Investigating Genetic Interactions Between Two Key Meiotic Genes *xnd-1* and *him-17,* in *C. elegans*

by

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Abstract

Meiotic chromosomes segregation errors in the oocyte are the principal risk factor in miscarriage, congenital birth defects and age-related fertility decrease. Aneuploidy from meiotic chromosomes segregation is a major underlying cause for human chromosomal abnormalities. Previous studies identified several key DNA recombination genes in meiosis and mitosis in both human and other species, including but not limited to SPO-11, MRE-11, RAD-50 etc. Mutations in these genes associate with cancers in human. Although mutations or variations of some meiotic genes in species, such as *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Caenorhabditis elegans*, are discovered to function in DNA recombination, no specific genes were studied in human meiotic nondisjunction.

Public Health Significance: Miscarriages, infertility and trisomy pregnancies are among the most common public health issues around the world. The goal of this research is to understand potential mechanism of meiotic chromosomal nondisjunction with *C. elegans* model. This research shows that HIM-17 and XND-1 interact in the same pathway when double-strand breaks (DSBs) are formed. Further studies should investigate how HIM-17 and XND-1regulate HIM-5 in this pathway and other pathways in regulating HIM-5.

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Preface

It is recommended that acknowledgments, nomenclature used, and similar items should be included in the Preface. It is with immense gratitude that I acknowledge the support and help of my advisor, Dr. Judith L Yanowitz. Without her expert guidance and persistence throughout the entirety of the project, this thesis would not have been possible.

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1.0 Introduction

Aneuploidy related infertility, miscarriage, congenital birth defects and age-related fertility decrease is a widespread medicine problem in the global community. Miscarriage is when an embryo and/or fetus is arrested by 20 weeks pregnancy. About half of all cases of miscarriage are caused by aneuploidy. Edwards syndrome, also known as Trisomy 18, occurs at an incidence rate of 1 in every 5,000 babies in the United States; Patau syndrome, also known as Trisomy 13, occurs at an incidence rate of 1 in every 16,000 babies in the United States and Turner syndrome, also known as Monosomy X, occurs at an incidence rate of 1 in 5,000 girls. Age-related fertility decrease relates to an increased rate of aneuploidy. A retrospective study on aneuploidy with comprehensive screening on 15,169 patients with blastocysts available for biopsy showed aneuploidy rates slightly increased from 2% to 6% in women aged 26 to 37 and the aneuploidy rates in women aged 42 and 44 are 33% and 53% respectively. The lowest aneuploidy rate is in the group of women aged 26 to 30. Both younger and older groups have higher aneuploidy rates (Franasiak et al.,2014).

The general fertility rate in the U.S. has gradually decreased from 1970 to 2018 to a fertility rate of lower than 60%. Birth rates in the U.S. decreased in women aged 15 to 34 and increase in women aged 35 to 44 from 1990 to 2018 (Martin et al., 2019). As more and more women give birth after age 30, aneuploidy-related infertility, miscarriage, congenital birth defects and age-related fertility decrease is emerging as a worldwide public health problem.

To reduce the rates of aneuploidy in human pregnancy, laboratory technologies have been developed in prenatal screening from the earliest Maternal Serum Alpha Fetoprotein (MSAFP) to the latest Cell-free Fetal DNA, chorionic villus sampling (CVS) and Amniocentesis. Unfortunately,

no therapies or preventions have been discovered to treat or decrease the rate of aneuploidy.

Scientists have attempted to use different animal models to disclose the mechanism of nondisjunction. Mice, *Saccharomyces cerevisiae*, *Drosophila melanogaster* and *Caenorhabditis elegans* are the most used animal models. Over 83% of *C. elegans* proteins have human homologs (Lai et al., 2000). *C. elegans* is a self-fertilizing hermaphrodite, which means that they are easy to manipulate genes in studies and mutant alleles can be maintained through self-reproduction in generations without mating.

This research study shows interactions of two key meiotic genes in meiotic process in *C. elegans* and may throw a light on chromosome nondisjunction in human beings.

1.1 Epidemiology of Aneuploidy in Human Pregnancy

Over 10% of human pregnancies result in chromosome aberrations and aneuploidy (Nagaoka et al., 2012). The incidence of chromosome aberrations and aneuploidy may exceed 50% when women are at an advanced age, over 40. Nondisjunction during meiosis results in the main chromosome aberrations: monosomy, trisomy, triploidy or tetrasomy. In 1890, German pathologist von Hansemann discovered aneuploidy in cancer cells. As early as 1907, German zoologist Theodor Boveri reported aneuploidy in his experiment with Double Fertilized Sea Urchin Eggs. The most common disease of aneuploidy, Down Syndrome, was described in a painting in 1515. In 1866, John Langdon Down named Down Syndrome as a disorder. However, it was not until 1956 that French physician Jérôme Lejeune observed 47 chromosomes in each cell of patients with Down Syndrome instead of the normal number of 46. Besides Down Syndrome, a handful of other disorders result from meiotic chromosomal nondisjunction, but most aneuploidies are

incompatible with embryonic survival. Clinical diagnostic techniques have been developed to screen for aneuploidies. In 2014, a comprehensive chromosomal screening, via quantitative polymerase chain reaction (qPCR) or single nucleotide polymorphism (SNP) array, was performed on 15,169 blastocysts from 2,701 patients in 3,392 cycles. There were 9,001 euploid results and 6,168 aneuploid results from 15,169 blastocysts (Table 1) (Franasiak JM.et al., 2014). Maternal age closely correlates to the incidence of aneuploidies in blastocysts(Figure 1) (Franasiak JM.et al., 2014). The lowest incidence of aneuploidies is from age 26 to 30. After age 30, there is a steady rise of incidence of aneuploidies which reaches 100% at age 47.

Table 1. Distribution of samples evaluated relative to the age of the female partner and the ensuing comprehensive chromosomal screening results.

				Euploid		Aneuploid	
Age (y)	Oocytes retrieved ($\mu \pm$ SEM)	Cohorts of embryos evaluated (n)	No. of biopsies evaluated (n)	n	Percentage of total	n	Percentage of total
22	23.5 ± 3.5	9	72	40	55.6	32	44.4
23	19.3 ± 1.7	12	76	45	59.2	31	40.8
24	21.5 ± 2.1	13	79	57	72.2	22	27.8
25	12.9 ± 1.1	17	90	50	55.6	40	44.4
26	15.1 ± 1.1	29	175	132	75.4	43	24.6
27	16.2 + 0.6	36	240	175	72.9	65	27.1
28	13.0 ± 0.7	57	335	259	77.3	76	22.7
29	13.9 ± 0.4	106	585	464	79.3	121	20.7
30	12.9 ± 0.4	126	802	616	76.8	186	23.2
31	13.9 ± 0.3	164	862	595	69.0	267	31.0
32	11.1 ± 0.2	193	1,023	705	68.9	318	31.1
33	13.9 ± 0.4	231	1,324	913	69.0	411	31.0
34	13.7 ± 0.4	221	1,156	794	68.7	362	31.3
35	11.6 ± 0.3	226	1,222	800	65.5	422	34.5
36	12.8 ± 0.3	267	1,284	828	64.5	456	35.5
37	10.1 ± 0.3	257	1,153	662	57.4	491	42.6
38	8.7 ± 0.2	280	1,123	585	52.1	538	47.9
39	10.5 ± 0.2	272	1,008	475	47.1	533	52.9
40	11.2 ± 0.3	249	953	398	41.8	555	58.2
41	9.2 ± 0.3	234	750	233	31.1	517	68.9
42	8.7 ± 0.2	150	453	113	24.9	340	75.1
43	6.0 ± 0.3	79	217	36	16.6	181	83.4
44	5.7 ± 0.2	41	85	10	11.8	75	88.2
45	8.0 ± 0.4	22	39	4	15.7	35	84.3
46	11.8 ± 0.5	4	43	12	27.9	31	72.1
47	6.3 ± 0.3	4	17	0	0.0	17	100.0
48	2	1	1	0	0.0	1	100.0
49	4	1	2	0	0.0	2	100.0
Total		3,301	15,169	9,001		6,168	

(Franasiak JM et al., 2014)

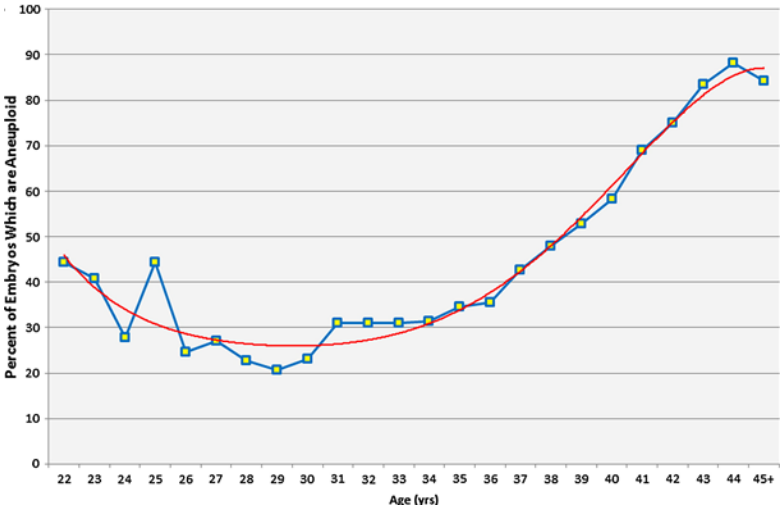


Figure 1. Aneuploidies and Maternal Ages.

（Franasiak JM et al., 2014)

1.2 DNA Double-Strand Breaks and Meiotic Repair

Meiosis is a highly conserved process among species. Meiosis includes two cycles of division – meiosis I and meiosis II- and results in four daughter cells with only one copy of each parental chromosome (haploid). Both meiosis I and meiosis II include four stages, named as prophase I, metaphase I, anaphase I and telophase I & cytokinesis and prophase II, metaphase II, anaphase II, telophase II & cytokinesis respectively. The accurate chromosomal segregation in meiosis I and meiosis II are required to prevent abnormal aneuploid daughter cells.

Briefly described, meiosis I starts with DNA replication. This is followed by sister chromatids connection, homolog pairing, the formation of double-strand breaks (DSBs), synaptonemal complex (SC) and DNA repair into crossovers (CO). Meiosis I ends with telophase I & cytokinesis where a parental cell divides into two daughter cells with half the number of chromosomes consisting of a pair of chromatids. Meiosis II occurs without DNA replication and ends up with four daughter cells each containing a haploid set of chromosomes (Figure 2).

