To the Graduate Council:

I am submitting herewith a dissertation written by Wendy Bradberry Stapleton entitled "Identification and Characterization of Genes Potentially Involved in Meiosis in *Drosophila melanogaster* Males." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biochemistry and Cellular and Molecular Biology.

Bruce D. McKee, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of the Graduate School

IDENTIFICATION AND CHARACTERIZATION OF GENES POTENTIALLY INVOLVED IN MEIOSIS IN *DROSOPHILA MELANOGASTER* MALES

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Wendy Bradberry Stapleton

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ABSTRACT

The process of meiosis is an essential mechanism in sexually reproducing organisms that allows the reduction of the genome size in gametes so that formation of a zygote from an egg and sperm leads to progeny with the proper chromosome complement. Although studies of the meiotic process have allowed determination of the steps that occur to reduce the genome complement, identification of all the gene products involved is not yet complete. Important cellular pathways are often highly conserved during evolution. Therefore, it is helpful to gain an understanding of complicated processes by studying simple model organisms.

Drosophila melanogaster is an ideal system for the genetic study of processes such as meiosis. A few of the many attributes of this organism are its well defined genetics, small genome size, and short generation time. Although numerous screens to obtain meiotic mutants have been performed over the last twenty-five years, there are still many gaps in our knowledge about gene products that function in the various meiotic pathways. Therefore, these studies represent additional attempts to identify factors involved in *Drosophila* meiosis. Although initial goals were to identify genes that function specifically in male meiosis, the nature of some of the factors obtained may lead into a study of the meiotic process in general, involving both male and female pathways.

Our search for *Drosophila* meiotic mutations has identified four genes that potentially act in meiosis. *homeless* is a gene initially identified by its female sterile

phenotype and its role in oogenesis has been characterized. This study reports the finding that Homeless is also important in spermatogenesis. Phenotypic characteristics of mutant flies resemble those previously characterized in *Suppressor of Stellate* (*Su(Ste)*$^-$) and XO males, suggesting that at least one of its meiotic functions is to repress the testis specific *Stellate* locus.

An additional factor, identified by observation of abnormal sex chromosome segregation in a deficiency stock, appears to affect both male and female meiosis. *nondisjunction in males and females (nmf)* causes chromosome nondisjunction in both sexes. The phenotypic characteristics of the mutation suggest the gene may be normally involved in segregation of achiasmate chromosomes in the male and female meiotic process. However, studies have not yet confirmed that both the male and female phenotypes are due to the same gene locus.

Gene products acting in cellular processes in an organism can often be obtained by identifying homologs of proteins known to function in that process in another organism. Homologs of proteins involved in mismatch repair in bacteria have been shown to have additional meiotic functions in yeast, mice, and potentially humans. We have identified two *Drosophila* homologs of the bacterial mismatch repair genes *mutL*, *hexB*, and yeast *Pms1*. Initial characterization supports meiotic roles for *dmlh1* and *dpms2*, but identification and study of mutations in these genes is necessary to confirm participation of their products in meiosis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NONDISJUNCTION IN MALES AND FEMALES (NMF): A FACTOR INVOLVED IN PROPER CHROMOSOME SEGREGATION DURING THE FIRST MEIOTIC DIVISION IN *DROSOPHILA MELANOGASTER*

PART I

General Introduction

CHAPTER I

MEIOSIS IN *DROSOPHILA MELANOGASTER MALES*

The purpose of the studies outlined in this dissertation was to identify and characterize meiotic mutations in *Drosophila melanogaster*. The focus was on defects in meiotic pairing and segregation during meiosis I. The specific aims of this work were as follows:

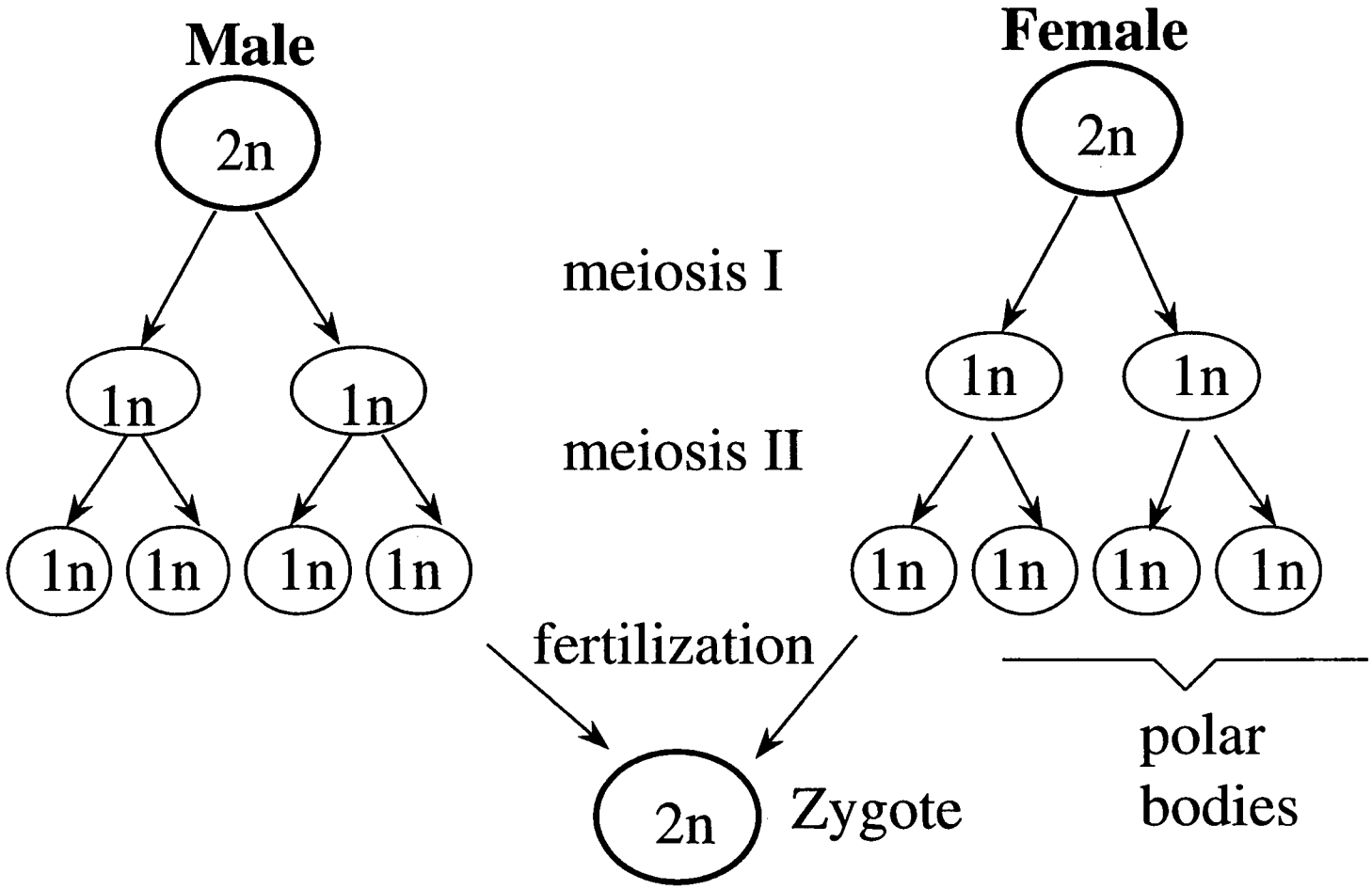
- To determine the function of *Homeless* in male meiosis.
- To characterize a newly identified male and female meiotic factor (*nmf*) located on the X chromosome.
- To identify and begin molecular characterization of *Drosophila melanogaster* homologs of the *E. coli mutL* gene in order to determine potential function in meiosis.

The general introduction will provide background information on what was known concerning meiosis in *Drosophila* before these studies.

General features of the meiotic process

Meiosis is a process in sexually reproducing organisms which is necessary to produce gametes that represent half the somatic genome complement in order to ensure that upon fertilization a zygote with an equal genome size to that of the parent will be produced. Figure 1 illustrates this process in an organism with a diploid genome complement. The meiotic process allows the production of haploid gametes in the

Fig 1 Illustration of the reduction in genome size from diploid (2n) to haploid (1n) during the process of meiosis. Adapted from Zubay, Geoffrey. Genetics. Mento Park, California; The Benjamin/Cummings Publishing Company, Inc. , 1987.


Male
2n
meiosis I
1n
1n
meiosis II
1n
1n
1n
1n
Female
2n
1n
1n
1n
1n
1n
1n
fertilization
2n
Zygote
polar
bodies

male and female organism which upon fertilization yields a diploid zygote. The production of gametes in higher eukaryotes is referred to as gametogenesis.

Meiosis involves a number of events which are separated into several stages. Two divisions occur in meiosis, with the first being the reductional division and the second being an equational division very similar to mitosis. Each of these divisions can be divided into four stages: Prophase, Metaphase, Anaphase, and Telophase. The stages of meiosis I are illustrated in Figure 2 and meiosis II in Figure 3. Briefly, during prophase chromosomes condense and undergo a number of events in preparation for the segregation of chromosomes. Metaphase marks a point in the process when chromosomes are aligned along a metaphase plate; during anaphase they begin separation from one another. New nuclear membranes form as a contractile ring divides the cell into two daughter cells during telophase.

During prophase I a number of events occur which make meiosis I unique from both mitosis and meiosis II. These events are a special focus in the studies that follow. The most obvious unique feature of meiosis I is that division results in daughter cells carrying only half of the genome complement of the parental cell. This is due to a mechanism by which homologous chromosomes pair and separate from one another. Homologs in a diploid organism are comprised of two copies of each chromosome, are originating from each of the two parents. These chromosomes contain the same genes but may differ in the alleles present. Normally, during prophase I, these homologs pair

Fig 2 The stages of the first meiotic division. The illustrated cell contains a total of four chromosomes at the beginning of meiosis.

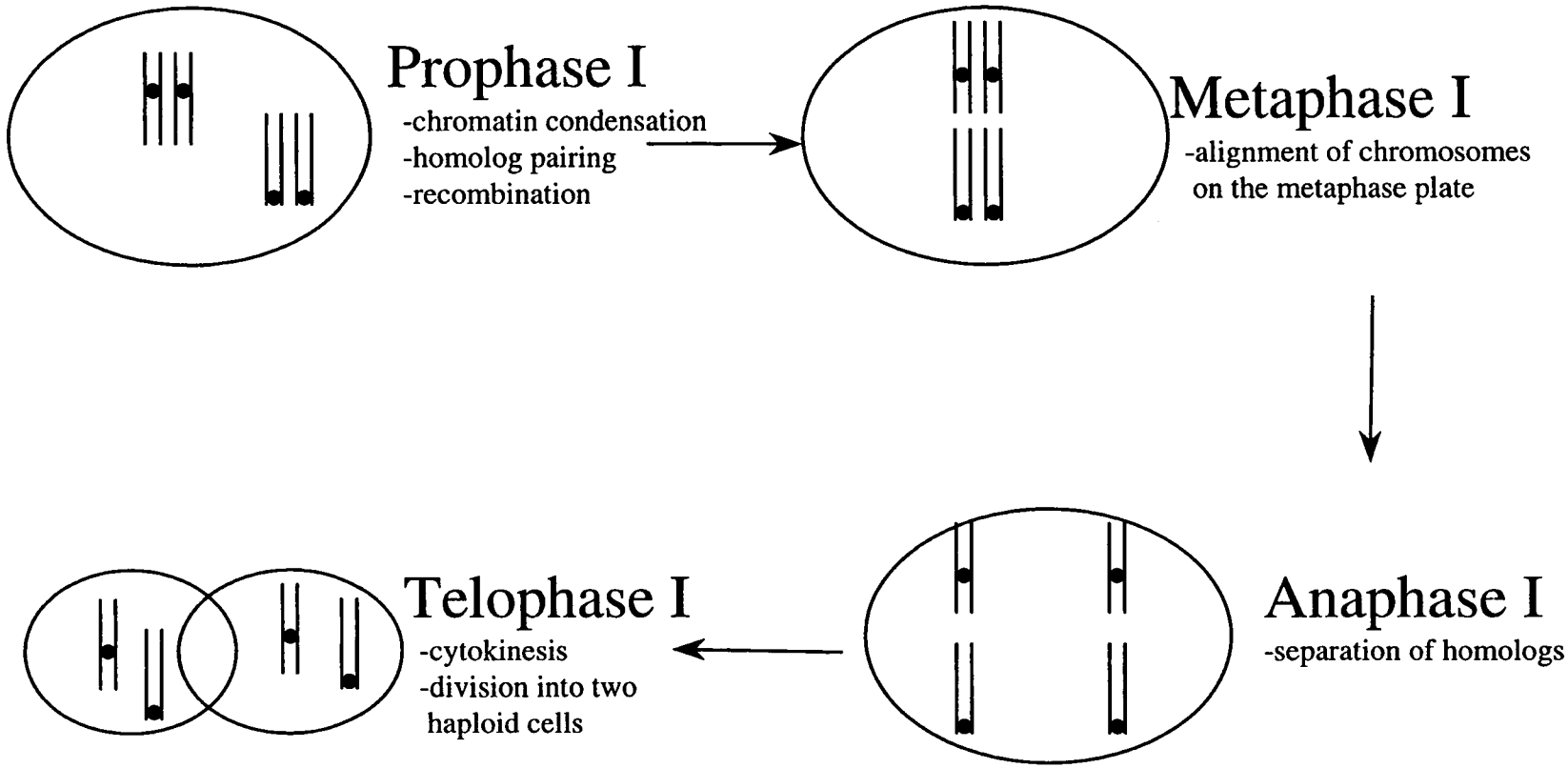
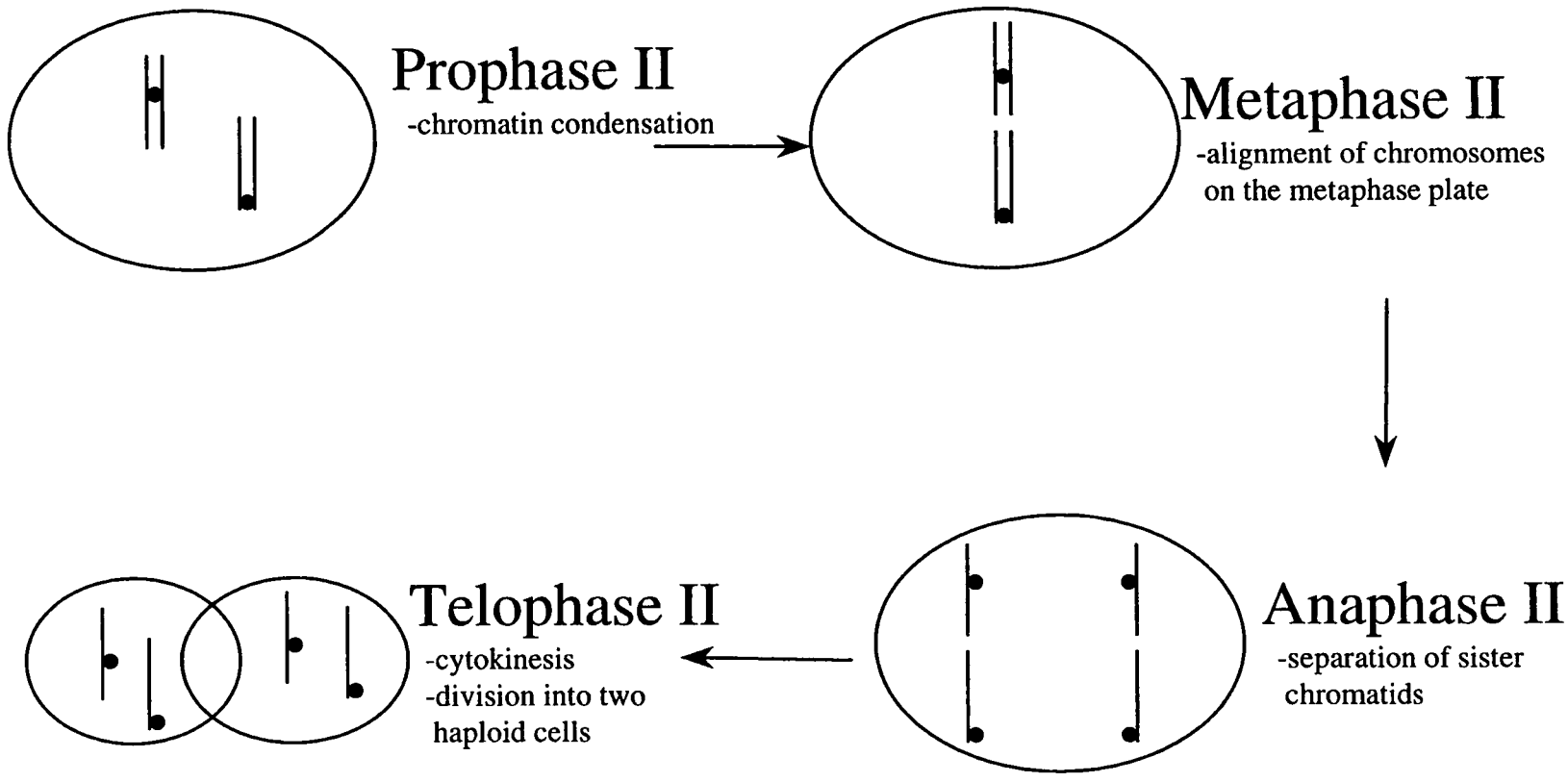

Prophase I
-chromatin condensation
-homolog pairing
-recombination
Metaphase I
-alignment of chromosomes
on the metaphase plate
Anaphase I
-separation of homologs
Telophase I
-cytokinesis
-division into two
haploid cells

Fig 3 The stages of the second meiotic division. The illustration begins with a single cell from the end of Fig 2 that has a total of two chromosomes after the first meiotic division.


Prophase II
-chromatin condensation
Metaphase II
-alignment of chromosomes on the metaphase plate
Anaphase II
-separation of sister chromatids
Telophase II
-cytokinesis
-division into two haploid cells

with each other and a structure unique to this stage, the synaptonemal complex (SC), forms between the two homologs. The synaptonemal complex is a proteinacious structure that is made of two axial elements and a central element located between the chromosome pair. Synapsis is a term used to describe the process by which homologs are held closely together with the SC between them. Prophase I is also the time at which high levels of genetic exchange occur. Exchange is necessary for both proper chromosome segregation and genetic diversity of the organism. Although recombination can occur in mitosis, it differs greatly in frequency from that deleted during the first meiotic division. The quantity and location of exchanges in meiosis are regulated so that in the majority of nuclei each chromosome pair has undergone at least one cross-over event. In contrast, mitotic nuclei generally contain no more than one pair that has undergone exchange and the majority have no exchanges at all (Kucherlapati and Smith 1988). The physical manifestations of crossing over are termed chiasmata, which are thought to be responsible for maintaining homolog alignment and pairing after dissolution of the SC. Conflicting evidence makes it unclear whether synapsis precedes genetic recombination or vice versa. Much of the conflict may reflect differences among species in timing of synapsis and recombination or in requirements for specific gene products. *S. cerevisiae* cells mutant for *spo11* are unable to recombine, and do not form synaptonemal complex, suggesting either a dual role for these gene products in recombination and synaptonemal complex formation or the requirement for a recombination event to initiate synapsis (Klapholz et al. 1985;

Giroux et al. 1989; Roeder 1997). On the other hand, *Drosophila* females homozygous for *mei-W68*, a gene which is a *spo11* homolog, form normal synaptonemal complex even in the absence of recombination (McKim et al. 1998). There is also the possibility that recombination and synapsis events are concurrent, each dependent on the other for efficient results. Regardless of the order, these two events are unique features of meiosis I that seem to facilitate the proper reduction of the genome size by segregation of homologs from one another.

Although there is little direct evidence, the remaining stages of meiosis I are thought to occur as in mitosis, with the exception that homologs rather than sister chromatids are segregated. The ability of homologs to segregate from each other is intrinsic to the chromosome rather than to the spindle since when a bivalent is placed on a meiosis II spindle, homologous chromosomes, not chromatids, will segregate as they would in meiosis I (Nicklas 1977). Meiosis II proceeds similarly to a mitotic division, with sister chromatids being separated from one another (Figure 3).

Problems associated with meiotic nondisjunction

As with any cellular process, the events of meiosis do not always occur without mistakes. Abnormal meiotic divisions can lead to consequence ranging from meiotic arrest to production of aneuploid progeny that are often inviable. Aneuploidy is the term used to describe a cell(s) that has more or less than the complete set of chromosomes. Chromosome loss or nondisjunction during the process of gametogenesis can lead to aneuploid gametes and potentially to the production of an

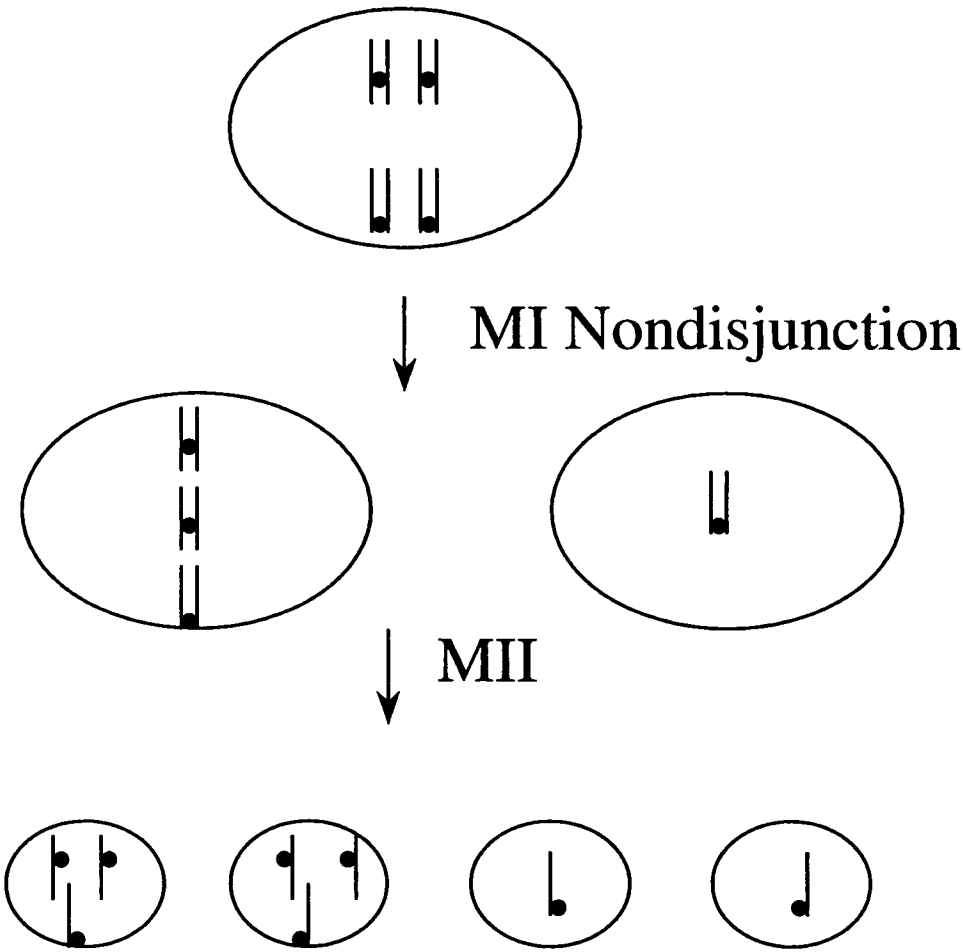
aneuploid zygote. The study of meiotic abnormalities is important clinically because of human medical conditions caused by aneuploidy. Mental retardation, sterility, and spontaneous abortions are commonly consequence of aneuploidy. Trisomy 21 or Down syndrome is a well-known example of aneuploidy, characterized by mental retardation and caused by nondisjunction of autosome 21 during meiosis. Human males with an extra X chromosome, XXY, suffer from infertility.

Figure 4 illustrates the consequences of a meiosis I nondisjunction event. Homologs fail to separate properly and the gametes produced are aneuploid, either carrying an extra chromosome or lacking a single chromosome. A zygote formed from such a gamete will be aneuploid and the severity of phenotype will depend on which chromosome is lost or gained.

Numerous kinds of defects can lead to homolog nondisjunction. Failure of homologs to pair or recombine, to form a synaptonemal complex, or to form and maintain chiasmata may increase the incidence of nondisjunction. A large number of proteins are associated with each of these processes and are necessary for proper function. Additionally, meiotic segregation requires the formation and function of complex spindle apparatus. Mutation in any one of these proteins could lead to a nondisjunction phenotype.

A phenomenon associated with some cases of chromosome nondisjunction is distorted recovery of sperm classes in a genotype-specific manner. This

Fig 4 Illustration of a chromosome nondisjunction event occurring in the first meiotic division. Prior to division, the cell has a total chromosome number of four. After a homolog nondisjoins in the first division, two secondary gametocytes are produced, one containing n+1 and the other containing n-1 chromosomes. A normal second division yields four aneuploid gametes; two gametes contain n+1 and two contain n-1.


MI Nondisjunction
MII
4 aneuploid gametes

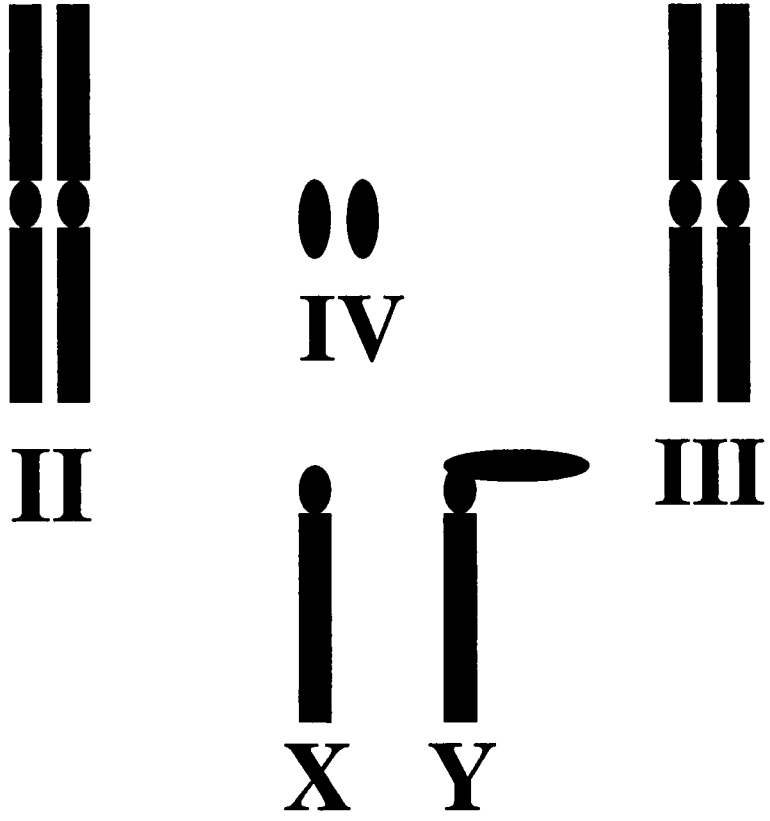
phenomenon is referred to as meiotic drive and is observed in *Drosophila* males deficient for XY pairing sites or deficient for the *Su(Ste)*$^{-}$ locus on the Y chromosome. In the case of males lacking the sex chromosomal pairing sites, an excess of progeny are recovered that carry the paternal X chromosome (with corresponding deficiency of those with the Y chromosome) and an excess of progeny from nullo-XY vs. XY sperm (Gershenson 1933; Sandler and Braver 1954; Peacock 1965; McKee 1984; McKee and Lindsley 1987). The same distortions are observed in *Su(Ste)*$^{-}$ males (Hardy et al. 1984; Hurst 1992; Hurst 1996). The cause of meiotic drive is unknown but there is a correlation between the amount of chromosome nondisjunction and the severity of drive.

Studies of spermatogenesis in *Drosophila melanogaster*

The fruitfly *Drosophila melanogaster* is a useful organism for the study of the meiotic process during spermatogenesis. Isolation of gene loci, molecular characterization, and cytological studies are all easily accomplished in this organism. The small size of the *Drosophila* chromosomal complement, only 4 pairs of chromosomes, is advantageous. The heterochromatic nature of the Y chromosome and the small size of the 4th autosomes make mapping of potential factors simple, with genes being most likely to be located on the X, 2nd, or 3rd chromosomes. Figure 5 shows the *Drosophila* chromosomal complement and illustrates how small the 4th chromosome is compared with the X and other two autosomes.

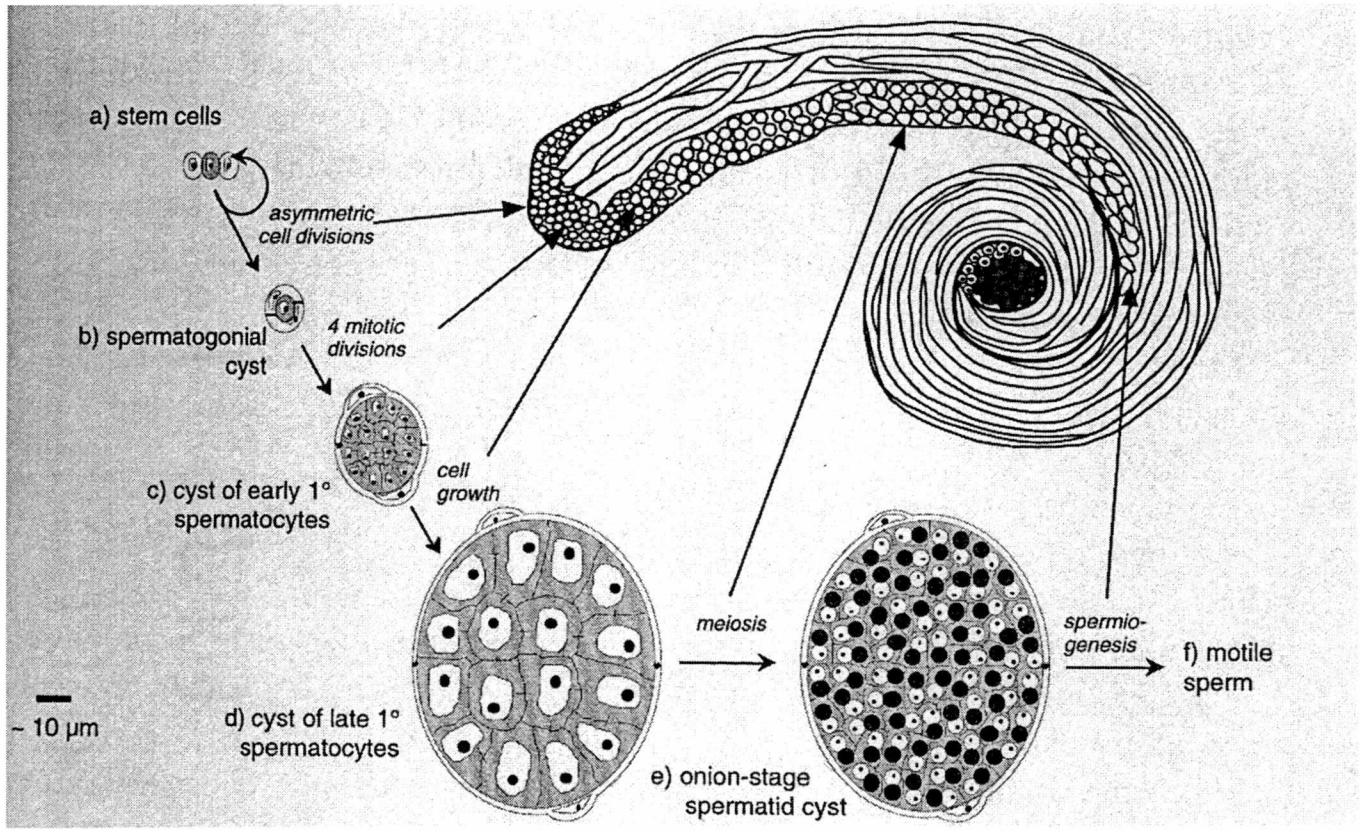
Fig 5 The chromosomal complement of *Drosophila melanogaster* males.

Drosophila melanogaster
Chromosome Complement



Since, the process of spermatogenesis is thought to be well conserved (Tokuyasu et al. 1972a and 1972b), findings in *Drosophila* might shed light on spermatogenic mechanisms in higher eukaryotes, such as mice and humans. An illustration of the events of spermatogenesis in *Drosophila melanogaster* males is shown in Figure 6. This process takes place in the tubules of the testes. The first event is an asymmetric division of a germ line stem cell to produce a blast cell, a spermatogonium, and a stem cell. Progenitor somatic cyst cells produce cells which surround the spermatogonium. Four mitotic divisions of the spermatogonium give rise to a cyst of sixteen primary spermatocytes. These cells undergo a premeiotic S phase DNA synthesis, followed by a growth phase during which high levels of transcription occur and the cell volume is increased by 25-fold. Within these primary spermatocytes chromosomes pair during meiotic prophase. However, unlike the typical prophase I described previously, there is no synaptonemal complex or recombination during meiosis I of *Drosophila melanogaster* males. In this anomaly lies the true advantage of studying chromosome pairing and segregation in this specific organism. In a typical meiosis, it can be difficult to distinguish among proteins involved in pairing, synapsis and recombination due to the overlapping nature of the mechanisms. However, in *Drosophila* males, pairing and segregation can be studied in the absence of synapsis and recombination.

Fig 6 Illustration of the process of spermatogenesis in *Drosophila melanogaster*. Illustration obtained from Maines and Wasserman 1998.


a) stem cells
asymmetric cell divisions
b) spermatogonial cyst
4 mitotic divisions
c) cyst of early 1° spermatocytes
cell growth
d) cyst of late 1° spermatocytes
meiosis
e) onion-stage spermatid cyst
spermio-genesis
f) motile sperm
~ 10 µm

The meiotic divisions result in a cyst with 64 cytoplasmically interconnected spermatids. These undergo the complex process of differentiation to yield mature haploid spermatids.

Aspects of *Drosophila* male and female meiosis

Analysis of mutations can indicate common mechanisms between male and female mitosis and meiosis II. However, there appears to be a divergence at some point during meiosis I. The major differences, as mentioned earlier, are the absence of synaptonemal complexes and recombination in males. Synaptonemal complexes (SC), a proteinacious structure between paired homologs, are thought to be important for proper homolog separation during the first division. Chiasmata, the physical manifestation of crossing-over, are important for holding homologs in close apposition following dissolution of the SC until the proper time to disjoin. Without these two key features, males must adopt some other primary mechanism to assure proper homolog disjunction. In order to dissect the pathways responsible for pairing and disjunction in males, it is necessary to study mutants that are affected at various steps in the meiotic process.

A secondary system in females operates to segregate achiasmate homologs and heterologs. It is similar to the male system in that it is responsible for the segregation of chromosomes that do not form chiasmata. The distributive system was first described to explain how noncrossover bivalents pair and segregate from one another (Grell 1962). The 4^{th} chromosome is an obligate member of the system since it is

always achiasmate. Other common members of the pool are achiasmate X chromosomes, free duplications and compound chromosomes. Grell's (1964) conclusion that the distributive mechanism of segregation was size-dependent was based on her studies of nonhomologs showing that those closest in size tended to segregate from one another under competitive circumstances. This was proposed to be completely independent of homology. However, the fact that small free X duplications are more likely to segregate from the X even though they are much closer in size to the 4^{th} autosome suggested that some dependence on homology existed within the distributive system. These observations led Whyte et al. (1993) to propose two mechanisms acting in the distributive system; one responsible for segregating achiasmate homologs and a size-dependent pathway for the separation of heterologs. This hypothesis has been strengthened by the finding of multiple mutations, which will be described, that are specific in the pathway they affect. The homology-dependent mechanism is believed to involve pairing associations in heterochromatic regions (Hawley and Theurkauf 1993). Studies involving a minichromosome (Dp1187) strengthened this argument since deletions within the heterochromatin region greatly affect the ability of two homologous minichromosomes to disjoin (Karpen et al. 1996). Interestingly, while Dp1187 contained the information necessary for proper disjunction in females, it segregated randomly in males, suggesting that the distributive system in females and achiasmate segregation in males are not the same (Karpen et al. 1996). Although one would postulate that proteins active in the female distributive system

would also function to segregate achiasmate chromosomes in males, none of the described mutations shown to affect distributive disjunction have any effect on segregation in males.

Although chromosome pairing occurs in both males and females during meiosis I, these pathways also differ between sexes in *Drosophila*. Autosomal pairing in both males and females appears to involve euchromatic regions (for review see McKee 1998). However, females have two distinct pairing mechanisms; one involving euchromatic regions for pairing of chiasmate bivalents and one heterochromatin-dependent pathway for alignment of achiasmate homologs (Hawley et al. 1993; Dernburg et al. 1996). Since the distributive system of females is more similar to male segregation with the absence of chiasmata and synaptonemal complex, one would suppose that pairing in males might parallel that seen for the female distributive system. However, autosomal pairing in males is restricted to euchromatic regions (Yamamoto and Miklos 1977; Yamamoto 1979; Hilliker et al. 1982, McKee et al. 1993). Although pairing sites for the sex chromosomes in males are located in heterochromatin, they also differ from that in females. Male pairing is dependent on the 240 bp IGS repeats within rDNA which are common to both the X and Y chromosomes (Merrill et al. 1992; McKee et al. 1992). In contrast, the rDNA region does not act in female sex chromosome pairing (Hawley 1988). Instead, the site of pairing action appears to be located in the region surrounding the X centromere (Karpen et al. 1996).

