females**

![Graph showing variation in fecundity index between *C. briggsae* and *C. nigoni* females when mated to *C. remanei* males.](image)

*C. briggsae*  *C. briggsae* wild-isolate from Kerala, India and lab derivative she-1 (v51)
JU1422  *C. nigoni* wild-isolate from Kerala, India
EG5268  *C. nigoni* wild-isolate from the Congo

A–D  F1 females;  A, from JU1422 males crossed to *C. briggsae* females, *C. briggsae* cytotype.  B, from *C. briggsae* males crossed to JU1422 females, JU1422 cytotype.  C, from EG5268 males crossed to *C. briggsae* females, *C. briggsae* cytotype  D, from *C. briggsae* males crossed to EG5268 females, EG5268 cytotype.


**Figure 24:** Variation in fecundity index between *C. briggsae* and *C. nigoni* females when mated to *C. remanei* males. Fecundity index: average brood size per cross-fertile female.

F1 and B2 females constructed from various crosses of *C. briggsae* and *C. nigoni* were mated to *C. remanei* males and scored for cross-fertilization. Error bars: 2x SEM.
Data from these experiments reveal variation in chemotaxis, cross-fertility, and fecundity between and within species of *Caenorhabditis*. The variation observed in these results provokes an inquiry into the strength of correlation between sperm chemotaxis and cross-fertility. This inquiry will determine how big of a role sperm chemotaxis plays in cross-fertilization and female fecundity between *C. remanei* males and *Caenorhabditis* females.

**V. Correlation of chemotaxis with cross-fertility**

There was low negative correlation ($r^2 = -0.018$) between the variation in cross-fertility and strong sperm chemotaxis in *C.nigoni* females [Figure 25a]. A low, positive correlative relationship is observed between *C. briggsae* and *C. nigoni* females; ($r^2 = 0.11$) [Figure 25b]. When all females were combined, the correlation between sperm chemotaxis and cross-fertility in all females is 0.012 [Figure 25c]. These low correlative values suggest that despite localization near the spermathecae, *C. remanei* sperm are still unable to fertilize oocytes. Furthermore, this indicates that other assortative fertilization mechanisms like receptor-ligand interaction and sperm-ovum fusion are also contributing to the variation in cross-fertilization when *C. remanei* males are mated to *C. briggsae* and *C. nigoni* females.
Correlation between chemotaxis and cross-fertility index

**Figure 25**: Correlation of cross-fertility and chemotaxis of *C. briggsae*, *C. nigoni* EG5268 and JU1422 strains, F1 and B2 hybrid females mated to *C. remanei* males.

Fecundity index: average brood size per cross-fertile female. Cross-fertility index: total percent females that laid at least one fertilized egg. A) Correlation for *C. nigoni* intra-specific hybrid females. B) Correlation for *C. briggsae* and *C. nigoni* inter-specific hybrid females. C) Correlation for all females.
VI. Correlation of chemotaxis with fecundity

There was low, positive correlation ($r^2 = 0.03$) between the variation in female fecundity and strong sperm chemotaxis in *C. briggsae* and *C. nigoni* females [Figure 26]. This implies that even if all *C. remanei* sperm are properly localized near the spermathecae the fecundity in the female is reduced. One possible explanation for the reduction of female fecundity despite the localization of sperm is an insemination reaction. An insemination reaction is *Caenorhabditis* characterized by female sterility in response to hetero-specific seminal fluid and sperm. An insemination reaction has been observed in *C. briggsae* hermaphrodites when mated to *C. nigoni* males. After 48 hours, there was a marked reduction in eggs laid and ectopic localization of *C. nigoni* sperm.

Species-specificity in the major sperm protein (MSP) could also explain the absence of a relationship between sperm chemotaxis and female fecundity. MSP is exocytosed by sperm while in the female uterus. MSP stimulates oocyte maturation and gonadal sheath contraction. If MSP was species-specific the *C. remanei* sperm would fail to stimulate hetero-specific oocytes to mature and ovulate into the spermathecae. In this example the fecundity of the female would be inhibited whether or not the sperm localized near the spermathecae.
Correlation between chemotaxis and fecundity indices of *C. briggsae* and *C. nigoni* females

\[ r^2 = 0.03 \]

**Figure 26:** Correlation between chemotaxis and fecundity in *C. briggsae, C. nigoni* EG5268 and JU1422 strains, F1 and B2 hybrid females mated to *C. remanei* males.