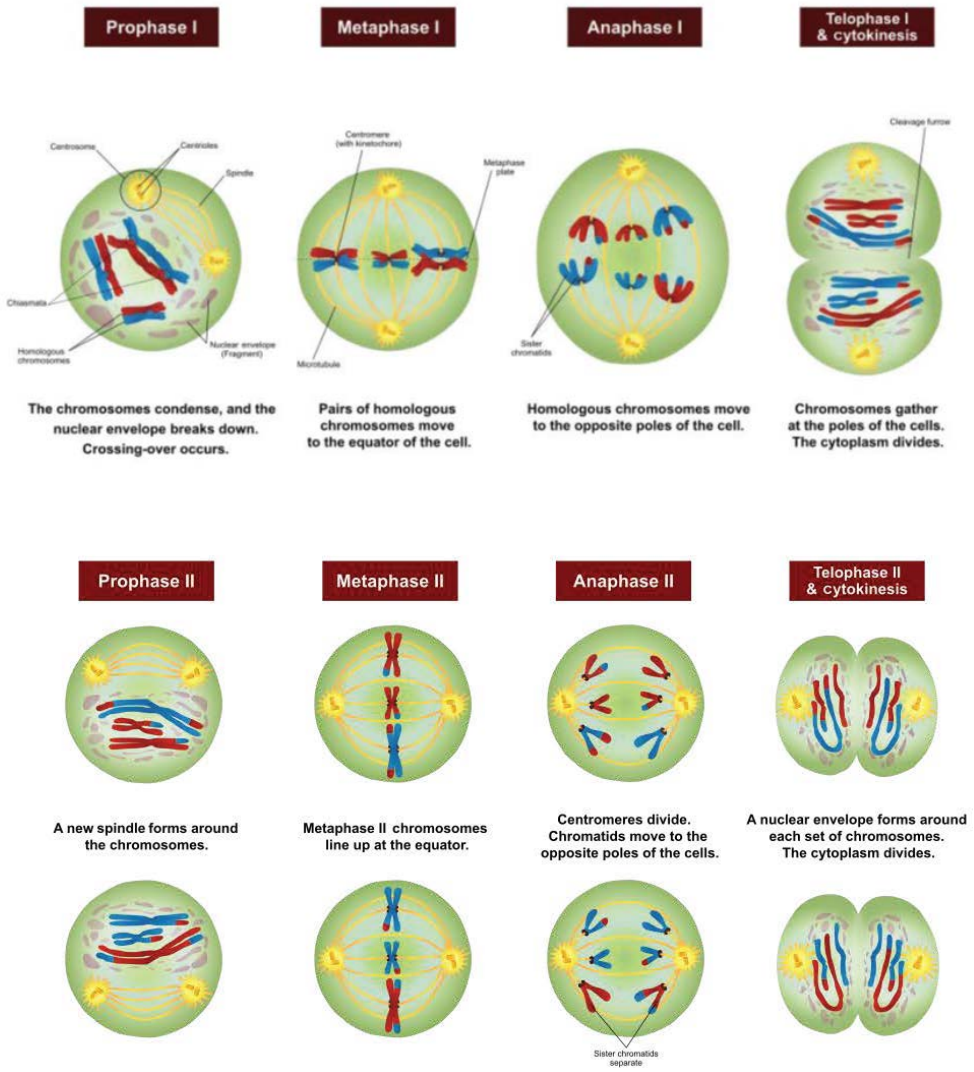


Figure 2. Meiosis I and Meiosis II.

(Credit: Ali Zifan, via Wikimedia Commons, CC BY-SA 4.0)

In most organisms, meiosis requires the accurate exchange of DNA between paternal and maternal chromosomes through homologous recombination. To start homologous recombination, DNA double-strand breaks (DSBs) must be created (Keeney, 2001). DSBs are formed by the action of a conserved meiosis-specific protein, Spo11, which functions like a topoisomerase and becomes covalently attached to the DNA end. In meiosis, cells have to generate at least one DSB

before crossover. After DSB formation, Spo11 is removed from DNA ends allowing the DNA ends to be chewed away, a process called resection. The overhanging 3' end of DSB invades a similar or identical homologous chromosome without a DSB to form a D-loop strand-exchange intermediate. After the invasion, two resolution pathways lead to different outcomes: crossover or non-crossover. In the crossover pathway, the homologous chromosome serves as a template to initiate DNA synthesis. The newly synthesized DNA strand is attached to the resected strand's end, forming a Holliday junction and indicating the crossover pathway (Figure 3).

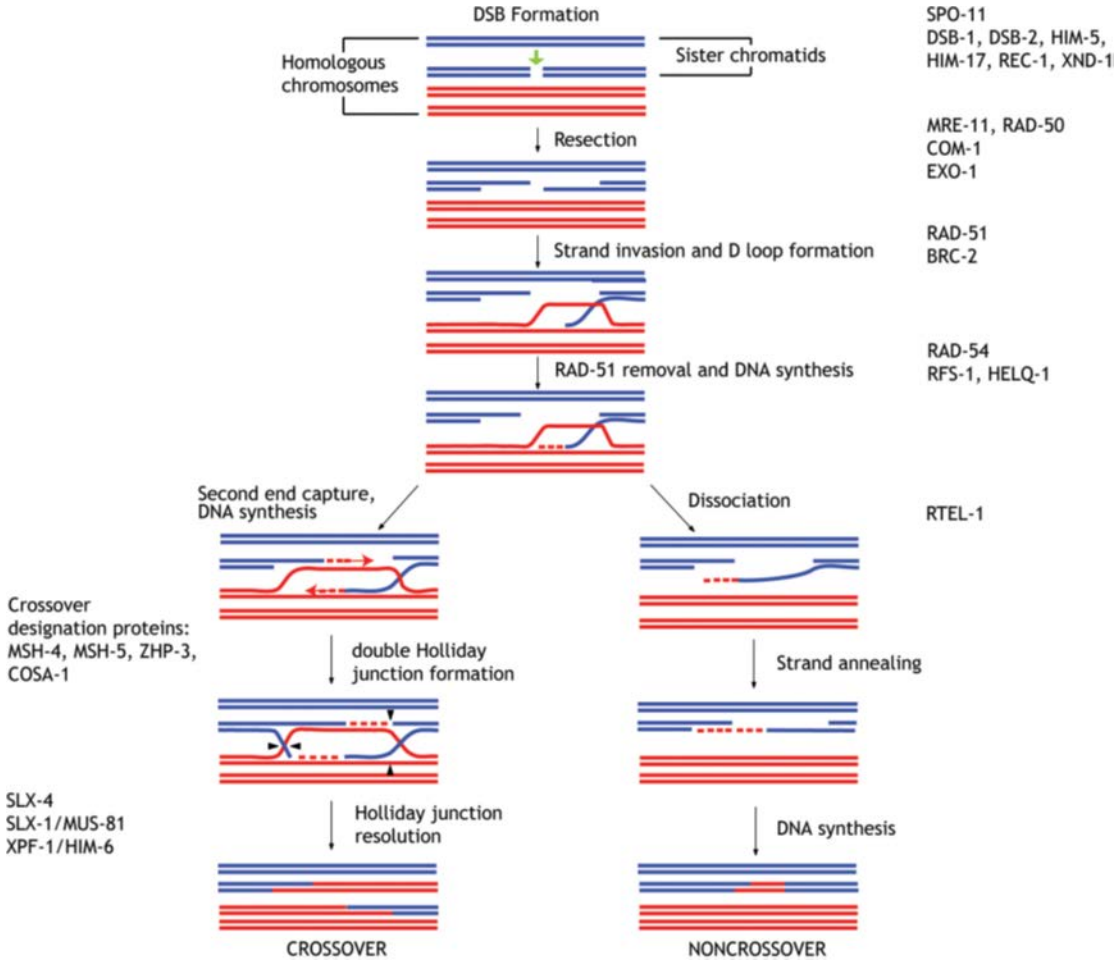


Figure 3. Model Depicting the Key Events of Meiotic Recombination.

(Hillers KJ. Meiosis. Wormbook, 2017)

1.3 *Caenorhabditis Elegans* as a Research Tool

C. elegans（*Caenorhabditis elegans*）is a member of the phylum *Nematoda* and an ideal animal model for molecular genetic studies because of it short and rapid life span and convenience of culture at 20℃ in the laboratory. They are grown on agar plates and fed bacteria *Escherichia coli.* A single wide type of *C. elegans* can produce 200 to 300 progenies. *C. elegans* is a self-fertilizing hermaphrodite with five autosomal chromosomes and one sex chromosome. Females or hermaphrodites have five pairs of autosomes and two X chromosomes (XX), while males have five pairs of autosomes and one X chromosome (XO).

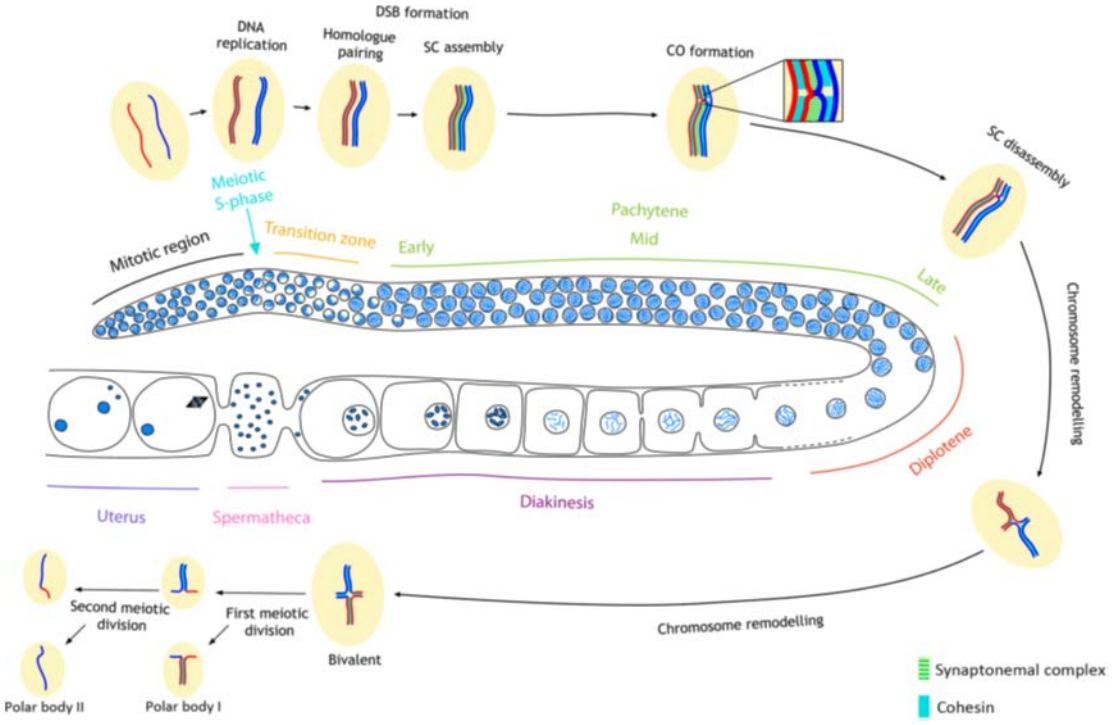


Figure 4. Diagram of Meiotic Events During Oogenesis in the *C. elegans* Germ Line.

(Hillers KJ. Meiosis. Wormbook, 2017)

C. elegans is a powerful animal model for reproduction research as its body is transparent, allowing visualization of the germ line, and it has reproducible cell positions that can be observed

during all stages of meiosis (Figure 4). Genetic balancers *C. elegans* have specific phenotypes for easy selection from the standard wild-type strain (called N2). Because meiosis is evolutionarily conserved across the species, *C. elegans* share at least 83% homologous genes in human (Lai et al., 2000), which makes *C. elegans* a powerful model organism in studying meiotic processes. In addition, the ease to freeze and recover *C. elegans* makes it possible to preserve mutant strains without losing important mutations or acquiring unwanted mutations.