Meiotic Mutations

With only seven exceptions thus far, all meiotic mutations of *Drosophila melanogaster* are specific to either male or female meiosis. Two of the exceptions that affect both sexes, *mei-S332* (Kerrebrock et al. 1992; Bickel et al. 1998) and *ord* (Mason 1976; Miyazaki 1992; Bickel et al. 1996), are defective in sister chromatid cohesion. The mutation *equational producer (eq)* (Schultz 1934), an X-linked gene, causes nondisjunction of the X chromosome at the 2nd division in males. Heterozygous females have some nondisjunction, but the division at which this occurs is unclear. *Horka* (Szabad et al. 1995) was originally isolated as a dominant female-sterile mutation with severe meiotic abnormalities, but also causes nondisjunction at the second division during spermatogenesis (Szabad et al. 1995). With the described mutations, nondisjunction is seen primarily at the equational division. A 2nd chromosome recessive mutation, *mei-G87*, causes nondisjunction at both the first and second division of meiosis, but the effect is specific to only the 2nd autosome (Gethmann 1984). Although the nondisjunction occurs during both divisions, the defect appears to be in sister chromatid cohesion. Thus, rather than a defect in homolog separation per se, precocious sister chromatid separation causes nondisjunction during the reductional division (Gethmann 1984). *mei-I3* causes nondisjunction of all chromosomes during the first division (Ivy 1981). However, in *mei-I3* mutants, the sex chromosomes also nondisjoin during the equational division

(Ivy 1981). Only one mutation has been described that appears to affect both male and female meiosis predominantly at the first division. The *Double or nothing (Dub)* dominant mutation on the second chromosome, causes nondisjunction of all chromosomes in both males and females predominantly during the first division (Moore et al. 1994). Thus, *Dub* may be only one of several genes that function in both male and female meiosis I, suggesting that some mechanisms of the first meiotic division might be shared.

Large scale screens for mutations affecting *Drosophila melanogaster* meiosis done by Sandler et al. 1968 and Baker and Carpenter 1972 have provided much of the material used for the last twenty years to study *Drosophila* meiosis. The Sandler et al. screen identified nine female-specific mutations, two male-specific mutations, and one mutation which showed effects in both males and females. These mutations were recovered from naturally occurring populations. On the other hand, Baker and Carpenter screened EMS-treated X chromosome lines and discovered twenty lines showing elevated X-Y nondisjunction in males and eleven which had increased X-X nondisjunction in females. Extensive analysis of the male mutants could not be performed due to a significant decrease in phenotype after a six month period. A more recent screen by Castrillon et al. (1993) revealed 83 P-element insertional mutations which cause complete or partial male sterility. Of these 83, only two appeared to affect the meiotic process. The mutations *double fault* and *bobble* give a phenotype of variable sized nuclei in spermatids suggestive of chromosome segregation abnormalities

during meiosis. These recovery rates highlight the difficulty in obtaining and characterizing male meiotic mutations. Therefore, study of available mutants is important to dissect the meiotic mechanisms of male pairing and segregation.

The two male specific meiotic mutations discovered in the Sandler (1968) screen showed very different phenotypes. mei-S8, a recessive on chromosome 2, showed a high level of 4^{th} chromosome nondisjunction, while having no effect on disjunction of the sex chromosomes. On the other hand, mei-081, a recessive on chromosome 3, altered the disjunction of all chromosomes, causing a genome wide increase in nondisjunction. These phenotypes indicate two separate mechanisms for chromosome segregation, one of which is chromosome specific and one of which has a more global impact. A later EMS screen performed by Sandler (1971) revealed another male-specific meiotic mutation, *mei-W5*, which was studied further and renamed *paternal loss (pal)* by Baker 1975. *pal* causes chromosome loss in homozygous males but no nondisjunction. The EMS-induced male meiotic mutations identified by Baker and Carpenter all showed similar phenotypes with frequent nondisjunction of the sex chromosomes, but no effect on the 4^{th} chromosome pair, further supporting the idea of chromosome-specific components of segregation in males. They also show an excess recovery of nullo-XY sperm as compared to XY sperm suggesting meiotic drive. Nineteen of these twenty mutations show no effect on the disjunction of sex chromosomes in females. mei-99, which showed aberrant

phenotypes in both males and females, may actually be due to two separate mutations present on the EMS-treated X chromosome.

Later screens by Gethmann (1974) and Ivy (1981) allowed identification of two additional male specific meiotic mutations. A recessive mutation on chromosome 2, *mei-G17*, affects nondisjunction frequencies in a chromosome-specific manner and causes meiotic drive with progeny showing excess XX females over XY males and XO males in excess of XXY females (Gethmann 1974). Homozygous males show increased levels of sex and second chromosome nondisjunction with no effect on the 3rd or 4th autosomes (Gethmann 1974). The nondisjunction is specific to the first meiotic division (Gethmann 1974). A recessive mutation located on the 3rd autosome, *mei-I1*, causes a genome-wide effect on chromosome segregation, with all chromosomes showing increased levels of nondisjunction (Ivy 1981). Nondisjunction in these males also occurs primarily during the first division. The defect appears to be in pairing since a high number of univalents are observed in primary spermatocytes (Ivy 1981). Also the male-specific mutations described in the two previous paragraphs, except *pal*, have been lost.

Two other loci have been shown to have male-specific effects. *Stellate* is located on the X chromosome and is composed of tandemly repeated copies of a sequence homologous to Casein Kinase II (CKII) (Livak 1984; Livak 1990). Expression of *Stellate* is normally repressed so that very little of the properly spliced mRNA is observed in XY males. However, in the absence of the Y chromosome, high

levels of the testis-specific *Stellate* transcript are present (Livak 1984). A deletion of the *Su(Ste)* (Livak 1990) or *crystal* (Pimpinelli et al. 1986) locus from the Y chromosome yields the same effect, implicating *Su(Ste)* in the mechanism of *Stellate* regulation. The *Su(Ste)* locus is also repetitive, and the repeat is highly homologous to the *Ste* repeat. Each *Su(Ste)* repeat consists of three regions: a *Stellate* homology region, a Y-specific fragment, and a mobile element that resides within the *Stellate* homology region (Kalmykova et al. 1998). Derepression of *Stellate* results in a number of meiotic abnormalities, including chromosome nondisjunction, chromatin bridging and fragmentation, abnormal chromatin condensation and meiotic drive (Lifschytz and Hareven 1977; Hardy et al. 1984; Palumbo et al. 1994). The severity of the defects is dependent on the number of tandem repeats present on the X chromosome (Palumbo et al. 1994; Hurst 1996). Lower copy number, referred to as a Ste^{+} allele, results in the phenotypes mentioned. The presence of a *Ste* allele results in higher amounts of the protein upon derepression and thus more severe defects. Males carrying a *Ste* allele and a deficiency for *Su(Ste)* are sterile; this is thought to be due to more severe defects in chromatin condensation and segregation (Palumbo et al. 1994). Furthermore, a cytological phenotype is observed in XO or $Su(Ste)^{-}$ males. Star-shaped crystals are visible in primary spermatocytes and spermatids in the presence of a *Ste* allele, while needle-shaped crystals manifest in the presence of the low copy number allele (Meyer et al. 1961; Livak 1984; Hardy et al. 1984; Palumbo et al. 1994). The crystals are composed of the Stellate protein (Bozetti et al. 1995).

The appearance of Stellate crystals in spermatocytes is a unique phenotype and has allowed the identification of other loci in *Drosophila* which apparently are also part of *Stellate* regulation. In a screen for male-sterile P element insertions, four loci were obtained that caused the appearance in primary spermatocytes of crystals that very closely resemble those seen in XO and *Su(Ste)*$^{-}$ males (Bozzetti et al. 1997). Two of these loci, *Stellate-interacting gene (sting)* and *scratch*, have been cloned and sequenced (Schmidt et al. 1999; Yue et al. 1999). Similar meiotic phenotypes to those seen due to *Stellate* derepression are also observed, specifically chromosome nondisjunction, meiotic drive and an inverse correlation between fertility and *Stellate* copy number in *sting* mutants (Schmidt 1999). The function of the gene product of *sting* is unknown, but *scratch* encodes a Hsp90 homolog which is important for spermatogenic processes that require microtubule interactions (Yue et al. 1999). Abnormal meiotic phenotypes include morphological alterations of nuclei and nebenkerns and spermatids that are abnormal in shape The Hsp90 protein appears to be required for proper microtubule dynamics (Yue et al. 1999).

Thus, several gene products necessary for *Stellate* regulation have been identified, but how they function to maintain *Stellate* repression is unknown. Many possibilities exist. Early supposition was that *Su(Ste)* controlled *Stellate* expression via an antisense-sense RNA interaction. Since *Stellate* and *Su(Ste)* are highly homologous, it was suggested that an antisense RNA copy was transcribed from the *Su(Ste)* locus which interacted with the sense strand of *Stellate* and thus prevented translation into

protein. However, evidence from sequencing cDNA copies of the *Su(Ste)* transcript suggest that the direction of transcription is the same for *Su(Ste)* and *Stellate* argues against such a mechanism (Kalmykova et al. 1998). Alternatively, competition for transcription or mRNA processing factors has been proposed to be a potential silencing mechanism. Both sequences contain introns which require splicing and *Su(Ste)* is known to affect both splicing and transcription of *Stellate,* making this competition a possibility (Livak 1990). Further supporting this idea is the observation that moderate levels of properly spliced *Stellate* RNA are observed when the X contains a *Ste* allele even in the presence of wild-type *Su(Ste)*(Livak 1990). However, although *Su(Ste)* mRNA has been identified in cDNA libraries by Kalmykova et al. (1998), attempts to visualize this mRNA using Northern blotting techniques has thus far been unsuccessful suggesting very low levels of *Su(Ste)* mRNA within the cell. This does not rule out the competition proposal, since the mRNA could be very unstable and, therefore, below the detection level of northern blots. Numerous other potential mechanisms can be discussed but further analysis is necessary to narrow the options. Hopefully, studies of the recently identified additional factors necessary for *Stellate* repression will shed light on the matter.

An additional unanswered question is how the presence of Stellate protein leads to the observed meiotic abnormalities. The homology of Stellate and Su(Ste) to the β subunit of CKII is an important consideration when discussing this topic (Livak 1990; Kalmykova et al. 1998). CKII is an enzyme which along with other target proteins is

known to phosphorylate Topoisomerase II (Topo II) (Ackerman et al. 1985). Topo II functions to relax DNA and is important for both chromatin condensation and chromosome segregation (DiNardo et al. 1984; Newport and Spann 1987; Uemura et al. 1987; Holm et al. 1989; Wood and Earnshaw 1990; Adachi et al. 1991; Downes et al. 1991; Shamu and Murray 1992). CKII phosphorylation of Topo II stimulates its DNA relaxing activity (Ackerman et al. 1985). The CKII beta subunit is thought to be responsible for regulating the activity of its catalytic α subunit (Takio et al. 1987). Therefore, interaction of Stellate protein with the CKII α subunit, due to its homology to the regulatory β subunit, could impair the inability of CKII to activate Topo II, which would then be unable to perform its meiotic functions. As a result, defects in chromatin condensation and segregation would be expected. Both of these phenotypes have been observed in *Su(Ste)*$^{-}$ males (Palumbo et al. 1994). A testis-specific form of the β subunit of CKII, Supressor of Stellate-like (SSL), has been identified (Kalmykova et al. 1997). SSL could be responsible for CKII substrates or levels of activity that are specific to spermatogenesis. It shows 53% identity to Stellate protein suggesting that Stellate may mimic this protein in its interaction with the catalytic subunit of CKII. However, Stellate lacks a conserved region of β CKII that is important for regulation of the target specificity and activity level of the α subunit and, therefore, it could interact but not function properly to control the catalytic activity of CKII (Kalmykova 1997). Interestingly, Hsp90 family members associate with Casein Kinase II (Miyata

and Yahara 1992; 1995). Thus, the manifestation of Stellate crystals in a *scratch* mutant background further ties Casein Kinase II into the *Stellate* and *Su(Ste)* dilemma.

Although it is obvious that male and female meiosis differ significantly, reflected in the number of sex specific meiotic mutations, it is nonetheless important to understand what is known about female meiotic mutations identified thus far. Prior to the screen of natural populations by Sandler et al., five meiotic mutations had been identified: *crossover suppressor in chromosome 3 of Gowen, C(3)G; claret nondisjunctional, (ca^{nd}); equational producer, (eq); Segregation Distorter, (SD);* and *Recovery Disruptor, (RD).* The effects of *eq* were described earlier. *C(3)G* and ca^{nd} are female specific mutations. While *C(3)G* mutations cause a complete absence of recombination in homozygotes leading to high levels of MI nondisjunction (Gowen and Gowen 1922; Hall 1972), ca^{nd} mutants show high MI nondisjunction with no apparent affect on recombination. Both are located on the 3rd autosome of *Drosophila melanogaster.* ca^{nd} is also known as *ncd (non-claret disjunctional)* and has been further characterized in Endow et al. 1990; Walker et al. 1990; McDonald et al. 1990; Knowles and Hawley 1991; Hatsumi and Endow 1992;. The *ncd* mutation exerts a genome-wide effect in that both exchange and nonexhange homologs show high levels of nondisjunction in homozygous females. Chromosome loss is actually the predominant cause of aneuploidy, with a much lower level due to actual nondisjunction. The Ncd protein has substantial homology to the motor domain of the kinesin heavy chain, suggesting it may function to move chromosomes along

microtubules during meiosis. *Segregation Distorter* (Sandler et al. 1957) and *Recovery Disruptor* (Novitski and Hanks 1961; Hanks 1964) both specifically affect male meiosis. Heterozygous mutations in the 2nd chromosome *SD* locus cause an increased recovery of progeny carrying *SD*. The *RD* mutation located on the X chromosome causes fragmentation of the Y chromosome and an excess recovery of female progeny.

Sampling of natural populations and testing for nondisjunction of the X and 4th chromosomes by Sandler et al. (1968) led to the discovery of a number of new female meiotic mutations. Two of the recovered mutations, *mei-S82* and *mei-T3*, cause virtual sterility in females. The mutation *mei-S51* causes a reduced amount of crossing over on the X, resulting in nondisjunction of the X at the first division. Noncrossover X chromosomes also interfere with 4th chromosome segregation, resulting in 44⇔XX segregations. Crossing-over is also reduced in *mei-S282* mutants with a concomitant increase in nondisjunction of all chromosomes at the first division (Parry 1973).

The *mei-41, mei-195, mei-218, mei-251,* and *mei-352* female-specific mutations identified in the EMS screen performed by Baker and Carpenter (1972) all cause male-specific abnormalities in recombination frequencies, and the *mei-218, mei-41,* and *mei-195* mutants all exhibit an increase in nondisjunction at the first division for all chromosomes. The mutations *mei-251* and *mei-352* cause nondisjunction of the sex and 4th chromosomes with an undetermined effect on the major autosomes. The identified mutant *mei-254* was actually found to have two separate meiotic mutations, one being allelic to *mei-9,* which causes a genome-wide decrease in recombination

leading to increased sex and 4th chromosome nondisjunction. Distributive disjunction, a female-specific pathway for segregation of noncrossover chromosomes, is not affected in these mutants. The second mutation of *mei-254* specifically affects distributive disjunction with high 4th chromosome nondisjunction and independent segregation of nonhomologs. This mutation has been renamed *nod* for *no distributive disjunction* and analyzed further (Carpenter 1973; Zhang and Hawley 1990; Zhang et al. 1990; Murphy and Karpen 1995; Afshar et al. 1995a; Afshar et al. 1995b). Mutations in *nod* cause an increase in nondisjunction for all chromosomes in homozygous females. The nondisjunction occurs mainly at the first meiotic division and predominantly affects noncrossover chromosomes. A large amount of 4th chromosome loss is also observed in *nod* females. Interactions between *nod* and ca^{nd} apparently occur since double heterozygotes show 10-50 fold higher amounts of X and 4th chromosome nondisjunction than either of the single heterozygotes. However these two gene products act independently since they cannot complement each other. The difference between the two mutants is that while ca^{nd} has a genome-wide effect as discussed earlier, *nod* is specific to the distributive system. The phenotype of the double heterozygote is specific to the distributive system (Knowles and Hawley 1991). Double homozygotes show the highest levels of nondisjunction with 77% X chromosome nondisjunction and 94% 4th chromosome nondisjunction (Knowles and Hawley 1991). The *nod* mutation does not appear to affect the processes of partner choice or coorientation in distributive pathway, but instead is specific to the

segregation step. Sequencing of the cloned *nod* cDNA revealed high identity with the motor domain of the kinesin heavy chain suggesting that Nod protein may act as a microtubule motor. Binding of Nod to both chiasmate and achiasmate chromosomes has been observed. Although its presence on chiasmate chromosomes may be redundant, it appears to be necessary for achiasmate segregation and may act to counterbalance the poleward force in the absence of chiasma.

Three other female-specific mutation identified in this screen, *mei-38, mei-99,* and *mei-160*, show changes in recombination patterns, MI disjunction and MII disjunction. Disjunction of both recombinant and non-recombinant chromosomes are affected by these mutations.

The *mei-9* mutation mentioned previously greatly reduces levels of meiotic exchange in homozygous females (Carpenter and Sandler 1974). Although synaptonemal complex formation and distribution of recombination nodules is normal in these females, high levels of chromosome nondisjunction and loss occur due to the decreased number of crossovers (Baker and Carpenter; 1972; Carpenter 1979; Kekelsky et al. 1995). Another mutation, *mei-W68*, actually causes a complete elimination of recombination in homozygous females (McKim et al. 1998a and b). Interestingly, synaptonemal complex and pairing appear normal in these recombination-deficient mutants (McKim et al. 1998a). This is in contrast to what has been described in yeast where mutations that eliminate recombination also prevent synaptonemal complex formation and subsequent synapsis of homologs (Klapholz et al. 1985; Giroux

et al. 1989; Roeder 1997). The *mei-W68* gene has now been cloned and found to be a homolog of yeast *spo11*, implicating the gene product in the initiation of recombination events. Spo11 is required for the double-strand break formation that is thought to be the initiating event of recombination (Cao et al. 1990). A second mutation affecting meiotic crossing-over was also reported with *mei-W68*. *mei-P22*, like *mei-W68*, eliminates both crossing-over and gene conversion but has no effect on synaptonemal complex formation (McKim et al. 1998a). The role which *mei-P22* plays in recombination is yet to be assigned.

The Axs^D mutation is an X-linked factor that specifically disrupts segregation of achiasmate homologs (Hawley 1989; Zitron and Hawley 1989). The mutation has no effect on the disjunction of non-homologous chromosomes. This and other findings have led to a model by Hawley and colleagues suggesting that the distributive system in *Drosophila* females consists of two separate mechanisms, one which is responsible for the segregation of achiasmate homologs and a separate mechanism for heterolog segregation. Axs^D causes an increase in the amount of 4^{th} chromosome nondisjunction, but only in the presence of high levels of X chromosome nondisjunction (Zitron and Hawley 1989) leading to the hypothesis by Whyte et al. (1993) that achiasmate homologs in Axs^D females are linked in a manner causing them to act as a single unit in the heterologous system of disjunction. The small size of the 4^{th} chromosome may result in lower levels of interlocking, so it is not affected significantly by the mutation. Axs^D appears to interact with at least two other meiotic mutations. The phenotype of

Axs^D is epistatic to the recessive *ald* mutation. A recessive allele of *Axs* when combined in a double heterozygote with the *ncd* mutation shows higher levels of both X and 4th chromosomal nondisjunction than either single heterozygote. Axs^D does not appear to interact with *nod* (Whyte et al. 1993).

The female specific *altered disjunction (ald)* mutation mentioned above causes increased X and 4th chromosome nondisjunction with no change in recombination (O'Tousa 1982). Ald protein function is specific to prevent nonhomologous disjunction of nonexchange sex and 4th autosomes in the distributive system since its mutation does not affect the segregation of the large autosomes (O'Tousa 1982).

Despite the large number of genetic screens performed to obtain *Drosophila* meiotic mutations, it is obvious that there are still many more yet undiscovered. A recent P-element meiotic screen performed by Sekelsky et al. (1999) recovered two new female recombination defective mutants in addition to a number of mutants showing abnormalities in the female distributive system. Although mutation that affected sex chromosome segregation in males could have been identified in their screen, none were. Several new alleles of already identified meiotic mutants were also identified, but in this particular screen, only one gene was hit more than one time suggesting that the genome was not covered fully. Therefore, it is important to continue the search for more of the factors that function to insure proper pairing and chromosome segregation in *Drosophila melanogaster.*

The majority of mutations described thus far were identified in screens that were designed to identify specifically factors involved in meiotic processes in *Drosophila*. This is considered a forward genetic approach, where a phenotype is first identified and then the gene responsible for the observed abnormality is isolated and characterized. Reverse genetic approaches are also useful in identifying genes of interest, especially considering the ever increasing information from various organisms. Important biological pathways are often conserved and, therefore, a protein that functions in one organism very likely has a similar function in a second organism. Therefore, it will be useful to obtain additional genes involved in *Drosophila* meiosis by identifying homologs of genes known to function in the meiotic process in other organisms.

Mismatch repair (MMR) homologs are one group of genes that would be of interest based on studies in yeast, mice, and humans. Mismatch repair is the process by which heteroduplex formed during replication or recombination can be corrected. Several of the proteins involved in this process have been shown to also have meiotic functions. A mismatch repair protein, Mlh1, has been shown to be important for meiotic crossing over in both *S. cerevisiae* and mice (Hunter and Borts 1997; Baker et al. 1996). Pms2 also has meiotic function in mice. Male mice deficient for Pms2 are sterile presumably due to a defect in chromosome synapsis during meiosis (Baker et al. 1995). Additionally, some proteins that are homologous to mismatch repair proteins appear to function specifically in meiosis. MSH4 and MSH5 in *S. cerevisiae* are

expressed specifically in meiotic cells and function in the process of genetic exchange (Ross-Macdonald and Roeder 1994; Hollingsworth et al. 1995). A homolog of MSH5 has been identified in mice and shown to have gonad-specific expression (Edelmann et al. 1999). Males and females deficient for MSH5 are sterile with phenotypic characteristics suggestive of a role in homolog pairing and/or synapsis (Edelmann et al. 1999). The conservation of meiotic function of some mismatch repair homologs may extend to humans since a MSH4 gene has been identified and shows expression only in the testis and ovary (Paquis-Flucklinger et al. 1997). Identification of *Drosophila* homologs of MMR proteins may further advance our general understanding of the meiotic process.

Four new genes potentially involved in meiosis

As emphasized by the previous discussion, the field is currently lacking in *Drosophila* male mutants defective in pairing and segregation. The previously identified meiotic mutations are listed in Tables 1,2 and 3. The studies here will describe four genes that may increase our understanding of these mechanisms. *Homeless* was previously identified (Gillespie and Berg 1995) as a female sterile mutation. Our studies show that this protein is also necessary in spermatogenesis for proper chromosome segregation. A second factor, *nmf*, may actually join the elite number of genes known to function in both male and female meiosis in *Drosophila*. It is even more unusual in that its effects are primarily exerted on homolog segregation in

Table 1. Female-specific meiotic mutations in *Drosophila melanogaster.*

Meiotic Mutations	**Sex Specificity**	**Phenotypic Characteristeics**
mei-S332	Female-specific	Defective sister chromatid cohesion
ord	Female-specific	Defective sister chromatid cohesion
C(3)G	Female-specific	Complete absence of recombination
cand *(ncd)*	Female-specific	MI nondisjunction
mei-S82	Female-specific	Sterile
mei-T3	Female-specific	Sterile
mei-218	Female-specific	Nondisjunction at the first division
mei-41	Female-specific	Nondisjunction at the first division
mei-195	Female-specific	Nondisjunction at the first division
mei-251	Female-specific	Nondisjunction of the 2nd and 4th chromosomes
mei-352	Female-specific	Nondisjunction of the 2nd and 4th chromosomes
mei-9	Female-specific	Decrease in recombination
nod	Female-specific	Specifically affects distributive disjunction
mei-W68	Female-specific	Elimination of recombination
mei-P22	Female-specific	Elimination of recombination
Axs	Female-specific	Specifically affects distributive disjunction
ald	Female-specific	Specifically affects distributive disjunction
mei-S282	Female-specific	Reduction in crossing over
mei-S51	Female-specific	Reduction in crossing over

Table 2. Meiotic mutations that affect *Drosophila* males and females.

Meiotic Mutations	**Sex Specificity**	**Phenotypic Characteristeics**
eq	Males and females	X chromosome nondisjunction
Horka	Males and females	Female-sterility and 2nd division nondisjunction in males
mei-G87	Males and females	Precocious sister chromatid separation
mei-I3	Males and females	Nondisjunction of all chromosomes at the first and second division
Dub	Males and females	Nondisjunction of all chromosomes at the first division

Table 3. Male-specific meiotic mutations in *Drosophila melanogaster.*

Meiotic Mutations	**Sex Specificity**	**Phenotypic Characteristeics**
Segregation Distorter	Male-specific	Sperm recovery distortion
Recovery Distorter	Male-specific	Y chromosome fragmentation
double-fault	Male-specific	Chromosome segregation defects
bobble	Male-specific	Chromosome segregation defects
mei-S8	Male-specific	4^{th} chromosome nondisjunction
mei-081	Male-specific	Nondisjunction of all chromosomes
mei-W5 (pal)	Male-specific	Chromosome loss
mei-G17	Male-specific	Sex and 2^{nd} chromosome nondisjunction
mei-I1	Male-specific	Nondisjunction of all chromosomes due to pairing defects
Stellate	Male-specific	Sterility, chromosome nondisjunction, fragmentation, and bridging
Su(Ste)	Male-specific	Sterility, chromosome nondisjunction, fragmentation, and bridging

meiosis I rather than sister chromatid separation in the second meiotic division. *dpms2* and *dmlh1* are two homologs of the bacterial *mutL* gene, which, based on functions in other organisms along with some initial data, may be important in *Drosophila* meiosis. The study of these four genes could lead to a better understanding of mechanisms of chromosome pairing in males and segregation in meiosis I and may also reveal ways to isolate and identify additional meiotic factors.

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PART II

Implications for Function of *homeless* in *Drosophila melanogaster* Male Meiosis

* This part is intended to be submitted for publication as Stapleton, W.B. and B.D. McKee (1999).

ABSTRACT

During meiosis I in *Drosophila melanogaster* males, homologous chromosomes must pair and segregate from one another in the absence of a synaptonemal complex (SC) and chiasma. The proteins which function in this process are largely unknown and, therefore, the study of proteins involved in proper homolog segregation in males could do much to clarify mechanisms of achiasmate segregation. Characterization of the phenotypes associated with mutations in the *homeless (hls)* gene suggest a function for this protein in male meiosis. Partial loss of function alleles of *hls* in the homozygous condition produce various defects in male meiosis. These defects include sex and autosomal chromosome nondisjunction, fragmented chromatin, tripolar meiosis, and chromatin bridges. When placed in trans over a deletion, some alleles result in male sterility. An interesting cytological phenotype is formation of crystals within primary spermatocytes, and this suggested some connection of *hls* phenotype with the derepression of the X-linked *Stellate (Ste)* gene. Phenotypes seen in *hls* males thus far are the same as abnormalities caused by derepression of *Stellate*. Additionally, overexpression of the *Stellate* transcript is seen in *hls* males confirming *hls* involvement in *Stellate* regulation. The pleiotropic phenotypes observed in males along with previous studies on the effects of *hls* in oogenesis suggest that *Homeless* protein may be required for processing, localization, or translation of a subset of mRNAs necessary during *Drosophila melanogaster* male and female gametogenesis. Our studies indicate a potential role of *Homeless* in meiosis, specifically in the regulation of *Stellate.*

CHAPTER I

INTRODUCTION

Although studies of the processes of meiotic homolog pairing, recombination, and segregation in *Drosophila melanogaster* females have been quite successful, there is still little known about the factors involved in the meiosis I events in males. Without the synaptonemal complex acting to hold homologs in close apposition (Meyer 1960) or a chiasma, the physical manifestations of crossover events, to provide stability until anaphase I reductional division (Cooper 1964), there must be some unique mechanism in males to ensure the proper chromosome distribution to haploid gametes. The mechanism of sex chromosome pairing in males has been shown to be dependent on *rDNA* sequences located on both the X and Y chromosomes (McKee and Karpen 1990). In contrast, autosomal pairing sites are located in euchromatic regions and appear to be widely distributed rather than focused in a specific region (McKee 1996). Other than this, little is known about the mechanism of chromosome pairing and segregation in *Drosophila* males.

Numerous screens have been performed to isolate *Drosophila* mutants in the process of meiosis (Sandler et al. 1968; Baker and Carpenter 1972; Castrillon et al. 1993; Sekelsky et al. 1999). Only seven mutations have been discovered in these screens that affect both male and female meiosis. Four of the five affect segregation of sister chromatids. *mei-S332* (Kerrebrock et al. 1992; Bickel et al. 1998), *ord* (Mason 1976; Miyazaki 1992; Bickel et al. 1996), *eq* (Schultz 1934), and *Horka* (Szabad et al.

1995) all cause equational nondisjunction of sister chromatids in males. With the exception of *Horka,* which causes female-sterility, these mutations have the same effect on 2nd division segregation defects in females. *mei-G87* causes precocious sister chromatid segregation during both the first and second meiotic division (Gethmann 1984). A genome wide segregation defect is seen in *mei-I3* homozygotes (Ivy 1981). All chromosomes nondisjoin during the reductional division, but the sex chromosomes also show abnormal segregation during the equational division (Ivy 1981). Only one mutation has been recovered thus far that affects the first meiotic division of both males and females. *Double or nothing (Dub)* (Moore et al. 1994) causes nondisjunction of all chromosomes during the reductional division in both sexes. The identification of *Dub* may indicate overlapping mechanisms for the segregation of homologs in males and females.

Additionally, several male-specific meiotic mutations have been identified. *mei-S8* and *mei-081* were identified in a screen of natural populations by Sandler et al. (1968). While *mei-S8* affects segregation of the 4th chromosome and not the sex chromosomes, *mei-081* shows segregation abnormalities for all chromosomes. Sandler (1971) also identified *mei-W5* or *paternal loss (pal)* (Baker 1975) which causes chromosome loss without an effect on disjunction. An EMS-induced mutatgenesis screen by Baker and Carpenter (1972) recovered several male mutants with similar phenotypes of nondisjunction of the sex chromosomes but no effect on the 4th autosome pair. *mei-G17* also shows chromosome-specific effects, causing increased

levels of sex and second chromosome nondisjunction in homozygous males (Gethmann 1974). On the other hand, *mei-I1* has a genome wide effect with defects in the segregation of all chromosomes and, in some cases, sterility (Ivy 1981). Unfortunately, most of these mutations are lost.

Lastly, nondisjunction of the X-Y and large autosomal pairs, along with meiotic drive and chromosome breakage and loss results from deletion of *Su(Ste)* (Livak 1990) or *crystal* (Pimpinelli et al. 1986), a locus on the Y chromosome that is necessary for the repression of the X-linked *Stellate* locus. Both loci are made up of tandemly repeated sequences containing an ORF that is homologous to the β-subunit of CKII (Livak 1984). The absence of *Su(Ste)* results in high expression of Stellate protein which accumulates in testis as crystals within primary spermatocytes (Bozzetti et al. 1995). The meiotic abnormalities seen in *Su(Ste)* males are thought to be due to overexpression of *Stellate*. The severity of the phenotype depends on the number of *Stellate* copies present. At high copy number, the presence of Stellate protein results in sterility while at lower copy number chromosome nondisjunction (Hardy et al. 1984; Palumbo et al. 1994), fragmented chromatin (Palumbo et al. 1994) , and chromatin bridging (Palumbo et al. 1994) are observed. An additional phenotype is the excess recovery of certain sperm classes in a genotype-dependent manner. This phenomenon is commonly referred to as meiotic drive. The meiotic drive seen in *Su(Ste)*$^{-}$ or XO males is independent of *Stellate* copy number and, therefore, it is not known if the

phenotype is caused by Stellate or another gene product that is regulated by *Su(Ste)*. The mechanism of Stellate regulation is not yet understood.

In an attempt to identify additional factors involved in male meiosis I, various mutations involved in other cellular processes were tested for their effect on sex chromosome nondisjunction. During this study, mutations in a gene originally named *spindle E* were found to cause high levels of sex chromosome nondisjunction (McKee et al. 1999). *spindle E*, and four other *spindle* genes, were initially identified in a screen for maternal effect mutants. Females homozygous for mutations in any of the five *spindle* genes were found to lay ventralised eggs (Tearle and Nusslein-Volhard 1987). *spindle E* has been extensively characterized with respect to its role in oogenesis under the alternative name of *homeless (hls)* (Gillespie and Berg 1995). The Homeless protein is necessary for proper axis formation and positioning of the oocyte during oogenesis. Females homozygous for any of a number of partial loss of function alleles are sterile. Additionally, deletions of the gene are lethal, suggesting functions in development as well as oogenesis. *hls* mutant females show mislocalization of several transcripts required during oogenesis for axis determination and oocyte positioning. Disorganization of the microtubule structure in *hls* oocytes is a likely cause of transcript mislocalization. Mutants also show reduced levels of *fs (1)K10, orb* and *gurken* proteins (Gillespie and Berg 1995; Gonzalez-Reyes et al.1997). The multiple phenotypes seen in *hls* females suggest a role of Homeless in RNA processing, localization, or translation. Furthermore, the amino-terminal portion of the deduced

sequence of the Homeless protein shows similarity to two yeast splicing factors and *maleless (mle)*, which all belong to a superfamily of RNA-dependent ATPases and helicases (Gillespie and Berg 1995).

The lethality associated with complete loss of *hls* function (Gillespie and Berg 1995) along with the data contained within this report suggest that Homeless functions in processes in addition to oogenesis. Our data suggest that it can be an essential factor in male meiosis since some *homeless* alleles cause complete male sterility in some genetic backgrounds. These results raise questions of whether Homeless is also involved in female meiosis and, if so, how a common protein can function in processes which differ significantly from each other.

CHAPTER II

MATERIALS AND METHODS

Fly stocks

The original *hls*^{E616} stock and all other stocks used, unless noted otherwise, were obtained from the Bloomington Stock Center at the University of Indiana. All other *hls* alleles were a generous gift of Dr. Celeste A. Berg. Stocks were maintained on cornmeal-molasses-yeast-agar medium at 25° C.

Cytological analysis

Orcein-stained meiotic chromosomes from adult testes were prepared according to Lifschytz and Hareven (1977). Briefly, testes were dissected in testis buffer (7% NaCl, Ashburner, 1989) and fixed in 45% acetic acid for thirty seconds. Staining of the testis in 3% orcein-60% acetic acid for five minutes was followed by squashing in a 1:1 mixture of 60% acetic acid and 2% lactic-acetic-orcein. Analysis and photographs were carried out using phase-contrast microscopy on a Zeiss Universal Axioplan photomicroscope and photograped with Kodak T-Max 100 color film.

Live testes were prepared by dissection in testis buffer followed by covering of the tissue with a siliconized cover slip and gentle tapping to release cells.

Molecular analysis

DNA extractions were performed by grinding 50 flies with a microhomogenizer in grind buffer (0.08M NaCl; 0.16M sucrose; 8 mM EDTA; 24mM Tris-HCl,pH8; 0.05M EDTA; 0.5% SDS; 0.1M Tris-HCl pH 9.2). Incubation at 65° C for thirty

minutes was followed by addition of twenty microliters of 8M potassium acetate and incubation for thirty minutes on ice. Phenol/chloroform extractions were performed to remove proteins followed by ethanol precipitation. Pellets were resuspended in Tris-EDTA (pH 8.0).

The polymerase chain reaction (PCR) parameters were 35 cycles of 94°C for 1 minute, 55°C for 1.5 minutes, and 72°C for 2 minutes in a Perkin-Elmer thermocycler. Reaction mixtures contained 1 nmol of each primer, 10 ng *Drosophila melanogaster* genomic DNA (Oregon R), 1.5 mM $MgCl_2$ and 5 U AmpliTaq polymerase (Perkin-Elmer Cetus) in a total volume of 50 microliters overlaid with an equal volume of mineral oil. Product size and purity was confirmed by electrophoresis in a 1% agarose gel. The Geneclean II™ kit from Bio101, Inc. (Vista, CA) was used to purify the PCR products for future use.

Northern blotting was performed by isolation of total RNA from the indicated tissue using the Perfect RNA™ kit from 5'↔3' (Boulder, CO) as described by the manufacturer. RNA was then denatured and run on a formaldehyde gel at 90 volts. Transfer to nitrocellulose paper was accomplished by overnight capillary transfer in 20X SSC buffer and the RNA was fixed by baking at 80 degrees for 2 hours. The blots were prehybridized and hybridized in 50% formamide, 5X Denhardt, 0.1M PIPES, 0.8M NaCl, 0.1% SDS, and 0.1 mg/ml salmon sperm DNA at 42° C. Probes were prepared using the Random Primers DNA Labeling System from GibcoBRL (Gaithersburg, MD). Washes were performed in 2XSSC for 10 minutes at room

temperature, 2XSSC and 1% SDS for two 30 minute washes at 65° C, and two washes in 0.1 SSC at room temperature for thirty minutes each.

Southern blotting (Southern 1975) was performed by isolation of total DNA as described then digestion with the appropriate restriction enzyme and separation on a 0.6% agarose gel. Overnight capillary transfer to Genescreen Plus (New England Nuclear; Wilmington, DE) was followed by fixation using UV irradiation. Prehybridization was performed in a 50% formamide solution. Probes and washes were done as described for Northerns.