Fecundity index: average brood size per cross-fertile female. Chemotaxis index is the total percent females with strong sperm localization of *C. remanei* sperm near the female spermathecae.
VII. Correlation of fecundity with cross-fertility

There was positive correlation \( r^2 = 0.30 \) between the variation in female fecundity and cross-fertility in *C. briggsae* and *C. nigoni* female [Figure 27]. This positive correlative value implies that even if a female lays one egg it does not guarantee she will continue to lay more eggs. This value further supports our hypothesis that other assortative fertilization mechanisms are affecting variation observed in cross-fertility and fecundity. Species-specificity in direct sperm-oocyte interactions like receptor-ligand interactions and sperm-egg fusion are preventing fertilization of large numbers of oocytes even when sperm are present. Additionally, an insemination reaction could explain the reduction in *C. briggsae* and *C. nigoni* female fecundity. Insemination reaction in *Caenorhabditis* females is characterized by female sterility 48 hours post mating. Any eggs laid were likely fertilized before 48 hours.
Correlation of fecundity and cross-fertility indices of *C. briggsae* and *C. nigoni* females

**Figure 27:** Correlation of fecundity and cross-fertility in *C. briggsae*, *C. nigoni* EG5268 and JU1422 strains, F1 and B2 hybrid females mated to *C. remanei* males. Fecundity index: average brood size per cross-fertile female. Cross-fertility index: total percent females that laid at least one fertilized egg.

$R^2 = 0.30$
VIII. Oocyte maturation and ovulation

The weak relationship value between cross-fertility and sperm chemotaxis suggests other assortative fertilization mechanisms are responsible for the observed variation in cross-fertility. One other mechanisms that may be responsible for the variation in cross-fertility is chemical signaling from sperm to oocyte. Some of the key components of fertilization are the presence of activated sperm and mature oocytes. After sperm are activated in the uterus they release the major sperm protein (MSP). The exocytosis of MSP initiates oocyte maturation and gonadal sheath contraction for ovulation. Mature oocytes are characterized by an increase in oocyte volume and the presence of a pronuclease. To determine if species-specificity of the MSP explains the variation in cross-fertility, we observed ovulation rates in unmated females and females mated to *C. remanei* males [Figure 28]. The ovulation index is the percent of total females with stacked oocytes in their proximal gonad.

In adult *C. remanei* one-day virgin females, stacked oocytes were not observed. All *C. remanei* 48 hr virgin females were observed with stacked oocytes in their proximal gonad. Stacking was also observed in *C. remanei* 24 hr and 48 hr virgin females that were mated to *C. remanei* males. In addition to stacked oocytes, these mated *C. remanei* females uteri were crowded with fertilized eggs yet to be laid. Two explanations for the stacking observed in mated *C. remanei* females are either the females were egg laying defective or the blocked uterus prevented oocytes from maturing. In *C.nigoni* strain EG5268 48 hr virgin females have a higher ovulation index than 24 hr virgin females. All
24 and 48 hr *C. nigoni* strain EG5268 mated females lacked stacked oocytes. *C. nigoni* strain JU1422 24 and 48-hr virgin females had high ovulation indices. The higher ovulation indices observed in 48 hr virgin *C. nigoni* females in comparison to 24 hr virgin females could be due to timing of female reproductive maturity. L4 females were picked and allowed to rest on an agar plate for 24 hrs. Some of those females may have reached reproductive maturity earlier than other 24 hr virgin females. *C. nigoni* strain JU1422 48-hr females mated to *C. remanei* males had a low ovulation index. One possible explanation of this ovulation index in 48hr virgin *C. nigoni* strain JU1422 females could be that an insemination reaction is preventing MSP signaling. An insemination reaction has been observed in *C. briggsae* hermaphrodites mated to *C. nigoni* males 48 hours after mating. *C. briggsae* AF16 sperm-depleted hermaphrodites did have stacked oocytes in their proximal gonad. When *C. briggsae* AF16 sperm-depleted hermaphrodites were mated to *C. remanei* males, ovulation resumed. Generally, species-specificity is not observed in MSP signaling and subsequent oocyte maturation within the Elegans-Group of *Caenorhabditis*. 60% of MSP DNA sequences and functions are largely conserved across all nematodes (Scott et al., 1989). This ovulation data coupled with the lack of relationship between chemotaxis and cross-fertility implies species-specificity in sperm-ova fusion is the likely cause of variation in cross-fertility between species of *Caenorhabditis*. 
Ovulation Index of females to *C. remanei* MSP

Figure 28. Ovulation indices of *C. briggsae* and both strains of *C. nigoni* females when mated to *C. remanei* males. Ovulation index is the percent of total females with stacked oocytes in their proximal gonad. Error bars: 2x SEM.
Discussion