1.4 Nondisjunction in *C. elegans*

As we discussed in 1.2, meiosis is crucial for population diversity. Meiosis is initiated by the formation of DNA double strand breaks (DSBs) by the conserved enzyme SPO-11, which is a member of topoisomerase II superfamily ((Dernburg et al., 1998), followed by crossovers (COs) between homologous chromosomes. Defects in formation and repair of DSBs promote genome instability. At least one CO is formed to ensure normal meiosis. In *C. elegans*, most chromosomes undergo only one CO in each meiosis. Distribution of COs does not occur randomly in *C. elegans*. COs occur in gene-poor regions towards the autosome ends (Barnes et al., 1995) and are few in the middle, gene-rich region in *C. elegans.* The X chromosome has a more even distribution of COs in *C. elegans*. At a local level, there are hotspots for COs (Nicolas et al., 1989; Baudat et al., 2010). These hotpots imply there are is genetic regulations for CO formation both in human and *C. elegans*.

Males account for 0.1% of *C. elegans* population which results from sex chromosomal nondisjunction during meiosis. Higher male progeny rates represent higher sex chromosomal

nondisjunction rates. Lower hatching rates may indicate higher autosomal chromosomal nondisjunction rates.

1.5 XND-1 and HIM-17 in *C. elegans*

X nondisjunction factor 1 (xnd-1) encodes an autosomally-localized protein, XND-1, an AT-hook containing protein with 702 amino acids, which is required for normal global CO distribution in *C. elegans*. XND-1 is involved in DSBs formation on the X chromosome through effects on chromatin structure genome-wide. In wild type, COs tend to occur towards chromosome ends, which contain fewer genes than centers. However, in *xnd-1* mutants, the distribution of COs is inverted, with more COs occurring towards chromosome centers. The loss of XND-1 function also decreases the number of DSBs, and hence COs, on the X chromosome, which in turn leads to increased frequency of males in progeny populations (Wagner, et al.,2010). This is called a High Incidence of Males or “him” phenotype and is characteristic of a group of meiotic mutations that increase X chromosome nondisjunction.

In addition to its role in meiosis, XND-1 also appears to function early in germ cell development. In the germline lineage, primordial germ cells (PGCs) are the precursor of sperm and eggs. XND-1 appears among the earliest proteins in new PGCs. Loss of *xnd-1* causes defects in PGC specification and differentiation (Mainpal et al., 2015), although the underlying cause of these defects are not well understood.

HIM-17 is a chromatin-associated protein that has roles in meiosis and more generally in the germ line. HIM-17 is required for DSB formation but not for homolog synapsis. SPO-11 dependent COs are inhibited in *him-17* null mutants and can be rescued by γ-irradiation induced

DSBs indicating that *him-17* is defective in making meiotic DSBs (Reddy and Villeneuve.,2004). Missense mutations also exhibit lower egg hatching rates and a high incidence of males among survivors, indicating a partial impairment of its meiotic functions. There are five *him-17* mutants reported: *him-17(ok424), him-17(me9), him-17(me24), him-17(e2806)* and *him-17(e2707)* (Figure 5, Reddy and Villeneuve,2004). Among these five mutants, *him-17(ok424)* is homozygous sterile, indicating it is null.

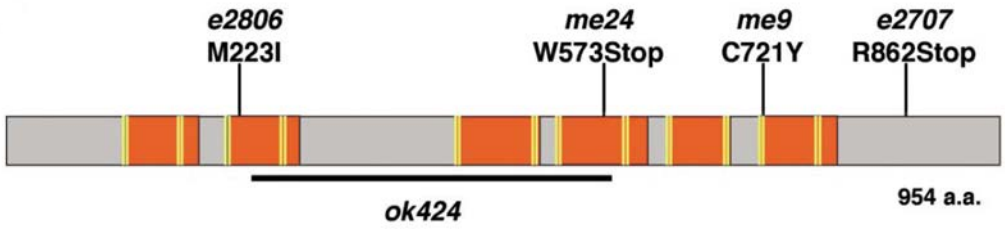


Figure 5. Modular Organization of the HIM-17.

(Reddy and Villeneuve.,2004)

Both *him-17* and *xnd-1* mutants show abnormal post-translational modifications of histones and impaired DSB formation (Reddy and Villeneuve.,2004); Wagner et al.,2010). Localization of HIM-5 is dependent on XND-1 and HIM-17 (Meneely et al., 2012).

HIM-5 is a protein of 252 amino acids and promotes DSB formation. HIM-5 plays a role in crossover distribution on both the X chromosome and autosomal chromosomes. Like XND-1, HIM-5 influences DSB formation mainly on the X chromosome (Meneely et al., 2012).

In this project, we studied the interactions between *him-17* and *xnd-1*, which collaboratively regulate *him-5*, to determine if they function in together to regulate meiotic crossovers.

1.6 Genetic Balancers

Many homozygous mutations are lethal or sterile to *C. elegans*. *C. elegans* strains carrying homozygous lethal or sterile mutations are hard to maintain and study. Genetic balancers are heterozygous chromosomes with significant rearrangements that can be maintained in the laboratories for researches. Balancers usually have unique phenotypes that allow them to be easily selected.

In this project, we used *qC1* as a balancer for *xnd-1(ok709)*, written as $\frac{xnd-1(ok709)}{qC1}$, and $\frac{hT2}{qC1}$. *qC1* is a very stable dominant crossover suppressor to use for strain maintenance. It is marked with a mutation that make the animals Roll (a helically left or right twisted body) on the plate. *hT2* is a stable reciprocal translocation between chromosome I and chromosome III and therefore balances parts of each chromosome including where *xnd-1* maps. (Figure 6, Wormbook). $\frac{hT2}{qC1}$ has a phenotype of rolling behavior (associated with *qC1*) with pharyngeal GFP element (green, on *hT2*). $\frac{xnd-1(ok709)}{qC1}$ has a phenotype of rolling behavior, whereas the wild type and *xnd-1(ok709)* homozygous genotype do not roll.

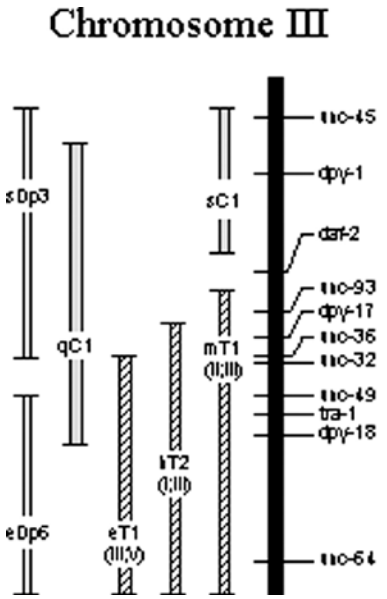


Figure 6. Balancers on Chromosome III.

(Hillers KJ. Meiosis. Wormbook, 2017)

1.7 The Project Goals

Evidence suggests both XND-1 and HIM-17 regulate *him-5* and are involved in DSB formation.

HIM-5 is a member of High Incidence of Males proteins in *C. elegans*. HIM-5 has strong effects on X chromosome exchange and recombination (Hodgkin et al. 1979; Broverman and Meneely, 1994). *him-5* mutations also alter recombination distribution and frequency. Previous studies showed that HIM-5 functions in the same pathway as *xnd-1* and that localization of HIM-5 is impaired in *xnd-1(ok709)* and *him-17(ok424)* mutant animals (Meneely et al., 2012). XND-1 has specific timing windows in DSB formation, regulates germ line cell cycle and primordial germ cell (PGC) specification and plays a role in histone modifications. *xnd-1* CO formation role is

through regulating *him-5* expression (Figure 7, McClendon et al.,2016). HIM-17 has overlapping functions with XND-1. Both HIM-17 and XND-1 have functions on histone modification and DSB formation and are required for HIM-5 localization.

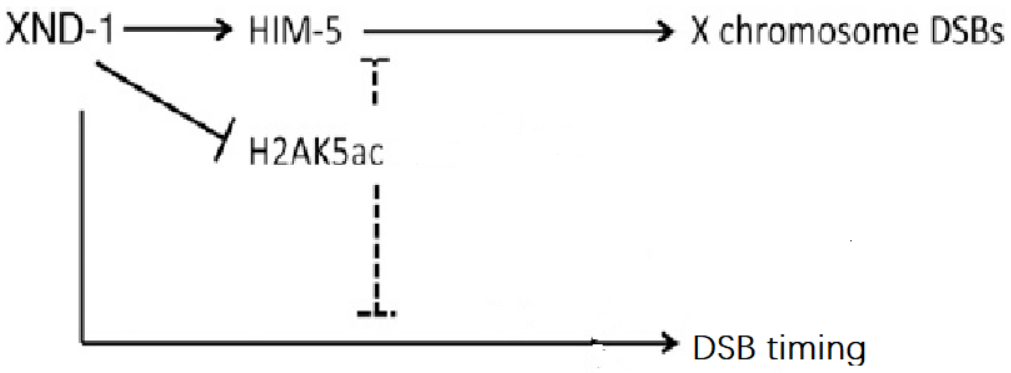


Figure 7. XND-1 Regulations and Mediators.

(McClendon et al.,2016)

HIM-17, by contrast, has no role in regulating the timing of DSB formation (Reddy and Villeneuve, 2004) and no known roles in PGC specification. However, no study shows mechanisms of relationships between *him-17* and *him-5* so far (Meneeley et al 2012).

Prior studies in the lab have suggested that these *xnd-1* and *him-17* may interact and work together for DSB formation (Meneely et al., 2012; McClendon et al.,2016). We are currently examining how these two genes work together to control various aspects of germ line development in *C. elegans*. We hypothesize that *xnd-1* and *him-17* work together to regulate *him-5* and influence the DSB formation in *C. elegans*. The *him-17(e2806)* mutant genotype has a weak phenotype in contrast to *him-17(e2707)*. To test this hypothesis, we have constructed double mutant strains with *xnd-1(ok709)* and two different partial loss-of-function alleles of *him-17(e2707)* and *him-*

17(e2806). We will evaluate clutch sizes, brood sizes, egg hatching rates, male incidence, and meiotic chromosome structures in *xnd-1(ok709); him-17(e2806)* and *xnd-1(ok709); him-17(e2707)* double mutant strains.

2.0 Materials and Methods

2.1 *C. elegans* Strain Information

Five strains of *C. elegans* are used in this project: N2, *him-17(e2707), him-17(e2806),* $\frac{xnd-1(ok709)}{qC1}$, and $\frac{hT2}{qC1}$ to create double mutated strains: $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2707),* $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806).*

N2 is a wild type of *C. elegans*. *him-17(e2707)* is a homozygote of *him-17(e2707)*, named and stocked as QP 0567. *him-17(e2806)* is a homozygote of *him-17(e2806)*, named and stocked as QP 0446. $\frac{xnd-1(ok709)}{qC1}$ is a *xnd-1(ok709)* mutation balanced with *q*C*1* with a phenotype of rolling behavior, named and stocked as QP 1042. $\frac{hT2}{qC1}$ is a strain originally made from a cross of $\frac{dpy-28}{qC1}$ strain and $\frac{xnd-1(ok709)}{hT2}$ strain with a phenotype of rolling behavior and pharyngeal expressed GFP transgene (green).

In this project, gene names without "+" and "-" represent mutated genes. "+" represents a wild type. Being unsure about a mutant was noted as "[ ]".