Statistical analysis

The parameters R_x and R_Y are used in the calculation of meiotic drive where R_x is an indicator of the viability of X-bearing sperm and R_Y of the viability of Y-bearing sperm relative to other sperm classes (McKee et al. 1998). Formulas for these parameters are: $R_x = (O_xO_{XY}/O_YO_O)^{1/2}$ and $R_Y = (O_YO_{XY}/O_XO_o)^{1/2}$. O_X, O_Y, O_{XY}, and O_O are the numbers of X-, Y-, XY- and nullo-XY-bearing sperm respectively. The formula for disjunction frequency, P, is $P= O_X + O_Y/O_X + O_Y + O_{XY} + O_O$. P, R_X, and R_Y values are equal to 1 in wild-type backgrounds.

Statistical comparisons for determination of significance were done using the z test (McKee and Karpen 1990). Variance is determined by the formula $V_j = P (1-P)/N$ where j = nondisjunction and P=disjunctional frequency. Pairwise comparisons were made using the statistical formula $z = (j_1 - j_2)/(V_{j1} + V_{j2})^{1/2}$. A z table was used for determination of significance (Kempthorne 1969).

CHAPTER III

RESULTS

Sex chromosome nondisjunction in *hls* males

Males homozygous for the EMS-generated $spnE^{hlsE616}$ mutation showed a high rate of sex chromosome nondisjunction when outcrossed to *y w/y w* females (McKee et al. 1999). In order to determine if this nondisjunction was due to the *hls* mutation or some background mutation within the stock, various independently generated alleles of *hls* were tested (Table 1). Since most of these alleles are known to be partial loss of function alleles (Gillespie and Berg 1995), they were tested in heterozygous combination with either a deletion allele, $hls^{\Delta 125}$, or a strong point mutation hls^{E616}, for nondisjunction of the sex chromosomes (see Figure 1A for crossing scheme). As shown in Fig. 1, the $hls^{\Delta 125}$ or hls^{E616} allele was always contributed by the mother of the tested male. Therefore, the X chromosome of the tested male was always derived from the $hls^{\Delta 125}$ or hls^{E616} stock. The importance of this will become evident. A marked Y, B^sYy^+, was used to measure the amount of sex chromosome nondisjunction. Males were crossed to chromosomally normal females marked with the *y* and *w* markers.

All alleles tested in combination with $hls^{\Delta 125}$, with the exception of hls^{DE3}, were male sterile. The hls^{DE3} allele when heterozygous with the $hls^{\Delta 125}$ deletion showed 16.9% nondisjunction of the sex chromosomes in comparison to only 0.06% nondisjunction seen in control males having only one copy of the deletion allele (Table 2). By contrast males heterozygous for a maternally derived hls^{E616} allele and any of the

Table 1. List of *homeless* alleles used in this study

Allele Name	Nature of Mutation
hls^{3987}	P element insertion; described in Berg 1995
$hls^{\Delta 125}$	Deletion generated by P element excision from hls^{3987}; described in Gillespie and Berg1995
hls^{E616}	EMS generated allele; described in Gonzalez-Reyes et al. 1997
hls^{CT4}	EMS generated allele; isolated in the lab of Celeste Berg
hls^{CT5}	EMS generated allele; isolated in the lab of Celeste Berg
hls^{E1}	EMS generated allele; isolated in the lab of Celeste Berg
hls^{W10}	EMS generated allele; isolated in the lab of Celeste Berg
hls^{DE3}	EMS generated allele; isolated in the lab of Celeste Berg
hls^{DE8}	EMS generated allele; isolated in the lab of Celeste Berg

A

P $+/B^sY\,y^+$; *hls*/TM3,Sb,e* ♂ *w/w*; $hls^{\Delta 125}$,*e/TM3,Sb,Ser,e* ♀

⇓

F1 *y w/y w* ♀ X *w*/$B^sY\,y^+$; *hls**/$hls^{\Delta 125}$,*e* ♂

⇓

F2 Score progeny for nondisjunction of the sex chromosomes

B

P $+/B^sY\,y^+$; *hls*/TM3,Sb,e* ♂ +/+; hls^{E616},*e/TM3,Sb,e* ♀

⇓

F1 *y w/y w* ♀ X $+/B^sY\,y^+$; *hls**/hls^{E616},*e* ♂

⇓

F2 Score progeny for nondisjunction of the sex chromosomes

Fig. 1 Crossing scheme for determination of the amount of sex chromosome nondisjunction for various *homeless* alleles when placed over (A) $hls^{\Delta 125}$ deletion or (B) hls^{E616} allele. *Each *homeless* allele, indicated by * , listed in Table 1 was tested in this crossing scheme.

Table 2. Sex chromosome nondisjunction observed with various combinations of *homeless* alleles.

Male Genotype	Total Progeny Scored	Avg. Progeny per Male	% Sex Chromosome Nondisjunction	% Y Breakage
$hls^{\Delta 125}$ heterozygous controls	1679	88	0.06%	0.00%
$hls^{DE3}/hls^{\Delta 125}$	1219	53	16.90%	0.16%
hls^{DE3}/hls^{E616}	431	43	1.20%	0.50%
$hls^{\Delta 125}/hls^{E616}$	2374	95	2.10%	0.50%
hls^{DE8}/hls^{E616}	773	70	2.30%	0.50%
hls^{E1}/hls^{E616}	529	59	2.80%	0.40%
hls^{E616}/hls^{E616}	1974	71	3.00%	1.20%
hls^{W10}/hls^{E616}	436	40	3.20%	0.70%
hls^{CT5}/hls^{E616}	990	71	3.50%	0.20%
hls^{CT4}/hls^{E616}	524	87	3.20%	0.38%
hls^{3987}/hls^{E616}	3787	86	4.80%	0.13%
hls^{3987}/hls^{3987}	875	67	6.30%	0.10%

other alleles were fertile (Figure 1B and Table 2). All of these males showed nondisjunction ranging in frequency from 2% to 5% as can be seen in Table 2. The only alleles that showed a significant difference from hls^{E616} homozygotes in combination with hls^{E616} are the P-element induced hls^{3987} allele and the EMS-induced hls^{DE3} allele. Males homozygous for hls^{3987} have a significantly higher sex chromosome nondisjunction percentage (Table 2; z test). Since hls^{DE3} has a milder effect (i.e. the only allele that is fertile) when placed in trans with $hls^{\Delta 125}$, it is not surprising that it shows significantly lower levels of nondisjunction as compared to hls^{E616} homozygotes (Table 2; z test). The sex chromosome nondisjunction observed in *hls* males is not due to an effect of the marked Y since control males carrying $B^{s}Yy^{+}$ showed no nondisjunctional progeny out of 371 counted progeny. These results demonstrate that mutations in *hls* cause a significant amount of sex chromosome nondisjunction in males.

Additionally, chromosome breakage occurs in these males. The marked Y used has a marker at each end of the chromosome, as illustrated in Figure 2. Therefore, identification of male progeny carrying only one of the two markers indicates chromosome breakage. The percentage of Y chromosome breakage for each genotype is also shown in Table 2.

An additional phenotype evident in these data is a difference in the recovery of reciprocal meiotic products. Table 3 shows the progeny numbers for the cross of $hls^{\Delta 125}/hls^{E616}$ males to *y w* females. The greater recovery of normal XX females vs.

Fig 2 Illustration of the marked Y chromosome used in crosses that allow genetic determination of the occurrence of breakage of the Y chromosome.

y^+
B^s

Table 3. Excess recovery of XX vs XY progeny and XO vs XXY progeny from $hls^{\Delta 125}/hls^{E616}$ males crossed to *y w* females is indicative of the occurrence of meiotic drive. Meiotic drive parameters are R_x and R_Y, the recovery of X or Y sperm in comparison to nullo-X or nullo-Y sperm respectively. The formulas are as follows: $R_x=(O_xO_{XY}/O_YO_O)^{1/2}$ and $R_Y=(O_YO_{XY}/O_XO_O)^{1/2}$ where O_X= number of X bearing sperm; O_Y= number of Y bearing sperm; O_{XY}= number of XY bearing sperm; and O_O= number of nullo XY sperm. The disjunction frequency (P) is determined by the following formula: $P= 1/[1+(O_{XY}O_O/O_XO_Y)^{1/2}$.

Total Progeny Scored	2374
Total XY Males	938
Total XX Females	1436
Total Nondisjunctional Progeny	50
% Nondisjunction	2.10%
Total XO Males	34
Total XXY females	16
Progeny with Fragmented Y	12
% Breakage of Y Chromosome	0.5%
R_x	0.85
R_Y	0.55
P	0.98

normal XY males and of XO males vs. XXY females suggests that meiotic drive is occurring in these mutant males. Similar recovery distortions are seen also in males deficient for the X chromosome pairing site and in *Su(Ste)*$^{-}$ males. In the former case, drive is associated with extensive spermatid mortality. Distortions in sperm recovery can also be caused by chromosome loss which results in an excess of XO progeny. However, XX females constitute well over 50% of total progeny (Table 3). This cannot be explained by chromosome loss confirming that meiotic drive is the cause of the distorted genotype dependent sperm recovery.

Autosomal nondisjunction in *hls* males

To determine whether the meiotic nondisjunction is sex chromosome-specific or is a genome-wide effect, disjunction for the 2nd and 4th autosomes was measured. Nondisjunction of chromosome 2 could be detected by crossing *hls*^{E616} homozygous males to *C(2)EN b,pr* females. Since C(2)EN b,pr females only produce nullo- or diplo-2 ova, only nondisjunctional sperm produce viable progeny. The number of viable progeny per male provides an estimate of the number of nondisjunctional gametes per male. Therefore, the relative amount of 2nd chromosome nondisjunction could be determined by counting the number of progeny per *hls*^{E616} homozygous male and comparing the number of progeny per male to a control with wild-type males (from an Oregon R stock). By this method, frequency of 2nd chromosome nondisjunction was found to be more than an order of magnitude higher in *hls* males than in wild-type

controls (Table 4). This increase is comparable in magnitude to the effect on sex chromosome nondisjunction.

C(4)EN *ci ey* females were used to determine the percentage of 4th chromosome nondisjunction in *hls* males. Progeny with the ci ey phenotype are derived from nondisjunctional nullo-4 sperm that fertilize an egg containing C(4)EN ci ey. Only half of the progeny arising from nondisjunction of the 4th chromosome during spermatogenesis can be scored since the reciprocal diplo-4 sperm would produce wild-type progeny which could not be distingushed from progeny receiving a single fourth chromosome from the father. Out of a total of 690 progeny scored, none were of the ci ey phenotype suggesting that little or no nondisjunction of the 4th chromosome occurs in hls^{E616}/hls^{E616} males. These data suggest that Homeless may be involved in a mechanism for segregation of the large autosomes and sex chromosomes, but is not involved in a potential separate pathway for segregation of the small 4th chromosome pair.

Cytological phenotypes of *hls* males

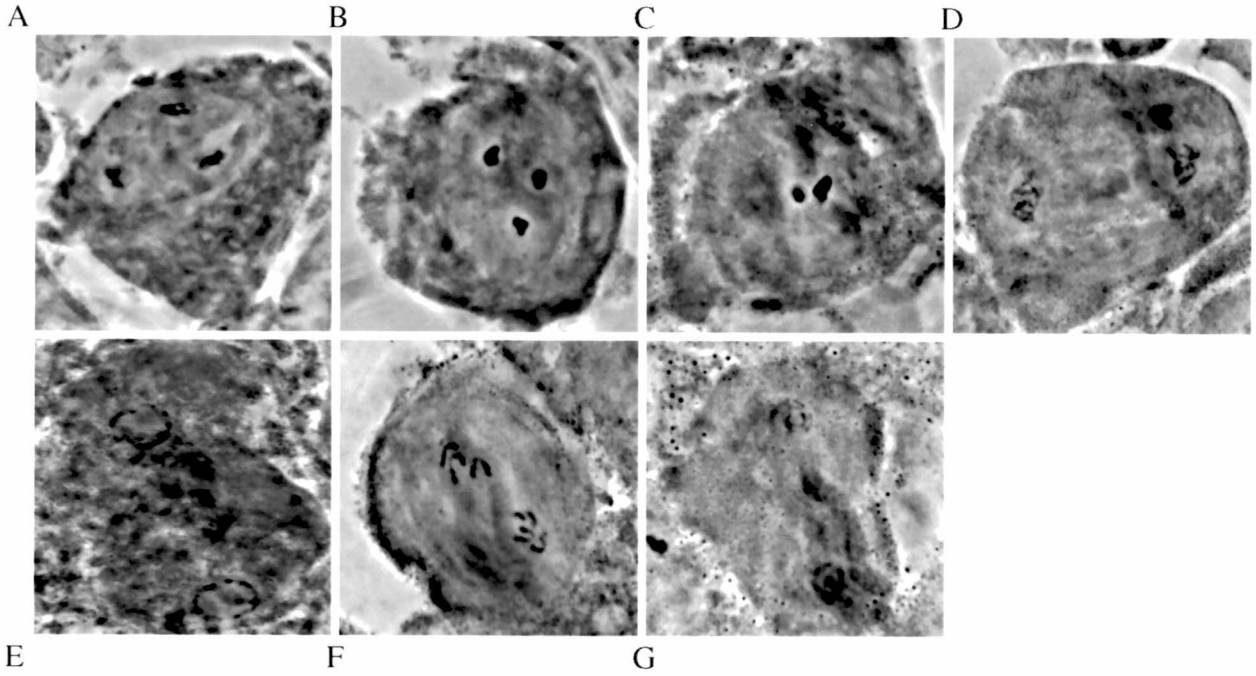
In order to assess the defects responsible for the observed nondisjunction phenotype, orcein stained preparations of dissected testis were analyzed to assess morphology of cells at various stages of the meiotic process. Figure 3 shows preparations from wild-type *Oregon R* testes illustrating cells at different stages in meiosis I and meiosis II. Numerous abnormal phenotypes were observed in primary

Table 4. Relative amount of chromosome 2 nondisjunction in *hls* males compared to wild-type Oregon R males

Male Type	Number of males scored*	Total number of progeny	Average number of progeny per male	Estimated nondisjunction frequency (per tested male)
Oregon R	100	5	0.05	0.1%
hls^{E616}/hls^{E616}	160	100	0.63	1.3%

* Crosses were set up with 10 males and 10 C(2)EN b,pr females per vial. Parents were discarded on day 10 and number of progeny were counted on day 20.

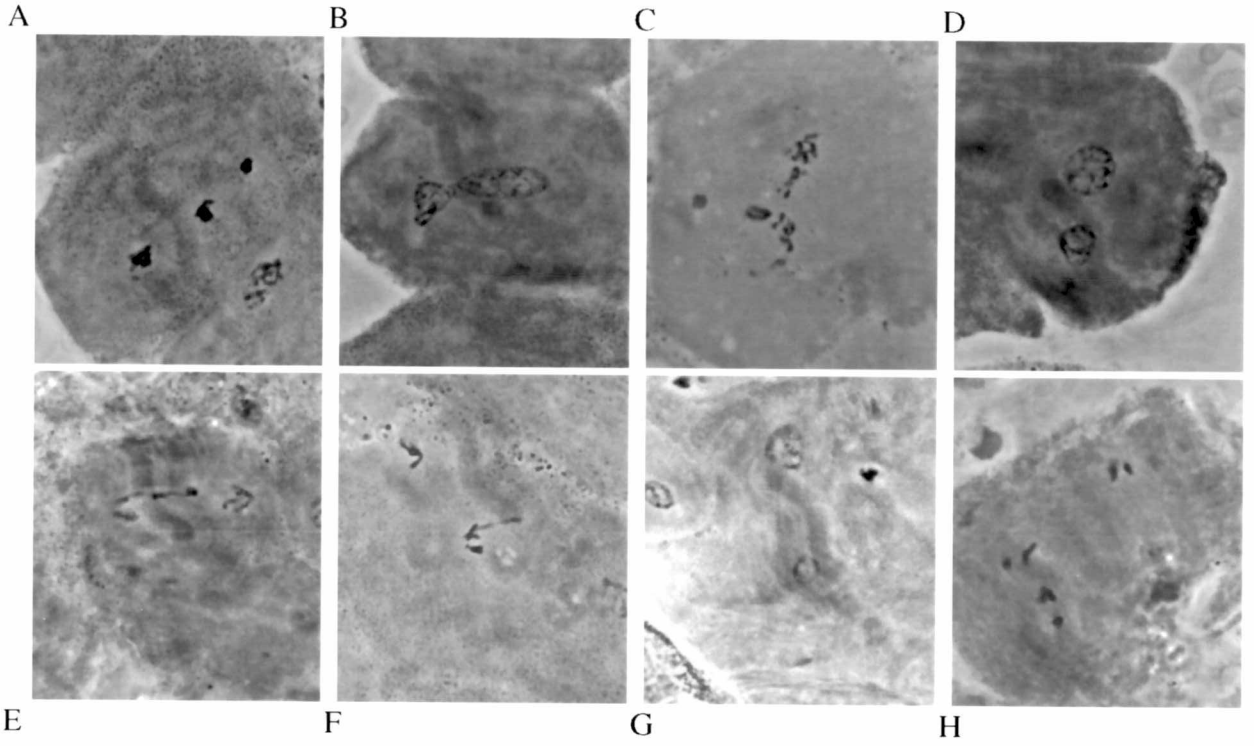
Fig 3 Orcein-stained wild-type (*Oregon R*) spermatocytes at various stages of meiosis. (A) Prophase I (B) Prometaphase I (C) Metaphase I (D) Anaphase I (E) Telophase I (F) Anaphase II (G) Telophase II.


A
B
C
D
E
F
G

spermatocytes of *hls*^{E616} homozygotes (Figure 4), including tripolar meiosis, fragmented chromatin, chromatin bridges, unequal separation of chromatin, incomplete chromatin separation, and lagging chromosomes. The approximate frequencies of the observed cytological phenotypes are, respectively, 0.4%, 0.4%, 1.1%, 0.4%, 0.4%, and 1.1% of the total meioses scored. Abnormalities can be seen in both of the meiotic divisions suggesting that the chromosome nondisjunction observed genetically occurs at both meiosis I and meiosis II. The defect does not appear to be in chromosome pairing since unpaired homologs were not observed. Prophase I, prometaphase I, and metaphase I all appear normal in mutant males. Instead, segregation of chromosomes appears dysfunctional. The cytological phenotypes suggest failure in release of that cohesion between the homologs or sister chromatids. This could be caused by improper chromatin condensation or impairment of a direct mechanism that releases attachment. As discussed previously, genetic data indicate the breakage of chromosomes (Table 2) which would be expected from the cytological observations of bridging and fragmented chromatin.

An interesting phenotype was observed in live testis preparations (see Materials and Methods) from *hls* males. In *hls*^{E616}/*hls*$^{\Delta 125}$ sterile males, live testis preparations showed star-shaped crystal structures within the primary spermatocytes (Figure 5A). *hls*^{E616} homozygote males, which are not sterile, show needle-shaped crystals within primary spermatocytes of the testis (Figure 5B).The crystals are identical in appearance to those seen in testes of XO males (Livak 1984; Hardy et al. 1984;

Fig 4 Orcein-stained hls^{E616}/hls^{E616} spermatocytes showing various abnormal phenotypes. (A) Tripolar meiosis I (B) Incomplete chromatin separation at telophase I (C) Fragmented chromatin in primary spermatocyte (D) Unequal sized nuclei at telophase I (E) Lagging chromosome at anaphase II (F) Chromatin bridge in a secondary spermatocyte (G) Unequal sized nuclei at telophase II (H) Aneuploid secondary spermatocyte.


A
B
C
D
E
F
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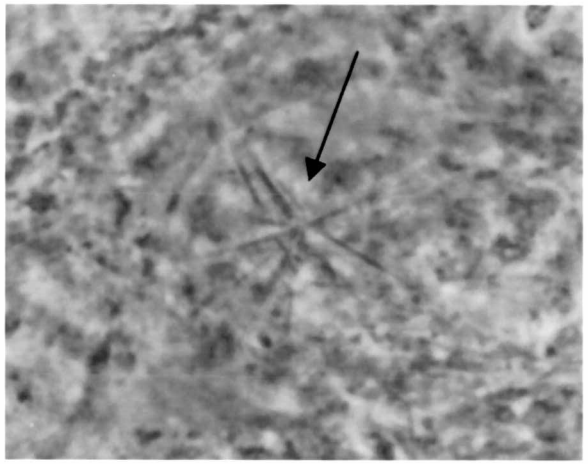
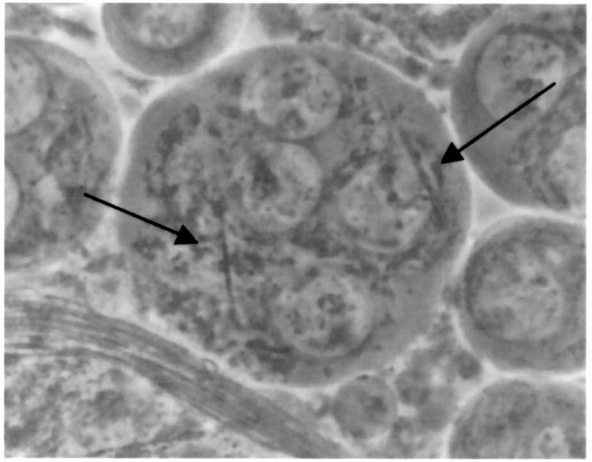
H

Fig 5 Live testes squashes of (A) $hls^{\Delta 125}/hls^{E616}$ (B) hls^{E616}/hls^{E616} males. Arrows indicate crystals within primary spermatocytes.

A



B



Palumbo et al. 1994) or in males carrying a Y chromosome deficient for the *Su(Ste)* locus (Meyer et al. 1961; Livak 1984; Hardy et al. 1984; Palumbo et al. 1994). As discussed above, these crystals in have been shown to result from overexpression of the X-linked *Stellate (Ste)* locus which is normally repressed by *Su(Ste)* (Hardy et al.1984; Livak 1990; Pimpinelli et al. 1986).

Table 5 compares the meiotic phenotypes of *Su(Ste)*$^-$ males to those associated here in *hls* males. In the presence of a low-copy number (*Ste*$^+$) *Stellate* allele, the sex chromosomes nondisjoin while the 4th chromosomes disjoin regularly and meiotic drive, chromosome breakage, fragmentation and condensation defects are also observed. The severity of these meiotic abnormalities (except for meiotic drive) is dependent upon the copy number of the *Stellate* repeats (Palumbo et al., 1994). In the presence of very high-copy-number (*Ste*) *Stellate* arrays, *Su(Ste)*$^-$ males are sterile. Table 5 shows a perfect correlation between the phenotypes of *Su(Ste)*$^-$ and *hls* males.

Effect of varying copy number of *Stellate* in *hls* males

In light of the above findings, the differences in fertility between $hls^{E616}/hls^{\Delta 125}$ and hls^{E616} homozygous males could be due to differences among the alleles in the amount or functionality of the Homeless protein (i.e., hls^{E616} could be a hypomorph) or could be dependent solely on the type of *Stellate* allele (*Ste* vs *Ste*$^+$) present. $hls^{E616}/hls^{\Delta 125}$ males were sterile when the X chromosome was derived from the $hls^{\Delta 125}$ stock. In order to determine the type of *Stellate* allele within this stock, XO males were generated by crossing $hls^{\Delta 125}$ stock males to *C(1)RM/O* females. Cytological

Table 5. Comparison of the meiotic phenotypes caused by *Stellate* derepression (Palumbo 1994) to the phenotypes seen in *homeless* mutant males

Meiotic Phenotype	***Stellate* Derepression**	***homeless***
Crystals in 1° spermatocytes	Yes[*Δ†]	Yes
XY nondisjunction	Yes[Δ†]	Yes
2nd chromosome nondisjunction	Yes[Δ†]	Yes
4th chromosome nondisjunction	No[†]	No
Meiotic Drive	Yes[Δ†]	Yes
Sterility	Yes[Δ†]	Yes
Fragmented chromatin	Yes[Δ]	Yes
Chromatin bridging	Yes[Δ]	Yes

[*]Meyer et al. 1961

[†]Hardy et al. 1984

[Δ]Palumbo et al. 1994

analysis showed the X chromosome of this stock to contain a *Ste* allele (data not shown). A similar analysis showed that the X chromosome in the hls^{E616} stock contained a Ste^{+} allele. To determine whether the different *Ste* alleles are solely responsible for the observed phenotypic differences between hls^{E616} homozygous and $hls^{E616}/hls^{\Delta 125}$ heterozygotes, males of these genotypes were generated in such a way as to reverse the *Ste* genotypes (i.e., Ste^{+} in $hls^{E616}/hls^{\Delta 125}$ and *Ste* in hls^{E616} homozygotes). As shown in Table 6, this reversal of *Ste* genotypes did indeed reverse the phenotpyes: hls^{E616} homozygous males carrying *Ste* are sterile and $hls^{E616}/hls^{\Delta 125}$ males carrying Ste^{+} are fertile. Moreover, the difference in nondisjunction frequencies between $hls^{\Delta 125}/hls^{E616}$ and hls^{E616}/hls^{E616} in the presence of a Ste^{+} allele is not significant (z test). Thus, there is no evidence for a phenotypic difference between hls^{E616} homozygous males and $hls^{E616}/hls^{\Delta 125}$ males, when the *Ste* genotype is held constant.

It is important to note that the comparison of *hls* alleles presented in Table 2 does take into account the allele of *Stellate* present. With only two exceptions, all males in the table carry the X chromosome from the hls^{E616} stock, which carries a Ste^{+} allele. Therefore, direct comparisons can be made between the phenotypes seen in these males since they all carry the same number of *Stellate* copies. The $hls^{\Delta 125}/hls^{DE3}$ males tested carry a *Ste* allele since the X is from the $hls^{\Delta 125}$ stock. The remaining alleles that were tested in heterozygous condition with $hls^{\Delta 125}$ and found to be sterile also carry the *Ste* allele and therefore further support a dependence of the strength of phenotype on the number of *Stellate* copies present. However, the fertility of

Table 6. Comparison of phenotypes in *hls* males with either a *Ste* or Ste^{+} allele present.

Male Genotype for *homeless*	***Stellate* Allele Present**	**Fertility**	**% Sex Chromosome Nondisjunction**
$hls^{\Delta 125}/hls^{E616}$	*Ste*	Sterile	-
$hls^{\Delta 125}/hls^{E616}$	Ste^{+}	Fertile	2.1%
hls^{E616}/hls^{E616}	*Ste*	Sterile	-
hls^{E616}/hls^{E616}	Ste^{+}	Fertile	3.0%

$hls^{\Delta 125}/hls^{DE3}$ males in the presence of *Ste* shows that there is some dependence on the allele of *homeless*. The males homozygous for hls^{3987} carry the X chromosome from this stock and the copy number of *Stellate* is unknown so no direct comparisons can be made between the phenotype of these males with others in the table.

<u>*Stellate* transcript expression in *hls* males</u>

If the meiotic phenotypes observed in *hls* males are indeed due to *Stellate* derepression, *hls* males which carry a normal Y and therefore are $Su(Ste)^+$ should nevertheless show high levels of *Stellate* transcript. Northern blot analysis confirms the presence of an abundance of *Ste* transcript in *hls* males (Figure 6). Although not shown, *Oregon R* XY males were tested and showed no *Stellate* RNA. Fifty pairs of testes were dissected and total RNA was isolated from each type of male. The Northerns were probed with a PCR-generated genomic fragment of *Stellate*. Testis RNA from $hls^{\Delta 125}/hls^{E616}$ males show a hybridizing fragment of 0.75 kb, the size expected for a properly spliced mRNA derived from the *Stellate* locus (Livak 1990). Comparison of Oregon R XO male expression of *Stellate* with the amount seen in $hls^{\Delta 125}/hls^{E616}$ XY males which carry a *Ste* allele show a similar level of expression (Figure 6A). Figure 6B shows a small amount of *Stellate* expression in hls^{E616} homozygotes containing a Ste^+ allele. This level is higher than seen in Oregon R XY males, but much reduced compared to the deficiency heterozygotes which carry a *Ste* allele. This lower amount of expression in the homozygote is expected since the meiotic phenotype is also milder.

Fig 6 Northern probed with PCR-amplified *Stellate* genomic DNA. 50 testes were dissected from the indicated males and total RNA was isolated, electrophoresed on a formaldehyde gel and transferred to Genescreen. An RNA ladder was used to approximate sizes of the observed bands. RNA loading was controlled by densitometry of ethidium bromide stained rRNA bands.

1.35kb
0.24kb
yw XO males
hls^{A125}/hls^{E616} males
hls^{A125}/hls^{E616} males
hls^{E616}/hls^{E616} males

Attempted rescue of the *homeless* phenotype with a c-myc construct

A final confirmation that a particular phenotype is due to a specific gene mutation is rescue of the mutant phenotype with a genetic construct that can be inserted into the mutant strain. Additionally, by tagging the insertion construct with a c-myc tag, antibodies against the fusion protein can give information about localization of the protein within the organism. A c-myc fusion construct was kindly supplied by Celeste Berg. The tag is added to the C-terminus of *homeless* cDNA and deletes about 13 amino acids of the protein. Rescue of fertility and proper disjunction by this construct would show proper function of the fusion protein. The construct contains a w^+ marker which when on a *white* background allows determination of insertion by eye color. The construct was inserted onto the X chromosome in the Berg lab. Using these insertion lines, crosses were done to determine if the construct rescued the mutant phenotypes seen in *homeless* males. Figure 7 shows the crossing-scheme used to determine if rescue was accomplished. In order to determine if the construct rescues fertility, the presence of a *Ste* allele would be necessary since sterility is seen only in *hls* males that carry a high copy number *Ste* allele. This is difficult because the construct insertion is on a X chromosome containing a Ste^+ allele as determined by observation of needle-shaped crystals in XO males (data not shown). However, recombination in the females of the cross will allow new linkages to be made between *Ste* and the insertion construct on the X chromosome. Therefore, some of the males should contain the *Ste* allele and thus answer the question of whether sterility is rescued by the

(A)

P $wP(w^+,hls^+\text{-}myc)/wP(w^+,hls^+\text{-}myc)$ ♀ X $w/B^sYy^+;\ hls^{\Delta 125},e/TM3,Sb,Ser,e$ ♂

⇓

F1 $wP(w^+,hls^+\text{-}myc)/w;\ +/TM3,Sb,Ser,e$ ♀ X $w/B^sYy^+;\ hls^{\Delta 125},e/TM3,Sb,Ser,e$ ♂

⇓

F2 $wP(w^+,hls^+\text{-}myc)/w;\ hls^{\Delta 125},e/TM3,Sb,Ser,e$♀ X $+/B^sYy^+;\ hls^{E616},e/TM3,Sb,e$ ♂

⇓

F3 *y w and C(1)RM/O* ♀ X $wP(w^+,hls^+\text{-}myc)/B^sYy^+;\ hls^{\Delta 125},e/hls^{E616},e$ ♂

(B)

P $w/w;\ hls^{\Delta 125},e/TM3,Sb,Ser,e$ ♀ X $+/B^sYy^+;\ hls^{E616},e/TM3,Sb,e$ ♂

⇓

F1 $wP(w^+,hls^+\text{-}myc)/w;\ +/TM3,Sb,Ser,e$ ♀ X $w/B^sYy^+;\ hls^{E616},e/TM3,Sb,Ser,e$ ♂
(from F1 in Fig 7A)

⇓

F2 $wP(w^+,hls^+\text{-}myc)/w;\ hls^{E616},e/TM3,Sb,Ser,e$ ♀ X $+/B^sYy^+;\ hls^{E616},e/TM3,Sb,e$ ♂

⇓

F3 *y w* ♀ X $wP(w^+,hls^+\text{-}myc)/B^sYy^+;\ hls^{E616},e/hls^{E616},e$ ♂

Fig 7 Crossing scheme to obtain males that contained the *homeless* c-myc fusion construct and either (A) deletion heterozygote or (B) hls^{E616} homozygous for determination of rescue potential of the fusion protein.

construct. In order to determine if some of the fertile males carry a *Ste* allele, individual males were doubly crossed to *C(1)RM/O* females and *y w* females. Observation of crystal shape in XO males from the *C(1)RM/O* cross will allow determination of whether the male contains a *Ste* or Ste^{+}. Progeny from the *y w* females can be scored for nondisjunction to compare the amount of sex chromosome nondisjunction when the *Stellate* allele is varied. Live testes preparations of the XO males generated from $wP(w^{+},hls^{+}\text{-}myc)/B^{s}Yy^{+}; hls^{E616}/hls^{\Delta 125}$ fathers allowed observation of Stellate crystals in primary spermatocytes. Three out of the eleven tested parents were proven to carry a *Ste* allele by the presence of star-shaped crystals in primary spermatocytes of XO sons. Thus, the construct rescues fertility of deletion heterozygotes. However, as shown in Table 7, the level of sex chromosome nondisjunction is much higher than observed in any of the previous *homeless* combinations. On the other hand, males that carry a Ste^{+} allele have levels of nondisjunction that do not significantly differ from those seen in control males that carry the fusion construct and are heterozygous for *homeless* (z test). Therefore, the conclusion can be made that the construct rescues fertility in males carrying a high copy number of *Stellate* repeats, but is unable to restore normal sex chromosome disjunction. In males with a low copy number of repeats, the fusion construct rescues disjunction to normal levels. These conclusions indicate that fertility and sex chromosome nondisjunction in *hls* males result from different levels of expression of the same protein (Ste).

Table 7. Partial rescue of *homeless* phenotype dependent on which *Stellate* allele is present.

Male Genotype	Total Progeny	Total Males	Total Females	Total NDJ Progeny	% NDJ	Total Progeny with Fragmented Y	% Y breakage
$wP(w^+,hls^+$-*myc*)/B^sYy^+; hls^{E616}/*TM3,Sb* control	489	216	273	0	0.0%	0	0.0%
Ste $wP(w^+,hls^+$-*myc*)/B^sYy^+; $hls^{E616}/hls^{\Delta 125}$	221	94	127	38	17.2%	0	0.0%
Ste^+ $wP(w^+,hls^+$-*myc*)/B^sYy^+; $hls^{E616}/hls^{\Delta 125}$	453	213	240	2	0.44%	4	0.88%

The lack of complete rescue by the cDNA construct could indicate a problem with expression of the fusion construct or that the last 13 amino acids of the protein are important for the involvement of Homeless in *Stellate* repression. A full-length construct is needed to determine if it is the C-terminal deletion which prevents rescue. However, our data indicate that the partial rescue is due to expression level since males carrying a low copy number of allele of *Stellate* show normal disjunction. The inability of the construct to completely rescue the observed male meiotic phenotype does not come as a complete surprise, since it is able to rescue oogenic defects in females in the Berg lab, but does not rescue fertility. The fact that a full-length construct rescues fertility in females suggests a C-terminal end function. The line containing this full-length construct was lost and, therefore, cannot be used to determine rescue of the male-specific phenotypes.

CHAPTER IV

DISCUSSION

Involvement of the Homeless protein in *Drosophila* oogenesis has been well documented (Gillespie and Berg 1995). The present data indicate a function for this protein in spermatogenesis as well. The phenotypes observed correlate well with those seen in *Su(Ste)*$^{-}$ males which result from high levels of *Stellate* expression. These phenotypes include Stellate crystals in primary spermatocytes, sex and 2nd chromosome nondisjunction, meiotic drive, fragmented chromatin, chromatin bridging, and sterility in certain genetic backgrounds. The 4th autosomes segregate normally which suggests its segregation is *hls* independent. A separate pathway for 4th chromsosome disjunction is not unexpected in males since the screen for meiotic mutants performed by Sandler et al. (1968) recovered the mutation *mei-S8* which caused high levels of 4th chromosome nondisjunction at the first meiotic division in males but had no effect on sex chromosome disjunction. Baker and Carpenter (1972) also recovered a number of male specific mutations in an EMS screen for potential meiotic mutations. Although the mutations were unstable and, therefore, not studied in detail, all showed altered disjunction of the sex chromosomes with no effect on the 4th chromosome. The disjunction patterns for the major autosomes were not tested in either screen. Therefore, it is not impossible for *homeless* to be involved in a pathway for chromosome disjunction that does not affect the 4th chromosome.

No meiotic phenotypes observed in *hls* males are different from those seen in *Su(Ste)* males, indicating that the primary role of *hls* in male meiosis may be in the regulation of the expression of the normally repressed Stellate protein. The regulation could be at the level of processing, localization, or translation of *Ste* mRNA. An early theory for *Su(Ste)* repression of *Stellate* was that an antisense mechanism was involved, whereby transcription from the *Su(Ste)* locus resulted in an antisense copy that could hybridize to *Stellate* transcripts and in this way prevent translation. However, recent evidence suggests that this is not the mechanism of repression. Sequencing of *Su(Ste)* cDNAs obtained from the screening of a testes cDNA library confirm that the transcriptional direction is the same as that for *Stellate*, which would not allow hybridization of the *Ste* and *Su(ste)* mRNA molecules (Kalmykova et al. 1998). The mechanism may instead involve *Su(Ste)* regulation of *Ste* transcript processing. Two introns are present in both the *Ste* and *Su(Ste)* transcription units (Kalmykova et al. 1998) and, therefore, control of splicing may be important in regulation. *XY* males show very low levels of *Ste* transcript and the transcripts that are present retain the first intron. In XY males with Ste alleles (high copy number of the *Ste* repeat), moderate levels of properly spliced *Ste* transcript are present, suggesting a competitive relationship may exist between *Ste* and *Su(Ste)* for transcriptional processing (Livak 1990). Therefore, the absence of *Su(Ste)* may produce Stellate protein by allowing the *Stellate* transcript to be properly processed. Unless the unspliced *Stellate* transcript is

unstable, splice regulation is unlikely to be the only level of *Stellate* regulation since the transcript abundance is dramatically higher in XO males as compared to XY males.