In sexually reproducing organisms, gametic interactions are necessary for the production of offspring. Sperm must locate an oocyte and fuse to the plasma membrane of the oocyte to induce a fertilization reaction within an oocyte to initiate embryogenesis. Embryogenesis is the development of fertilized oocytes into viable progeny. When two species are able to mate but unable to produce viable offspring, incompatibilities in gametic interactions are responsible for this dysfunction. Assortative fertilization encompasses any genetic mechanism affecting gametic interactions. These mechanisms include sperm chemotaxis, receptor-ligand interactions and sperm-ova fusion. Species-specific sperm chemotaxis has been observed between species of *Caenorhabditis* (Hill and L’Hernault, 2001). *C. remanei* male sperm were able to localize near the *C. elegans* ‘female’ spermathecae. Despite this proper localization of sperm, this cross is infertile. In reciprocal crosses, *C. elegans* male sperm was unable to localize near the spermathecae (Hill and L’Hernault, 2001). Additionally, the localization of *C. briggsae* sperm within *C. remanei* females was inferred through cross-fertility data. Sperm chemotaxis experiments revealed a low percentage of *C. remanei* females with strong chemotaxis of *C. briggsae* male sperm. One of the main goals of this project was to determine how much of the observed variation in cross-fertility is explained by chemical signaling between gametes. If signaling between gametes does not explain variation in cross-fertility then other assortative fertilization mechanisms are negatively influencing these gametic interactions.
In my study, the segregation of assortative fertilization mechanisms were assessed by cross-fertility and fecundity indices within and between sister species of *Caenorhabditis*. *C. briggsae* and *C. nigoni* share a common ancestor 10^7 generations ago (Cutter et al., 2010). *C. nigoni* strains used to construct intra-specific hybrid were JU1422 and EG5268. *C. nigoni* strain JU1422 is an inbred-lab derivative of JU1325 a natural isolate from Kerala, India. *C. briggsae* strain used to construct inter-specific hybrids were AF16 a natural isolate from Kerala, India. To investigate the impact species divergence has on allelic variants associated with assortative fertilization mechanisms intra and inter-specific hybrids were constructed.

**Intra-specific *C. nigoni* hybrid females.**

Intra-specific *C. nigoni* hybrids were constructed using both strains of *C. nigoni*: EG5268 and JU1422. Cross-fertility data suggests *C. nigoni* strain EG5268 allelic variants are dominant in intra-specific hybrid females. This was observed in the high cross-fertility indices of F1 females and backcross females with the highest proportion of EG5268 genome. In general, it does appear that time since divergence of *C. nigoni* strain EG5268 and JU1422 has affected cross-fertility and sperm chemotactic indices. Based on intra-specific data for cross-fertility and sperm chemotaxis, no pattern of segregation for allelic variants associated with both phenotypes was apparent.

**Inter-specific *C. briggsae* and *C. nigoni* hybrid females**

Variation was observed in sperm chemotaxis, cross-fertility and fecundity for initial crosses of *C. briggsae* and strains of *C. nigoni* females mated to *C. remanei* males. C.
*C. briggsae* females had higher chemotaxis and fecundity indices than either *C. nigoni* strain. *C. nigoni* strain EG5268 had the highest cross-fertility index than all other females scored for cross-fertility with *C. remanei* males. *C. briggsae*: EG5268 F1 females had higher cross-fertility and chemotaxis indices than their *C. briggsae*: JU1422 F1 female counterparts. The increase in *C. briggsae*: EG5268 F1 female fitness suggests overdominance due to heterozygote advantage or additive alleles. In contrast, it appears *C. briggsae* allelic variants associated with chemotaxis and cross-fertility are dominantly expressed in *C. briggsae*: JU1422 hybrid female. This is evident in similar chemotaxis and cross-fertility indices between *C. briggsae* and *C. briggsae*: JU1422 hybrid females. *C. briggsae*: EG5268 and *C. briggsae*: JU1422 B2 hybrids are not significantly different than one another. Two conflicting hypotheses for variation observed in cross-fertility and chemotaxis for both strains of *C. nigoni* are suggested: overdominance and dominance. These conflicting data suggests that transgressive segregation of allelic variants affecting chemotaxis and fertility is responsible for the variation observed in inter-specific hybrids.