2.2 *C. elegans* Culture Maintenance

Petri plates with Nematode Growth Medium (NGM) agar is mostly used in *C. elegans* maintenance in the laboratory (Brenner, 1974). In this project, small petri plates (35 mm diameter)

were used to cross, large petri plates (60 mm diameter) were used to maintain strains. Commercial NGM was used (HIMDIA). 5 mg/mL cholesterol in 100% ethanol was added to the medium base solution to a final concentration of cholesterol in the NGM at 5 mg/L prior to autoclaving. *Escherichia coli* (*E. coli*), OP50, was used as a feeder host for *C. elegans*.

Two worm transferring methods were used in this project: 1) single worm or multiple worms transferred with a platinum worm pick and 2) “chunking” where a sterilized scalpel was used to move a chunk of agar with starved worms from an old plate to plate with fresh OP50. Transfer frequency depends on the purpose of experiment. For worm maintenance, I transferred worms before they were starved. For progeny counting, I transferred worms every 18 to 24 hours.

C. elegans can be maintained between 16°C and 25°C, but grows faster at higher temperatures, 2.1 times faster at 25°C than at 16°C, and 1.3 times faster at 20°C than at 16°C (Maniatis et al., 1982). However, maximum progeny production is at 20°C, so for this project, all *C. elegans* strains were maintained at 20℃. *him-17* mutants also have mitotic proliferation defects the higher temperature (Bessler JB et al., 2017), so we used the permissive temperature of 20°C.

The original stocks used from thaws of frozen stocks maintained in liquid nitrogen. After thawing from liquid nitrogen, worms in cryotubes were poured to plates with one cryotube with one plate. The plates then were put into 20℃ in incubator for one day to recover. Alive worms were transferred to fresh plates after recovery.

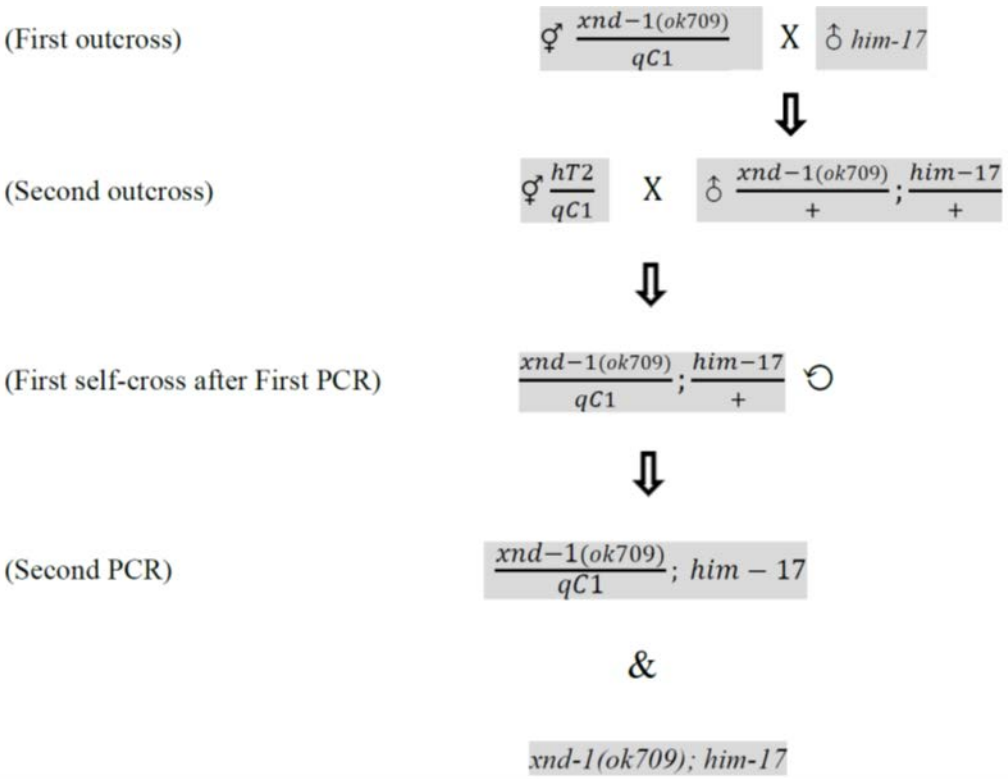
The genetic and phenotype information of all new created strains of *C. elegans* from this project were recorded into the lab “NEMASTOCK” database with unique names starting with “QP”. All these strains were stored in 1.8 ml cryotubes (Thermo scientific) and frozen at −80 °C

2.3 *C. elegans* Crosses

To create $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2707),* $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806)* , *xnd-1(ok709); him-17(e2707)* and *xnd-1(ok709); him-17(e2806)* double mutant strains, three strains were used for outcross: $\frac{xnd-1(ok709)}{qC1}$, *him-17(e2707), him-17(e2806)* and $\frac{hT2}{qC1}$.

For outcross, three adult males and one L4 hermaphrodite were picked to each small cross plate in each outcross step, 3 to 5 plates for each outcross. For self-cross, one L4 *C. elegans* was placed to one plate, 24 to 30 plates for each self-cross. For outcross, a higher rate of males in progenies indicates a success cross.

After cross, hatched single L4 hermaphrodites were transferred to small seeded plates individually. Genotype these hermaphrodites 2 to 3 days later when they laid over 50 eggs.



2.4 PCR Genotyping

PCR was applied to select $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2707)* genotype, $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806)* genotype, *xnd-1(ok709); him-17(e2707)* genotype and *xnd-1(ok709); him-17(e2806)* genotype. Four sets of primers were used in this project to select and verify desired mutant strains. Stand PCR was applied to select and verify *xnd-1(ok709)* mutant. Allele specific PCR was applied to select and verify *him-17(e2707)* and *him-17(e2806)* mutants. The primers used in these studies are included in the Appendix A.

xnd-1(ok709) allele was run on 1.5% agarose gel with our lab's customized DEL40EXT PCR protocol. *him-17(e2707)* and *him-17(e2806)* alleles were run on 3% agarose gel with customized DEL40 PCR protocol on PCR instruments (MJ Research PTC-200 Thermal Cycler and TC 9639 Thermal Cycler by Benchmark Scientific). The details of customized DEL40EXT and DEL40 are included in Appendix A.

2.5 Brood Analysis

N2, *him-17(e2707), him-17(e2806), xnd-1(ok709), xnd-1(ok709); him-17(e2707)* and *xnd-1(ok709); him-17(e2806)* were analyzed for clutch sizes (total number of eggs), brood sizes, hatching rates, and male rates. Clutch sizes were calculated by counting all fertilized (refractile) eggs laid by a single worm every 18 hours until the worm died or no more eggs were laid in 2 consecutive days. Brood sizes were calculated by counting all alive progenies hatching from fertilized eggs laid by a single worm 3 or 4 days after eggs were counted. Hatching rates were calculated as the number of alive progeny divided by the number of fertilized eggs from a single

worm with the format of xx.xx%. Unfertilized eggs were excluded from counting. Genotyping by PCR was performed on each individual worm to confirm the genotype after eggs were depleted. Male rates were calculated as the number of male progenies divided by the number of total progenies. Males and hermaphrodites were counted 3 to 4 days after eggs were laid.

3.0 Results

3.1 Creating *xnd-1* and *him-17* Double Mutants

I was able to successfully construct the desired double mutant strains that are analyzed below. Genotypes of $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2707)*, $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806)*, *xnd-1(ok709); him-17(e2707)* and *xnd-1(ok709); him-17(e2806)* were confirmed multiple times by with PCR testing both the heterozygous parents and the homozygous progeny.

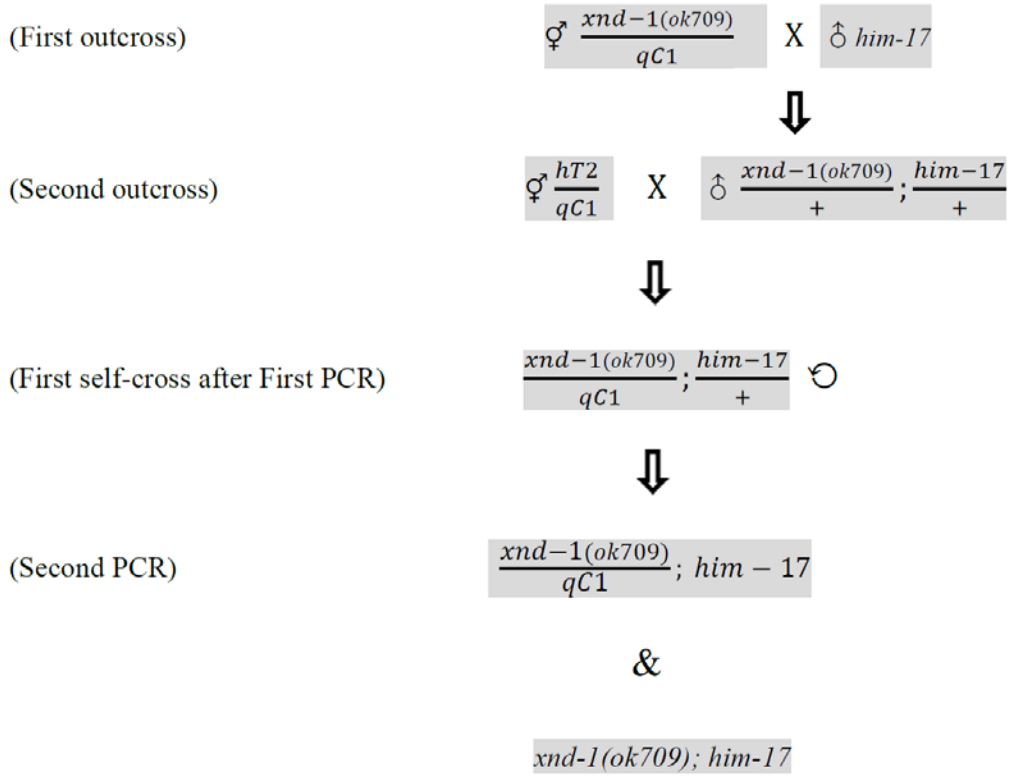


Figure 8. *C.elegans* Crossover and Experiment Procedure.

The first outcross from stains of $\frac{xnd-1(ok709)}{qC1}$ hermaphrodites and him-17 males gave progenies with genotypes of $\frac{[xnd-1(ok709)]}{qC1}$; $\frac{[him-17]}{+}$ and $\frac{[xnd-1(ok709)]}{+}$; $\frac{[him-17]}{+}$.

Pick $\frac{[xnd-1(ok709)]}{+}$; $\frac{[him-17]}{+}$(non-roller) males to outcross with $\frac{hT2}{qC1}$ hermaphrodites.

The first PCR was run to select and verify $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17}{+}$(Figure 9 and Figure 10). 6 worms were confirmed as $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17(e2707)}{+}$ out of 30 $\frac{[xnd-1(ok709)]}{qC1}$; $\frac{[him-17(e2707)]}{+}$ worms. PCR for *xnd-1(ok709)* failed to identify *xnd-1(ok709)* mutant in $\frac{[xnd-1(ok709)]}{qC1}$; $\frac{[him-17(e2806)]}{+}$ worms(Figure 11), so we continued with the project with 19 $\frac{[xnd-1(ok709)]}{qC1}$; $\frac{him-17(e2806)}{+}$ worms out of 30 $\frac{[xnd-1(ok709)]}{qC1}$; $\frac{[him-17(e2806)]}{+}$ worms (Figure 12).