In addition to Homeless four other loci involved in *Stellate* regulation have been identified (Bozetti et al. 1997). *Scratch* is located on the third chromosome (cytological position 63 B11-C1) along with three other crystal-inducing loci, *most*, *sting* and *1006*. *Sting* and *1006* are cytologically located at positions 32D and 35BC respectively (Bozetti et al.1997). Based on these mapping results and mapping of *homeless* to the third chromosome (Gillespie and Berg 1995), *hls* is not an allele of any of these newly identified crystal-inducing loci. Two of these loci have been further characterized (Schmidt et al. 1999; Yue et al. 1999). Information obtained about these mutations may aid in determination of *homeless* function since they may together be involved in a pathway to regulate expression of *Stellate*.

One of these, *scratch*, encodes an Hsp90 protein family member and corresponds to the *hsp83* locus (Yue et al. 1999). Hsp90 family members associate with many different proteins including Casein Kinase II. The proteins recognize targets by tertiary structure rather than amino acid sequence. *Drosophila* spermatogenesis is very sensitive to changes in the level of Hsp90 protein and it is enriched in testes. Immuno-staining of Hsp90can be seen throughout spermatogenesis, mainly in the cytoplasm with only weak staining in the nuclei of primary spermatocytes. Males homozygous for *scratch* are viable but male sterile. Females carrying this P element insertion mutation are fertile. The insertion is within an intron which causes a

reduction in protein level rather than complete absence. All viable *hsp83* mutants show defects in spermatogenic processes mediated by microtubules, including both mitotic and meiotic divisions (Yue et al. 1999). Stellate crystals are seen in primary spermatocytes and greater than 90% of spermatocytes have defective meiotic divisions. The catalytic subunit of CKII is stabilized by Hsp90 (Miyata and Yahara 1992; 1995) so the derepression of Stellate may be the result of alteration of CKIIα function. Some heteroallelic *hsp83* combinations are male sterile yet do not show crystals, suggesting meiotic roles for this protein other than Stellate repression. Meiotic abnormalities include alteration of nuclear and nebenkern structure, abnormal size, shape and number of spermatids, and crystals within spermatids. The final stages of sperm development also show abnormalities. These studies are suggestive of Hsp90 having a role in microtubule dynamics and maintenance of spermatogenic signal transduction protein function.

Sting (*stellate-interacting gene*)was identified in a screen for male-sterile mutation, and originally referred to as *Ms(2)32D* (Schmidt et al. 1999). Stellate crystals are seen in *sting* spermatocytes and the presence of the 750 nt *stellate* mRNA was demonstrated. Additionally, Schmidt et al. (1999) observe two higher molecular weight bands (8 kb and 1.2 kb) that are present in tissues other than testes (brain, salivary glands, and ovaries for example). While these bands disappear in *sting* testes with the concomitant appearance of the 750 nt band, this high molecular weight *Stellate* bands are present in other tissues regardless of whether *sting* is heterozygous

or homozygous. The 8 kb somatic tissue band has not been observed previously and was not seen in the Northern blot reported here for *homeless*. Further analysis needs to be done to determine if this higher molecular weight species disappears in a *homeless* background. Male fertility is inversely correlated with *Stellate* copy number in *sting* mutants. Less extreme phenotypes include sex and 2nd chromosome nondisjunction. Sequence homology to Sting was found in 28 GenBank proteins, only two of which have known functions. Argonaute and Zwille are *Arabidopsis thaliana* genes known to regulate self-perpetuation of shoot meristem. Expression of Sting is normally restricted to the gonads in males and females, but mutants show deregulation of expression so the *sting* transcript is present in tissues outside the gonads. Specifically, in spermatogenesis, Sting is normally present only in spermatogonia and early spermatogenesis, but mutation allows expression at later stages of the meiotic process.

Further studies are needed to determine where *hls* fits in the *Stellate* regulation pathway. A possibility that should be explored is how Homeless interacts with the other four mutations that have been identified to induce *Stellate* crystal formation in spermatocytes. No homology is observed between *homeless* and either *sting* or *scratch* when analyzed by the Bestfit program of GCG. Homeless could play a role in the regulation of one of these proteins. Since *sting* (Schmidt et al. 1999) and *scratch* (Yue et al. 1999) have been cloned, interaction of Homeless with these proteins could be tested by using the yeast two hybrid system. Additionally, the expression and processing of the *sting* and *scratch* RNAs could be examined in testes of *homeless*

males. Lastly, genetic combinations of these three mutations would lead to determination of the phenotypic effects when they are placed in the same background. It has been postulated that the Stellate protein affects meiosis by interacting with the active domain of CKII through its region homologous to the CKII regulatory subunit, thereby interfering with normal CKII activities, which include the regulation of Topo II (Livak 1990). Without TopoII, chromosomes remain intertwined causing nondisjunction, chromatin bridging and chromatin fragmentation (DiNardo et al. 1984; Newport and Spann 1987; Uemura et al. 1987; Holm et al. 1989; Wood and Earnshaw 1990; Adachi et al. 1991; Downes et al. 1991; Shamu and Murray 1992). Interference with Topo II meiotic function could explain the lack of Stellate effect on 4^{th} chromosome segregation. If inability to release chromosome entanglement causes nondisjunction, chromosome size could affect the severity of the defect. Since the 4^{th} autosome in *Drosophila* is very small compared to other chromosomes, the likelihood of entanglement would be much less, so it would be less affected by the absence of Topo II.

The finding that Scratch also induces crystal formation and similar meiotic abnormalities suggests that CKII may somehow be involved in Stellate regulation. Hsp90 has been shown to stabilize CKII (Miyata and Yahara 1992, 1995) so the *scratch* mutation would presumably result in unstable CKII α subunit and this could be the cause of the derepression of *Stellate*. It would be interesting to determine if there is an interaction between Homeless and CKII RNA or protein using gel shift and yeast

two-hybrid systems respectively. CKII and Stellate are very likely to interact in some way, but it is unclear which is regulating and which is responding. The presence of the properly spliced *Stellate* mRNA in *sting* testes suggests that the Sting protein may be involved in preventing *Stellate* processing. The *sting* mutations causes the protein to be incorrectly expressed in additional tissues and at later stages of the meiotic process. Therefore, its proper function requires very strict expression patterns. Homeless could be involved in regulation of Sting expression.

Alternatively, Homeless may act independently of these other crystal-inducing loci. There are a number of different possibilities. The *Homeless* protein may act either directly with *Su(Ste)* to repress *Stellate* transcription, or indirectly through regulation of *Su(Ste)*. Considering the homology between Homeless and splicing factors, one might postulate that Homeless is involved in splicing of the *Ste* or *Su(Ste)* transcripts. Since Homeless absence causes an overabundance of *Ste* transcript, it is unlikely that it is needed for proper *Ste* processing. However, it might hinder the proper splicing by acting as a competitive inhibitor of a splicing factor, thereby allowing proper splicing only in its absence. Alternatively, it may be needed for proper *Su(Ste)* mRNA splicing and, in its absence, lack of proper splicing may cause a loss of *Su(Ste)* protein and, therefore, an increase in the amount of *Ste* mRNA. A change in *Su(Ste)* mRNA processing or expression could be determined by using RT-PCR to generate cDNA copies of *Su(Ste)* mRNA that is present in normal backgrounds and in testes of *homeless* males. A variation in processing should be easily recognized by a change in

the size of the mRNA molecule. A possible way to show that Homeless directly regulates the processing of either *Stellate* or *Su(Ste)* RNAs is to use gel shift experiments in which the RNA of interest is mixed with Homeless protein and then electrophoresed. A decrease in the rate of migration would suggest interaction with Homeless protein. While high amounts of *Stellate* RNA should be easily obtained for such an experiment, *Su(Ste)* RNA would be more difficult considering the low amounts probably present within the cell (not detectable in Northern blot analysis). It may be necessary to transcribe *Su(Ste)* in vitro for use in this study. A role of Homeless in the regulation of *Su(Ste)* processing would suggest that *Su(Ste)* RNA or protein is needed to repress *Ste*. If the repression is due to a competition for transcription factors or splicing factors, then the mature *Su(Ste)* RNA or protein itself would be unnecessary. Other potential functions of Homeless in *Stellate* regulation include an involvement in proper localization of the *Su(Ste)* transcript to allow its repression of *Stellate* or translational control of *Su(Ste)* or other proteins needed for *Stellate* repression. Studies of the *Su(Ste)* transcript in *hls* males should add data to support or refute some of these possibilities.

Localization of Homeless within the cell will also aid in the determination of its function. If it is directly responsible for RNA processing or localization, it would be expected to be present within the nucleus, while a role in translational regulation would only require Homeless in the cytoplasm of the cell. Since the c-myc hls^{+} construct does partially rescue *homeless* phenotype in its restoration of fertility to $hls^{\Delta 125}/hls^{E616}$ males

with a *Ste* allele and disjunction to those with a Ste^{+} allele, antibodies to the myc tag could be used to localize the fusion protein within the cell.

Although *homeless* fertility was shown to be dependent specifically on the allele of *Ste* present, it is unclear how much of the remaining meiotic phenotypes are due to the *Ste* derepression. Although all the phenotypes observed thus far have been previously described in *Stellate*, it is possible that *hls* is involved with the regulation of other meiotic genes in male meiosis. Two attractive models that would explain *hls* downregulation of *Stellate* are that Homeless protein is involved in either the packaging of repeats in heterochromatin-like configurations or the specific silencing of the sex chromosomes during meiosis. Tandemly repeated sequences are known to be packed tightly into chromatin that resembles the state of heterochromatin (reviewed in Henikoff 1998). This type of packaging leads to a silencing of the gene activity. *Stellate* repeats could be part of this system which, when released by defects in *hls*, allows expression of Stellate protein and other sequences which are normally inactive. This hypothesis could be examined by using stocks that contain tandem repeats of a reporter that are normally silenced due to heterochromatin packaging. Genetic crosses to place these constructs together with *homeless* would allow the determination of whether repeat-induced silencing is released in a *hls* background.

Alternatively, the silencing of the sex chromosomes has been shown to occur during meiosis when they exhibit differential chromatin packaging (reviewed in McKee and Handel 1993). Homeless could be involved in this process and its absence could

allow many X-linked genes including *Stellate* to be expressed in spermatocytes causing abnormal meiotic phenotypes. Northern blotting could be used to probe for genes known to be located on the X. RNA could be isolated from testes of mutant males that are arrested in spermatogenesis so the majority of cells are primary spermatocytes so that variation in expression of X-linked genes during meiosis in a *homeless* background could be examined.

Our study focuses on the involvement of *hls* in male meiosis; however, the possibility of *hls* involvement in female meiosis has not yet been ruled out. In fact, evidence suggests that this may be the case. A *hls*-c-myc construct created by the Berg lab is able to rescue the oogenic defects in *hls* mutants; yet the females are still sterile. Although not establishing a meiotic defect, this does suggest other as yet unstudied roles for Homeless in females. The construct contains a full length cDNA with the exception of the last thirteen amino acids which are truncated for c-myc epitope tag insertion. The construct also contains upstream regions which contain putative regulatory sequences. A full-length cDNA without the c-myc fusion is capable of oogenic rescue and almost complete rescue of fertility (60%) (personal communication from Celeste Berg). These unpublished results suggest that the last thirteen amino acids may be necessary for specific functions of Homeless. The inability of this construct to completely rescue male meiotic phenotypes suggests either a problem in the expression of the fusion protein or an importance for the very C-terminal end of the protein in certain meiosis-related phenotypes. It is unclear whether the rescue of

fertility but inability to prevent sex chromosome nondisjunction by the fusion construct is due to separate roles of the Homeless protein or a need for different levels of protein activity. The two phenotypes could define two different functions of the protein one of which, chromosome segregation, requires the C-terminal end and the other, fertility, is independent of this domain. This is unlikely since infertility in *Su(Ste)*$^{-}$ males appears to be directly caused by an increase in Stellate protein from the *Ste* allele and thus increased levels of nondisjunction and abnormal condensation (Palumbo 1994). It is more likely that low levels of Homeless protein are capable of rescuing fertility but higher levels are needed to prevent chromosome nondisjunction. Low levels of wild-type Homeless from the construct could be due to instability of the fusion protein or low expression levels. Alternatively, high levels of the fusion protein could be present, but its level of activity could be too low for full rescue.

If *Homeless* is indeed involved in both male and female meiosis, it becomes one of a very small number of proteins known to function in both. Most proteins identified that function in male and female meiosis are involved in sister chromatid cohesion showing that this is a common mechansim in the sexes. Involvement of *Homeless* in both would suggest other common pathways since *homeless* has effects in the first division as well as the second division in male meiosis. The further study of such proteins will allow the determination of which aspects of female and male meiosis are conserved and which differ.

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Part III

Nondisjunction in males and females (nmf): A factor involved in proper chromosome segregation during the first meiotic division in *Drosophila melanogaster*

***This part is intended to be submitted for publication as Stapleton, W.B. and B.D. McKee (1999).**

ABSTRACT

There are significant differences in the processes by which male and female *Drosophila melanogaster* accomplish proper homolog segregation during the first meiotic division. While females use the well-characterized recombination-based system involving synaptonemal complex and chiasmata to ensure proper chromosome alignment and disjunction, males have a little understood achiasmatic non-recombinogenic mechanism for homolog pairing and segregation. Because of these drastic differences, it has been often presumed that different sets of proteins act in the two sexes to assure the proper chromosome complement after meiosis I. Indeed most meiotic mutations in *Drosophila* are sex-specific. However, the data presented here document an X chromosome factor (or factors) important in both males and females for proper chromosome segregation during meiosis I. Although the chromosomal location is not yet confirmed in females, *Nondisjunction in males and females (nmf)* is located on the X chromosome in males. The gene product(s) is necessary for proper sex chromosome disjunction in both males and females. Additionally, *nmf* appears to be important in 4^{th} chromosome segregation in males and may act in the female distributive system to assure proper segregation of noncrossover bivalents. Cytological phenotypes indicate an inability to release homolog cohesion which results in both chromosome fragmentation and nondisjunction. Further characterization of *nmf* may expose common features between male and female meiosis I in *Drosophila melanogaster.*

CHAPTER I

INTRODUCTION

Although the mechanism of pairing and segregation during the first meiotic division of female meiosis in *Drosophila melanogaster* has been well-studied, the workings of the achiasmatic meiosis I in males are less understood. Numerous proteins have been identified that affect segregation in females, but very few have been shown to be active during male meiosis. This differential use of proteins presumably reflects the lack of recombination, and the absence of the synaptonemal complex (SC) and chiasmata in males which are essential to the female mechanism for homologous chromosome segregation. Different mechanisms often require different sets of proteins for function.

However, there is some overlap in protein usage in males and females during the parts of meiosis that are similar between the two sexes. For example, sister chromatid cohesion is an important component in both male and female meiosis. During the first division, the sisters must remain attached while the homologs segregate and then become released during the second division. Since this process is common to both males and females, it is not surprising to find that mutations that affect sister chromatid cohesion show defects in both males and females. Mutations in *ord* cause premature separation of sisters in both males and females (Mason 1976). *Mei-S332* mutations cause a similar effect with slightly increased levels of MI nondisjunction and very high levels of MII nondisjunction. The Mei-S332 protein appears to be important

in the maintenance of sister chromatid cohesion after the metaphase I/anaphase I transition (Kerrebrock et al.1992). *mei-G87* also appears to be involved in sister chromatid cohesion since homozygous males and females show precocious sister chromatid segregation in the first and second meiotic division (Gethmann 1984).

Other than these proteins involved in sister chromatid cohesion, only two mutations have been identified which have effects in both *Drosophila* male and female meiosis. *Double or nothing (Dub)* causes nondisjunction of all chromosomes in both sexes, primarily during the reductional division (Moore et al. 1994). *mei-I3* causes genome-wide nondisjunction during the first division and the sex chromosomes also nondisjoin during meiosis II (Ivy 1981). Additional proteins are expected to act in both male and female meiosis I since females have a back-up system, called the distributive system, which operates to segregate achiasmate chromosomes (Grell 1962). Since the first meiotic division in males requires the proper disjunction of achiasmate chromosomes, one might presume that some of the same proteins could function both in the female distributive system and in male meiotic I segregation. The sex chromosomes in females are nonrecombinogenic in approximately 6% of meiosis, yet exhibit only 0.5% nondisjunction in wild-type backgrounds due to their inclusion in the distributive system of segregation (Bridges 1916; Weinstein 1936). Additionally, the 4^{th} chromosome is always achiasmate and, therefore, necessarily part of the distributive pool (Grell 1964). Moreover, heterologous non-exchange chromosomes will segregate efficiently from each other especially if well matched in size. It has been proposed that

there are two separate pathways within the distributive system for the segregation of achiasmate chromosomes, one of which involves a homology-dependent pairing mechanism which would be responsible for proper segregation of homologs and a second mechanism based on spindle morphology and dynamics which is responsible for the segregation of heterologs at the first meiotic division. The two mechanisms appear separate since several mutations affect the segregation of achiasmate homologs but not of heterologs (Hawley and Theurkauf 1993).

Even though the female distributive system and male segregation each operate by a nonrecombinational based mechanism, all mutations recovered in the distributive system thus far are female-specific. Sixteen mutations in distributive segregation have been described. *Altered disjunction (ald)* (O'Tousa 1982) affects the first of three steps in distributive disjunction, namely, identification of the proper participants in the distributive pool. Normally, exchange tetrads would not participate in distributive interactions; however, in *ald* homozygous females, exchange pairs are allowed to participate in the distributive pool and disjoin from nonhomologs. Mutations in *ald* also cause incorrect partner choice within the distributive system. Only the homology-dependent mechanism of distributive disjunction is affected in *ald* mutants. *Aberrant X segregation (Axs)* also alters distributive partner choice causing frequent nonhomologous segregation of members of the pool and affects only the homologous pathway (Hawley 1989). The *mei-S51* (Robbins 1971) synthetic mutation may participate in the pairing process for both homologs and members of the distributive

pool since mutations cause both reduced levels of exchange and improper partner choice in the distributive system. Mutations in *nod*, a kinesin-like motor, cause nonexchange chromosomes to nondisjoin and affects both homologous and heterologous achiasmate disjunction (Carpenter 1973). Twelve additional genes affecting achiasmate segregation were found in a recent screen by Sekelsky et al. (1999). Two of these were alleles of previously identified *Trl* and *CycE* which are known to affect heterochromatin formation (Dorn et al. 1993; Lilly and Spradling 1996) and suggest the involvement of heterochromatin in the mechanism of distributive segregation. The remaining ten mutations fall into two classes. One class produces phenotypes similar to those described for *Axs*. The second class appear to affect a chromosome-specific mechanism which is responsible for the proper disjunction of non-recombinant X chromosomes.

Although twenty-seven male meiotic mutations have been identified in screens, characterization of the majority of these was not possible. Twenty of these mutations were identified by Baker and Carpenter (1972) and all exhibited similar phenotypes with nondisjunction of the sex chromosomes, meiotic drive, and normal 4^{th} chromosome segregation. Further analysis of these mutants was not possible since a time lapse resulted in loss of the phenotype. *mei-S8* and *mei-081* are male-specific meiotic mutations which cause chromosome specific nondisjunction of the 4^{th} chromosome or genome wide nondisjunction respectively (Sandler et al.1968). Sandler (1971) uncovered a third meiotic mutation, *mei-W5*, or *paternal loss (pal)* (Baker

1975). Chromosome loss without any nondisjunction is the phenotype seen in *pal* males. Castrillon et al. (1993) obtained the P-element induced mutations *double fault* and *bobble*. Cytological examination of testes from mutant flies revealed variable sized nuclei within spermatids suggestive of aneuploidy resulting from meiotic nondisjunction or chromosome loss. *mei-I1* (Ivy 1981) causes abnormal segregation of all chromosomes, while *mei-G17* (Gethmann 1974) is chromosome specific in its effects, causing only sex and 2nd chromosome nondisjunction. Both cause nondisjunction primarily at the first division. Thus, even though attempts to identify and describe *Drosophila* male meiotic mutants began over twenty years ago, we still know little about the mechanism and factors involved with homolog pairing and segregation.

Both chiasmate segregation of females and achiasmate segregation of males require proper homolog pairing during meiosis I to proceed normally. However, the rules for pairing site selection also differ between males and females. The rDNA repeats located on the X and Y chromosomes have been found to be important in XY pairing in males. While this repeated locus is located in the heterochromatin, pairing sites for the autosomes in males appear to be spread throughout the euchromatic regions. In females, there appear to be two different types of pairing, one for chiasmate chromosomes and one for distributive pairing. Chiasmate pairing sites are located within euchromatic regions, while distributive pairing for the 4th and X chromosomes is mainly in heterochromatin. Although the X heterochromatin contains sites involved in

male and female meiotic pairing, these appear to be quite different. A review of this information can be found in McKee (1998).

Here, we report the identification and characterization of a recessive factor(s) on the X chromosome, *nondisjunction in males and females (nmf)*, which affects segregation of chromosomes at the first meiotic division in both males and females. Aberrant phenotypes in females are suggestive of a role for *nmf* in the distributive system in females. While the sex chromosomes and 4th chromosome pair showed increased levels of nondisjunction in *nmf* males, the 2nd chromosome disjunction was normal which indicates chromosome specific mechanisms for segregation in male meiosis I.

CHAPTER II

MATERIALS AND METHODS

Fly stocks

The *y*^{1}*w*^{1}*/Dp(1;Y)y*$^{+}$*;Df(2R)XTE-18/CyO* and other stocks used in crossing schemes were obtained from the Bloomington Stock Center at Indiana University. All stocks were maintained at 18° Celsius on a molasses, agar medium.

Cytological analysis

Orcein-stained testis slide preparation was done according to Lifschytz and Hareven (1977). Briefly, testes were dissected in testis buffer (7% NaCl, Ashburner, 1989) and fixed in 45% acetic acid for thirty seconds. Staining of the testes in 3% orcein-60% acetic acid for five minutes was followed by squashing in a 1:1 mixture of 60% acetic acid and 2% lactic-acetic-orcein. Analysis and photographs were done using phase-contrast microscopy on a Zeiss Universal Axioplan photomicroscope and photographed with Kodak T-Max 100 color film.

Genetic crosses

Unless otherwise specified, the male or female being tested was crossed singly to multiple flies of the specified genotype for the experimental cross. Parents were removed from the food vial ten days after the cross is initiated. Progeny were counted between days 18 and 22 to avoid the presence of the next generation.

CHAPTER III

RESULTS

Identification of nondisjunction phenotype

During a routine examination of the *y*^{1}*w*^{1}*/Dp(1;Y)y*$^{+}$*;Df(2R)XTE-18/CyO (Df(2R)XTE-18)* stock obtained from Bloomington Stock Center, a high frequency of sex chromosome aneuploidy was observed. The stock contains a *y*$^{+}$*Y* chromosome and an X chromosome marked with *y* and *w* (white eyes) which allows the Y chromosome to be followed based on the *y*$^{+}$ body color phenotype. Examples of the *y* and *y*$^{+}$ body color phenotypes can be seen in Figure 1. The exceptional progeny were males that lacked the *y*$^{+}$ marker and, less frequently, females that carried this marker. These observations suggested a high amount of sex chromosome nondisjunction leading to the exceptional progeny. The genotypes of the exceptional progeny would be XO males that lacked the marker and XXY females that carried the Y chromosome phenotypic marker (Figure 2). Exceptional *y* males were tested for fertility and found to be sterile, confirming that they are XO in genotype. In order to confirm that the exceptions were indeed due to sex chromosome nondisjunction rather than contamination of the stock, genetic crosses were conducted.

Quantitation of sex chromosome nondisjunction

In order to determine if the observed sex chromosome nondisjunction was occurring in males, females, or both, males from the stock were outcrossed to *y/y; ry/ry* females and females from the stock were outcrossed to +/*B*^{s}*Yy*$^{+}$ males (Figure 3). This

Fig 1 Examples of the (A) y^+ and (B) *y* body colors of *Drosophila melanogaster.*

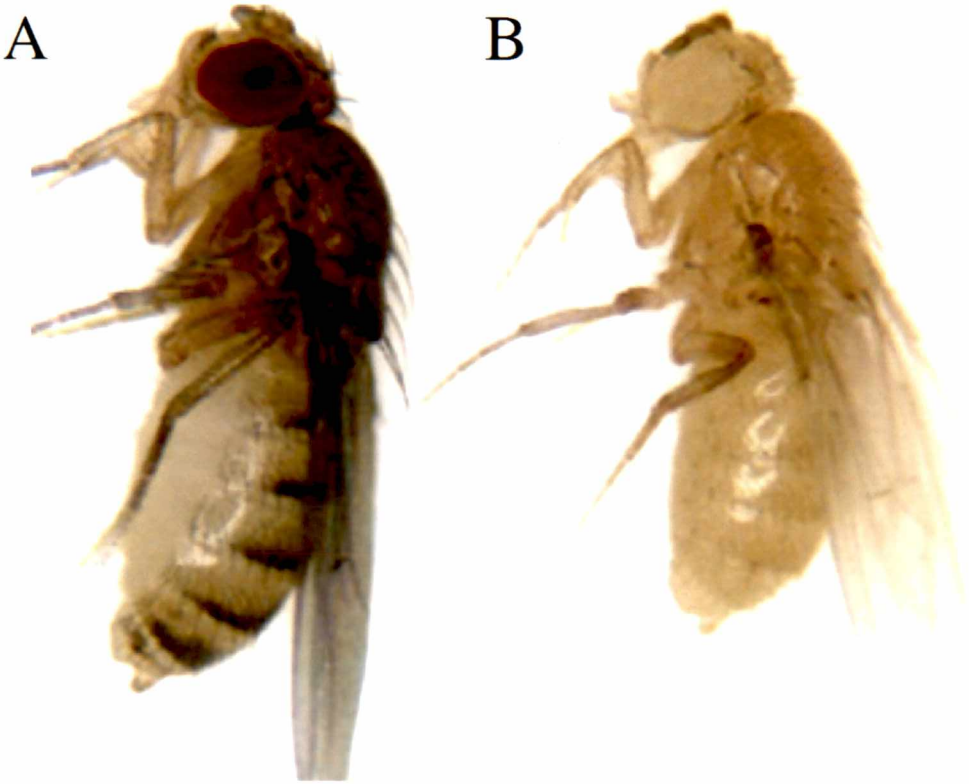
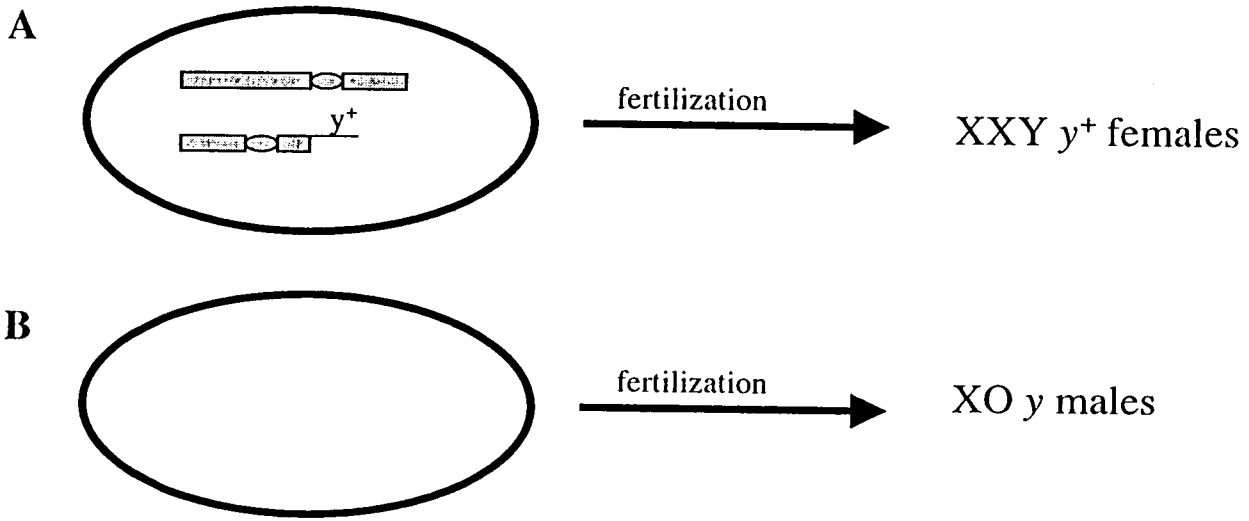

A
B

Fig 2 Illustration of sex chromosome nondisjunction. (A) An XY bearing sperm upon fertilization of a normal XX bearing egg results in the production of XXY y^+ females. (B) A sperm lacking a sex chromosome upon fertilization of a normal XX bearing egg results in the production of XO *y* males.


A
y+
fertilization
XXY y+ females
B
fertilization
XO y males

Males

P *y w/y*$^{+}$*Y; Df(2R)XTE-18/Cy* ♂ X *y;ry* ♀

⇓

F1 Score for *y*$^{+}$ females (XXY) and *y* males (XO)

Females

P *y w; Df(2R)XTE-18/Cy* ♀ X *y/B*^{s}*Yy*$^{+}$ ♂

⇓

F1 Score for *B*^{s}*Yy*$^{+}$ females (XXY) and *y* males (XO)

Fig 3 Crossing scheme for determination of amount of sex chromosome nondisjunction occurring in *y*^{1} *w*^{1}*/Dp(1;Y)y*$^{+}$*; Df(2R)XTE-18/CyO* stock males and females.

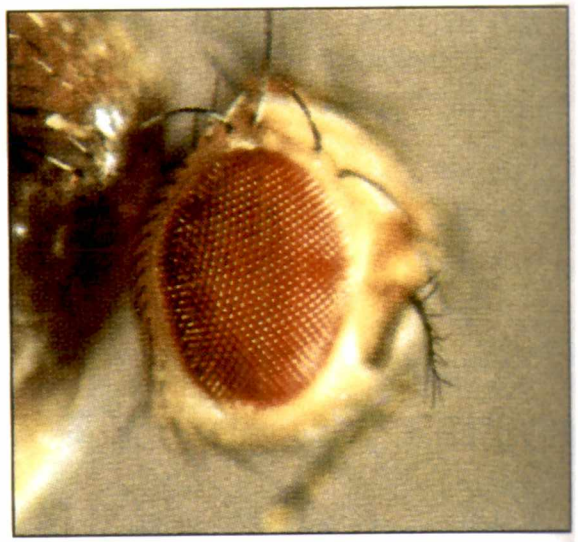
crossing scheme allowed quantitation of the sex chromosome nondisjunction occurring in the stock. Sex chromosome nondisjunction in males could be quantified by using the y^+Y as previously shown. The males used to quantify sex chromosome nondisjunction in females carried a Y chromosome marked with both y^+ and the B^s eye marker (Figure 4). Results indicated that the nondisjunction was occurring in both males (Table 1A) and females (Table 1B), although to a lesser extent in females. The 7.5% nondisjunction seen in males and the 2.6% observed in females are both high compared to rates in wild-type flies which are almost always lower than 0.5% (Bridges 1916; Weinstein 1936). Due to the effects in both males and females, the factor has been named *nondisjunction in males and females (nmf).*

Chromosomal mapping of the nondisjunction phenotype

The nondisjunction phenotype was initially presumed to be due to the deficiency on chromosome 2R which includes the locus of *dpms2*, a homolog of yeast *PMS2* and mouse *pms2*. Mutations of Pms2 and Mlh1 in mice and yeast have been shown to cause meiotic disruption (Baker 1995; Baker 1996; Hunter and Borts 1997). (Further discussion of the isolation and characterization of *dpms2* can be found in the subsequent Part IV.) Genetic crosses were performed to determine if the sex chromosome nondisjuction seen in males segregated with the deficiency chromosome (Figure 5). In these crosses, the tested males carried the y^+Y chromosome along with either *Df(2R)XTE-18* or the *CyO* balancer chromosome from the original stock, but had an X chromosome derived from a wild-type laboratory stock. Analysis of the

Fig 4 Illustration of the B^s phenotype used to follow inheritance of the Y chromosome. (A) nonbar wild-type eye (B) B^s eye. Pictures taken from Klug and Cummings 1994.

A



B

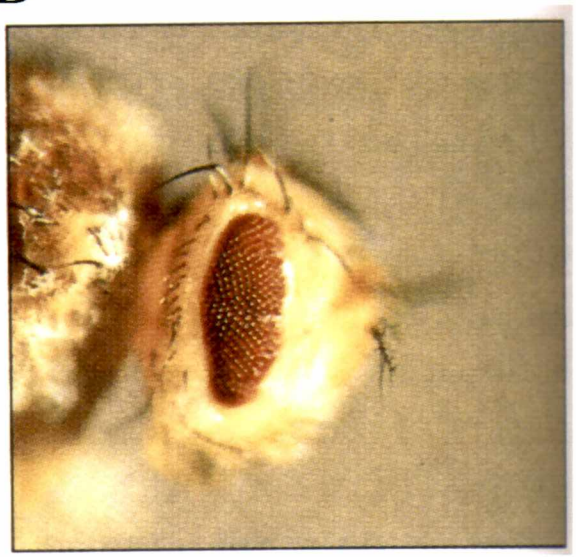


Table 1. Amount of sex chromosome nondisjunction occurring in *nmf* males and females.

Gamete Type	***nmf***
A. *nmf* Males	
Sperm Type	*nmf males*
Regular sperm	
X	836
Y	765
Nondisjunctional sperm	
XY	24
O	105
Total Progeny Scored	1730
% Nondisjunction	7.5%
B. *nmf* Females	
Ova Type	*nmf* females
Regular Ova	
X	1338
Nondisjunctional Ova	
XX	10
O	34
Total progeny scored	1681
% Nondisjunction	2.6%

P *y w/y*$^{+}$*Y; Df(2R)XTE-18/Cy* ♂ X *y;ry* ♀

⇓

F1 *y/y*$^{+}$*Y; Df(2R)XTE-18/+* or *y/y*$^{+}$*Y; Cy/+* ♂ X *y w* ♀

⇓

F2 Score for y^{+} females (XXY) and *y* males (XO)

Fig 5 Crossing scheme to determine if the *Df(2R)XTE-18* chromosome is responsible for the observed male sex chromosome nondisjunction.

resulting data from males showed that although a small amount of nondisjunction remained, it was not linked to the presence of the deficiency as equal frequencies were seen in males carrying the *Cy* balancer chromosome as well (Table 2). The 1.9% nondisjunction seen in these males has not at this point been characterized further.

To determine if the female phenotype is linked to the *Df(2R)XTE-18* chromosome, the crosses shown in Figure 6 were used. The data in Table 3 suggest that the factor is recessive since outcrossing of females results in a loss of phenotype. Outcrossed females show normal disjunction of the sex chromosomes. However, it can not be determined from these crosses if the nondisjunction is linked to the *Df(2R)XTE-18* chromosome since the *CyO* chromosome could carry the same recessive mutation. The results in females also suggest that the 1.9% sex chromosome nondisjunction seen with both the *CyO* and *Df(2R)XTE-18* chromosomes is male specific. These data suggest that the *nmf* phenotype is due to some factor located on the X, 3rd, or 4th chromosome.

Since the majority of the phenotype did not appear to be linked to the second chromosome, the third chromosome was tested for linkage with the genetic crosses shown in Figure 7. Results in Table 4 show that males and females carrying only the 3rd chromosome and the Y from the original *nmf* stock have normal levels of nondisjunction. This eliminates the possibility of the factor being located on the 3rd chromosome; it also rules out a male-specific factor on the Y.

Table 2. Amount of sex chromosome nondisjunction in males carrying either the *Cy* balancer chromosome or the *Df(2R)XTE-18* chromosome.

Sperm Type	*Cy*/+ ♂	*Df(2R)XTE-18*/+ ♂
Regular Sperm		
X	1006	736
Y	926	843
Nondisjunctional Sperm		
XY	3	7
O	37	23
Total Progeny Scored	1972	1609
% Nondisjunction	1.9%	1.9%

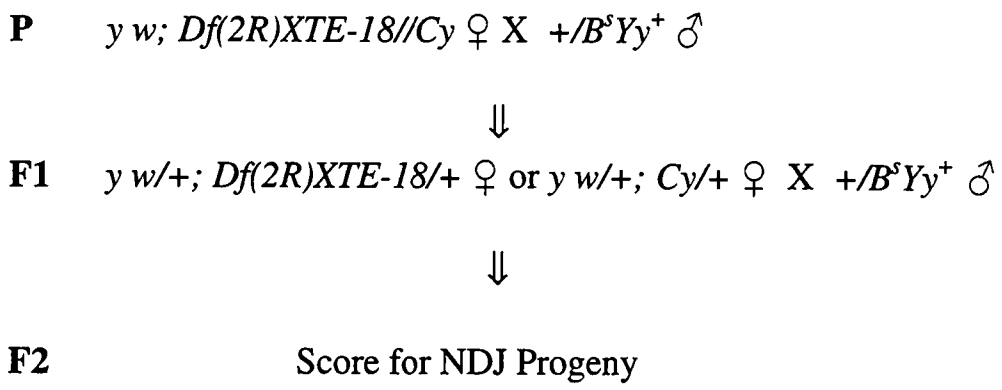


Fig 6 Crossing scheme to determine if the female sex chromosome nondisjunction segregates with the *Df(2R)XTE-18* chromosome.

Table 3. Quantitation of sex chromosome nondisjunction in females with either the *Df(2R)XTE-18* or *Cy* chromosome.