**Why is there little if any correlation between cross-fertility and sperm chemotaxis?**

**Random walks**

In some females, fluorescently-stained sperm were scattered throughout the uterus. This observation of sperm behavior is puzzling. If *C. remanei* sperm are localizing near the spermathecae in response to oocyte-derived signals then why do some females have weak sperm chemotaxis? Weakly chemotactic females were characterized by *C. remanei* sperm reaching the spermathecae and randomly scattered throughout the uterus.
The ‘random walk theory’ could explain the occurrence of a small amount of sperm localizing near the spermathecae. This mathematical theory accounts for randomness in the succession of events. Under this scenario each event or moment is independent of past or future events. In the context of this theory, the probability of a sperm reaching the spermathecae is unpredictable. The ‘random walk’ theory would explain higher rates of cross-fertility observed in females with weak sperm chemotaxis. If fertilization is based solely upon the presence of sperm near an unfertilized egg then we can assume our indices for cross-fertility would be higher than chemotaxis. My chemotactic index is the percentage of females with complete localization of all fluorescently-labeled sperm near the spermathecae divided by the total number of females scored for sperm chemotaxis. In weakly chemotactic females, the sperm reaching the spermathecae is more likely due to random localization than chemically-directed localization.

**Volume of uterus**

If cross-fertility is based solely upon the presence of sperm near the spermathecae, then it is surprising there is not a stronger correlation between cross-fertility and strong sperm chemotaxis. This suggests other assortative fertilization mechanisms are responsible for the observed variation in cross-fertility. *C. remanei* sperm is twice the size of *C. elegans* sperm (Hill and L’Hernault, 2001). *Caenorhabditis* females have a confined cylindrical uterus. If sperm are not responding to chemotactic signaling and are crawling along the uterus at the same speed and localizing near the
spermathecae by chance than higher rates of localization of *C. remanei* sperm than *C. elegans* sperm just based upon size alone is expected.

**Single embryo random distribution of sperm sufficient**

The random of distribution of sperm does appear to be sufficient to fertilize oocytes. This is evident in the higher rate of cross-fertility observed in *C. nigoni* strain EG5268 females with a comparatively lower rate of strong sperm chemotaxis. The difference between these rates could only be explained by the random localization of a few sperm near unfertilized oocytes in females with weak chemotaxis resulting in a fertilized egg. This theory that randomly localizing sperm can sufficiently fertilize oocytes is contradicted by the higher rate of sperm chemotaxis than cross-fertility in observed in *C. briggsae* and *C. nigoni* strain JU1422 females. *C. briggsae: C. nigoni* strain JU1422 hybrid female data suggests that despite the presence of sperm, direct sperm-oocyte interactions are defective and prevent fertilization.

**Ability of sperm to regain position after ovulation**

After fertilization, an embryo is pushed through the uterus and out of the female through the vulvae. The size of the fertilized embryo is large enough that it may displace sperm crawling along the uterine wall. If oocyte-derived prostaglandin signals are directing sperm towards the spermathecae then sperm should be able to re-attain their position within the female. If oocyte-derived chemotactic signaling is species-specific then sperm should not be able to re-attain their position within the uterus. ‘Randomly-walking’ sperm would have the same probability of reaching the spermathecae before and
after displacement because their localization is not chemically-directed. In my study, all sperm was contributed by *C. remanei* males which have larger sperm than *C. elegans* and *C. briggsae* male sperm. This difference in sperm size suggests that *C. remanei* sperm would have a higher probability of being displaced in comparison to smaller sperm. The size of sperm coupled with species-specificity in chemotactic signaling could reduce the amount of sperm localizing near the spermathecae after displacement.

**Why is fecundity of females reduced when mated to *C. remanei* males?**

Even in the most fecund of females the average brood size is small in comparison to within species mating. Reproductively mature *C. remanei* female have an average brood size of 328 progeny when mated to *C. remanei* male (Diaz, 2008). *C. nigoni* females have an average of 259 progeny when mated to *C. nigoni* males. In my study, the highest fecundity indices (16.76) were observed in F1 females constructed from *C. briggsae* males mated to *C. nigoni* strain EG5268 females. Not only is the cross-fertility variable between species of *Caenorhabditis* but the fecundity of each cross-fertile female is also drastically reduced. One mechanism that may explain the low fecundity observed in hetero-specific crosses is an insemination reaction (Kozlowska, et al., 2011). This has been observed in *C. briggsae* hermaphrodites 48 hours after mating with *C. nigoni* males. The sterility observed in *C. briggsae* hermaphrodites induced due to heterospecific sperm presence in their reproductive tract would inhibit female fecundity (Kozlowska et al., 2011). In our experiments females were isolated from *C. remanei* males and then scored 72 hours later. An insemination reaction may explain the positive relationship between
fecundity and cross-fertility. Future experiments would observe the brood schedule of females mated to *C. remanei* males to determine if an insemination reaction is causing a reduction in female fecundity.