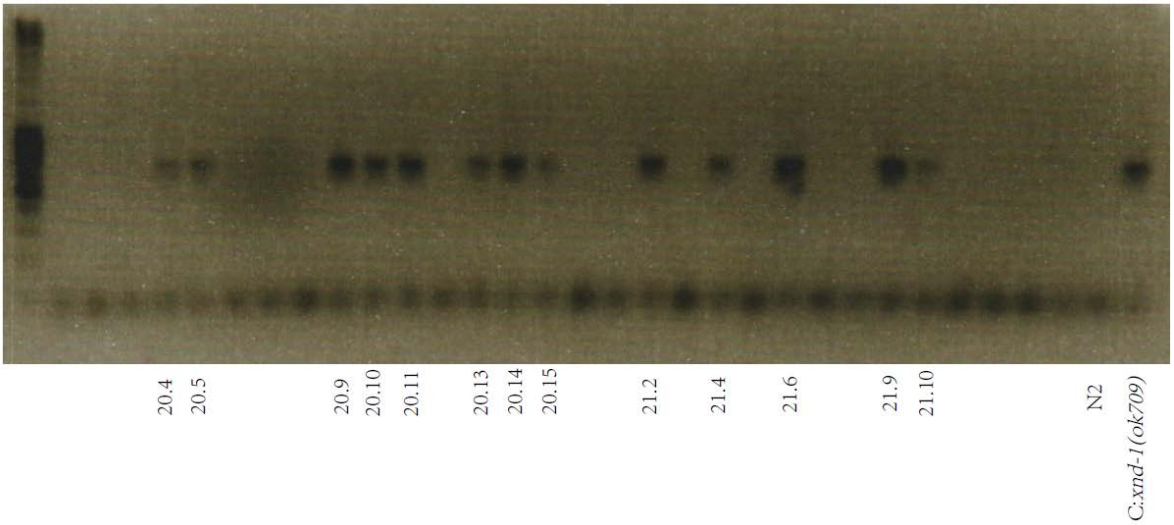


Figure 9. *xnd-1(ok709)* Primers to Select $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17}{+}$. 13 worms were detected as $\frac{xnd-1(ok709)}{qC1}$ out of 30 worms.

The plate names are underneath the gel lane. Expected size of *xnd-1(ok709)* procuct: 700 bp

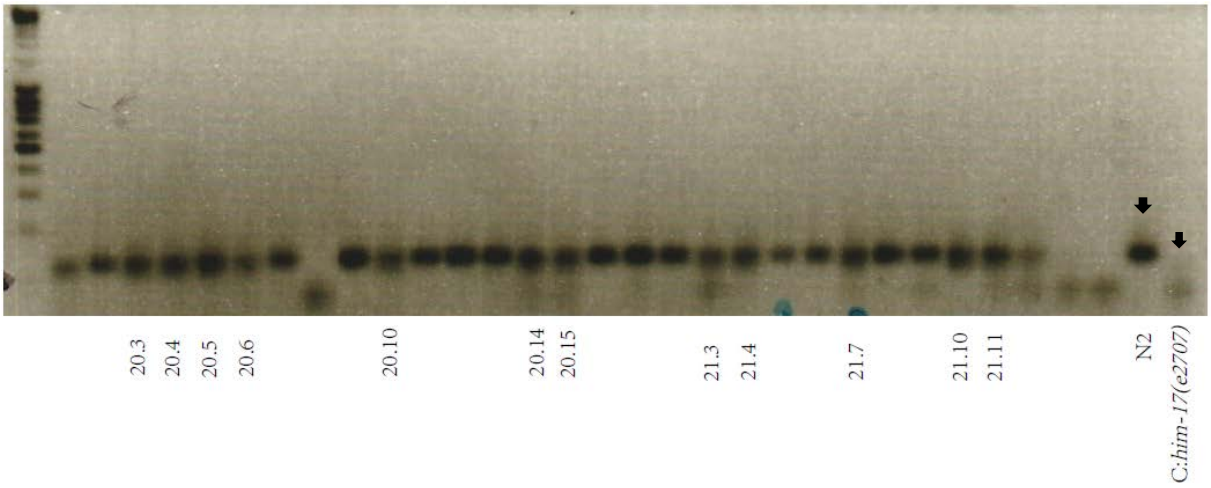


Figure 10. him-17(e2707) Primers to Select $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17(e2707)}{+}$. 12 worms were detected as $\frac{him-17(e2707)}{+}$ out of 30 worms.

The plate names are underneath the gel lane. Expected size of N2 product: 120 bp; expected size of him-17(e2707) product: 100 bp.

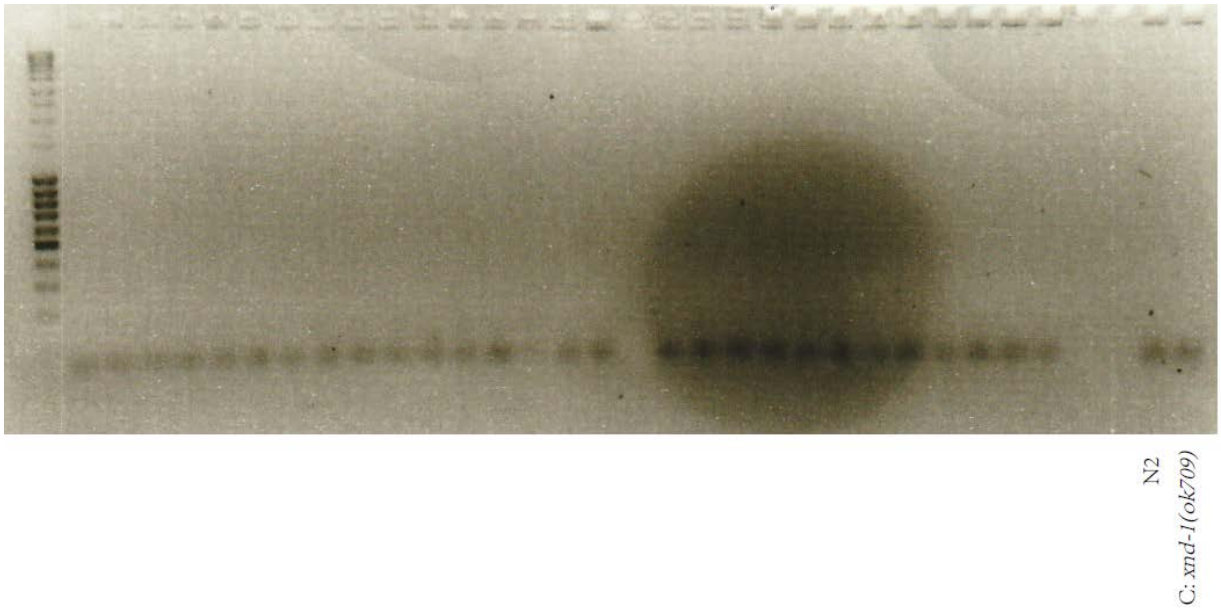


Figure 11. *xnd-1(ok709)* Primers Failed to Select $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17\ (e2806))}{+}$.

Expected size of *xnd-1(ok709)* procuct: 700 bp

This is presumably because the PCR did not work at all and needed to be repeated. Since the stocks were balanced, I was able to check for xnd-1 in the Rolling progeny at the next generation.

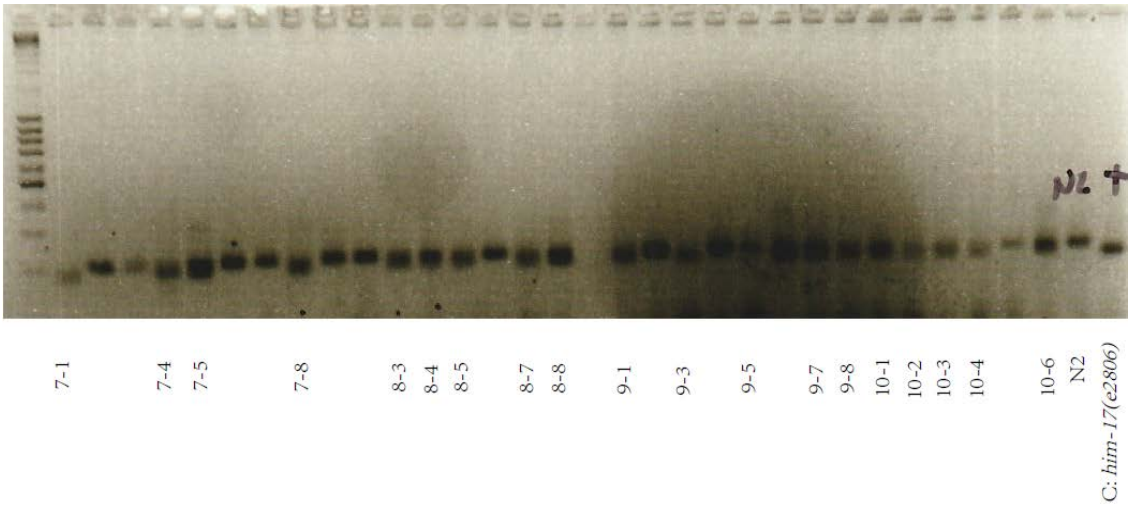


Figure 12. *him-17(e2806)* Primers to Select $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17(e2806)}{+}$. 19 worms were detected as $\frac{him-17(e2806)}{+}$ out of 30 worms.

The plate names are underneath the gel lane. Expected size of N2 product: 195 bp; expected size of him-17(e2806) product: 175 bp.

Second PCR was to select and confirm $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2707),* $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806), xnd-1(ok709); him-17(e2707)* and *xnd-1(ok709); him-17(e2806)* with *him-17(e2707), him-17(e2806), xnd-1(ok709)* and *xnd-1* wild-type primers.

As shown in Figure 13, $\frac{xnd-1(ok709)}{qC1}$;*him-17(e2707)* and *xnd-1(ok709); him-17(e2707)* were confirmed by PCR to have the *him-17(e2707)* mutation. $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806)* was confirmed by PCR to have the *him-17(e2806)* mutation, while *xnd-1(ok709); him-17(e2806)* was not. However, I believed it was a sampling error, because in this genotyping, *xnd-1(ok709); him-17(e2806)* worm was a progeny of $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806)*

In Figure 14, we see the results for genotyping with *xnd-1* wild-type primers and *xnd-1(ok709)* primers. Bands with both *xnd-1* wild type primers and *xnd-1(ok708)* primers indicated genotype of $\frac{xnd-1(ok709)}{qC1}$. Only one band with *xnd-1(ok709)* primers indicated genotype of *xnd-1(ok709)*. Upon attaining the balanced stocks $\frac{xnd-1(ok709)}{qC1}$; *h*im-17(e2707) and $\frac{xnd-1(ok709)}{qC1}$; *him-17(e2806)* stocks, they were maintained at 20°C throughout the course of my experiments.