Ova Type	*Df(2R)XTE-18/+* ♀	*Cy/+* ♀
Regular Ova		
X	1212	984
Nondisjunctional Ova		
XX	3	4
O	2	3
Total progeny scored	1217	991
% Nondisjunction	0.40%	0.70%

P *w; TMS/Dr* ♀ X *y w/y*$^{+}$*Y; Df(2R)XTE-18/Cy; 3***/3** ♂

⇓

F1 *w/y*$^{+}$*Y; Cy/+; Dr/3** ♂ X *w;TMS/Dr* ♀

⇓

F2 *w/y*$^{+}$*Y; TMS/3** ♂ X *w; TMS/3** ♀

⇓

F3 *w/y*$^{+}$*Y; 3***/3** ♂ X *y w* ♀ *w;3***/3** ♀ X *y w/B*^{s}*Yy*$^{+}$ ♂

⇓

F4 Score for nondisjunction

Fig 7 Crossing scheme used to determine if the *nmf* phenotype is linked to the 3rd chromosome in males and/or females. * indicative of 3rd chromosome from the *nmf* stock.

Table 4. Quantitation of sex chromosome nondisjunction in males and females homozygous for the 3rd chromosome of the *nmf* stock.

	$3^{*}/3^{*}$ males	$3^{*}/3^{*}$ females
Normal males	987	469
Normal females	1122	555
Nondisjunctional progeny	1	2
Total Progeny Scored	2110	1026
% Nondisjunction	0.05%	0.20%

Very different results were obtained when the X chromosome was tested for linkage to the male sex chromosome nondisjunction phenotype. The crosses shown in Figure 8A were used to ascertain the possibility of the factor being located on the X chromosome. The tested males carried the X chromosome along with either *Df(2R)XTE-18* or *CyO* from the stock but had a Y chromosome and one set of autosomes from a wild-type stock. Results in Table 5 show that the majority of the nondisjunction seen in stock males is also present in F1 males that carry the X chromosome from the *y w/y*$^{+}$*Y; Df(2R)XTE-18/Cy* stock. However, earlier data had shown that some nondisjunction occurs in the absence of the X but in the presence of either the *Df(2R)XTE-18* or *CyO* from the *nmf* stock (Table 3). Therefore, the crosses shown in 8B allowed the separation of the X chromosome from the stock to test males that lack both the 2nd chromosomes from the stock. These males show a sex chromosome nondisjunction frequency of 4.7%. In order to confirm that this observed nondisjunction was not due to the *B*^{s}*Yy*$^{+}$ chromosome present in these males, control +/*B*^{s}*Yy*$^{+}$ males were tested and showed no nondisjunctional progeny out of 371 total progeny. The effect caused by the X chromosome and either of the 2nd chromosomes from the stock appears to be additive. Thus, the major component of the nondisjunction phenotype present in the stock males is X-linked, but both second chromosomes also contribute.

Another important question concerns whether *nmf* is homozygous within the stock. When the distribution of the percentage of nondisjunction among males from

A.

P *y w; Df(2R)XTE-18/Cy* ♀ X *+/B^{s}Yy^{+}* ♂

⇓

F1 *y w/B^{s}Yy^{+}; Df(2R)XTE-18/+* and *y w/B^{s}Yy^{+}; Cy/+* ♂ X *y w* ♀

⇓

F2 Score progeny

B.

P *y w; Df(2R)XTE-18/Cy* ♀ X *+/B^{s}Yy^{+}* ♂ *y w; Df(2R)XTE-18/Cy* ♀ X *FM6/Y* ♂

⇓

F1 *y w/B^{s}Yy^{+}; Cy/+* ♂ X *C(1)RM/B^{s}Yy^{+}* ♀ ⇓

⇓

F2 *y w/B^{s}Yy^{+}; +/+* ♂ X *yw/FM6; Cy/+* ♀

⇓

F3 *y w/B^{s}Yy^{+}; +/+* ♂ and *y w/y w ; +/+* ♀ (Stock)

Fig 8 Crossing scheme to determine if the male sex chromosome nondisjunction phenotype segregates with the X chromosome. (A) Scheme to obtain males with the X and one of the 2nd chromosomes from the *nmf* stock. (B) Scheme to obtain a stock containing the X from the *nmf* stock but neither of the 2nd chromosomes.

Table 5. Quantitation of sex chromosome nondisjunction in males carrying the X chromosome from the *nmf* stock

Sperm Type	*yw nmf/B^{s}Yy$^+$;Df(2R)XTE-18/+* ♂	*yw nmf /B^{s}Yy$^+$; Cy/+* ♂	*yw nmf/ B^{s}Yy$^+$; +/+* ♂
Regular Sperm			
X	942	658	1125
Y	833	585	1096
Nondisjunctional Sperm			
XY	29	27	39
O	67	51	70
Total Progeny Scored	1871	1321	2330
% Nondisjunction	5.1%	5.9%	4.7%

the cross in 8B is plotted, the resulting schematic in Figure 9 suggests that a lack of homozygosity in the stock is a real possibility. The majority of males show either normal disjunction of the sex chromosomes (0% nondisjunction) or between 7.0% and 8.0% sex chromosome nondisjunction suggesting that some males may have *nmf* while others do not. In order to test this hypothesis, single male and female crosses from the *nmf/nmf*; +/+ stock were set up and F1 males were chosen from parents that showed high levels of nondisjunction to determine if all their sons also exhibited this phenotype. The levels of nondisjunction seen in F1 males were compared to those in the parental generation (Table 6). There is some correlation seen between parents and F1 sons. In five out of six cases, high sex chromosome nondisjunction in the parental cross corresponds to a high rate in the F1 sons. However, in vial number 5, the highest level of parental nondisjunction occurs and F1 males show a relatively low level of sex chromosome nondisjunction. Therefore, although nonhomozygosity for *nmf* seems unlikely, the possibility of one or more segregating modifiers remains. To generate a homozygous stock, these tests can be redone and male and female F1 progeny from parents with high nondisjunction can be crossed to each other to generate several stocks to be tested for a more even distribution of nondisjunction among individuals.

An important note is that the nondisjunction in the parental generation shown in Table 6 is much higher than what was observed in males from this stock (4.7%, Table 5). Presumably this is due to nondisjunction that occurs in the females of the stock. So although not directly tested, this suggests that *nmf* is located on the X in females since

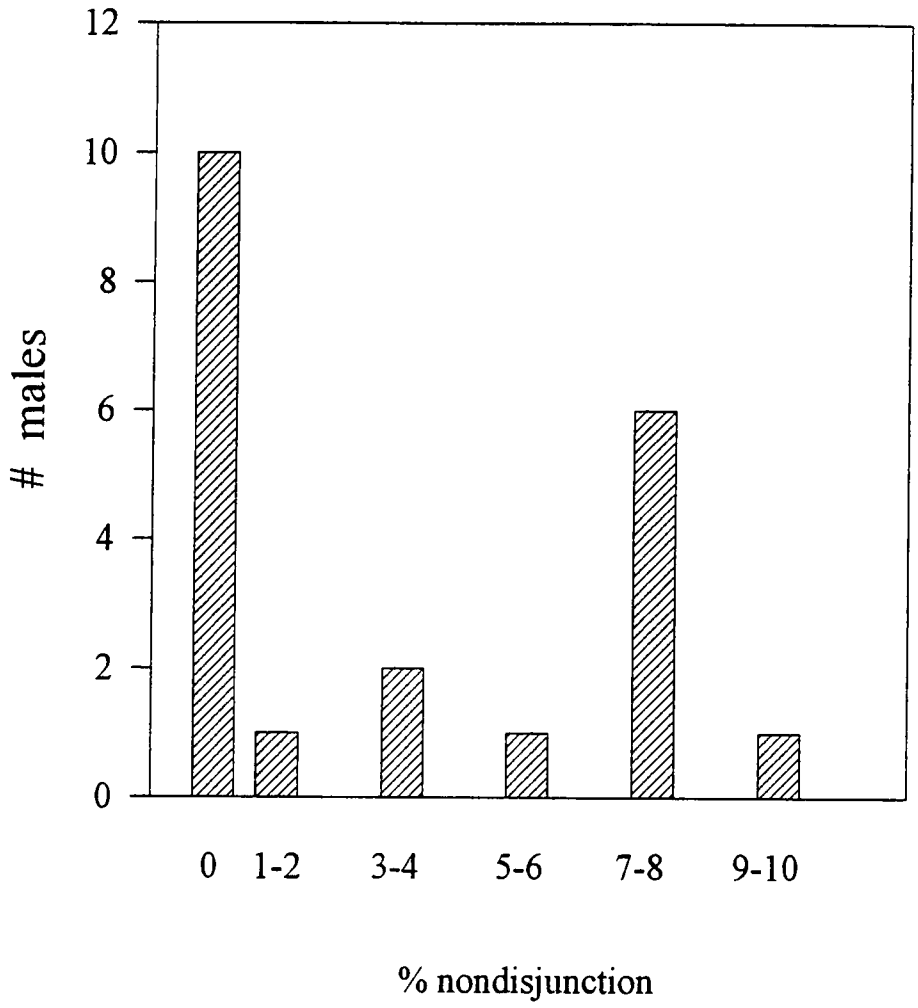


Fig 9 Distribution of percent nondisjunction in *nmf* males. The X axis shows the percent nondisjunction of the sex chromosomes and the Y axis shows the number of males that exhibited each amount of nondisjunction.

Table 6. Correlation of sex chromosome nondisjunction in parents with nondisjunction in F1 males. Each vial contained a single male and single female from the *nmf/nmf;+/+* stock. Therefore, nondisjunction occurring in the parental generation is presumably from both the male and female. Three to five of the F1 males were scored for nondisjunction to determine correlation of nondisjunction in the F1 generation with that seen in the parental generation.

Vial Number	**Parental % Nondisjunction**	**F1 % Nondisjunction**	**# F1 Males with Nondisjunction/Total Males Scored**
1	13.3%	11.2%	4/4
2	18.2%	10.6%	4/4
3	4.8%	1.7%	1/3
4	6.0%	5.4%	3/5
5	23.7%	5.5%	3/5
6	13.6%	10.5%	3/5

females within this stock do not contain either of the 2nd chromosomes from the original *Df(2R)XTE-18*.

Evidence that the *nmf* mutation is located on the X chromosome suggests the possibility that nondisjunction could be due to a lack of rDNA pairing sites. However, a lack of rDNA repeats would lead to a bobbed phenotype in XO males. When *nmf* males were outcrossed to *C(1)RM/O* females and F1 XO sons examined, no bobbed phenotype was observed (data not shown).

Testing for Autosomal Nondisjunction (NDJ)

Previously discussed data explored only disjunction of the sex chromosomes. In order to determine if the effect is chromosome specific or genome wide, stock males and females were tested for autosomal NDJ. *C(2)EN b,pr* stocks were used as testers for 2nd chromosome nondisjunction. Crosses contained 10 *C(2)EN b, pr* males or females and 10 *nmf* males or females from the original *Df(2R)XTE-18* stock. Since all of the eggs from these *C(2)EN* females carry either O or 2 copies of chromosome 2, only nondisjunctional gametes being diploid or nullo for chromosome 2 will lead to viable progeny. Table 7 shows that the number of progeny produced from *nmf* males did not differ from wild-type numbers suggesting that normal disjunction of the 2nd chromosome occurs. In contrast, *nmf* females do show a marked amount of 2nd chromosome nondisjunction. The number of progeny per *nmf* female is an average of 1.6 which predicts a nondisjunction frequency of 3.2% since only half the nondisjunctional progeny are recovered. This is much higher than the previously

Table 7. Quantitation of 2nd chromosome nondisjunction in control males and *nmf* males and females based on the number of progeny resulting from crosses to *C(2)EN b, pr.*

	Oregon R control ♂	*nmf* stock ♂	*nmf* stock ♀
Total tested	100	380	280
Total no. progeny	5	6	451
Average progeny per tester	0.05	0.016	1.6

measured 0.01% 2nd chromosome nondisjunction seen in wild-type females (Carpenter 1973). Stock females carry a 2nd chromosome balancer, *Cy*, which prevents crossing-over between homologs. A 2nd chromosome balancer does increase the amount of nondisjunction in wild-type females but only to 0.172 progeny per female or .344 % nondisjunction as reported in Carpenter (1973). Therefore *nmf* causes a nine-fold increase in 2nd chromosome nondisjunction in the presence of a balancer as compared to wild-type females also carrying a 2nd chromosome balancer. Nondisjunction of the 2nd chromosome in these females suggests a potential defect in the distributive system of disjunction.

Secondly, 4th chromosome disjunction was tested in *nmf* males and females. C(4) EN ci ey stocks were used to assess by phenotype the amount of 4th chromosome nondisjunction. Since these C(4) flies generate only gametes containing 0 or 2 copies of chromosome 4, normal 4th chromosome disjunction leads to haplo 4 Minute progeny and phenotypically wild-type triplo 4 progeny. The Minute phenotype is caused by hemizygosity for M(4), one of about 40 haplo-insufficient Minute loci in the genome. Minute flies have numerous morpological abnormalities, and their recovery is sporadic; therefore, they are omitted from data collections. Diplo- nondisjunctional sperm or ova upon fertilization of a *C(4)RM ci ey* gamete result in lethality while fertilization of a nullo-4 gamete results in wild-type progeny indistinguishable from progeny in normal fertilizations. On the other hand, fertilization of nullo-4 nondisjunctional gametes and *C(4)RM ci ey* gametes leads to *ci ey* progeny which are

phenotypically distinguished by reduced eye size and gaps in the wing veins. Note that in this cross, only the nullo sperm or ova will produce progeny phenotypically different from wild-type sperm and, therefore, the actual amount of nondisjunction could be double the reported amount due to uncounted diplo-4 sperm or ova. Table 8 shows the high level of 4th chromosome nondisjunction in *nmf* males. The normal level of 4th chromosome nondisjunction is 0.1% (Hawley 1989). Surprisingly, *nmf* females showed no significant amount of 4th chromosome nondisjunction. The 4th chromosomes are always in the distributive pool and might be expected to show high nondisjunction if *nmf* causes defects in the distributive system.

Analysis of previous mutants has also shown that while 4th autosomes may not normally nondisjoin in the mutant background, the presence of an X balancer chromosome increases the frequency of 4th nondisjunction due to the sex chromosomes being added to the distributive pool (Hawley 1989). In order to test this possibility with *nmf* females, crosses to *FM6* males were used to generate females of the genotype *y w/FM6; Df(2R)XTE-18/+*. These females were crossed to *C(4)RM, ci, ey* males to quantitate 4th chromosome nondisjunction. These females had a calculated sex chromosome nondisjunction rate of 9.7%. In addition, as can be seen in Table 8, they also had an increased 4th chromosome nondisjunction frequency of 2.8% in the presence of the X chromosome balancer. There is a significant difference between the 4th chromosome nondisjunction observed in these females as compared to *nmf*

Table 8. Quantitation of 4th chromosome nondisjunction in *nmf* stock males and females as well as females carrying an X chromosome balancer.

	nmf ♂	*nmf* ♀	*nmf/FM6; Df(2R)XTE-18/+* ♀
Total no. vials scored	46	20	36
Total no. progeny	1698	226	351
Total no. 4th nondisjunctional progeny	77	1	10
% 4th NDJ	4.5%	0.44%	2.8%

homozygous females lacking the balancer as determined by the z test. The reported percentage for 4th chromosome nondisjunction in wild-type females containing an X chromosome balancer is 0.20% (Zitron and Hawley 1989). Thus, the presence of *nmf* results in a 14-fold increase.

Since both the XY and 4th autosomes nondisjoin in *nmf* males, an important question is whether they are segregating independently from one another or if they actually segregate from each other in some cases. Therefore, crosses involving the same technique as those previously discussed to measure 4th chromosome nondisjunction were set up, but females were used that carried a marked X along with the *C(4) ci ey* chromosome. Therefore, both 4th and XY nondisjunction could be measured at the same time. Initial findings (Table 9) suggest a very interesting phenomenon. First, all progeny identified as resulting from a 4th chromosome nondisjunction event in spermatogenesis also showed the effects of a sex chromosome nondisjunction event. Secondly, only progeny resulting from XY, nullo-4 sperm were obtained. No progeny from nullo-XY, nullo-4 sperm were observed. This results in all progeny lacking a paternally derived 4th chromosome being XXY females. It was noted in the previous results shown in Table 8 that most if not all progeny lacking a paternally derived 4th chromosome were female, but this was not quantitated. The presence of only XY,nullo-4 sperm and not nullo- XY, nullo-4 sperm indicates that the sex chromosome pair is actually segregating from the 4th autosome pair. This is similar to

Table 9. Correlation between sex and 4th chromosome nondisjunction in *nmf* males.

Total Scored Progeny	531
Sex chromosome nondisjunction only	36
4th chromosome nondisjunction only	0
4th and sex chromosome nondisjunction	6
% sex nondisjunction	7.9%
% 4th nondisjunction	1.1%

what occurs in females with defects in components of the distributive system which will be discussed later. The sex chromosome nondisjunction and 4th chromosome nondisjunction shown in Table 9 differs from that seen in the results in Table 8. These differences are probably due to the low progeny counts since only 9 individual crosses have been performed for the results in Table 9. Therefore, the confirmation of a correlation between sex and 4th chromosome nondisjunction in *nmf* males awaits higher progeny counts.

MI vs MII nondisjunction in *nmf*

Although the presence of high levels of XY nondisjunction in *nmf* males confirms the occurrence of MI nondisjunction, it does not address the question of whether nondisjunction is occurring at both divisions. In order to determine if the male and female nondisjunction occurs during both MI and MII, the genetic crosses shown in Figures 10 and 11 were performed. Data from these studies, shown in Table 10 shows that the nondisjunction in both sexes is occurring specifically at the first meiotic division. Notice that one class of nondisjunctional progeny in both the male and female crosses cannot be classified as MI or MII nondisjunction since either could result in the phenotype. However, since the progeny class that is specifically derived from MII nondisjunction was completely absent, it is assumed that the total nondisjunction is equal to the amount of 1st division nondisjunction. In *y w nmf/FM6; Df(2R)XTE-18/+* females, 100 of the total 234 nondisjunctional progeny are XXY females which must be derived from first division nondisjunction. 65 of the 161 are XXY in *y w nmf/FM6;*

P *C(1)RM/O* ♀ X *y w nmf/y*$^{+}$*Y; Df(2R)XTE-18/Cy* ♂

⇓

F1 Score progeny

Normal progeny: *y w/O* ♂ and *C(1)RM/y*$^{+}$*Y* ♀

MI nondisjunctional progeny: *y w/y*$^{+}$*Y* ♂ and *C(1)RM/O* ♀

MII nondisjunctional progeny: *y w/y w* ♀ and *C(1)RM/O* ♀

Fig 10 Crossing scheme to determine if sex chromosome nondisjunction occurs at the second meiotic division in *nmf* males.

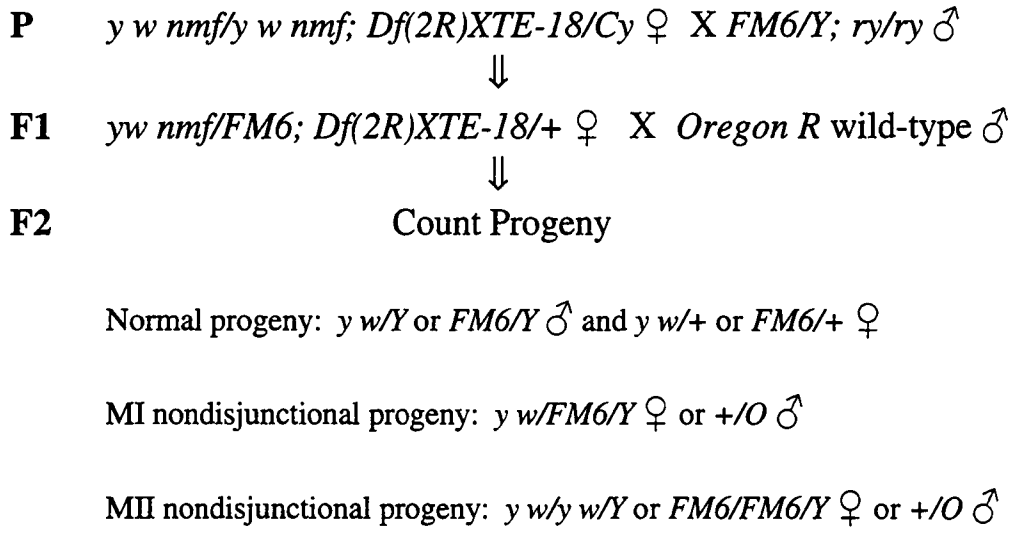


Fig 11 Crossing scheme to determine if sex chromosome nondisjunction occurs at the first or second meiotic division in *nmf* females.

Table 10. Quantitation of MI and MII sex chromosome nondisjunction in *nmf* stock males and females carrying *nmf*, an X chromosome balancer, and either *Df(2R)XTE-18* or *Cy*. Controls for females carrying an X chromosome balancer or an X and 2nd chromosome balancer are shown.

	y w nmf/y⁺Y; Df(2R)XTE-18/Cy ♂	*y w nmf/FM6; Df(2R)XTE-18/+* ♀	*y w nmf/FM6; Cy/+* ♀	*y w/FM6* control ♀	*y w/FM6; Cy/+* control ♀
Total no. vials	56	110	41	46	20
Total progeny	1802	4213	1722	2722	856
Total NDJ progeny	146	234	161	8	55
Total MII NDJ progeny	0	0	0	0	0
Total % NDJ	8.1%	5.5%	9.3%	0.29%	6.4%
% MII NDJ	0.00%	0.00%	0.00%	0.00%	0.00%

CyO/+ females. In the males tested, forty-four of the one hundred forty-six nondisjunctional progeny are of the class that is specific to reductional division nondisjunction. The presence of progeny known to be derived from first division nondisjunction and the lack of any MII nondisjunction specific progeny strongly supports the idea that nondisjunction is specific to the reductional divison with none or very little occurring during the equational division.

Balancer effect on *nmf* females

In studies to determine if MII nondisjunction occurred in *nmf* females, an X chromosome balancer, *FM6*, was present in the tested females. Surprisingly, the presence of this balancer appears to greatly enhance the sex chromosome nondisjunction phenotype in females heterozygous for *nmf* (Table 10). In fact, levels of nondisjunction in these females are even higher than those seen in females homozygous for *nmf*. The presence of a balancer chromosome prevents crossing-over. Control females carrying the *FM6* balancer show no significant sex chromosome nondisjunction confirming that *nmf* and not the balancer itself is the cause of the segregation abnormalities. These results suggest that the suppression of exchange on the X chromosomes sensitizes X-X segregation to a reduction in dose of *nmf* by ½. An alternative possibility is that *nmf* is totally recessive but that *FM6* also carries an *nmf* allele; arguing against this is the fact that *FM6* does not show elevated nondisjunction in males.

Females discussed above that show high amounts of nondisjunction in the presence of an X chromosome balancer also carry the *Df(2R)XTE-18* chromosome as can be seen in Figure 10. In order to confirm that the nondisjunction was not due to a factor located on this deficiency, sex chromosome nondisjunction frequencies of females that carried the *Cy* chromosome from the P generation in Figure 10 were compared to those carrying the deficiency. The results also shown in Table 10 show that these females show an even higher level of nondisjunction. However, the control females carrying both an X and 2nd chromosome balancer also show elevated levels of sex chromosome nondisjunction suggesting that the presence of two balancers in an otherwise wild-type background perturb chromosome segregation. But the difference between the *nmf* and controls are significant (z test).

Effect of *nmf* on recombination in females

The sex chromosome nondisjunction in *nmf* females could be caused by a reduction in the amount of recombination between homologs. In order to test this possibility, the crosses shown in Figure 12 were performed, allowing quantitation of recombination between four markers located on the 2nd chromsome in females homozygous for *nmf*. The number of F3 flies with the various phenotypes are shown in Table 11. Calculation of map distances gives a recombination distance of 43 map units between *al* and *b*; 17 map units between *b* and *c*; and 27 map units between *c* and *sp*. Comparing these distances to those for wild-type flies given in Lindsley and Zimm

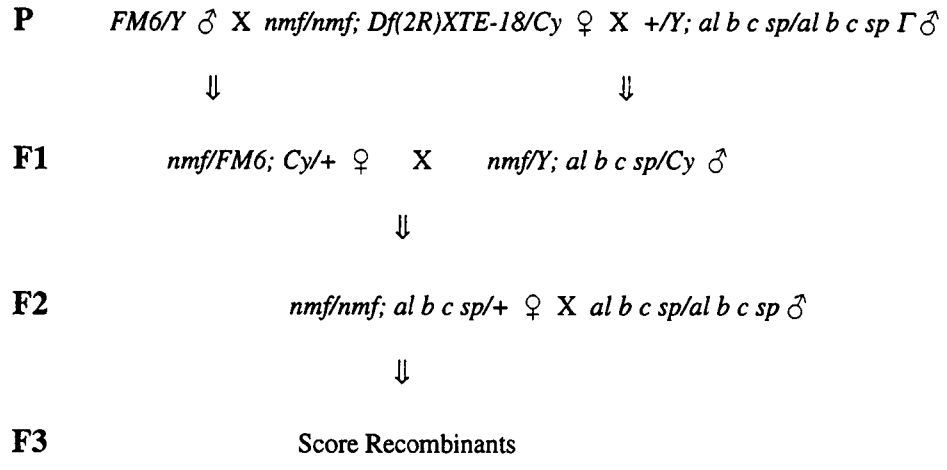


Fig 12 Crossing scheme to determine effect of *nmf* on 2nd chromsome recombination in females.

Table 11. Quantitation of 2nd chromosome recombinant progeny from *nmf* homozygous mothers.

Recombinant Phenotype	Number Counted	% of Total Progeny Counted
$al^+ b^+ c^+ sp^+$	136	30.5%
$al\ b^+ c^+ sp^+$	46	10.3%
$al^+ b^+ c^+ sp$	62	13.9%
$al\ b^+ c^+ sp$	17	3.8%
$al\ b^+ c\ sp^+$	4	9.0%
$al^+ b^+ c\ sp$	16	3.6%
$al\ b\ c^+ sp^+$	24	5.4%
$al\ b\ c\ sp^+$	15	3.4%
$al\ b\ c\ sp$	42	9.4%
$al^+ b\ c\ sp$	36	8.1%
$al^+ b\ c\ sp^+$	13	2.9%
$al\ b\ c^+ sp$	1	2.2%
$al^+ b\ c^+ sp$	3	6.7%
$al\ b^+ c\ sp$	10	2.2%
$al^+ b^+ c\ sp^+$	5	1.1%
$al^+ b\ c^+ sp^+$	16	3.6%

(1992) the differences are not significant. The p value is greater than 0.05 (approximately 0.1) suggesting that the observed differences could have occurred by chance. These results indicate that *nmf* has no general effect on recombination in females.

Effect of *nmf* on recombination-deficient females

If *nmf* is involved in the distributive system in females, presumably increasing the number of chromosomes within the pool would greatly disrupt the meiotic process in female homozygous for *nmf*. *mei-W68* mutant females have been shown to completely lack recombination (McKim et al. 1998a and 1998b). In order to determine the effect of *nmf* on recombination-deficient females, crosses shown in Figure 13 were performed to generate females homozygous for both *nmf* and *mei-W68*. These females are very weak with many dying before producing any progeny. Only 50% of the obtained females produced progeny and these produced an average of only 24 progeny per female. The amount of sex chromosome nondisjunction in these females is 20.5%. Results are shown in Table 12. As was expected, the level of nondisjunction is also increased in the presence of an X chromosome balancer and *mei-W68* (16%). The control with *nmf/nmf* and *mei-W68* with a balancer shows no sex chromosome nondisjunction. This is somewhat confusing considering the data in Table 6 which suggested that these females should show sex chromosome nondisjunction. Therefore, eliminating recombination does cause an increase in the amount of nondisjunction and may suggest involvement of *nmf* in an alternate segregation mechanism. However, this

P *y/y⁺Y; meiW68/CyO* ♂ X *FM6/53B,X-1; Sp/+* ♀

⇓

F1 *FM6/y⁺Y; mei-W68/Sp* ♂ X *nmf/nmf; Df(2R)XTE-18/Cy* ♀

⇓

F2 *nmf/y⁺Y; Cy/Sp* and *nmf/y⁺Y; mei-W68/Cy* ♂ X *FM6/nmf; mei-W68/Cy* ♀

⇓

F3 *nmf/nmf; mei-W68/mei-W68* and *nmf/nmf; mei-W68/Cy* or *Sp* ♀ X *+/B^sYy⁺* ♂

and

nmf/y⁺Y; mei-W68/mei-W68 ♂ X *y w* ♀

⇓

F4 Score fertility and/or nondisjunction

Fig 13 Crossing scheme to obtain males and females homozygous for *nmf* and heterozygous or homozygous for *mei-W68*. Tester females were crossed to males in order to determine if they were fertile and, if so, the amount of sex chromosome nondisjunction. Tester males were crossed to females carrying a marked Y to score the amount of sex chromosome nondisjunction.

Table 12. Amount of sex chromosome nondisjunction in females with both *nmf* and *mei-W68*.

Genotype	Total Progeny	Progeny per Parent	% Nondisjunction
nmf/FM6; mei-W68/mei-W68 ♀	50	25	16.0%
nmf/nmf; mei-W68/Cy ♀	270	67.5	0.00%
nmf/nmf; mei-W68/mei-W68 ♀	195	24	20.5%
nmf/y$^+$Y; mei-W68/mei-W68 ♂	714	89	4.3%

is not definitve because the level of nondisjunction in *mei-W68* females has not been reported. Therefore, conclusive answers await the testing of *mei-W68* homozygotes in the absence of *nmf* to determine what nondisjunction frequencies result when recombination is eliminated. Males carrying *nmf* and *mei-W68* were also tested and showed no change compared to *nmf* males without *mei-W68* in the amount of sex chromosome nondisjunction (Table 12).

Further mapping of *nmf*

The crosses shown in Figure 14 allowed recombination of the X chromosome containing *nmf* in F1 females and subsequent scoring of F2 males to determine linkage of the male-associated phenotype to any of the four markers used. The markers used were *white (w)*, *cross-veinless (cv)*, *vermillion (v)*, and *forked(f)*. The *w* and *v* are eye-color markers while *cv* is a wing variation and *f* is a bristle phenotype. Table 13 shows the resulting mapping data. Comparisons of sex chromosome nondisjunction percentages with respect to the different alleles of the four markers used showed the following results: *w* vs w^+; 2.6% and 2.6% respectively; *cv* and cv^+; 2.3% and 2.7% respectively; *v* and v^+; 1.6% and 3.5% respectively; *f* and f^+; 0.55% and 4.0% respectively. The largest difference is between *f* and f^+ with a weaker link to v^+ suggesting a linkage of *nmf* to f^+ as shown in Figure 15. Note that in this cross, males that were *w* could not be scored for the *vermillion* phenotype reducing the numbers for comparison of *v* and v^+.

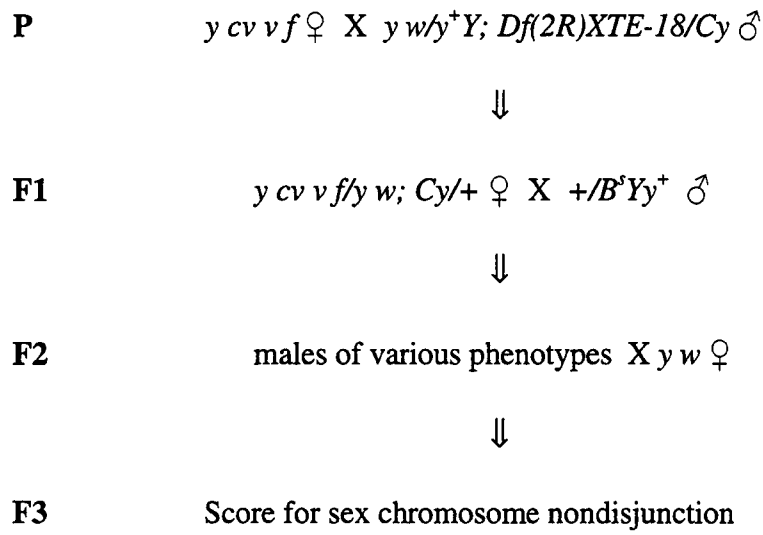


Fig 14 Crossing scheme to map *nmf* on the X chromosome.

Table 13. Recombinational mapping of *nmf* in males

	w cv$^+$ *f*$^+$	*w cv*$^+$ *f*	*w cv f*	*w cv f*$^+$	*w*$^+$ *cv v f*	w$^+$ cv v f$^+$	*w*$^+$ *cv*$^+$ *v*$^+$ *f*	*w*$^+$ *cv v*$^+$ *f*$^+$	*w*$^+$ *cv*$^+$ *v*$^+$ *f*$^+$
Regular Sperm									
X	1638	1489	264	256	206	386	102	284	333
Y	1560	1377	227	221	219	383	109	261	286
NDJ Sperm									
XY	28	3	0	4	2	3	2	4	5
O	128	4	0	21	6	8	5	15	19
No. vials scored	38	29	5	5	5	7	3	7	9
Total Progeny Scored	3354	2873	491	502	433	780	218	565	643
% Nondisjunction	4.7%	0.24%	0.0%	5.0%	1.8%	1.4%	3.2%	3.4%	3.7%

Fig 15 Illustration of the chromosome from the *nmf* stock and the tester chromosome with four markers spanning the majority of the X chromosome. Data from Table 9 indicated that *nmf* is located between the *vermillion* and *forked* loci being closer to *f* as shown above.

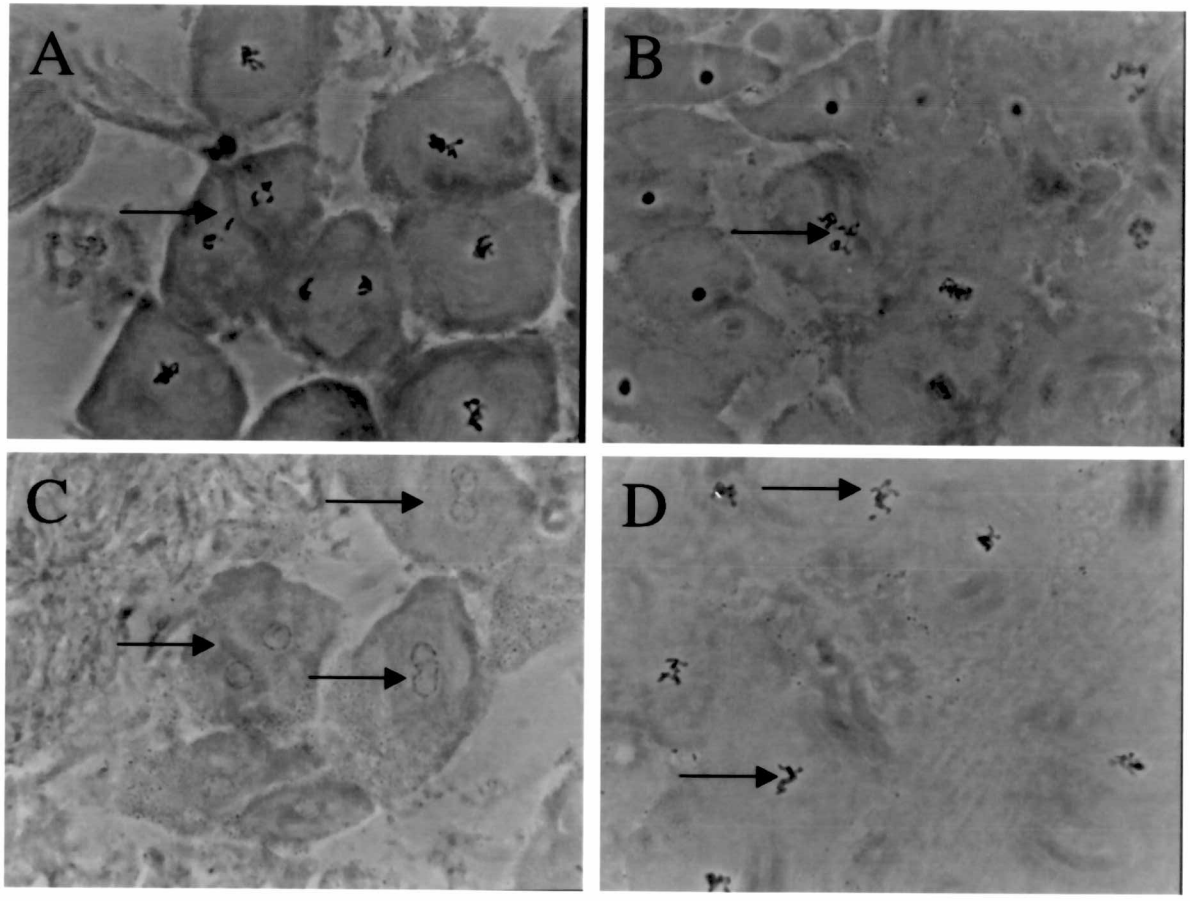

y w
nmf


y
cv
v
f

Cytological features of *nmf*

In order to further characterize the defects occurring in *nmf* males, testis dissections and staining were performed as described in Materials and Methods. Cytological examination of *nmf* spermatocytes showed no apparent defects in pairing. Prophase I and metaphase I appear normal. Abnormal cytology was observed beginning in anaphase I when lagging chromosomes (Figure 16A) and chromatin bridging (Figure 16B) can be seen. Telophase is also disrupted as can be seen by incomplete chromatin separation (16C). The cytological manifestation of the observed genetic chromosome nondisjunction is aneuploid spermatocytes seen in Figure 16D. Chromatin bridging was observed in approximately 1% of meiotic cells and incomplete chromatin separation in 0.3%.