Species-specificity in sperm-derived signaling for oocyte maturation and ovulation

Conservation of MSP function across *Caenorhabditis*

In *Caenorhabditis*, males ejaculate sperm through the vulvae into the female uterus. Sperm activation occurs when spermatocytes are mixed with male seminal fluid during ejaculation (Marcello et al., 2013). Amoeboid sperm crawl along the uterine wall towards the spermathecae in response to oocyte-derived prostaglandin signals. Male sperm will exocytose the major sperm protein (MSP) to initiate oocyte maturation and gonadal sheath contraction for ovulation (Marcello et al., 2013). In my study, species-specificity of MSP function was not observed. *C. remanei* male sperm were able to initiate oocyte maturation and gonadal sheath contraction in hetero-specific females. 60% of the function and DNA sequences are identical throughout the genus of *Caenorhabditis* (Scott et al., 1989). These ovulation results along with the weak correlation between chemotaxis and cross-fertility eliminates chemotactic signaling between gametes as a cause of variable fertilization observed. These data further suggests receptor-ligand interactions and/or sperm-ova fusion may be the assortative fertilization mechanisms inhibiting fertilization. Future studies should focus on cell-surface interactions between gametes. Several candidate oocyte receptors and sperm ligands have been identified egg-1, egg-2, spe-9 and etc. (Kandandale et al., 2005; Marcello et al., 2013). But no definitive
relationship between a receptor and its corresponding ligand has been identified between *Caenorhabditis* gametes. Furthermore, species-specificity of receptor-ligand interactions of *Caenorhabditis* is still largely a mystery.

Sperm-ova fusion could be responsible for the variation in fertilization. A well-known sperm-ova fusion mechanism is observed in flagellated sperm. Flagellated sperm have a cap-like acrosome at the anterior portion of the head. When flagellated sperm detect female hormones or other oocyte derived chemical signals they shed their acrosome and their membrane fuses with the oocyte plasma membrane. This is commonly referred to as an acrosome reaction. This has been studied in *C. elegans* and no evidence found suggests *C. elegans* sperm have an acrosome. Future investigations will focus on investigating if species-specificity in receptor-ligand interactions and other sperm-ova fusion mechanisms explain the variation in the cross-fertility.
Conclusion

Previously it was assumed chemotaxis of sperm was an important mechanism for fertilization. This assumption was largely based on animals that employed external fertilization. In sea urchins and blue mussels, chemotactic signaling is necessary for gametes to transverse wide tracts of water. In animals with internal fertilization the importance of sperm chemotaxis is diminished. Fruit fly and nematode sperm does not have to travel far to fertilize an oocyte because it is already in the female reproductive tract. Selection for allelic variants associated with chemotactic signaling should be stronger in animals with external fertilization.

_C. elegans_ fat-2 mutant females are unable to produce chemo-attractant signals for sperm (Kubagawa et al., 2006; Edmonds et al., 2010). Despite this inability to produce sperm chemo-attractant signals, _fat-2_ females do produce fertilized eggs albeit at a greatly diminished rate in comparison to wild-type females. The diminished fecundity of _fat-2_ females is due to self-sperm being pushed out of the spermathecae during ovulation and inability of the sperm to re-attain a position within the female reproductive tract (Kubagawa et al., 2006). Amoeboid sperm are unable to re-attain a position for fertilization due to the lack of sperm chemo-attractant directing localization. In _Caenorhabditis_, female fecundity is sperm limited (Baird et al., 1992). The exocytosis of MSP is necessary for ovulation and maturation (Han et al., 2010). Therefore, the absence of sperm would significantly decrease female fecundity. Selection may maintain sperm
chemotaxis in *Caenorhabditis* to ensure female fecundity and sperm localization for fertilization.

Based on the effect of sperm presence in female fecundity and cross-fertility data, Hill and L’Hernault (2001) assumed sperm localization near the spermathecae in an inter-specific cross between *C. briggsae* males and *C. remanei* females. When Hill and L’Hernault’s experiments were replicated defects in sperm chemotaxis were observed. Furthermore, chemotactic index of *C. remanei* females when mated *C. briggsae* males is 25%. Upon further experimentation, defects in chemotaxis and variation in chemotactic indices were also observed between *C. remanei*, *C. nigoni*, and *C. briggsae*. My correlative analysis revealed how the small role sperm chemotaxis has in cross-fertility and female fecundity. The advantages of sperm chemotaxis are not apparent between species of *Caenorhabditis* and it appears chemotaxis is a poor barrier to isolation. Future studies will focus on allelic variants associated with gametic cell surface interactions as an explanation for the assortative fertilization observed in *Caenorhabditis*. 


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