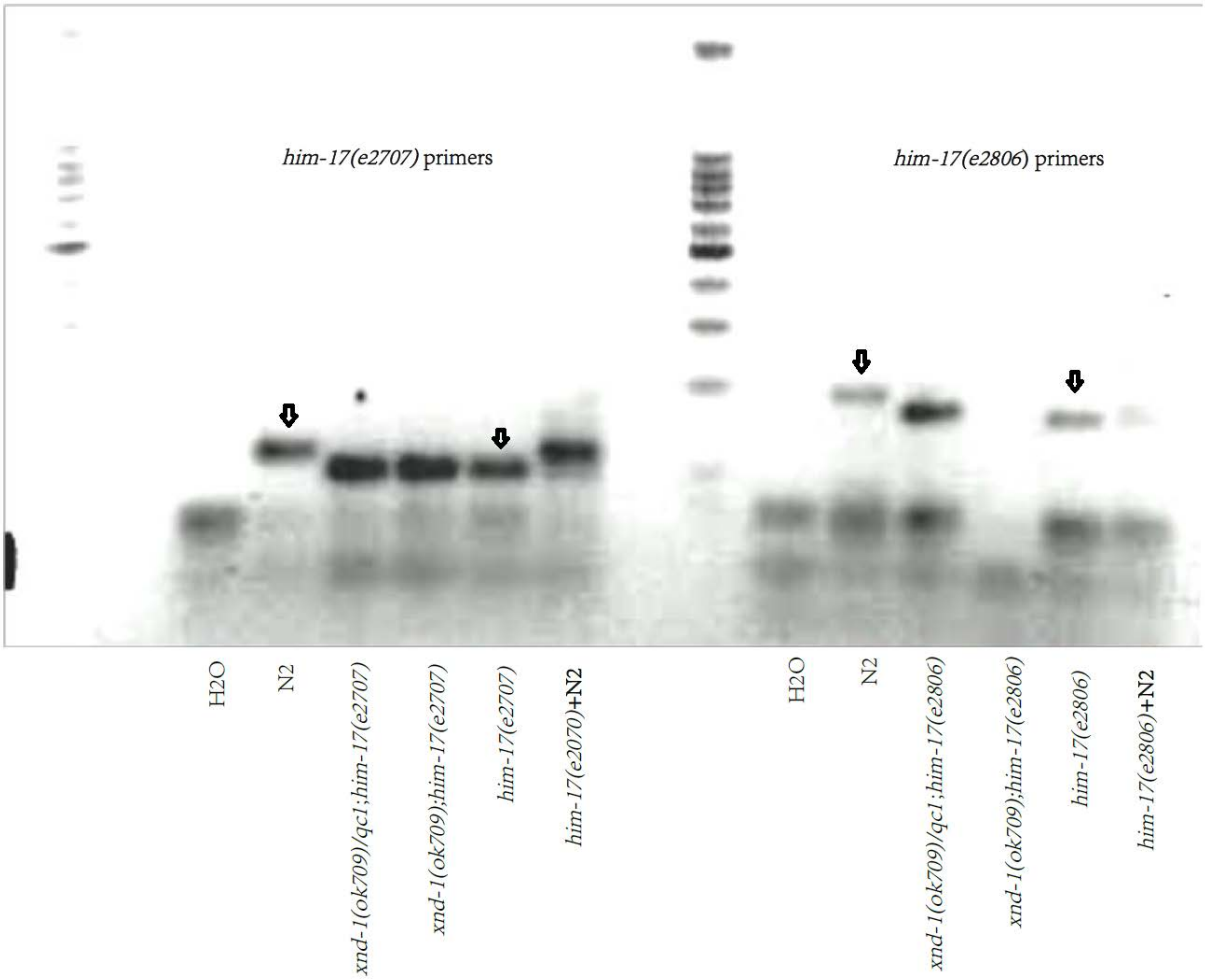


Figure 13. Genotyping of *him-17(e2707)* and *him-17(e2806)*. *him-17(e2707)* primers.

Expected size of N2 product: 120 bp; expected size of *him-17(e2707)* product: 100 bp. *him-17(e2806)* primers: Expected size of N2 product: 195 bp; expected size of *him-17(e2806)* product: 175 bp;

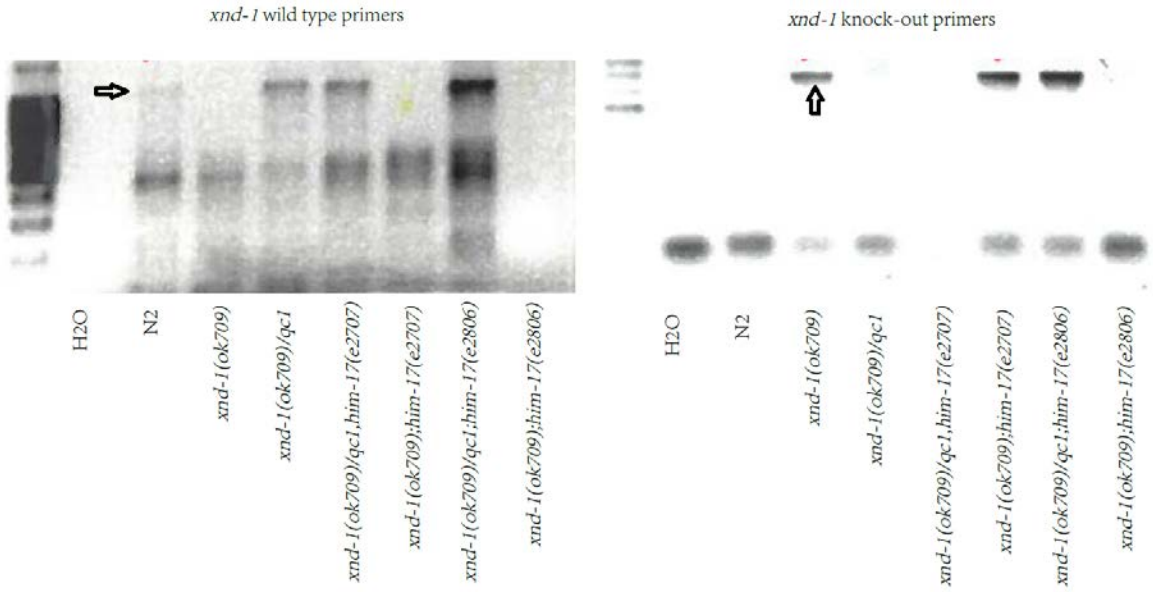


Figure 14.Genotyping of *xnd-1(ok709)*.

3.2 Hatching Rates and Male Rates

To determine if there is a genetic interaction between *xnd-1* and *him-17*, I next wanted to perform detailed brood studies of the single and double mutants. Average clutch size, average brood size, average hatching rate and average male rate for all genotypes were calculated as described in material and methods (Table 2). The data are presented as the average± SEM. Clutch size is the total number of eggs laid by a single worm. Brood size is the total number of hatched laid eggs by a single worm. Hatching rate is the ratio of brood size to clutch size, which indicates the degree of the meiosis success. Male rate is the ratio of the number of males to the number of progeny which indicates the degree of X chromosomal nondisjunction.

Table 2. Characteristics of Genotypes Used in this Project.

Genotype	N	Avg. Clutch size ± SEM	Avg. Brood size ± SEM	% Hatching ± SEM	% Male ± SEM
N2	15	219.2±12.91	216.07±12.89	98.57±0.46	0.09±0.05
xnd-1(ok709)	8	93.50±22.21	28.75±10	30.75±8.29	16.09±3.76
him-17(e2707)	13	69.69±11.99	21.00±5.15	30.13±4.65	40.66±5.09
xnd-1(ok709); him-17(e2707)	14	75.07±15.47	12.21±4.21	16.27±7.17	38.01±5.50
him-17(e2806)	12	142.50±16.82	123.00±17.33	86.32±6.3	6.17±2.16
xnd-1(ok709); him-17(e2806)	14	150.93±19.58	71.79±15.93	47.56±7.90	27.77±4.03

t-tests were applied to compare differences between pairwise combinations of genotypes. As expected, the wild type N2 is different from *xnd-1(ok709), him-17(e2707),* and *him-17(e2806),* as well as the respective double mutants, *xnd-1;him-17(e2707)* and *xnd-1;him-17(e2806)*(Figure 15 and Figure 16). This was true for all parameters analyzed: clutch size, brood size, hatching rate and frequency of males.

In Figure 15, when we look at brood sizes, *xnd-1(ok709)* is not statistically different than *him-17(e2707)* and *xnd-1;him-17(e2707),* but *him-17(e2707)* is different from *xnd-1;him-17(e2707). xnd-1; him-17(e2707)* has a higher brood size than *him-17(e2707).* However, although the brood sizes are different between *him-17(e2707)* and *xnd-1;him-17(e2707),* the calculated hatching rates of these two genotypes does not have a significant difference. I observed significant individual variances in terms of clutch size, brood size and male rate, indicating that a larger number of animals should be counted in future studies.

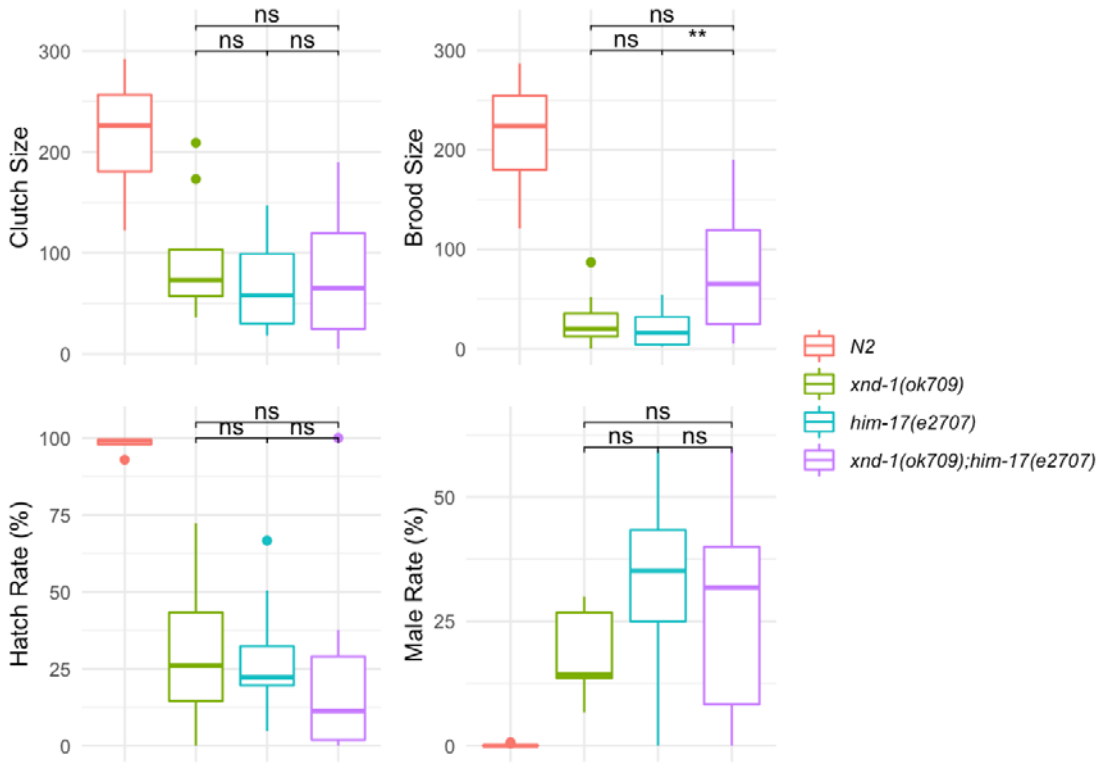


Figure 15. Characteristics of *xnd-1(ok709)*, *him-17(e2707)* and *xnd-1(ok709);him-17(e2707)*; N2 as a control. The only statistical difference is between brood size of *him-17(e2707)* and *xnd-1(ok709);him-17(e2707)*.