Fig 16 Abnormal cytological phenotypes in spermatocytes of *nmf* males. (A) Lagging chromosome (B) Chromatin bridging (C) Left arrow shows normal telophase separation and right arrows show incomplete chromatin separation at telophase (D) Aneuploid spermatocytes from nondisjunction. Top arrow indicates a spermatocyte containing 4 chromosomes and bottom designates a spermatocyte containing only 2 visible chromosomes. Wild-type spermatocytes generally contain 3 visible chromosomes with the 4^{th} chromosome being too small to visualize.


A
B
C
D

CHAPTER IV

DISCUSSION

The identification of a factor(s), *nondisjunction in males and females (nmf)*, that potentially acts in the segregation of achiasmate chromosomes in both *Drosophila* males and females may link two pathways that were formerly thought to be completely separate. *Drosophila* males do not recombine and lack synaptonemal complexes and chiasmata which are present in female meiosis to ensure proper segregation of homologs during the first meiotic division. The distributive system in females is a back-up mechanism for insuring the segregation of nonexchange bivalents. The 4^{th} chromosomes are obligate members of the distributive pool since they never undergo an exchange event. Although one would postulate that the distributive system in females and the mechanism for homolog segregation in males would have proteins that overlap, none have been identified thus far. *nmf* could represent the first of such factors. The mutation *Double or nothing (Dub)* (Moore et al. 1994) is the only factor identified thus far to function in both males and females during the first meiotic division. However, it causes nondisjunction of all chromosomes in males and females suggesting it is not specifically involved in the distributive system of females (Moore et al. 1994). *mei-G87* mutants show nondisjunction of all chromosomes at the first division and second division but the defect is on sister chromatid segregation rather than any homolog segregation defect (Gethmann 1984). *mei-I3* also shows a genome-

wide effect at the first division but the sex chromosomes also nondisjoin in the second division (Ivy 1981).

Males hemizygous for *nmf* exhibit genetically identified abnormalities of sex chromosome and 4th chromosome nondisjunction. Segregation of the 2nd chromosome is normal. Cytological defects include lagging chromosomes, incomplete separation of chromatin and chromatin bridging. Although *nmf* phenotypes differ in males and females, they do suggest a common involvement in the segregation of noncrossover bivalents.

Data from females that support this hypothesis are the observation of much higher levels of chromosome nondisjunction in the presence of a balancer chromosome. Presumably, this is due to the inclusion of these chromosomes in the distributive pool since balancers prevent crossing over. The rate of sex chromosome nondisjunction in females heterozygous for *nmf* and *FM6*, an X chromosome balancer, is even higher than that observed in *nmf* homozygotes. 2nd chromosome nondisjunction is also elevated in a background containing *Cy*, a 2nd chromsome balancer. 4th chromosome segregation is normal in *nmf* females except in the presence of elevated levels of X nondisjunction induced by an X chromosome balancer, which has been observed also in other mutants known to be defective in distributive disjunction (as will be discussed). Further supporting the notion of overlapping functions in the female distributive system and male segregation is the observation that 4th chromosome nondisjunction in *nmf* males appears to result from the 4th autosome pair segregating from the XY pair

which is similar to the effects in females where a X chromosome balancer causes an increase in the amount of 4th chromosome nondisjunction. The confirmation that *nmf* is a single factor functioning in the segregation of nonexchange chromosomes in males and females awaits first the localization of both phenotypes to a single gene locus and secondly further data indicative of a defect in the female distributive system.

The male associated *nmf* phenotype has been mapped to the X chromosome as described in the Results section. Its relative location was further defined using reombination-based assays which mapped *nmf* between two markers. This still is a broad area and further recombination assays are necessary which will use chromosomes containing additional markers in the area of interest to further narrow the area of study. Once the area in which *nmf* resides has been narrowed, the flybase can be searched to identify lines with P elements inserted in the area of interest. These lines can be obtained and tested for phenotypes similar to those seen in *nmf* males. If a line is identified, sequencing of the area surrounding the P element would allow probes to be made for screening genomic and cDNA libraries to pull out the *nmf* gene. Although *nmf* in females is known not to be on the 3rd autosome, its presence on the X has not yet been confirmed. There still exists the possibility that it is located on the 2nd autosome. Studies of females homozygous for the X chromosome from the *Df(2R)XTE-18* stock but lacking either of the 2nd chromosomes from the stock should determine if *nmf* is indeed on the X. If it is not, stocks will be generated and tested that contain only the 2nd chromosomes from the *Df(2R)XTE-18* stock. Once chromosomal

location of the female *nmf* phenotype has been determined, deficiency kits will be ordered from Bloomington Stock Center (Indiana University). These kits include stocks that contain deficiencies (heterozygous deletions) covering the chromosome of interest. Each deficiency can be tested in heterozygous condition with an X chromosome balancer to see if it exhibits high levels of sex chromosome nondisjunction. Positive lines will be tested in heterozygous condition with *nmf* to confirm it is the gene of interest rather than a second gene with the same effect on sex chromosome disjunction. P element rescue experiments can then be used as described for males to clone the gene.

Another mapping question concerning the phenotype observed in *nmf* females is whether the dominant effect on X chromosome nondisjunction is due to the same defect as that which causes the lower levels of nondisjunction seen in homozygotes. This question may be answered in the deficiency studies. Identification of a deficiency which causes high levels of sex chromosome nondisjunction in the presence of a balancer but not when placed in homozygous condition with *nmf* would lead to further studies to determine the locus causing this specific effect.

The second concern to be addressed is whether *nmf* phenotypes in females are due to a defect in the distributive system. The critical test is to determine whether nondisjunctional chromosomes are always or mostly nonexchange. This will require generating a fully marked X chromosome that contains *nmf* by recombination. The *nmf* nondisjunctional progeny can then be scored to determine if a recombination event

occurred. Combination with other mutations known to be involved in distributive segregation will be one way to determine a pathway of involvement for *nmf*. If abnormalities are unchanged when two mutations are combined, it is often suggestive that they are involved in the same pathway while additive effects suggest two alternative pathways. Additionally, the distributive system is important for the segregation of both heterologs and nonexchange homologs. It should be determined if *nmf* is involved in both of these pathways or is specific to one. The mechanism of heterolog segregation is dependent on size with similar sizes being more likely to segregate from each other. Thus, free duplications are more likely to interfere with segregation of the 4th chromosome if they are similar in size (Grell 1964). The heterolog system can be tested in *nmf* females by introduction of free duplications of various sizes to determine if interference with 4th segregation is still size-dependent. Additionally, nondisjunction of 4th chromosomes in *nmf* females in the presence of nondisjoining X chromosomes suggests that the X and 4th chromosomes are segregating from each other via an intact heterolog system. Markers for both the X and 4th chromosomes will allow visualization of the nondisjunction of the two chromosomes simultaneously in order to determine if they are disjoining from each other.

It is important to compare the phenotypes observed in *nmf* females with abnormalities seen in previously identified mutants. The altered phenotype seen in *nmf* females is similar to *nod* with nondisjunction occurring mainly at MI. However, in a

nod mutant background, there is a very high level of nondisjunction and loss of the 4th chromosome. Females homozygous for *nmf* show normal levels of 4th chromosome disjunction unless an X chromosome balancer is present. Additionally, the *nod* transcript has been shown to be present in females but not in males (Zhang et al. 1990). However, it is seen in embryonic, larval and pupal stages of development of both sexes suggesting roles in processes other than female meiosis.

The phenotype of the *Axs^{D}* mutation described initially in Hawley (1989) is similar to the phenotype seen in *nmf* females. This semidominant mutation specifically affects achiasmate homolog segregation. It has no affect on segregation of heterologs within the distributive system. Mutant females also show no disruption of 4th chromosome segregation except in the presence of a high degree of X chromosome nondisjunction (Whyte et al. 1993). *nmf* shows this same pattern of 4th chromosome segregation defects. A number of EMS-induced revertants have been produced which do not exhibit the dominant phenotype of *Axs^{D}*. However, in homozygous condition, they exhibit meiotic defects similar to those seen in *Axs^{D}* mutants (Whyte et al. 1993). The amount of 4th chromosome nondisjunction observed in the presence of an X chromosomal balancer varies between 3.6% and 7.7% depending on the *Axs* allele present. This is similar to the 2.8% frequency of 4th chromosome nondisjunction in females heterozygous for *nmf* and *FM6*. The *Axs^{D}* mutation is located just distal to *forked* putting it within the region of localization of the male associated phenotype of *nmf*. The major difference is that *Axs* is female specific while *nmf* mutants show

segregational defects in both sexes. Therefore, if *nmf* is a single mutation at the Axs^D locus that causes the phenotypes observed in both sexes, it is an as yet undescribed allele which affects both males and females.

A screen of EMS-induced mutants by Baker and Carpenter (1972) revealed a number of X chromosome linked mutations which affected disjunction of the sex chromosomes, but not of the 4th chromosome in males, differentiating them from *nmf* which affects both. Therefore, if *nmf* is made of two mutations, one male-specific and the other female-specific, the male component is unlikely to be one of these previously recovered mutants. Nineteen of the twenty factors were male-specific. The one recovered X chromosome which showed effects in both sexes, *mei-99*, may have had two mutations, one female-specific and the other male-specific. The same screen also generated the female-specific mutants *mei-41, mei-195, mei-218, mei-251,* and *mei-352* all of which show a polarized reduction in recombination with the proximal regions being more strongly affected. *mei-218, mei-41,* and *mei-195* increase first division nondisjunction of the entire chromosome complement. Both *mei-251* and *mei-352* increase sex and 4th chromosome disjunction in females with an undetermined affect on the other autosomes. Two mutations are present in *mei-254*, one of which is a *mei-9* homolog which does not affect distributive pairing but causes nondisjunction of both the sex and 4th chromosomes. The second *mei-254* mutation is essential for distributive disjunction with a low amount of X chromosome nondisjunction and a very high level of improper 4th chromosome disjunction. An increase in nondisjunction for all

chromosomes mostly at the first meiotic division is observed in *mei-38, mei-99, and mei-160* mutants. Recombination is altered in all three with an increase in nonexchange 2nd chromsomes for *mei-99* and *mei-160* and decrease in *mei-38.*

None of the mutants recovered in this screen seem to correspond to *nmf.* First, no single factor was found to affect both male and female disjunction at the first division. Secondly, none of the male mutants had both sex chromosome and 4th chromosome nondisjunction as is seen in *nmf* males. A male-specific mutant, *mei-S8*, discovered in a screen of naturally occurring mutations shows high levels of nondisjunction of chromosome 4 but no effect on sex chromosome disjunction differentiating it from *nmf* (Sandler et al. 1968). Two other male specific mutations, *mei-I1* and *mei-G17,* are different from male *nmf* phenotypes since *mei-I1* causes a genome-wide effect and *mei-G17* affects only the second and sex chromosomes (Ivy 1981; Gethmann 1974). Female mutations known to affect distributive disjunction including *ald* (O'Tousa 1982), *mei-S51* (Sandler et al. 1968), , *nod* (Carpenter 1973; Zhang and Hawley 1990), and *mei-254* (Baker and Carpenter 1972) all cause significant levels of 4th chromosome nondisjunction or loss which is not seen in *nmf* females. *Axs* is similar to the phenotype seen in females and needs to be explored as the cause of female-specific abnormalities if *nmf* is shown to actually be the result of two independent mutations, one male-specific and one female-specific.

Previous analysis of meiotic mutants in males and females has suggested that the meiotic pathways acting in the two sexes diverge during meiosis I and then

converge again during meiosis II (Sandler et al. 1968). However, if continued analysis of *nmf* proves it to be a single factor responsible for the phenotypes seen in males and females, it will be suggestive of some overlapping systems acting in both sexes. The cytological defects seen in *nmf* males suggest a role of *nmf* in the release of homolog interactions. Pairing appears normal and defects are observed only after the stage at which homologs should separate. The chromatin bridging, lagging chromosomes, and incomplete chromatin separation all point to an inability of the chromosomes to come apart. Very little is known about the mechanism of release at this point. The study of *nmf* may reveal the cellular processes that result in attachment release. This would necessarily involve interaction with cell cycle machinery to allow proper timing of release. Therefore, future studies will need to identify interactions between the Nmf protein and cell cycle machinery.

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PART IV

Mismatch Repair and Meiosis

* This part is intended to be submitted for publication with additional work as Stapleton, W.B., Borromeo, D., Hong, C.S., and McKee, B.D.

ABSTRACT

As more information becomes available about eukaryotic mismatch repair (MMR) proteins, it becomes increasingly evident that these proteins have evolved new functions in the process of meiosis. Proteins originally identified as homologs of MMR components have now been shown to be important in meiosis-specific processes such as homolog pairing and crossing-over. In order to determine if MMR proteins function in *Drosophila melanogaster* meiosis, we have cloned and begun molecular characterization of two homologs of the *E. coli mutL* gene. Sequencing of the clones obtained revealed putative reading frames with the highest identity to hPms2 and hMlh1; therefore, the *Drosophila* genes are referred to as *dpms2* and *dmlh1* respectively. Localization by in situ hybridization confirms that these are single copy genes within the *Drosophila* genome. However, Southern analysis suggests a gene family with as yet unidentified members of the MMR protein family. Expression patterns in the ovaries and testis along with potential differential transcriptional start sites support the possibility of specific meiotic functions. Attempts to obtain mutations are currently underway and will be described in the text.

CHAPTER 1

INTRODUCTION

Mismatch repair is a mechanism by which errors that occur during replication and other processes such as recombination may be corrected. The long-patch methyl-directed pathway in *E. coli* requires the involvement of three key proteins: MutH, MutL, and MutS. The current model for this pathway suggests that MutS is responsible for recognizing and binding to the mismatch. MutH then binds to a GATC site up to 1 kb from the mismatch and makes an incision on the unmethylated, newly synthesized strand, thereby determining strand specificity. MutL is thought to act as a "molecular matchmaker" (Sancar and Hearst 1993) between MutS and MutH to allow the activation of the latent endonuclease activity of MutH. The system has been extensively reviewed elsewhere (Modrich 1991; Schaaper 1993; Claverys and Lacks 1986; Modrich 1996). The eukaryotic system, although more complex, appears to function in a similar manner to that of prokaryotes. Eukaryotic MutS and MutL homologs act as heterodimers rather than homodimers but perform similar cellular functions (Prolla 1994a; Prolla 1994b; Habraken 1997; Palumbo 1996; Gradia 1997; Iaccarino 1996). No MutH homolog has been identified in eukaryotes, but a nick is sufficient to allow strand discrimination suggesting that the basic mechanism is the same as that seen in prokaryotes. Although a nick may initiate the process of excision and subsequent repair in the eukaryotic system, some other protein must function in formation of the incision. Therefore, MutL homologs could still act as a matchmaker

but between MutS and the unidentified protein responsible for making the initiating incision.

One of the repair functions of MMR genes is in the correction of heteroduplex created in recombination intermediates. Conversion events are the result of MMR correction of these intermediates so that rather than a normal 4:4 segregation pattern, a 6:2 pattern is seen due to the replacement of one allele with the other during the repair event. Yeast mutants showing a decrease in conversion events and a concomitant increase in post-meiotic segregation (5:3) had a defect in this pathway of recombination intermediate correction. The first of these, *PMS1*, was eventually shown to be a homolog of the bacterial *mutL* gene (Williamson et al. 1985; Kramer et al. 1989a; Kramer et al. 1989b; Borts et al. 1990; Schar et al. 1997). The yeast *mutS* homolog, *MSH2* and two other *mutL* homologs, *MLH1* and *PMS2* show the same phenotype and are thought to function in the same pathway to repair mismatches present in recombination intermediates (for review see Modrich and Lahue 1996). This is one well-defined role for MMR proteins in the meiotic process.

Additional MMR homologs have evolved in eukaryotes to play other roles in meiosis. Meiosis-specific MutS homologs have been identified in yeast, mice and humans (Paquis-Flucklinger et al. 1997; Ross-Macdonald and Roeder 1994; Edelmann et al. 1999; Hollingsworth et al. 1995). *S. cerevisiae* MSH4 was originally identified in a screen for genes expressed specifically in meiotic cells (Ross-Macdonald and Roeder 1994). The upstream region of MSH4 contains a URS1 element thought to be

involved in the regulation of meiosis-specific genes, further supporting meiotic-specific roles for MSH4. It was, therefore, not too surprising to find that mutations of MSH4 have no effect on MMR in somatic cells. The fact that mutations also has no effect on the repair of recombination intermediates suggests that it may be very specific in the role it plays in meiotic events rather than functioning as the only MMR homolog in meiotic cells. The specific role appears to be in the actual mechanism of exchange itself since the number of crossovers in the mutant strains was decreased by one-half which leads to an increase in nondisjunction at the first meiotic division.

Mutations in Zip1, a synaptonemal complex protein, and Mer2 cause the same phenotype suggesting the three proteins may be involved in the same process. The MSH4 protein localizes to discrete sites on synapsed pachytene chromosomes in a distribution pattern that correlates well with the occurrence of recombination nodule distribution. MSH4 has amino acid substitutions in very conserved residues of the potential DNA binding sites found in MutS proteins which may allow the recognition of a meiosis-specific DNA configurations like the conformation produced by an invading strand into a second duplex rather than heteroduplex DNA (Ross-Macdonald and Roeder 1994). However, there has been some evidence that the helix-turn-helix motif found in MutS homologs does not function as a DNA binding site, but instead acts as a site for interaction with other proteins (Alani et al. 1997). Therefore, the changes could be responsible for interaction of MSH4 with meiotic proteins rather than repair proteins.

A second meiosis-specific MutS homolog has been identified in yeast. MSH5 was found in a screen for meiotic homolog recombination mutants (Hollingsworth et al. 1995). Like MSH4, MSH5 mutants showed no defect in MMR activities. The phenotype of MSH5, although slightly more severe, resembles that of MSH4 mutants with decreased spore viability, decreased crossing over, and a high amount of meiosis I nondisjunction. The double MSH4/MSH5 mutant has the same phenotype as the MSH5 single mutant, suggesting that they are involved in the same pathway. Since these mutations do not completely eliminate crossing over, there is potential for a second MSH4/MSH5 independent pathway to account for the remaining crossovers. Msh5 also contains a URS1 element suggesting transcriptional regulation; however, its limitation to meiotic expression has yet to be shown.

A mouse MSH5 homolog has been identified and a knockout has been studied (Edelmann et al. 1999). The wild-type protein was shown to be expressed in the gonads of both male and female mice and the timing of expression is coincident with the onset of meiosis in the two sexes. MSH5 -/- males and females are sterile. The phenotypes suggest a role for this protein in homolog pairing and/or synapsis. The role of the mouse protein appears to be earlier than that of yeast MSH4 and MSH5 since chromosomes never pair or synapse. Although no role of human MMR proteins in meiosis has been established, the human MSH4 gene has been identified and was found to be expressed only in the testis and ovary (Paquis-Flucklinger et al. 1997).

A *S. cerevisiae* MutL homolog, Mlh1, has also been shown to function in meiotic crossing over (Hunter and Borts 1997). In addition to the expected mutator phenotype due to defects in MMR, Mlh1 deficiency has been shown to result in a decrease in crossing over. Tetrad analysis indicates high levels of missegregation and nondisjunction at the first meiotic division. Mlh1 and Msh4 appear to act in the same pathway for crossing over since the double mutant has the same phenotype as the Msh4 single mutant which is more severe than the Mlh1 single. The recombination activity of Mlh1 is meiosis specific. Yeast Pms1, Msh2, Msh3 and Msh6 do not appear to play a role in crossing over. The fact that Mlh1, but not Pms1 functions in crossing over suggests that these two proteins are not always complexed in a heterodimer to perform functions.

Mlh1-deficient mice also show defects in meiotic homolog crossing over. Mlh1 -/- mice are male and female sterile. In males, the lack of round spermatids and spermatozoa suggests an arrest during pachytene or metaphase of meiosis I. Deficient females have smaller ovaries with low numbers of follicle cells and an increase in the percentage of stromal cells. Mlh1-deficient males show normal pairing and synapsis. However, early separation of the X-Y pair and autosomes occurs resulting in univalents during metaphase I. A reduction in the number of chiasmata is probably the cause of the precocious separation of homologs. Wild-type Mlh1 protein is localized to the synapsed portion of the synaptonemal complex in males and females. The number of foci seen corresponds well with the expected number of chiasmata further supporting

Mlh1 involvement in the process of crossing-over or in the stabilization of the configuration (Baker et al. 1996).

Mice deficient in the MutL homolog, Pms2, show a meiotic phenotype (Baker et al. 1995) in addition to the common MMR-deficient phenotypes (Narayanan et al. 1997). Male mice deficient for Pms2 are sterile. Pms2 -/- males show low numbers of abnormal spermatozoa and the number of spermatids is also greatly reduced suggesting disruption of spermatogenesis during meiosis I. Frequent abnormalities are seen in homolog synapsis. Both asynapsis and nonhomologous synapsis are observed. The different meiotic phenotypes for the Pms2 and Mlh1 mice suggest that these two proteins are not functioning together in meiosis as they do in MMR. They appear to have evolved functions in two separate pathways.

Drosophila melanogaster offers useful advantages as a model organism to further study the meiotic functions of mismatch repair homologs. First, *pms2* deficiencies in mice show problems in pairing and synapsis (Baker et al. 1995). *Drosophila* males lack the complicated features of recombination and synaptonemal complexes. Studies of the effect of *pms2* mutations in males may further define the exact role of the PMS2 protein since an effect in these organisms would suggest a function in pairing rather than the protein being a part of the synaptonemal complex structure itself. Furthermore, females do recombine and would allow study of mismatch repair genes involved in this process. The well-defined genetics of the organism and quick generation times would aid in the study of these processes. In

order to determine if MMR homologous proteins have acquired meiotic functions in *Drosophila melanogaster*, two *mutL* homologs have been cloned and sequenced. Molecular characterization of these homologs suggests potential meiotic function. Mutant screening has produced two potential mutations for one of the proteins. The mutants were identified as male steriles when placed over a deficiency for the gene of interest.

Based on evidence from other organisms, it seems likely, first of all, that MMR homologs are present within the *Drosophila melanogaster* genome and, secondly, that these proteins may have meiotic function in addition to expected roles in repair of mismatched bases. *Drosophila* male meiosis differs from the typical mechanism found in females that utilizes the synaptonemal complex and chiasma for stability of homologs until the appropriate time for segregation. Neither of these structures is seen in males and the replacement mechanism is unknown. The role of identified MMR proteins in meiosis of other organisms supports the hypothesis that these proteins are likely candidates to be involved in meiosis and may lead to identification of the mechanism by which males can properly halve the genome size in the absence of structures normally functioning in this process.

CHAPTER II

MATERIALS AND METHODS

Amplification of *mutL* homologous sequence

Degenerate primers (Oligos, etc.) were designed for the polymerase chain reaction (PCR) amplification of potential *Drosophila melanogaster MutL* homologs using conserved regions of *E. coli mutL* and *hexB* and *S. cerevisiae Pms1*. The sequences of the primers are: MLF1(forward primer) CGG GAT CCG TNA ARG ARY TNG TNG ANA AY; MLR1 (reverse primer) GGA ATT CAR NGC YTC NCC NCK RAA NCC. Sequence symbols are as follows: N=(G,A,T,C); R=(A,G); Y=(C,T); K=(G,T).

Reaction mixtures contained 1 nmol each primer, 10 ng genomic DNA (Oregon R), 5 units of AmpliTaq polymerase (Perkin-Elmer Cetus), and 3 mM Mg^{++} in a total 50 µl volume overlaid with 50 µl of mineral oil. Cycle parameters were 94° C for 1 min, 55°C for 1 min and 72° C for 3 min for a total of 40 cycles. Analysis of the PCR products was performed by gel electrophoresis through 1% agarose gels containing ethidium bromide. Amplification from *Drosophila* genomic DNA produced two products of 250 bp and 300 bp in size. These two fragments were subcloned into pBluescript KS^- (Stratagene) using the method of Sambrook et al. 1989 and are subsequently referred to as L25 and L3 respectively.

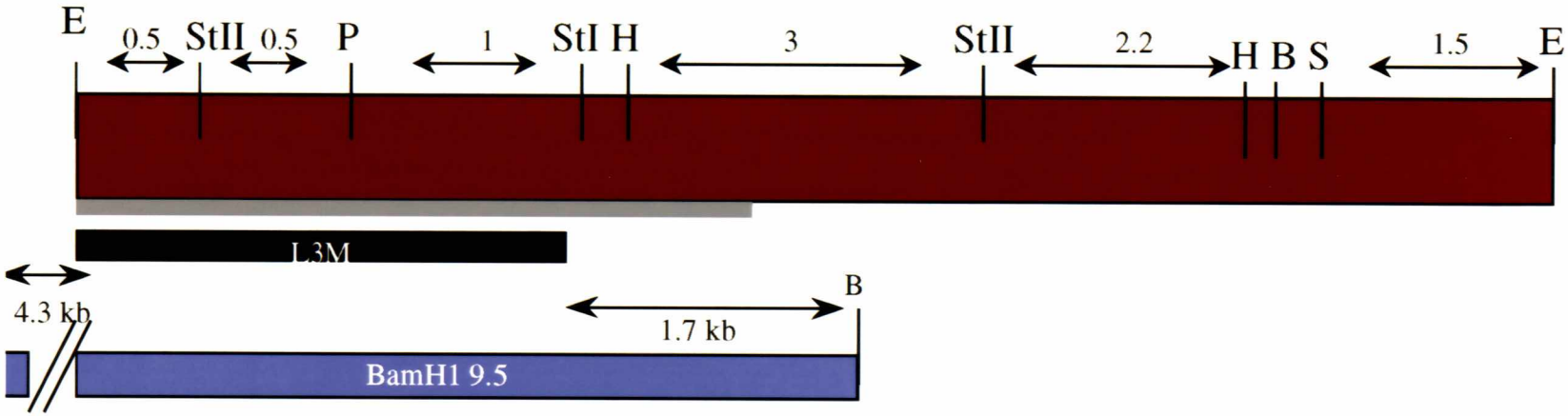
Screening of genomic libraries

A *Drosophila Canton S* genomic library was prepared and screened as described in McKee et al. (1996). L25 and L3 were used as probes in the library screen. Two genomic clones were recovered, one obtained using L25 as a probe and the other using L3. Restriction analysis and mapping of the two clones and subsequent Southern blotting allowed determination of smaller fragments that contained sequence homologous to L25 and L3 (Figures 1 and 2). Southern blot analysis showed a 2.4 kb SstI EcoRI fragment to contain the sequence homologous to the L3 probe. This fragment was subcloned into pBluescript and called L3M. Two fragments from the L25 homologous genomic clone were subcloned; a 1.8 kb SstI, XhoI fragment (L1800) and a 3.7 kb XhoI, EcoRI fragment (A1). Sequencing of the two genomic subclones showed A1 to contain the entire open reading frame of *dmlh1*. L3M did not contain the 3' end of *dpms2* and, therefore, a second round of genomic library screening was used to pull out a 12 kb BamHI fragment which contained the entire *dpms2* gene. Sequencing using primers to the 3' end of L3M revealed the remaining part of the open reading frame.

DNA sequence analysis

Double-strand DNA sequencing of the L3M and L25 clones was carried out by the method of Sanger et al. (1977). Supercoiled plasmids were used as templates and the Sequenase enzyme (U.S. Biochemical) was used in the reaction according to the directions of the manufacturer. Sequencing of the 3' end of *dpms2* was done in the

Fig 1 Restriction digest map of the 9 kb genomic clone containing L3M. Letters indicate sites of restriction enzyme cut sites. Gray stippled box indicates region that has been sequenced. The black filled box indicates the fragment containing L3M which was subcloned into pBS. The blue bar indicates the BamH1 9.5 subclone which contains the entire gene. Arrows with numerical values indicate distances between sites in kb. Distances above BamH1 9.5 indicate the amount of sequence found within this subclone in addition to the L3M sequence.

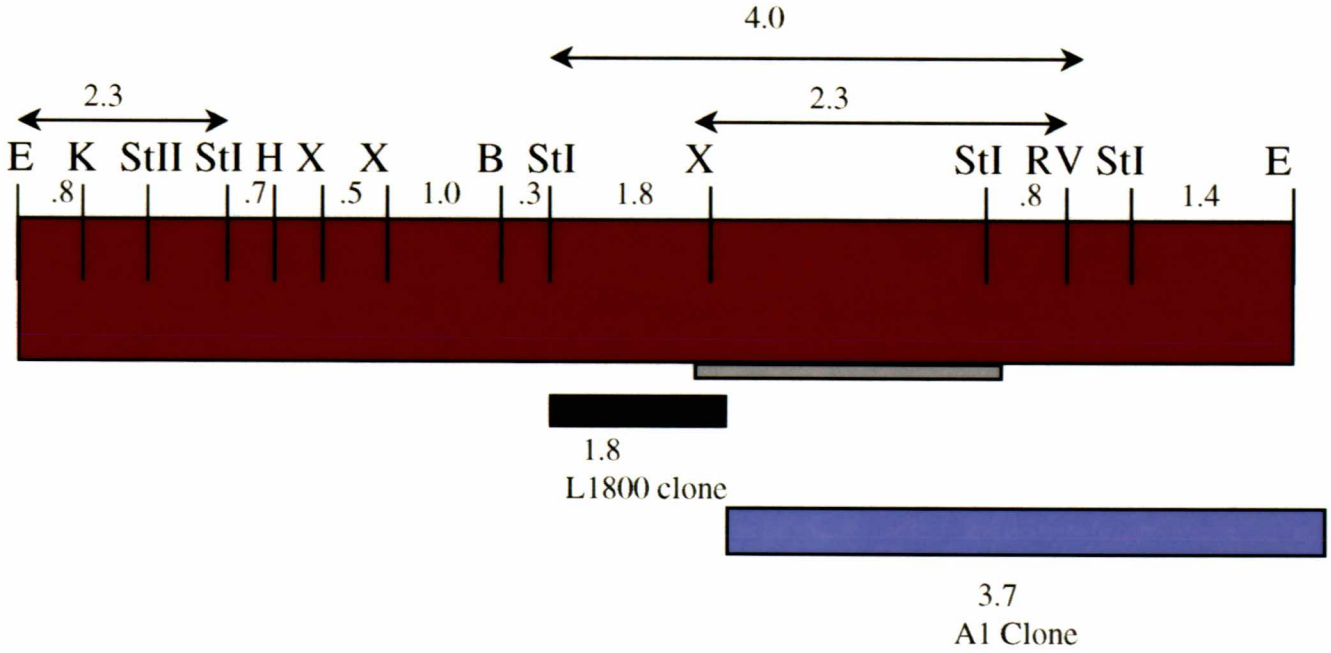


H=HindIII
E=EcoRI

StII=SstII
StI=SstI

B=BamHI
P=PstI

Fig 2 Restriction digest map of the genomic clone containing sequence for L25. Letters indicate sites of restriction enzyme recognition sequences. Numbers indicate distances between sites in kb. Gray stippled box indicates region sequenced. The filled black box indicates the L1800 clone that was subcloned into pBS. The blue filled box indicates the A1 clone that was subcloned into pBS.



K=KpnI
X=XhoI
H=HindIII
RV=EcoRV

E=EcoRI
StII=SstII
StI=SstI
B=BamH1

Automated Sequencing Lab at the University of Tennessee, Knoxville. The GCG program (Wisconsin Genetics Group, Madison) and DNASTAR™ were used for sequence alignments and analysis.

In situ hybridization

In situ hybridization was performed as described in Engels et al. (1991). Salivary glands were dissected from *Oregon R* 3rd instar larvae in 45% acetic acid on subbed slides. After three minutes in 45% acetic acid, a siliconized cover slip was placed on the salivary glands. Spreading was accomplished by gentle tapping with a pencil erasure. After overnight refrigeration at 4° C, the coverslips were removed by freezing in liquid nitrogen. Slides were soaked in ¾ methanol: ¼ acetic acid for 10 minutes. Subsequent dehydration in 50%, 70% and 95% EtOH for 10 minutes each followed. 2XSSC washes at 65° C for 30 minutes was followed by a room temperature 2XSSC wash for 2 minutes. The slides were then immersed in two liters of 100 mM TEA and 10 ml of acetic anhydride was added during vigorous stirring. After dissolution of the acetic anhydride, stirring was halted and the slides remained in the solution for 10 minutes. Two five minute 2XSSC washes followed and then dehydration with two 70% EtOH and two 90% EtOH washes of 5 minutes each. 70mM NaOH was used to denature DNA at room temperature for 3 minutes. Another series of EtOH dehydrations was followed by air drying and then probe hybridization procedures. DNA probes were prepared using the BioNick labeling system (Gibco-BRL) and purified using Sephadex-50 columns. Boiling for 5 minutes denatured the

probes and then 10 microliters was placed on the slides for hybridization. Siliconized coverslips were placed over preparations and sealed with rubber cement. Hybridization was carried out at 50° C in a moisturized glass container overnight. The following day, coverslips were removed by removal of the rubber cement and soaking in 2XSSC at 53° C for 3 washes. Two 10 minute PBS washes at room temperature were followed by a 20 minute 2% BSA in TMN1- triton X-100 wash at 42° C and a 10 minute soak in the same at room temperature. Detection of the hybridized probe was accomplished using the BluGene™ Nonradioactive Acid Detection System (Gibco/BRL). Briefly, 50 microliters of a SA/AP solution (2 µl streptavidin/alkaline conjungate in one ml TMN1, triton X-100) was placed on the slides for 5 minutes. 50 microliters more was added for a second 5 minute incubation. TMN1, triton X-100 was used to rinse slides. The slides were then washed in TMN1, triton X-100 twice and in TMN2 twice for 3 minutes each. 30 µl of nitroblue tetrazolium (NBT)/5-bromo-4chloro-3-indolylphospate (BCIP) solution was then added to each slide. Coverslips were added and slides were placed in the dark for 2 hours at room temperature. Distilled water was used to rinse slides. Coverslips were placed over chromosomes for viewing with a Zeiss Universal Axioplan photomicroscope and photos taken using Kodak T-MAX 100 film.

Genomic blot analysis

Genomic DNA was digested with the indicated restriction enzymes (GIBCO/BRL) and electrophoresed in 0.5% agarose gels. Transfer to Genescreen Plus (New England Nuclear) was accomplished by Southern blotting (Southern 1975) in 10X SSC. ^{32}P-labeled probes were made using the Random Primers kit from GIBCO/BRL as indicated in the manufacturers' instructions. High stringency prehybridization and hybridization were performed at 42° C in a 50% formamide solution. High stringency washes were 65° C in 0.2XSSC. Low stringenecy prehybridization and hybridization were performed at 42° C in a 25% formamide solution. Washing was performed at room temperature in 1XSSC.

RNA analysis

Poly(A)$^+$ RNA for *dmlh1* analysis was obtained from the indicated tissues by first grinding in 4M guanidium isothiocyanate (5 Prime ↔3 Prime), then passage over an oligo(dT) column (5 Prime↔3 Prime). Total RNA was prepared for *dPms2* analysis using the Perfect RNA™ kit (5 Prime↔3 Prime) as described by the manufacturer. For northern blot analysis, RNA was electrophoresed on denaturing formaldehyde gels. RNA degradation was analyzed by assaying rRNA bands through staining with ethidium bromide. RNA was transferred to Genescreen Plus (New England Nuclear) by capillary action in 20X SSC. Prehybridization and hybridization were performed in a 50% formaldehyde solution. ^{32}P-labeled probes were made using

the Random Primers kit from GIBCO/BRL as indicated in the manufacturers instructions. High stringency washes were performed at 65° C in 1% SDS, 2X SSC.

RT PCR

Reverse transcription reactions were performed by first isolation of total RNA from the indicated tissues using the Perfect RNA™ kit from 5'→3' as suggested by the manufacturer. RNA was then treated with Dnase I and reverse transcription reactions were performed using the SUPERSCRIPT™ Preamplification System for First Strand cDNA Synthesis from GIBCO/BRL as described by the manufacturer.

CHAPTER III

RESULTS

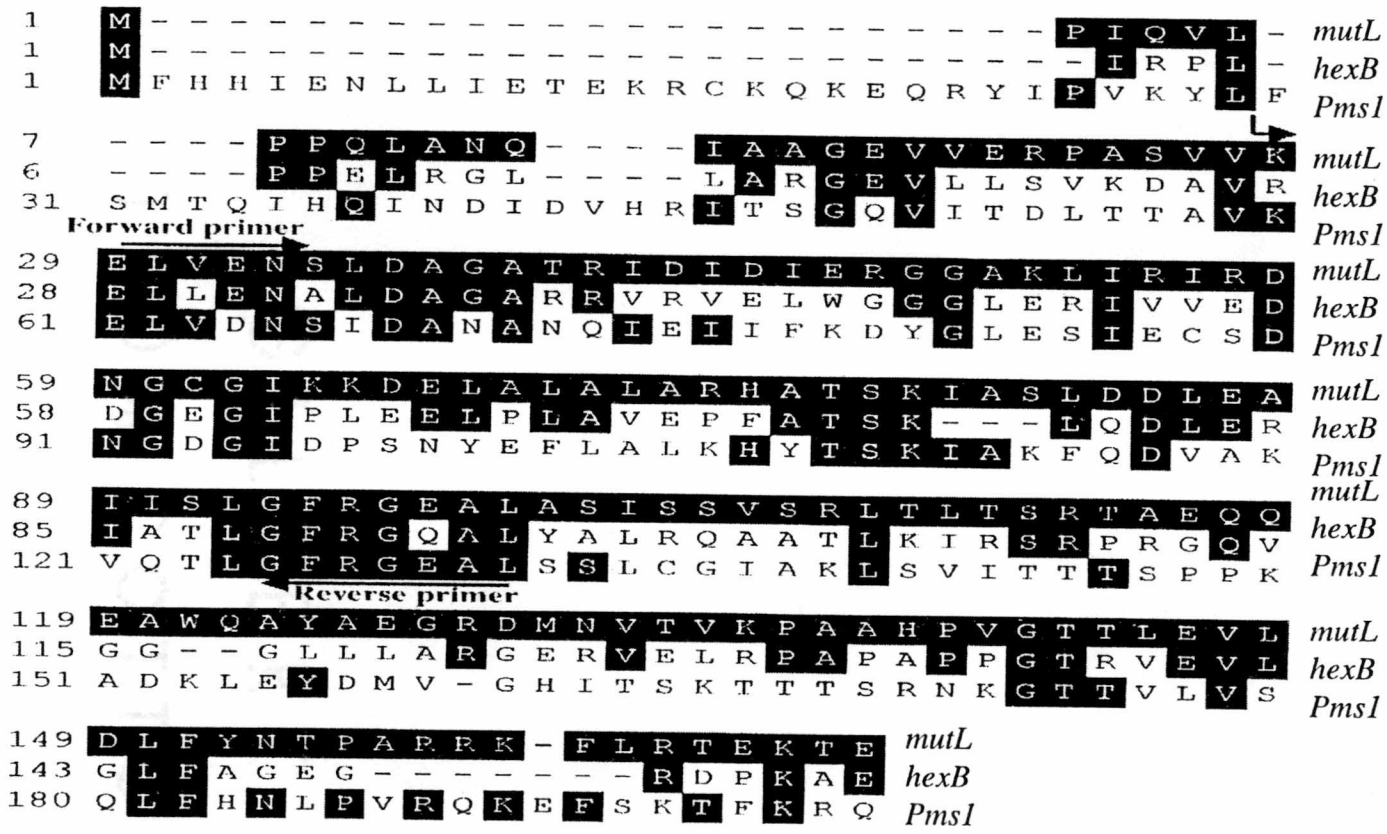
Isolation and cloning of two *mutL* homologs

Degenerate primers designed using the conserved sequences of bacterial *mutL* and *hexB* genes and yeast *Pms1* gene (Figure 3) were used to amplify two fragments (250 bp and 300 bp) from *Drosophila* genomic DNA. Cloning of these fragments into pBS resulted in L25 and L3 clones respectively which were used as probes for genomic library screening. Restriction mapping and Southern blots were used to isolate smaller genomic subclones from the obtained genomic library clones as described in Materials and Methods. The two subclones obtained were L1800 and A1 from the L25 probe and L3M from the L3 probe (Figures 1 and 2 respectively).

Sequencing and alignment

Sequencing of the clones revealed the complete open reading frame of a *Drosophila mutL* homolog within the A1 clone, but only a partial gene sequence of a second homolog within the L3M clone. The lack of a stop codon within the ORF at the 3' end of L3M and the absence of the highly conserved C-terminal end suggested that L3M did not contain the carboxyl terminus of the protein. In order to obtain the remaining portion of the gene, a second library screen was conducted. An approximately 1 kb genomic PCR fragment from the coding region of L3M was used as a probe to screen a genomic library. This resulted in the identification of a phage

Fig 3 Amino acid sequence of the amino terminal end of bacterial *mutL* and *hexB* genes along with yeast *Pms1*. Identical residues are shaded. Location of the degenerate primers made for use in PCR are identified.


Forward primer
Reverse primer
mutL
hexB
Pms1

containing a 12 kb SalI insert. Restriction enzyme digests and Southern blotting showed a 9.5 kb BamH1 fragment to contain the region of interest. Therefore, this fragment was subcloned into pBS. Restriction enzyme digests confirmed the presence of the entire L3M fragment plus an additional 4.3 kb upstream and 1.7 kb downstream. Figure 1 shows the location of the 9.5 kb BamH1 fragment in relation to the original genomic clone and L3M subclone. Primers designed to the 3' end of L3M were used for automated sequencing of the remainder of the gene. The three introns at the 3' end were identified using RT-PCR, subcloning of cDNA fragments, and automated sequencing. Comparison to genomic sequence allowed exact splice sites to be determined.

The genomic sequences of the two genes are shown in Figures 4 and 5. Conceptual translation of the sequences revealed two genes highly homologous to *mutL*. The A1 subclone shows the highest homology (52% amino acid identity) to human Mlh1 and is, therefore, referred to as *dmlh1* (Figure 6; GenBank accession number AF068257). The second clone is most similar to human Pms2 (43% amino acid identity) and is named *dpms2* (Figure 7; GenBank accession number AF068271). The deduced amino acid identity between the obtained *Drosophila* clones and their human homologs is not spread throughout the length of the proteins as can be seen in figures 6 and 7. The highest degree of identity is seen at the amino and carboxyl ends with a less conserved region between these domains. The amino terminal 200 amino acids of dMlh1 shows 70% identity to the corresponding end of human Mlh1 and the

Fig 4 Genomic sequence of *dmlhl*. Start codon and stop codon at positions 60-62 and 2106-2108 respectively are marked in bold and the single intron is underlined.

1 ctccgcgcaa caacggcaca ctacttttgt tacattaaac ttcgcatgaa gatttgccca

61 tggcggaata tttgcagcct ggagtcatcc ggaagctgga cgaggtggtg gtgaaccgca

121 tagccgccgg cgagattatt cagcgacccg ccaacgcccc aaaagagctg ctcgagaaca

181 gtttggacgc ccaatcgacc cacatccagg tgcaggtgaa ggccggtggg ctgaagctgc

241 ttcaaatcca ggacaatggc acagggatac gacgcgagga tctggccatc gtctgcgagc

301 ggttcaccac ctccaagctg acccgcttcg aggatttgtc ccagatagca acatttggct

361 tcagaggaga ggccttggcc agcattagtc atgtggccca cctaagcatc cagaccaaga

421 cggctaagga gaagtgcggc tacaaggcca cctacgcgga tggcaagctc cagggtcagc

481 cgaagccgtg tgccggaaac caaggcacga tcatttgcat agaggatctg ttctacaaca

541 tgccgcaaag gaggcaggca ctccgttcgc cggccgagga gttccagaga ttgtccgagg

601 ttctggctag gtacgctgtc cacaatccaa gggtgggctt cactctccgc aagcagggag

661 acgcgcaacc tgccctgcgg acaccagtgg ccagttcccg atccgaaaac atccgcatca

721 tttacggggc agcaatttcc aaggagctgc tggagtttag ccaccgcgat gaggtttaca

781 agttcgaggc cgagtgtctg atcactcagg ttaactattc ggccaaaaag tgtcagatgc

841 tgctgttcat caaccagcga ctggtggagt ccacaggttt gtgagtctaa acatcttctc

901 attcagaaaa cgaatccaat atatatcccc tagctctcag gacctccgtg gactcgattt

961 atgccacata cctgccccgt ggccaccatc ctttcgtata tatgagcctg acgctgccgc

1021 cacaganctt ggacgtgaat gtccatccca ccaagcatga ggtgcacttt ctctaccagg

1081 aagagattgt ggatagcatt aagcagcagg ttgaagcccg actcctaggc agcaatgcca

1141 cgaggacgtt ctacaaacag ctaaggctac caggagcccc cgacctggat gaaacccagt

1201 tggctgataa aacgcaacgc atttatccca aggagatggt gcgtactgac tccacagagc

1261 aaaagctgga taagttttg gcgcctctgg tcaaangtga ctcaggcgta tcctccagtt

1321 cctcccagga agcgtctcgt ctgccagang agagttttcg cgttacagcg gcaaaaaaat

1381 cgcgagaagt gcgtttgagc agcgtgctag acatgaggaa gcgcgtggag cggcagtgca

1441 gcgttcagct gaggagcacg ctcaaaaact tggtctatgt gggctgtgtg gatgaggagc

1501 gagccctgtt ccagcatgaa acgcgccttt atatgtgcaa cacacggtcg tttagtgagg

1561 aactgttcta tcagcggatg atttacgagt ttcaaaactg ctcggaaatc acaatatgtc

1621 cgccattgcc tctcaaggag ctgctaatct tgtcgctgga aagcagggcc gccggctgga

1681 cgcccgaaga cgaggataag gctgagctgg cggacggtgc tgcggacatt cttctgaaaa

1741 aggcnccccat catgagagag tatttcgggc tgcgcatctc ggaggatgga atgctcgaat

1801 ctctgcccag tttgctgcac cagcatcgac ctgtgtggcc ccacctgcct gtttacctgc

1861 tccgcttggc caccgaagtg gactgggagc aggagacacg atgcttcgag acattctgta

1921 gggaaactgc tcgattttac gcgcaattgg attggcggga aggtgcgact gcggtctttt

1981 ccagatggac aatggaacac gttctatttc ctgccttaa aaaatatctt ctgccaccac

2041 gcatcaagga tcagatctac gagctcacta acctgcccac tctgtacaag gtgttcgagc

2101 ggtgctagtt taggttgttc acttccagaa ctttaaatat tgattatatt tggtattcta

2161 aacatacata tttgcgctt tggttattgt ggtaaggata tgatcacagg atcatataaa

2221 cttctaacta caa

Fig 5 Genomic sequence of *dpms2*. Start codon and stop codon at position 1113-1115 and 4086-4088 respectively are marked in bold. The seven introns are underlined.

1 gagctccatt aaatctatct cttatcaaga agtagccata atctaggtct tttataaaag
61 agcaagtatt caaaatcatt tttagttccg aaatgcggat catcatgaaa ttgctggtga
121 gtaagcttgt aagtgaaaaa agtatcataa gctaaaatta aatctccgc aaattgtcgt
181 ccttgggca ttattggtg ttgtttcttt cacatcaaat agtctacata aaatgtatgg
241 caatatcgcc tggtcctgcc cgccatggtg tagcaattaa atggcgagaa aaagagatac
301 aaaaggttca actgtagttg ttctcgatgt cttaataaaa agtgaaatac tttgacttg
361 tgcattaact tttattata attgattta ataaaatgtt tgcgcaatat attactcaca
421 taaattgcga cammcccttt agcgctttg cattctacaa tggtgcggaa ctatagctaa
481 tcccattgtc caccattaaa tccgatctct tatcttactt agtgtatctc aaatcgtagc
541 tcaaatataa aagrgttcaa cataattttc agttctaaaa ttcaccttat tgtgaaattc
601 ctggtgaatt atctagtcag tgaaaattat cttacgctta cattccatcc cacttagaat
661 ttgctttgg cattattagc tgcttgtggc gttttaatgt tatccacgtg aatacgataa
721 ttcaaaatga gataatattg tttaactgta gttgttctca atgcctaata aaaagtataa
781 tacttttaac ttgctttatt tattgtaggc aacgattgaa atgtatcgaa aacataatat
841 ttatattgac gacagcgccc tctagcgtat ttccgatata caacttttat attccgtatc
901 cgattagtta agtcatataa tctggtcaca cagcaaccta aagagcgcca taatttgtg
961 aattagtgca ctcgttgcat ttaagccacn attatgttgg aaaccaatta caatgctgac
1021 acggaggaag agccggatgt gcctccacca accaccgcgt tgtcgggtca gataaaggcg
1081 attggcaagg atacagtgca caaaatatgc tcgggacaag tgagatacaa aattatttt
1141 ttttcgttc aagttcctta gacctggact caattcattt taggtggttc tcagcttggc
1201 tgtggctgtc aaggagctag tggagaactc aatagatgcg ggcgcaacgc tggtggagat
1261 cnagctaaag gatcagggac tgcagagcgt ggaggtcagc gataatggca gtggcgtgga
1321 ggaaatgaat ctggaaggca tgagtaagtg cagccaaatc tacaatgatg cggaacaata
1381 gttaatccca ctgcacacag ctgcaaaata ccacacctca aaaatccggg agttcgtgga
1441 cctcctgggc gtggaaacct ttggattccg tggagaggct ctgagttcac tgtgtgcctt
1501 gtcggatatg gttatccaaa cccggcacaa gtctaccgat gtgggtgagt tatggacgga
1561 accctacgtt taacgctaca tttattccat taaaaacgtc caccttttta gggggttaag
1621 gtggaactgg accatgnggg caggatcaag aagcgatctc ctgtgcccg tggagttggc
1681 accaccgtcc ttctggcnaa tcttttttcc acactgccag ttcgtcggcg tgacttiacc
1741 cggnacatca agaaggagtt caccaagatg tgccagattt tgcaggccta ttgtctagtt
1801 accaaaggtg tccgcatcct ttgcagcaat cacactccca aagggccaaa actattgtgc
1861 tacaaacgca tggtgaccag aaggtcatgg ccaatatatc agccatatcg gagcacgcca
1921 ggccgcggat ctgtgccttt aaagtctcct tttggccagg gccagctgac cgaggcggaa
1981 ttgcgggctg atctagaatc tggtgcagat ttagccgata ctacttctcc acaaatcagc
2041 accgaggatg tagagcgtct taatcaggcg gacttccaac tcgagggatt catttccagt
2101 tgccgacatg gtgctggacg ctctagcaga gatcgccagt tcttttttgt cnactcgagg
2161 ccctgtgacc ctaaaaatgt gagacaaacc cctttaaaat gcttaaataa agtaatatta
2221 tattcagata gccaaggtga tgaacgaggt ttaccatcgc tataacgtcc aacagcaacc
2281 ctttatttat ctcaacatca tcacggcccg ttctgatgtg gatgtgaatc tcacgcccga
2341 caaacgccna ttgctgataa acaacgagcg aattcttctg ctggcgctga aaaaatccct
2401 gctggacacc tttggccaaa ctccagctac atttcaaatg caaaacacca caattgtgag
2461 catgctggna cccaagacaa attctggtaa aactaaattc cctaaagaat caagtaagga
2521 ggaccaacac gaagaagtat ccgnagaaga tgtacccact acctccacgc aaaggtttat
2581 ggatgtgctc acccagtggc gacgcactgg cgatacaaaa ggtacagcac cgtcagttcc
2641 agtaaagcgc cggtgcagtg aatccgaaga attggttacc cgaacgttaa aaatgcaaaa
2701 gattcataca tttctcagcc aagaggagag ttccaaatgc gaggcagaat ctgatgcagc
2761 aagtgatgtt gacatggcaa aaaaagacaa gacgcaagtc tccttggata atagtttcct
2821 gaacctgnaa gagctggcta aggagtccga gggtgaggaa gcttaactat catatcattc
2881 gttaataaat tttaatttat tcaaattggt tacagcttat gatcttttaa cgaaaccaca
2941 gaatgcacct cgcattgatt gcaaggttct gaccccaatt aaaagtcgtc gcagtatttt
3001 cgagtttaag actgatgcta aggcattaga caaacaggaa tcaccggcag aactcaattc
3061 ccaaccgcct agtttcactc agctgtcgga agaaactgat attgctagcg acgaagatga
3121 ccacattgag ctgcccactc ggattgagtt cgatgaagag atggaggagg gtctgccatc
3181 aaatttttca agcggggaaa taactacttc tctagaggag atcgcatctt ctttaaaggc
3241 ccacgagcaa cagcagcgtg atcggaggac tcgaaccaaa cttcagcgtc tgcgcttcna
3301 gacggaaatt aatccgaacc agaacaccag tgctgaagca gaacttcggc gtgagatcga
3361 taaggaggat ttcgccccga tggnaatant aggacagttt aatcttggct ttatcatagt
3421 cnagctngng gatgatctat tcatagtaga tcagcatgcc actgatgaaa agtacanctt
3481 tgaaacgctg cagcgcacaa ctcnattgga gtatcagcgc ttggcagtgc cgcaaaatct
3541 cgaactaact gcggttaacg agatggtgct attgaaccac attgatgtgt tcgagaagaa
3601 tggcttcaag tttgaagtag accacgaagg tgggattica actgacctic tgttatttta
3661 cttataaacg attgccattt cagctcctgc cacgaagaag gtgcgtctgt tgggcaagcc
3721 gcacagcaag cgatgggnat tcggcaagga ggacattgat gagctcattt ttatgctgca
3781 agatgcgccc gaaggcacca tctgtcgccc gtctagggtg cgagctatgt ttgcctcacg
3841 tgcttgccga aaatcggtga tgattggcac agctcttagc aggaatacaa cgatgagaag
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3961 gaattaagaa ttgcaaattc tcagttttat agaactgtcc acatggacgt cctacgatgc
4021 gacacctcat aaacatcgca atgctaatta atagtgacga aaacgatgag caagagcctc
4081 cggcctaata gaaacgacta tgtgtaaaaa atcagttgtt

Fig 6 Multiple alignment of Mlh1 homologs. The predicted protein sequence of dMlh1 (GenBank accession number AF068257) was aligned with human Mlh1 (Bronner et al. 1994) and *S. cerevisiae* Mlh1p (Prolla et al. 1994b) using the MEGALIGN™ program of the Lasergene™ Software for Windows. The BestFit program of the GCG sequence analysis software package was used to determine identity among the proteins. dMlh1 showed 52% identity to hMlh1 and 47% identity to yMlh1p. Residues identical to dMlh1 are shaded.

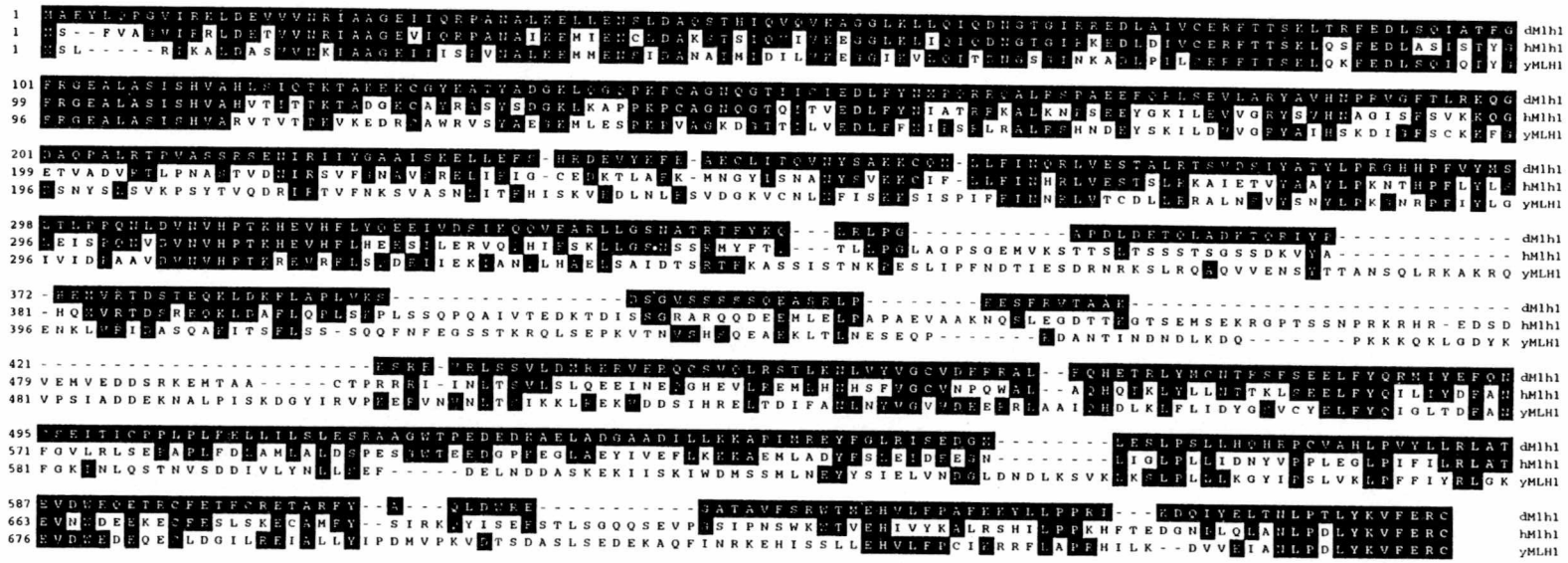
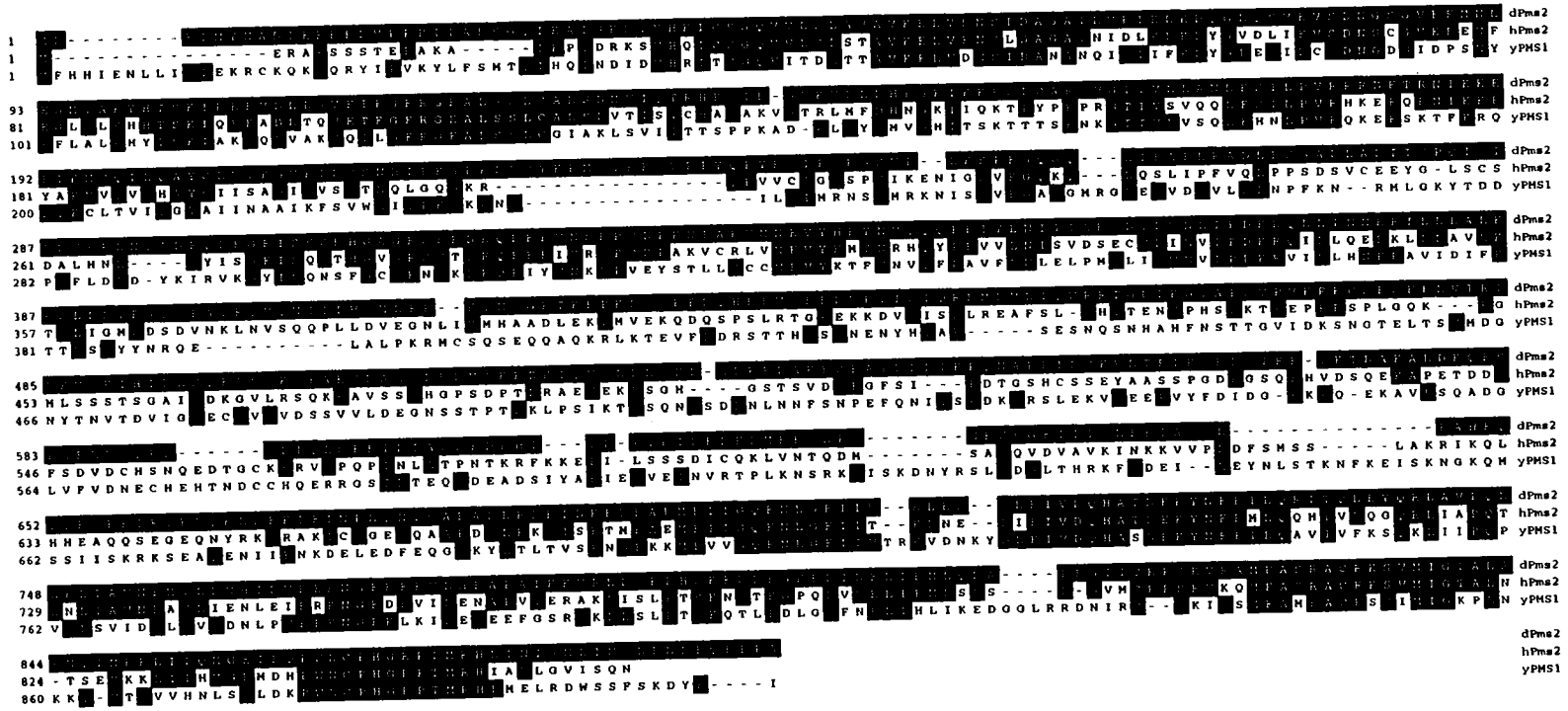


Fig 7 Multiple sequence alignment of Pms2 homologs. The predicted protein sequence of dPms2 (GenBank accession number AF06827) was aligned with human Pms2 (Nicolaides et al. 1994) and *S. cerevisiae* Pms1p (Kramer et al. 1989b) using the MEGALIGN™ program of the Lasergene™ Software for Windows. The BestFit program of the GCG sequence analysis software package was used to determine identity among the proteins. dPms2 showed 43% identity to hPms2 and 34% identity to yPms1p. Residues identical to dPms2 are shaded.


dPms2
hPms2
yPMS1

carboxy terminus beginning with dMlh1 amino acid 623 shows 51% identity. The first 191 amino acids of dPms2 show 65% identity to human Pms2 while the carboxy terminal 198 amino acids show 66% identity. These high levels of amino acid identity at the ends of the proteins suggest some conservation of function in these regions. During evolution deletions and insertions have led to divergence within the internal regions of the proteins, but the functional regions were conserved. Using the GCG program, no domains of known function could be found within the deduced amino acid sequences of dMlh1 or dPms2. This is not unexpected since other known *mutL* homologs also lack obvious functional domains. The two proteins do contain the GFRGEAL sequence which has been termed the signature motif of *mutL* homologs since it is 100% conserved in all identified thus far.

Localization of *dmlh1* and *dpms2* within the genome

In situ hybridization to *Drosophila melanogaster* polytene chromosomes showed both *dmlh1* and *dpms2* to be represented as single copy genes within the genome. *dmlh1* and *dpms2* localize to the right arm of the second chromosome with *dmlh1* located at position 44B (Figure 8A) of the polytene map and *dpms2* at position 51F (Figure 8B). Although in situ results reveal that both *dmlh1* and *dpms2* are single copy genes, Southern blotting reveals the likelihood of both genes being a part of a larger gene family within the *Drosophila* genome (Figure 9). Figures 9A and 9C show Southerns using a *dmlh1* and *dpms2* probe respectively and washed at high stringency. The probes used were the original 250bp and 300 bp PCR subclones identified in the

Fig 8 In situ hybridization localizes (A) *dmlh1* to position 44B on the polytene chromosome map and (B) *dpms2* to 51F.

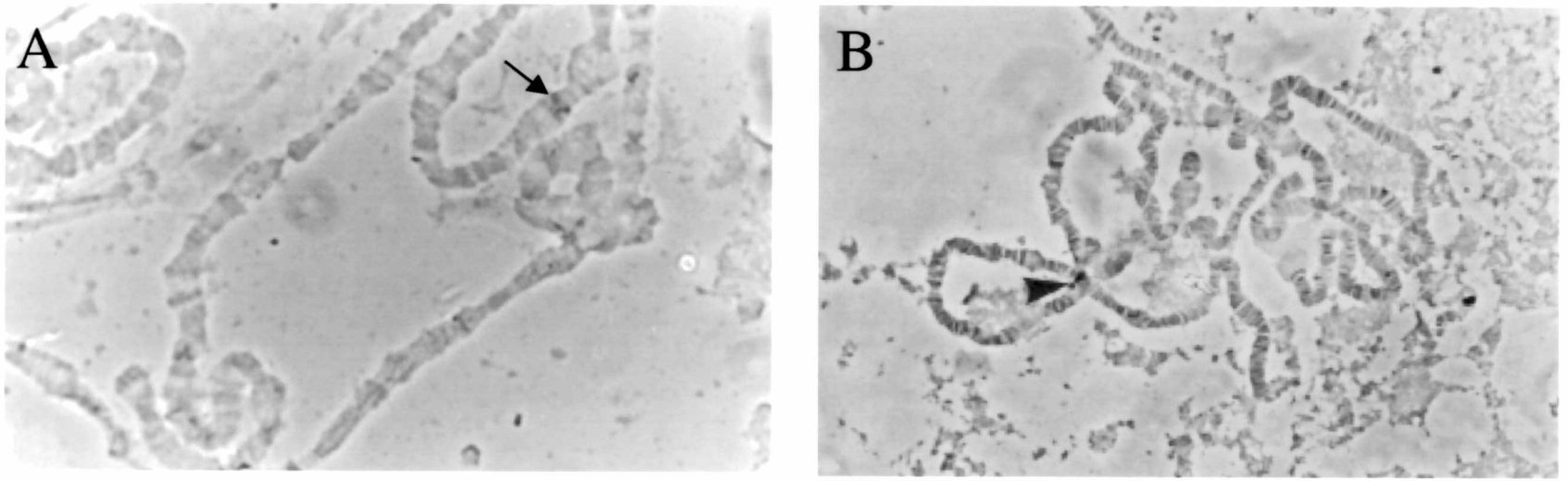

A
B

Fig 9 Southern blots washed at high or low stringency. (A) Southern probed with L3 clone washed at high stringency. (B) Southern probed with L3 clone washed at low stringency. (C) Southern probed with L25 washed at low stringency. For (A) and (B) genomic DNA was digested with BamH1 (Lane 1), EcoRI (Lane 2), HindIII (Lane 3), or SalI (Lane 4). For (C) and (D) genomic DNA was digested with SalI (Lane 1), BamH1 (Lane 2), EcoRI (Lane 3), or HindIII (Lane 4).

A
Lane 1
Lane 2
Lane 3
Lane 4
B
Lane 1
Lane 2
Lane 3
Lane 4
C
Lane 1
Lane 2
Lane 3
Lane 4
D
Lane 1
Lane 2
Lane 3
Lane 4

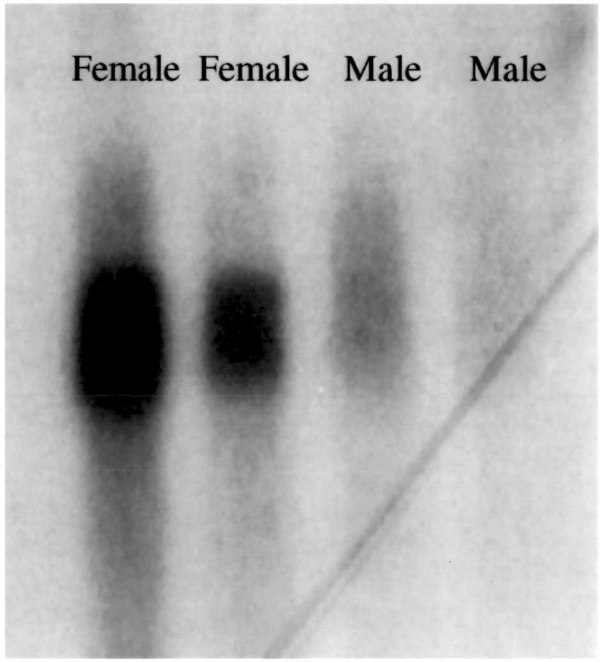
initial screen for *mutL* homologs. These fragments do not contain any of the restriction enzyme sites used to cut the DNA before the Southern blotting was performed. Therefore, the film exposed to the radioactive filter would be expected to reveal only a single band for each of the four digests. However, although a major band is always obvious, additional bands can be seen suggesting the presence of other homologous sequences within the genome. These additional bands are seen even when the procedure is done with high stringency (Figure 9A for *dpms2* and Figure 9B for *dmlh1).* The presence of these additional homologies would suggest the presence of additional genomic sequences homologous to the PCR probes. Figures 9B and 9D are washed at low stringency and no further bands are seen. The appearance of these gene family members even at high stringency shows that the family is highly conserved at least in the region for which the filter was probed.

Expression patterns of *dmlh1* and *dpms2*

Northern analysis revealed a single 2.37 kb mRNA species for *dmlh1* that is highly expressed in females and ovaries and, to a lesser extent, in males (Figure 10). No apparent band can be seen in total RNA isolated from testis. *dpms2* is expressed as a single 2 kb band and is present in both somatic and germline tissues of males and females (Figure 11). The obtained sequences reveal a 2 kb coding region for *dmlh1* and a 2.6 kb coding region for *dpms2*. Therefore, the 2.37 kb mRNA of *dmlh1* is of the expected size, but the 2.0 kb band observed for *dpms2* is smaller than would have been predicted. This could indicate some unidentified processing of the mRNA.

Fig 10 Northern analysis for *dmlh1*. (A) Male and Female total RNA probed with the A1 clone DNA. (B) Ovary and Testis total RNA probed with the A1 clone DNA.

A



B

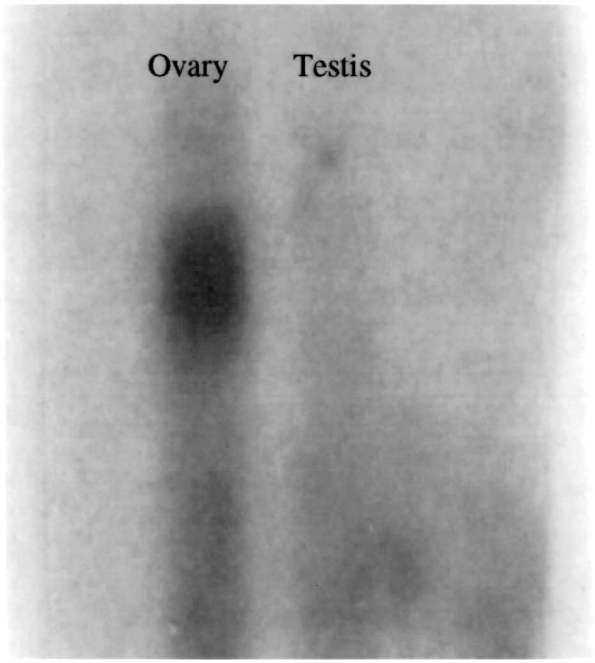
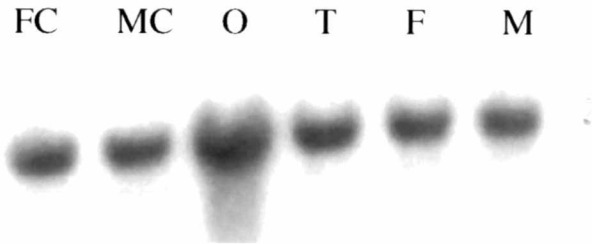


Fig 11 Northern analysis of total RNA using L3M as a probe. (FC) Females Carcass, (MC) Male Carcass, (O) Ovary, (T) Testis, (F) Female, (M) Male.


FC
MC
O
T
F
M

Differential transcription start sites for *dpms2*

RT-PCR results suggest sex-specific *dpms2* transcription start sites. Using the primers ML3F7 and ML3R1 shown in Figure 12, RT PCR was performed. The expected product was seen from male and testis cDNA, however, no product was seen from female and ovary cDNA (Figure 13A). In order to confirm the presence of female and ovary cDNA, the same samples were used in a PCR reaction with the primers ML3F10 and ML3R2 which are internal to the *dpms2* coding region. These primers amplify a region 3' to the translation start site and the product contains two introns. The primer set showed the presence of correctly spliced cDNA as compared to the genomic Oregon R control (OR) (Figure 13B). Figure 13C shows the PCR products from Oregon R (genomic), male cDNA, female cDNA, testis cDNA and ovary cDNA using the ML3F19 and ML3R1 primer sets. As seen in Figure 12, ML3F19 is 3' to ML3F7. This primer set gave the correctly spliced cDNA product for all samples. These data suggest that the male and testis transcription start site is located 5' to ML3F7 while the female and ovary transcription start site is located between primers ML3F7 and ML3F19. Studies by Nicolaides et al. (1994) on human PMS2 suggest that it also has alternate start sites. Attempts to map the 5' end of hPMS2 were unsuccessful due to complex patterns due most likely to the presence of multiple transcripts with different start sites.