In Figure 16, we look at the other double mutant genotypes, *xnd-1(ok709); him-17(e2806)*. Here, in terms of brood size, hatching rate and male rate, we find *xnd-1* is not statistically different from *xnd-1(ok709);him-17(e2806)*, while *xnd-1(ok708)* is statistically different from *him-17(e2806)* and *him-17(e2806)* is statistically different from *xnd-1(ok709); him-17(e2806)* . *xnd-1(ok709)* has a lower brood size rate and hatching rate and a higher male rate than *him-17(e2806)*. *him-17(e2806)* has higher brood size and hatching rate in contrast to *xnd-1(ok709); him-17(e2806)*.

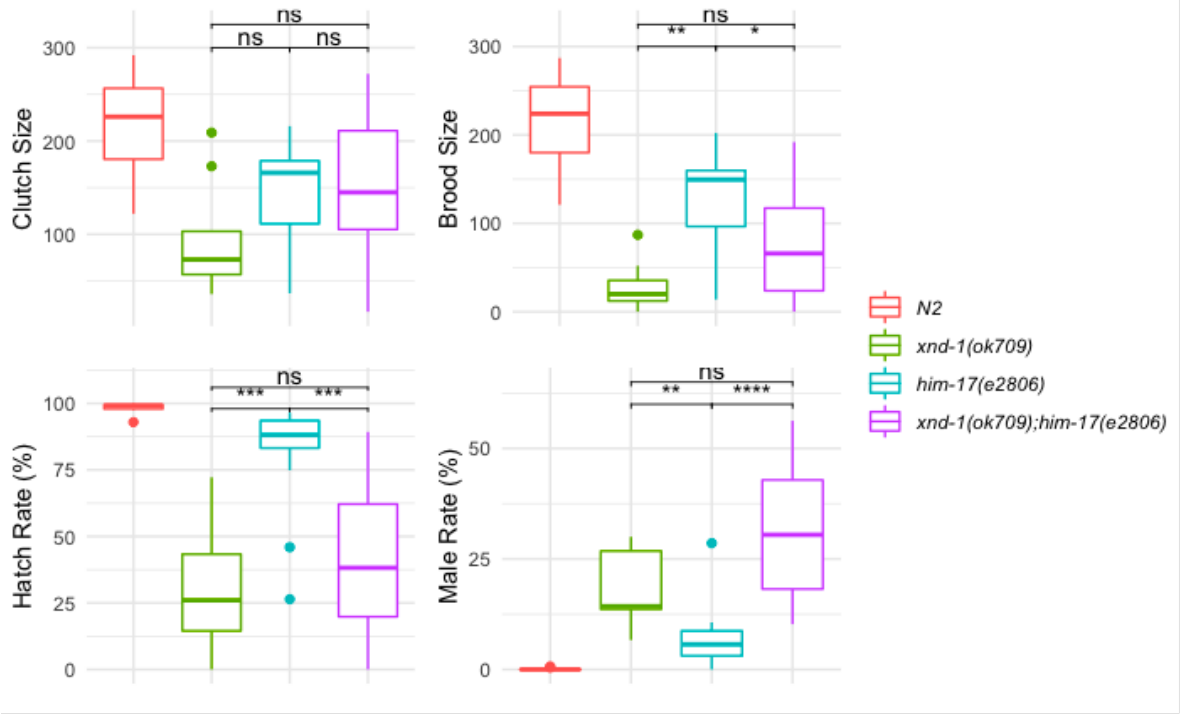


Figure 16. Characteristics of *xnd-1(ok709)*, *him-17(e2806)* and *xnd-1(ok709);him-17(e2806)*, N2 as a control.

***xnd-1(ok709)* is statistically different from *him-17(e2806)* in terms of brood size, hatching rate and male rate.**
***him-17(e2806)* is statistically different from *xnd-1(ok709);him-17(e2806)* in terms of brood size, hatching rate and male rate.**

4.0 Discussion

Miscarriage and infertility are a public health problem all over the world. Meiotic nondisjunction is a main cause of miscarriage, trisomies and monosomies. Cytogenetic analysis of 132 villous tissues of miscarriage showed 58% trisomy,7.9% monosomy, 10.1% polyploidy, 1.1% double trisomy and 15.7% mosaic (I Horiuchi et al., 2019). Nondisjunction can occur in either meiosis I or meiosis II. Crossovers create physical connections between homologs that ensure their alignment and segregation during the first meiotic division.

The well-studied *him-5* gene mutations in *C. elegans* reduce the frequency of crossovers on X chromosome and changes the crossover distributions on both X chromosomes and autosomes (Meneely et al. 2012). *xnd-1* and *him-5* are in the same functional pathway (Meneely et al. 2012).

him-17(e2707), him-17(e2806), xnd-1(ok709), xnd-1(ok709);him-17(e2707) and x*nd-1(ok709);him-17(e2806)* genotypes have significant differences compared to N2 in clutch size, brood size, hatching rate and male rate, consistent with the known roles of these genes in crossover formation. XND-1 was reported to localize to autosomal chromosomes and influences distribution of crossovers on autosomal chromosomes and frequencies in sex chromosomes, either of which results in a higher lethal rate and male rate (Wagner, et al.,2010). We also know *him-17* mutants exhibit a Him phenotype with lower hatch rates compared to wild type. From the data I observed, low clutch sizes and hatching rates and higher male rates may develop from a complicated relationship between *xnd-1* and *him-17*.

him-17(e2707) and *him-17(e2806)* have significant differences in clutch size, brood size, hatching rate and male rate, which verifies that these two mutations have very different levels in

abilities to regulate crossovers formation. This is consistent with published data on these genotypes (Reddy and Villeneuve,2004). The different effects between *him-17(e2707)* and *him-17(e2806)* may be because *him-17(e2707)* is a nonsense mutation (R862Stop) that shortens and changes the structure of HIM-17, while *him-17(e2806)* is a missense mutation (M223I) that may not affect HIM-17 structure and functions.

We are interested in determining whether XND-1 and HIM-17 function together or in the same pathway to influence meiotic processes. Therefore, the most important interactions to analyze are the differences between the single and double mutant genotypes.

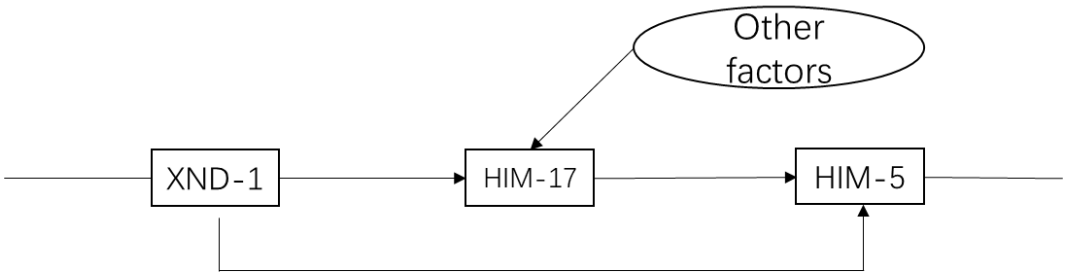


Figure 17. Interactions Among *xnd-1, him-17* and *him-5.*

If *xnd-1(ok709)* mutation and *him-17* mutations work together to increase the rate of nondisjunction though HIM-5, then double mutant could show 1) higher male rates and lower hatching rates than both single mutants, 2) higher male rates and lower hatching rates than only one of the two single mutants or 3) no significant difference than either of the single mutants. If 1) then we would interpret that *xnd-1* and *him-17* are complementary genes. If 2), we would interpret *xnd-1* is an epistatic gene of *him-17* or vice versa. If 3), we would interpret that *xnd-1* and *him-17* are redundant genes. In *xnd-1;him(e2707)* double mutants, we saw no significant

difference from either of the single mutants, therefore it could be interpreted that *xnd-1* and *him-17* are in the same pathway. However in *xnd-1;him(e2806)* double mutants, we saw higher male rates and lower hatching rates than *him-17(e2806)* mutants, therefore we might interpret that *xnd-1* is epistatic to *him-17*. How do we reconcile these different interpretations for how *xnd-1* and *him-17* function?

I think my data is best described by the model in Figure 17. *xnd-1* both works through *him-17* and independently regulates *him-5*. There may be other factors regulating *him-17*. When *xnd-1* and *him-17* are both mutated, *xnd-1* cannot regulate *him-17* and because *him-17(e2806)* is a weaker allele, it has a partial function in regulating *him-5*. Other factors may regulate *him-17(e2806)* to have some functions. This may explain why there is no significant difference between *xnd-1(ok709)* and *xnd-1(ok709); him-17(e2806)*. Since *xnd-1(ok709)* is significantly different than *him-17(e2806)*, I think this confirms that in *xnd-1* mutant animals, *him-17(e2806)* retains some function to regulate *him-5*. In contrast to *him-17(e2806)*, both *xnd-1* and other factors regulate *him-17* so that *him-17(e2806)* gave better results in brood size and hatching rate than *xnd-1(ok709)*. The significant difference between *him-17(e2806)* and *xnd-1(ok709); him-17(e2806)* verifies this interaction again. For *him-17(e2806)*, both *xnd-1* and other factors can regulate *him-17* to have some partial functions to work on *him-5*, while for *xnd-1(ok709);him-17(e2806)*, other factors but not *xnd-1* can regulate *him-17* to have some partial functions to further work on *him-5*, which explains why *him-17(e2806)* had a higher brood size and hatching rate than *xnd-1(ok709);him-17(e2806)*.

Brood size and hatching rate give an overall measure of egg viability, which is at least in part, a product of the meiotic success that preceded egg and sperm fusion. Another measure of meiotic success is the frequency of males. HIM-17 is required for DSB formation on both

autosomal and the X chromosomes (Reddy and Villeneuve ,2004) and XND-1 influence DSB formation mainly on X chromosome (Wagner, et al.,2010). *xnd-1(ok709)* has no difference compared to *xnd-1(ok709);him-17(e2707)* and *xnd-1(ok709);him(e2806)* in terms of male rate; *him-17(e2707)* is different from *xnd-1(ok709);him-17(e2707)* and *him-17(e2806)* is different than *xnd-1(ok709);him-17(e2806)*, indicating *xnd-1* is an epistatic to *him-17* for nondisjunction of the X chromosome. Although t-test gives the conclusion that *xnd-1(ok709)* has no significant difference from *him-17(2707)* in hatching rate, the raw data seem very different in % Male ± SEM: 16.09±3.76 (*xnd-1(ok709)*) vs 40.66±5.09 (*him-17(e2707)*). I think it is because only 8 animals of *xnd-1(ok709)* were counted, fewer than other genotypes. Further, when we look at male rates of *him-17(e2806)* and *xnd-1(ok709);him-17(e2806)*, *him-17(e2806)* has a lower male rate than *xnd-1(ok709);him-17(e2806)*. This may be interpreted by that partial loss of function for HIM-17 in *him-17(e2806)* still works on *him-5*. However, when *xnd-1* is knocked out in double mutants, *xnd-1* cannot regulate *him-5* through *him-17* and cannot regulate *him-5* independently. Double mutant with *him-17(e2707)* mutation is different from double mutant with *him-17(e2806)* mutation in hatching rate and male rate. I interpret this as the weak allele function of *him-17(e2806)* and factors in other pathways may influence *him-17* functions on X chromosome. I found some differences in male rates between single mutants and double mutants, indicating that *xnd-1* and *him-17* work together to regulate *him-5* to influence DSBs on X chromosome.

Overall, my studies suggest that there is a complex relationship between *him-17* and *xnd-1* and that the interaction between the two genes may depend strongly on which phenotype is being measured. This is expected because both genes have roles both in meiotic break formation and in other germ line functions including mitotic cell division. As individual differences in

characteristics were observed, future studies should count more worms to decrease variance. Further analysis of whether there are alternative pathways would be interesting.

Appendix A Materials Used for Polymerase Chain Reaction

Primers used in this project (all primers are written in the direction of 5'→ 3'):

1. xnd-1(ok709) knock out primers
1.
JY-JLY-717: CCAGATTTGAGTCGAATCGAACCAA

JY-JLY-722: CCACTGCAGTTTCTACTGGTTGCG

Expected sizes:

N2: no band

xnd-1(ok709): 700 base pairs

2. him-17(e2707) primers:

ZK270: reverse primer: ATTAGGCATATCGGCATAGA

ZK271: wild type forward primer:

GTTGCAAGTTGTTACTAGTTACCGAGTTGAACTTCCACGGTC

ZK272: mutant forward primer: GACCGAGTTGAACTTCCACGCAT

Expected sizes:

N2: 120 base pairs

him-17(e2707): 100 base pairs

3.him-17(e2806) primers
4.
JY-JY-152: CTCCGCCTCGTAATGCATGG

JY-JY-153: wild-type SNP; mismatch: T to C one base 5' to the terminal 3'G

CCTAGACGCGTACTAGTCGACTGGCCCTCTTGCAACCACG

JY-JY-154: mismatch A to G two bases 5’ of the terminal 3’ A

CTCGGCCCTCTTGCAACCGTA

N2: 195 base pairs

him-17(e2806): 175 base pairs

4. *xnd-1* wild type primers

CQC-3: Forward primer to *xnd-1* wt.

GCTCTGGAGATGACGAAAAACGCC

CQC-4: Reverse primer to *xnd-1* wt.

AGCCATCGATGCCTGATTAACTGAG

N2: 1100 base pairs

xnd-1(ok709): no band

1.5% agarose gel for xnd-1

1.1.5g agarose in 100 mL1xTAE in a 250 mL flask and microwave for 1 to 2 minutes
2.Cool agarose solution to about 50 °C
3.Add 2.5 mL ethidium bromide (EtBr) to agarose solution
4.Pour the agarose solution into a tray with well combs
5.Wait for 30 minutes at room temperature until the gel completely solidifies
6.Fill gel tray with 1xTAE until the gel is covered and add 4 mL ethidium bromide (EtBr) to 1xTAE solution

3% agarose gel for him-17(e2707) and him-17(e2806)

1.3g agarose in 100 mL1xTAE in a 250 mL flask and microwave for 2 to 3 minutes
2.Cool agarose solution to about 50 °C \
3.Add 2.5 mL ethidium bromide (EtBr) to agarose solution
4.Pour the agarose solution into a tray with well combs
5.Wait for 30 minutes at room temperature until the gel completely solidify
6.Fill gel tray with 1xTAE until the gel is covered and add 4 mL ethidium bromide (EtBr) to 1xTAE solution

Worm lysis before PCR

Worm lysis materials

•Proteinase K

•Worm Lysis Buffer

50 mM KCl

10 mM Tris-HCl

2.5 mM MgCl2

0.45% Nonidet P-40

0.45% Tween-20

0.01% gelatin

Worm lysates Procedure

1.Add 100 uL worm lysis buffer into a 1.5 mL Eppendorf tube
2.Add 0.5 uL proteinase K into worm lysis buffer and mix

3.Aliquot 12 ul worm lysis buffer to each PCR tube

4.Pick single worm (or multiple worms) into lysis buffer

5.Spin down to the bottom of each PCR tube

6.Run on PCR instrument with WORMLYS program

WORMLYS program for worm lysis

1.65℃ for 60 minutes

2.95℃ for 20 minutes

3.Cool to 4℃

DEL40 for him-17(e2707) and him-17(e2806):

1.95℃ for 2 minutes

2.95℃ for 15 seconds

3.55℃ for 30 seconds

4.72℃ for 1 minute

5.Repeat step 2 to 4 for 39 times

6.72℃ for 5 minutes

7.12℃ for ever

DEL40EXT for xnd-1(ok709) and *xnd-1* wt:

1.95℃ for 2 minutes

2.95℃ for 15 seconds

3.55℃ for 30 seconds

4.72℃ for 2 minute

5.Repeat step 2 to 4 for 39 times

6.72℃ for 5 minutes

7.10℃ for 10 seconds

Appendix B Detailed Protocols Used in the Project

C. elegans Maintenance Protocol

Nematode Growth Medium (NGM) agar Preparation

Materials:

1. Worm medium base (HIMDIA)
2. OP50: Escherichia coli (E. coli)
3. OP50 liquid culture to grow and store OP50
4. Small petri plates (35 mm diameter) and large petri plates (60 mm diameter).

Prepare OP50 liquid culture

1. 48g Tryptone, 30g Yeast Extract and 15g Sodium Chloride in to a 5-liter flask
2. Add deionized water to 3 liters
3. Add a stir bar and dissolve at room temperature
4. Aliquot to small jars (~100 mL) and autoclave with Liq60 for about 1.5 hours
5. Cool to room temperature, tight the lid and store at room temperature.
6. Pick a colony of OP50 from a LB agar plate and dip into OP50 liquid culture
7. Cap and loose the lid to allow the bacteria to grow overnight at 37 ℃
8. Tight the lid and store liquid culture with OP50 at 4℃ready for use

Preparation and seeding of NGM plates:

1. A bottle of worm medium base (HIMDIA) into a 5-liter flask.
2. Add 3 liters of deionized water into the flask
3. Add 3ml 5 mg/mL Cholesterol in Ethanol into the medium base solution
4. Add a stir bar into the medium base solution and swirl to mix well at room temperature.
5. Autoclave the medium base solution with Liq60 program for about 1.5 hours
6. Cool autoclaved medium base solution at about 55 ℃ with a stir bar.
7. Dispense solution into small (35 mm diameter) and large (60 mm diameter) petri plates, fill plates 2/3 full of agar
8. Cool the plates for 3 days at room temperature
9. Add 3 drops of OP50 to large petri plates or 1 drop of OP 50 liquid

culture to small petri plates and spread to create a lawn for *C. elegans* maintenance

10. Add 1 drop OP50 liquid culture in the center of small petri plates and do not spread to make *C. elegans* cross plates
11. Allow OP50 to grow at room temperature for 2 or 3 days and store the seeded plates at 4 ℃

Recovery of C. elegans stocks

1. Leave a vial of frozen *C. elegans* at room temperature until all ice turn into liquid
2. Pour the liquid onto a large seeded plate
3. Transfer 10 to 15 worms individually to separate small seeded plates after 2 to 3 days
   Allow worms to grow and reproduce

C. elegans **Crosses Protocol**

C.elegans outcross *for* $\frac{xnd-1(ok709)}{qC1}$; $him-17(e2707)$ *and xnd-1(ok709); him-17(e2707)*

1. Pick 3 L4 *him-17(e2707)* males and one L4 $\frac{xnd-1(ok709)}{qC1}$ hermaphrodite on a cross plate, to increase cross success rate, create 3 to 5 cross plates

2. Observe the rate of male progenies on the plates after 3 to 4 days, if many male progenies (more than 1% male) are observed, that indicates a success cross.

3. Pick 3 L4 $\frac{xnd-1(ok709)}{+}$; $\frac{him-17(e2707)}{+}$ (non-roller) males and one L4 $\frac{hT2}{qC1}$ hermaphrodite on a cross plate, to increase cross success rate, create 3 to 5 cross plates

4. Pick at least 24 L4 $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17(e2707)}{+}$ (non-green rollers) to hermaphrodites individually to separate small seeded plates
5. Genotype every hermaphrodite after they lay over 50 eggs

6. Keep $\frac{xnd-1(ok709)}{qC1}$; $him-17(e2707)$ (non-green rollers) as stock and *xnd-1(ok709); him-17(e2707)* for experiment

C.elegans outcross for $\frac{xnd-1(ok709)}{qC1}$; $him-17(e2806)$ *and xnd-1(ok709); him-17(e2806)*

1. Pick 3 L4 *him-17(e2806)* males and one L4 $\frac{xnd-1(ok709)}{qC1}$ hermaphrodite on a cross plate, to increase cross success rate, create 3 to 5 cross plates

2. Observe the rate of male progenies on the plates after 3 to 4 days, if many male progenies (more than 1% male) are observed, that indicates a success cross.

3. Pick 3 L4 $\frac{xnd-1(ok709)}{+}$; $\frac{him-17(e2806)}{+}$ (non-roller) males and one L4 $\frac{hT2}{qC1}$ hermaphrodite on a cross plate, to increase cross success rate, create 3 to 5 cross plates

4. Pick at least 24 L4 $\frac{xnd-1(ok709)}{qC1}$; $\frac{him-17(e2806)}{+}$ (non-green rollers) to hermaphrodites individually to separate small seeded plates

5. Genotype every hermaphrodite after they lay over 50 eggs

6. Keep $\frac{xnd-1(ok709)}{qC1}$; $him-17(e2806)$ (non-green rollers) as stock and *xnd-1(ok709); him-17(e2806)* for experiment

C. elegans Counts Protocol

In order to calculate clutch size, brood size, hatching rate and male rate, numbers of eggs, progenies and males were counted for every single worm.

1. At 0 hour: place a single L4 hermaphrodite worm(mother) on a small seeded plate at 20 ℃
2. After every 18 to 20 hours: transfer the mother to a fresh small seeded plate and count numbers of eggs left on the old plate until no eggs are laid or the mother are arrested. Always place plates at 20 ℃
3. Count hatched worms and category their sexes to hermaphrodite or male 3 or 4 days after eggs are laid
4. If necessary, genotype the mother after all eggs are laid

C. elegans **Storage Protocol**

Freezing buffer:

1X worm freezing buffer	1L
NaCl	20 ml of 5 Molar
KH2PO4 anhydrous	6.8 gm
Glycerol	150 ml
1xM9 media	500 ml
10N NaOH	560 µl
1M MgSO4	600 µl
dH2O	to make 1L

Sterilize by 0.2-micron filter and store at 4 C

Freeze and store C. elegans

1. Pick 10 L4 hermaphrodites on a large NGM plate
2. Starve worms starve for 5 to 7 days
3. Add 1ml of freezing buffer and wash the plate to cryotubes
4. Leave the plate at -80℃ for 48 hours
5. Put the cryotubes in Liquid Nitrogen

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