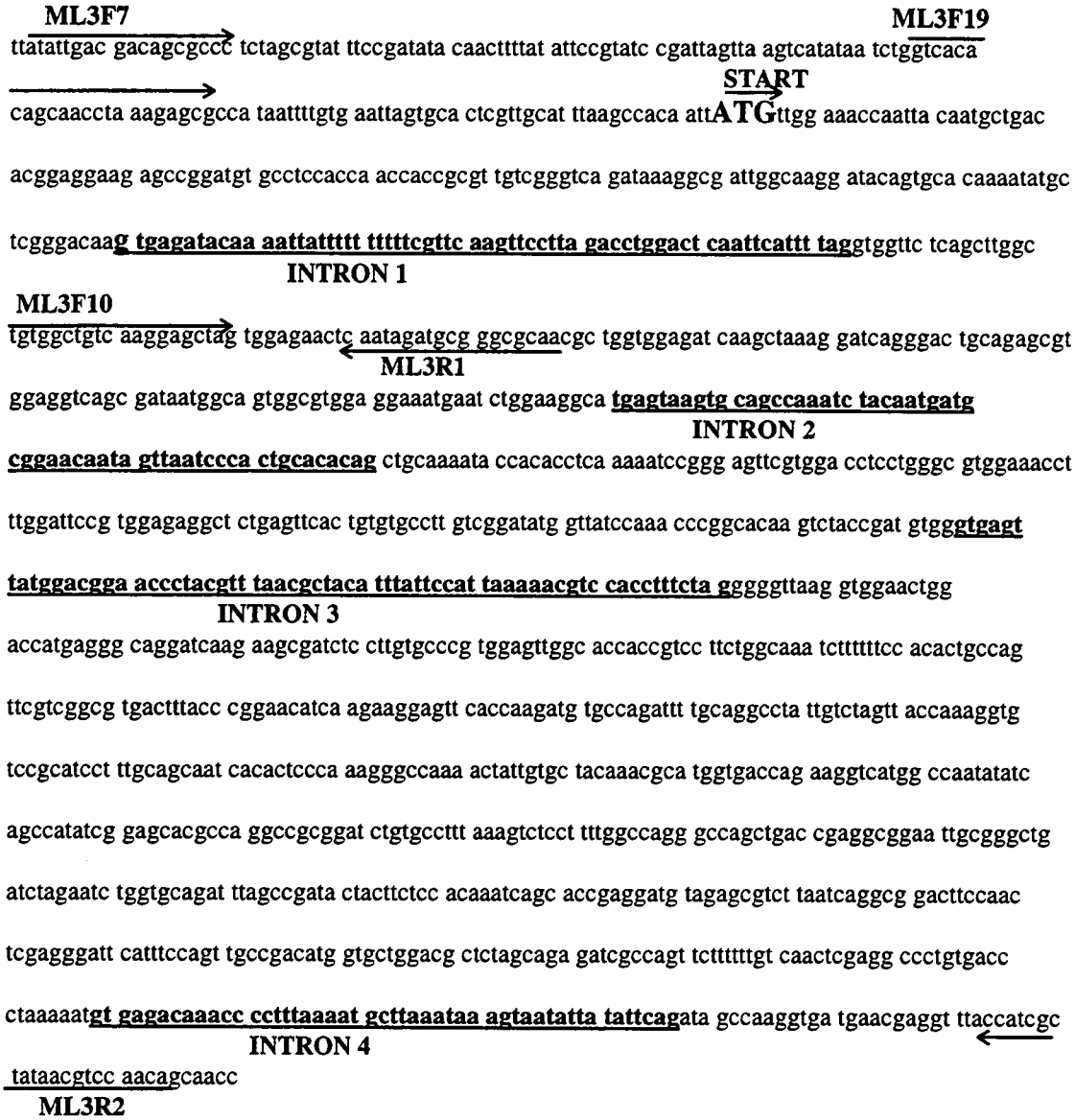
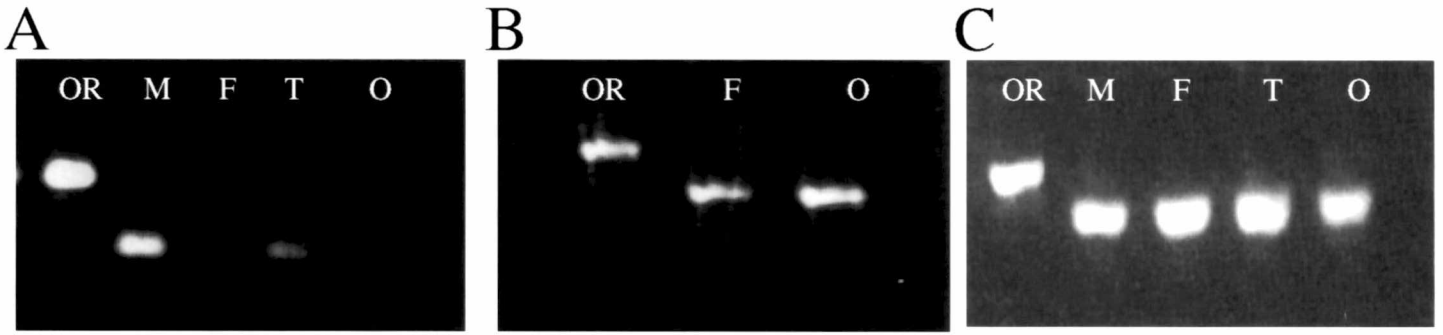


Fig 12 Sequence of the 5' end of *dpms2* showing the start codon, primers used in RT-PCR reactions, and position of introns. Primer sequences for the forward primers (ML3F7, ML3F19, and ML3F10) are identical to the shown nucleotide sequences and reverse primers (ML3R1 and ML3R2) are complementary. Note: The primer ML3R2 has been previously referred to as ML3F1 within the McKee laboratory.

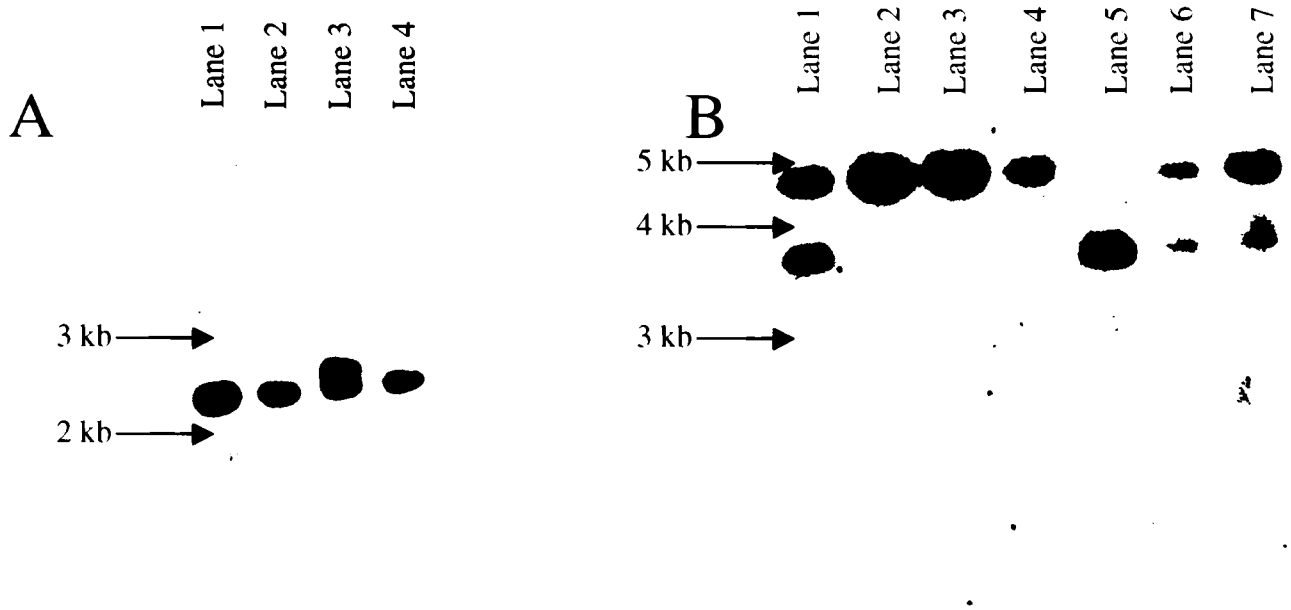
Fig 13 Ethidium bromide stained agarose gels containing electrophoresed samples of RT-PCR reactions using various primers described in the text. (A) F7R1 primer set, (B) F10R2 primer set, (C) F19R1 primer set.
OR= *Oregon R* genomic DNA (positive control)
M= Male total RNA
F= Female total RNA
T= Testis Total RNA
O= Ovary total RNA


A
OR M F T O
B
OR F O
C
OR M F T O

Identification of *dmlh1* and *dpms2* deficient lines

Deficiency lines that carry a heterozygous deletion of a large region of the 2nd chromosome are available. Lines that contained deficiencies in the chromosomal regions containing either *dmlh1* or *dpms2* were obtained from the Bloomington Stock Center at Indiana University. Southern blot analysis was then used to quantitate the amount of DNA present in each line for the specific gene of interest. Equal loading was accomplished by running a small aliquot on an agarose gel, staining with ethidium bromide, and using a negative image to compare concentrations using a densitometer. The amount of each sample loaded was determined by equalizing the concentration of all samples based on the densitometer readings. Thus a reduction by one-half of the signal on the audoradiogram indicates the lack of one copy of the gene of interest. Figure 14A shows a Southern probed for *dpms2*. One line, $w^a\ N^{fa\text{-}g}$; *Df(2R)Jp4/CyO* (*Df(2R)Jp4*), can immediately be ruled out as a deficient line for *dpms2* since two bands are present when the genome is cut with SstI and EcoRI and probed for *dpms2*. The two bands represent two different alleles of the area of interest which results in a difference in size. Lines $y^1\ w^1$*/Dp(1;Y)*y^+; *Df(2R)XTE-18/CyO* (Df(2R)XTE-18, Lane 2 Figure 14A) and $w^a\ N^{fa\text{-}g}$; *Df(2R)Jp1/CyO* (Df(2R)Jp1, Lane 4 Figure 14B) both are deficient for *dpms2* based on densitometry readings. Of the total signal, Df(2R)XTE-18 and Df(2R)Jp1 represent only 22% and 14% respectively while *Oregon R* and Df(2R)Jp4 make up the remaining 64% with 34% and 30% respectively. Since the signals are approximately one-half of the wild-type signal for Df(2R)XTE-18 and

Fig 14 Southern blotting to determine lines deficient in *dmlh1* or *dpms2*. (A) Southern blot probed with L3M clone shown in Fig 1. Genomic DNA digested with SstI and EcoRI from wild-type *Oregon R* (Lane 1), *Df(2R)XTE-18* (Lane 2), *Df(2R)Jp4* (Lane 3), and *Df(2R)Jp1* (Lane 4). (B) Southern blot probed with A1 clone shown in Fig 2. Genomic DNA digested with XhoI and EcoRI from *Df(2R)cnS6* (Lane 1), *Df(2R)44C-E* (Lane 2), *Df(2R)cn9* (Lane 3), *Df(2R)CA53* (Lane 4), *Df(2R)cn83c* (Lane 5), *Df(2R)cn87e* (Lane 6), *Df(2R)H3C1* (Lane 7).


A
Lane 1
Lane 2
Lane 3
Lane 4
3 kb
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B
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Lane 4
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Lane 6
Lane 7
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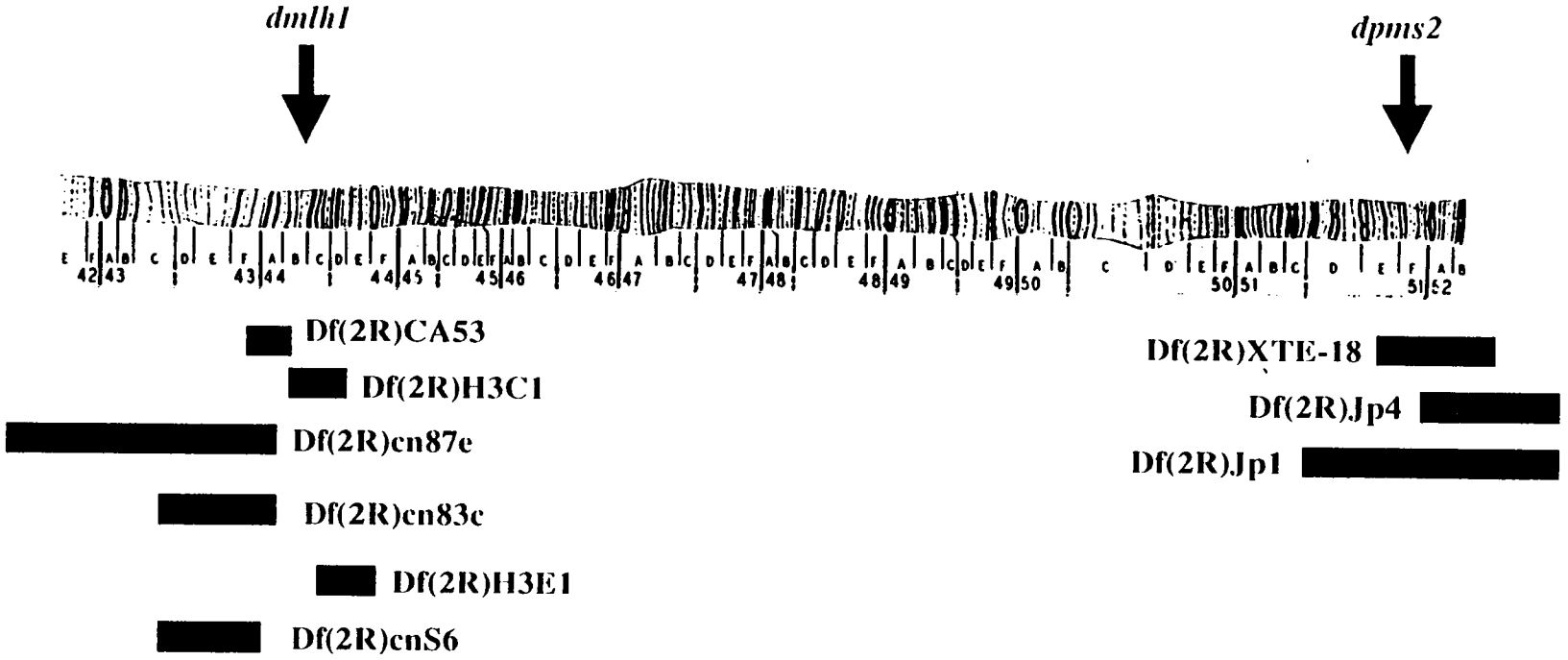
4 kb
3 kb

Df(2R)Jp1, this indicates that the *Df(2R)XTE-18* and *Df(2R)Jp1* lines are haplo-insufficient for the gene of interest, *dpms2*. Line *w*; Df(2R)CA53/CyO* (Df(2R)CA53, Lane 4 Figure 14B) was the only line showing a deficiency for *dmlh1*. Lines *In(2LR)O + Df(2R)cnS6,Cy* dp^{lul} *pr cn/*cos^{5} *cn* (Df(2R)cnS6, Lane 1 Fig 14B), *Df(2R)cn87e/In(2LR)*$bw^{V1}b^{1}$ (Df(2R)cn87e, Lane 6 Figure 14B), and *Df(2R)H3C1/CyO* (Df(2R)H3C1, Lane 7 Figure 14B) all have two alleles of the region probed, confirming they are not deficient for *dmlh1*. When *Df(2R)CA53* is compared by densitometry to the remaining three lines, it is only 6% of the total signal while *Df(2R)44C*-E, *Df(2R)cn9*, and Df*(2R)cn83c* account for 37%, 34%, and 21% respectively. Thus, the much lower signal of *Df(2R)CA53* confirms it contains only one copy of the gene of interest. Known cytological map positions of these deficiencies are illustrated in Figure 15. The reported breakpoints for *Df(2R)H3C1* were 43F and 44D. These breakpoints would include all of *Df(2R)CA53* which showed a deficiency for *dmlh1*. But *Df(2R)H3C1* certainly does not have a deficiency since it has two alleles of the gene. Therefore, salivary gland squashes were done to check the reported breakpoints and the proximal breakpoint was found to be incorrect. The bands representing 43F and 44A are present. Therefore, the breakpoint is in 44B or 44C. The distal breakpoint appears correct. The proper breakpoints are shown on the map in Figure 15. *Df(2R)cn9* has the same problem with its reported breakpoints showing that it includes all of *Df(2R)CA53* yet does not show *dmlh1* deficiency. Presumably this is due to an incorrect breakpoint mapping, but it is yet to be confirmed so this

Fig 15 Chromosomal location of deficiencies. A salivary gland map is shown with cytological boundaries. Cytological locations of dmlh1 and dpms2 are designated by arrows. Deficiency names are listed beside the bar illustrating their location on the map.


dmlh1
dpms2
Df(2R)CA53
Df(2R)H3C1
Df(2R)cn87e
Df(2R)cn83c
Df(2R)H3E1
Df(2R)cnS6
Df(2R)XTE-18
Df(2R)Jp4
Df(2R)Jp1

deficiency is not shown on the map. The distal breakpoint of *Df(2R)cn83c* is not well defined and maps somewhere between 44B6 and 44C1, but it must end prior to the distal breakpoint of *Df(2R)CA53* since it is not deficient for *dmlh1* so it is represented as doing so on the map.

Potential mutations

In order to obtain mutant lines for *dmlh1* and *dpms2*, EMS-treated 2nd chromosome lines created in the lab of Charles Zucker were used in mutant screens. The screens involved placement of the EMS-treated 2nd chromosome over a deficiency for either *dmlh1* or *dpms2*. Deficiency lines Df(2R)CA53 and Df(2R)XTE-18 were chosen to use in a screen for *dmlh1* and *dpms2* respectively. The crossing scheme for screening for *dmlh1* and *dpms2* mutants is shown in Figure 16 and Figure 17 respectively. The phenotype chosen for screening was male and/or female sterility. Evidence in mice showing male and female infertility in Mlh1 knockouts and male infertility in Pms2 knockouts (Baker et al. 1995; 1996) led to our choice of sterility as a likely phenotype in *Drosophila dmlh1* and *dpms2* mutants. 723 chromosomes have been screened to determine if their presence over a *dpms2* deficiency results in sterility. Of these, primary screens have yielded 11 potential lines to be studied further. Screening of 267 chromosomes has yielded 9 potential lines showing male sterility when placed in trans over a *dpms2* deficiency. 502 and 159 chromosomes have been screened for female or male sterility respectively when placed in heterozygous

A

P *cn bw*/Cy* ♂ X *w/w; Df(2R)CA53/Cy* ♀

⇓

F1 *w/+; Df(2R)CA53/cn bw** ♀ X *Oregon R* ♂

⇓

Determine female fertility

B

P *cn bw*/Cy* ♂ X *w/w; Df(2R)CA53/Cy* ♀

⇓

F1 *w/+; Df(2R)CA53/cn bw** ♂ X *Oregon R* ♀

⇓

Determine male fertility

* indicates 2nd chromosome treated with EMS mutagen

Fig 16 Crossing scheme to screen for *dmlh1* mutants. (A) Crosses to generate females that carry the EMS treated 2nd chromosome and a deficiency containing *dmlh1* (B) Crosses to generate males that carry the EMS treated 2nd chromosome and a deficiency containing *dmlh1*

A

P *cn bw*/Cy* ♂ X *y w/y w; Df(2R)XTE-18/Cy* ♀

⇓

F1 *y w/+; Df(2R)XTE-18/cn bw** ♀ X *Oregon R* ♂

⇓

Determine female fertility

B

P *cn bw*/Cy* ♂ X *y w/y w; Df(2R)XTE-18/Cy* ♀

⇓

F1 *y w/+; Df(2R)XTE-18/cn bw** ♂ X *Oregon R* ♀

⇓

Determine male fertility

* indicates 2nd chromosome treated with EMS mutagen

Fig 17 Crossing scheme to screen for *dpms2* mutants. (A) Crosses to generate females that carry the EMS treated 2nd chromosome and a deficiency containing *dpms2* (B) Crosses to generate males that carry the EMS treated 2nd chromosome and a deficiency containing *dpms2*

condition with a *dmlh1* deficiency. The female screens yielded 14 potential mutations and the male screen identified 3 to be further studied. Two potential *dmlh1* mutants have been characterized further. Line *70-187 cn bw/Cy* and Line *4189 cn bw/Cy* are male sterile and female fertile when placed over the *Df(2R)CA53* deficiency. Homozygotes from these stocks were tested and while the 4189 homozygotes showed the same pattern of male sterility and female fertility, the 70-187 stock was homozygous male and female sterile.

The deficiencies used in this screen contain a heterozygous deletion for several genes. Therefore, the observed phenotype could be due to a mutation in the EMS line of any one of these genes. In order to further support that the mutation was likely to be in *dmlh1*, several deficiencies within the region that did not contain *dmlh1* were tested with the EMS stock. The deficiencies tested are w^{118}; *Df(2R)H3C1/CyO*; w^{118}; *Df(2R)H3E1/CyO*; *Df(2R)cn87e/In(2LR)*bw^{V1}, b^{1}; and *Df(2R)cn9/SM6b,* Cy^{1} Roi^{1}. Table 1 shows no other deficiency causes the infertility seen with *Df(2R)CA53*. Numerous males of each genotype were tested for fertility with *y w* females to confirm a complete sterile phenotype rather than reduced fertility. These two lines must now be further tested to determine if they contain a mutation within *dmlh1*. Northern analysis will reveal if *dmlh1* is expressed correctly within these lines.

Table 1 Results of fertility tests of two potential *dmlh1* mutants when placed in trans with deficiencies near or containing *dmlh1*.

Genotype	Male Sterile	Female Sterile
70-187/Df(2R)CA53	Yes	No
70-187/Df(2R)H3C1	No	No
70-187/Df(2R)H3E1	No	No
70-187/Df(2R)cn87e	No	No
70-187/Df(2R)cn9	No	No
70-187/70-187	Yes	Yes
4189/Df(2R)CA53	Yes	No
4189/ Df(2R)H3C1	No	No
4189/ Df(2R)H3E1	No	No
4189/ Df(2R)cn87e	No	No
4189/ Df(2R)cn9	No	No
4189/4189	Yes	No

CHAPTER IV

DISCUSSION

Using degenerate PCR, we have identified two *mutL* homologous sequences in *Drosophila melanogaster*. Sequence analysis and alignment of putative protein sequence shows one, dMlh1, to be most similar to mouse Mlh1 and dPms2 to human Pms2. In situ hybridization localized *dmlh1* and *dpms2* to the right arm of the second chromosome at cytological positions 44B and 51F respectively. Northern blot analysis showed a single mRNA species for each gene. While *dpms2* is equally expressed in males, females, testis, and ovaries, *dmlh1* shows a much higher level of expression in females and ovaries than that seen in males and testis. Based on the functions of the Mlh1 and Pms2 proteins in mice, these results might be expected (Baker et al. 1995; Baker et al. 1996). Mouse Mlh1 knockouts show defects in their ability to crossover. This leads to high levels of chromosomal nondisjunction and sterility in both sexes. However, homologous chromosomes in *Drosophila* males do not recombine. Therefore, a protein involved in meiotic crossing-over would not be expected to be necessary in male meiosis. Mlh1 also functions in mismatch repair in mice. A function in MMR in *Drosophila* would explain the low levels present in males and testis while a much increased level is seen in females and ovaries due to the additional function in meiosis. Pms2, on the other hand, appears to have some involvement in homolog pairing in mice. Although *Drosophila* males differ from females in that they do not require synaptonemal complexes and chiasmata for homolog pairing stability, this does

not rule out the possibility that some proteins are common in the pairing processes of males and females.

Although confirmation of meiotic function awaits the analysis of *dpms2* and *dmlh1* mutants, some evidence supports the potential role of these genes in *Drosophila* meiosis. First, the high level of *dmlh1* expression in ovaries suggests some function in addition to the normal MMR activities of *mutL* homologous proteins. Secondly, the differential transcriptional start sites for *dpms2* suggest a tissue specific regulation of the protein in meiotic tissues.

In order to study protein expression of the identified MMR homologs, antibodies against synthetic peptides have been produced for both dMlh1 and dPms2. dPms2 antibodies were produced by immunizing a New Zealand White rabbit with a synthetic peptide (Research Genetics) of the sequence LETNYNADTEEEPDV. Antibody to dMlh1 was produced by Research Genetics, Inc. to the peptide sequence KKSREVRLSSVLDMRKRVER. The peptides used were chosen by using the program to determine peptides with the highest antigenicity. Antibodies will be used in future studies to localize and quantitate protein expression in *Drosophila* male and female meiotic tissues. Localization of the protein to meiotic tissues in males and females would further support their involvement in meiotic processes. A low level of expression would be expected throughout the organism if the proteins function in DNA repair, but higher levels in the gonads would support additional roles in these tissues.

If the proteins function in pairing or recombination, they might also localize to chromosomes. The antibody will also be used to determine if this is the case.

An additional question is what other proteins interact with dMlh1 and dPms2 during meiotic processes. Co-immunoprecipitation experiments could determine if these two proteins interact. To identify interacting proteins, the yeast two-hybrid system would be a valuable tool. A cDNA library that is produced specifically from ovaries or testes can be used in the assay to increase the chances of identifying factors that specifically interact with dMlh1 and dPms2 during their meiotic roles rather than roles in repair.

Screens of EMS-induced mutations on the 2nd chromosome have yielded two potential mutants for *dmlh1*. Both of these mutants are male sterile when placed over a deficiency containing *dmlh1*. Further analysis of these lines is necessary to determine if they do indeed carry a mutation in the *dmlh1* gene. The expression of *dmlh1* will first be tested in these lines to check for an absence of expression or variation in the size or tissue specificity of the mRNA. Secondly, antibodies produced as described previously can be used to determine if the dMlh1 protein is properly expressed in these lines. If variation in protein migration is seen, sequencing of the *dmlh1* gene from these mutant lines may be necessary to determine the exact nature of the aberration. The final test to confirm mutation in a gene of interest is rescue studies. A clone of *dmlh1* can be used for microinjection into mutant embryos. Rescue of the sterile phenotype would confirm that sterility is due to mutation of the *dmlh1* gene. Once a mutant has been identified,

further phenotypic studies can be instigated. Testes preparations will allow determination of where the defects occur during spermatogenesis. For instance, are cells arrested at a certain stage which would allow identification of the time at which dMlh1 is required to act. Secondly, chromosome staining of spermatocytes can allow visualization of how meiosis proceeds in these mutants. For instance, does pairing occur? Are chromosomes properly condensed? Do they align at the metaphase plate? Does chromatin bridging occur? Observation of meiotic stages in mutants is necessary to pinpoint the purpose of dMlh1 and dPms2 in meiosis.

The previously described screens for mutants rely on the correctness of the hypothesis that mutation in *dmlh1* or *dpms2* would result in male and/or female sterility. This is based on results in other organisms and, therefore, may not hold true in *Drosophila*. Thus, it is possible that the mutant screen will produce no lines with mutations in either *dmlh1* or *dpms2*. In order to determine the phenotype of *dmlh1* and *dpms2 Drosophila* mutants, double-stranded RNA will be injected into wild-type embryos. This technique has been shown to transiently inactivate genes specifically in *Drosophila* (Kennerdell and Carthew 1998). RNA for this procedure has already been produced as shown in Figure 18. Primers were generated (IDT) that would amplify a portion of the open reading frame from *white*, *dmlh1*, *or dpms2*. The primers contain an overhang with the sequence of the T7 RNA promoter. Primers are used in PCR to form DNA templates that are used in in vitro transcription reactions to form RNA. Since both primers contain the T7 RNA Promoter, the transcription of both strands can

Fig 18 Annealed double-stranded RNA that will be used for injection into embryos. The lanes contain 1.5 microliters of the RNA preparation from *white*, *dmlh1*, and *dpms2* genes.


white
dmlh1
dpms2

be carried out in the same reaction. Products are then boiled and allowed to anneal at room temperature. 1/10 of the resulting reactions was electrophoresed on an ethidium bromide stained gel (Figure 18). This RNA will be used to inject embryos. The *white* RNA will serve as a control since injection should result in white eyes in the injected progeny. The *dmlh1*- and *dpms2*- injected flies will be scored for fertility and chromosome nondisjunction.

Once mutants for *dmlh1* and *dpms2* have been obtained, studies should determine if the meiotic phenotypes observed in mice and yeast are conserved in *Drosophila*. If so, the functions of the gene products will give us additional information on the mechanisms of chromosome pairing and segregation. Additionally, other MMR homologs could be cloned from *Drosophila* to see where they fit in such a pathway.

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PART V

General conclusions and future directions

CHAPTER 1

STUDIES OF MALE MEIOSIS IN *DROSOPHILA*

Meiosis is an important mechanism in sexually reproducing organisms. The process allows production of gametes with reduced chromosome number to yield zygotes of the proper ploidy. Although the steps in the meiotic process are well-described, the mechanisms by which these steps occur are less understood. Mistakes that occur during meiosis can lead to various problems including sterility and lethality. These problems occur in diverse organisms from yeasts to fruitflies to humans. It is important to study these mechanisms in a model organism in order to gain knowledge to explore treatment options for humans, where aneuploidy is a costly financial and emotional issue. *Drosophila melanogaster* males offer an excellent system for the study of chromosome pairing and segregation. In addition to the well-characterized genetics of this organism, males lack the processes involved in synaptonemal complex formation and recombination which complicate determination of protein function due to the overlapping nature of these pathways. Although numerous screens have been performed to identify meiotic mutants in *Drosophila*, very few mutants have been identified with defects in the pairing and segregation of chromosomes in males. Therefore, the purpose of our studies was to identify as yet uncharacterized factors that function in these processes.

Involvement of *homeless* in male meiosis

The first mutation described in our studies was initially identified as a female-sterile (Tearle and Nusslein-Volhard 1987). Females carrying hypomorphic alleles of *homeless (hls)* caused either by P-element insertion or EMS treatment showed female sterility while deletions of the gene made by P element excision were lethal (Gillespie and Berg 1995). Certain aspects of the study suggested the possibility of meiotic phenotypes in addition to the oogenic defects. A c-myc tagged fusion construct which lacked the C-terminal thirteen amino acids of *homeless* rescues the observed oogenic defects, but not fertility, suggesting *hls* may have effects on female meiosis (personal communication C. Berg).

In order to determine if *hls* males had problems in pairing or segregation, they were tested for chromosome nondisjunction by monitoring segregation of an X-Y pair in which the Y chromosome was genetically marked. All alleles tested showed sex chromosome nondisjunction when tested in trans with the EMS-induced allele hls^{E616} (see Figure 2 Part II). Genetic studies showed the chromosome nondisjunction was not genome-wide, but instead affected the sex chromosomes and 2nd autosome pair but not the 4th. A cytological manifestation of meiosis I nondisjunction was observed in orcein-stained meiotic chromosomes from *hls* males in the form of aneuploid secondary spermatocytes. Other cytological phenotypes seen in spermatocytes from *hls* males include tripolar meiosis, incomplete chromatin separation, fragmented chromatin, lagging chromosomes, and chromatin bridging.

The P-element allele hls^{3987} showed the highest levels of sex chromosome nondisjunction when placed in trans with hls^{E616} (Part II Table 2). hls^{3987}, when placed over a deficiency, shows stronger oogenic phenotypes suggesting, at least in females, that it is a partial loss of function allele (Gillespie and Berg 1995). The difference between hls^{3987}/hls^{E616} males and hls^{E616} homozygotes was significant (as determined by the z test). With the exception of hls^{DE3}, which showed lower levels of sex chromosome nondisjunction, none of the EMS-generated alleles differed significantly from hls^{E616} homozygotes when placed in trans with this allele. The stronger phenotype of hls^{3987} suggests that all of the EMS alleles are also hypomorphs. This corresponds to results in females that showed when hls^{E616} was placed over a deficiency it exhibits a stronger phenotype than when homozygous; furthermore, this phenotype is weaker than hls^{3987} over the deficiency. Interestingly, a deletion allele, $hls^{\Delta 125}$ when placed in trans with hls^{E616} did not differ significantly from hls^{E616} homozygotes (z test). This deletion allele is lethal in the homozygous state and was shown to contain a deletion of the entire P element (inserted to make the hls^{3987} allele) with an additional 6 kb 3' to the insertion site (Gillespie and Berg 1995). The deletion includes the majority of the 4.6 kb transcript observed in females, but since the male transcript has not yet been identified, it is unknown if the deletion completely removes the male ORF. But a 6 kb deletion within the gene locus should cover a significant portion of the male transcript. Thus, it is surprising that a deletion which should remove the majority of the coding does not show a stronger phenotype than EMS induced point mutations. Although

unlikely, this could suggest that *hls*^{3987} and some of the EMS mutants are dominant negatives, whereby presence of the mutant actually shows a worse defect than complete absence of the protein. The observation that the phenotype is stronger in the presence of a deficiency could suggest that a small amount of the protein is capable of normal function. Northern blot analysis by the Berg lab showed very little of the expected 4.6 kb mRNA in ovaries of females homozygous for *hls*^{3987}. However, the mutation could produce an unidentified mRNA of a different size.

The presence in this genome of a construct containing *hls* cDNA failed to rescue the sex chromosome nondisjunction seen in *hls* males. This could be interpreted as meaning that in the presence of the mutant protein, the wild-type Homeless is unable to function properly. The fact that lethality is not rescued in *hls*$^{\Delta 125}$ homozygotes argues against this scenario. It is also unlikely that a P element insertion would cause a gain-of-function phenotype since these transposons are approximately 3 kb in size.

Instead, the inability to rescue may be due to the fact that the construct is lacking the C-terminal 13 amino acids and this or an inability to express the construct properly could be the reason for the lack of rescue. Experiments performed in the Berg lab using this same construct in females rescued the oogenic phenotypes but failed to restore fertility. Additionally, a full-length *homeless* cDNA when placed behind a heat-shock promoter showed this same partial rescue phenotype. However, if upstream DNA is added to the construct, fertility is rescued to 60% of that seen in wild-type. A full-length cDNA construct along with the *homeless* promoter also rescues to 60%

fertility (personal communication C. Berg). These observations suggest upstream regulation is important for the control of differential functions of the Homeless protein. Additionally, northern blot analysis has not yet shown *homeless* expression in testes; therefore, the size of the mRNA is unknown. When probed with a cDNA copy of the 4.6 kb female and ovary specific transcript, an additional 11 kb band is observed in these tissues along with a 9 kb band that is also present in male and female carcass RNA (Gillespie and Berg 1995). The rescue construct used contains the 4.6 kb transcript and thus may not be the proper form of *homeless* needed to rescue testis-specific phenotypes. Although testis expression of *homeless* is not yet confirmed, its presence is expected since in situ staining for lacZ expression from a reporter placed in control of the *hls* promoter is observed in testes as well as ovaries (personal communication C. Berg).

An important clue to the role of Homeless in spermatogenesis was the presence of crystals within primary spermatocytes of *hls* males. These crystals are identical in appearance to the crystals described in spermatocytes of XO males and X/Y *Su(Ste)*$^{-}$ males which are composed of large amounts of the normally repressed testis-specific protein Stellate (Ste) (Hardy et al. 1984; Livak 1984; Livak 1990; Palumbo 1994). Northern blot analysis confirmed the presence of *Stellate* mRNA within testes of *hls*^{E616} homozygous males and males heterozygous for a deletion and the *hls*^{E616} allele. The abnormalities observed when *Ste* is derepressed very closely parallel those seen in *homeless* males, as was shown in Table 4 of PART II. Common defects observed in

both *homeless* and *Su(Ste)*$^{-}$ males include 2nd chromosome nondisjunction, sex chromosome nondisjunction, meiotic drive, undercondensed chromosomes, chromatin bridging and fragementation, and an inverse correlation between fertility and the copy number of the *Stellate* allele present on the X chromosome (Palumbo et al. 1994). The chromosome nondisjunction occurs at both the first and second division and cytological abnormalities are also observed at both divisions (Palumbo et al. 1994). Pairing of homologs appears unaffected in these mutants. Therefore, we conclude that Homeless is involved in the pathway responsible for preventing the expression of Stellate and that mutations that prevent proper function in Homeless result in high levels of Stellate protein which in turn causes numerous meiotic abnormalities. Homeless is thus part of a regulatory pathway that also includes the *Su(Ste)* locus and the products of the *sting*, *scratch*, *most*, and *1006* genes (Livak 1990; Bozetti et al. 1997; Yue et al. 1999; Schmidt et al. 1999).

The best explanation thus far for the meiotic abnormalities observed when Stellate is derepressed is that its homology to the β subunit of CKII (Livak 1990) allows it to interact and interfere with the activities of the catalytic α subunit. A testis-specific homolog of the β subunit has been identified (Kalmykova et al. 1997) supporting an important role of the CKII enzyme in spermatogenesis. One of the many targets of CKII phosphorylation is TopoII (Ackerman et al. 1985). This enzyme is important for relaxing and unwinding DNA. These activities have been shown to be important for chromatin condensation and chromosome segregation(DiNardo et al.

1984; Newport and Spann 1987; Uemura et al. 1987; Holm et al. 1989; Wood and Earnshaw 1990; Adachi et al. 1991; Downes et al. 1991; Shamu and Murray 1992). Thus, the observed defects in *Homeless* and *Su(Ste)*$^{-}$ males would corroborate a theory of Stellate protein interfering with CKII activities, specifically with its regulation of TopoII.

The mechanism by which Homeless or other proteins regulate *Stellate* is not yet clear. However, there are methods by which some potential mechanisms could be examined. If Homeless regulates *Stellate* via a pathway responsible for silencing of tandem repeats then it would be expected to be involved in the silencing of repeats throughout the genome and constructs containing normally silent reporter repeats could be examined in a *homeless* background. By contrast, if the protein specifically regulates the packaging of the X and Y during spermatogenesis, then other X-linked genes would be affected and their transcripts could be observed in *homeless* spermatocytes.

The phenotypes induced in *homeless* females suggest other potential functions of Homeless that can be explored. Studies in females showed that the microtubule array was disorganized in oocytes of *homeless* females and phenotypes observed closely resemble those seen in mutations which affect microtubule structures (Gillespie and Berg 1995). It is expected that the RNA mislocalization seen in *homeless* oocytes is due to improperly functioning microtubule systems. Homeless may interact with tubulin since 50% of the c-myc tagged protein is present in the microtubule pellet

(personal communication C. Berg). Therefore, one important avenue of exploration is to visualize the spindle morphology in *homeless* males by using antibody to tubulin. These studies have begun but are not yet conclusive.

Clues to protein function are often originally based on analogy to known functions of homologous gene products. In the case of Homeless, similarity to *Drosophila maleless (mle)* and yeast *Prp16* and *Prp2* has been demonstrated (Gillespie and Berg 1995). The two yeast proteins both function in splicing of pre-mRNA molecules (Chen and Lin 1990; Burgess et al. 1990). Homology with these proteins raises the possibility of Homeless involvement in processing of *Stellate* or *Su(Ste)* pre-mRNA molecules. Interactions between Homeless protein and these mRNAs can be tested using gel shift assays. *maleless* is involved in sex determination and has been proposed to act in chromatin remodeling (Lee et al. 1997). This would correlate with the hypothesis discussed earlier where Homeless is necessary for a chromatin conformation necessary for silencing of tandem repeats.

maleless, *Prp2*, and *Prp16* are members of the DEAH-box subfamily of ATP-dependent helicases. Members of this family function in a variety of ways including those mentioned above as well as DNA repair (Ellis et al. 1995) and chromosome transmission and cell cycle progression (Gerring et al. 1990). Interestingly, a yeast family member, SGS1, has been shown to interact with TopoII (Gangloff et al. 1994). These results are especially interesting considering the link between *Stellate* and Topo II. Stellate has homology to the regulatory subunit of CKII and it is thought to disrupt

regulation of this protein when expressed at high levels. One known target of CKII action is Topo II. Since Topo II is necessary for proper chromosome condensation, the interference of Stellate with its regulation is thought to be the cause of the meiotic defects observed when *Stellate* is derepressed. The fact that *scratch*, one of the other crystal inducing loci, is an Hsp90 homolog further supports the importance of CKII and Topo II in *Stellate* regulation. Hsp90 proteins have been shown to stabilize CKII. Thus, these proteins somehow fit together in a pathway but the function of each in the regulation of *Stellate* and the male meiotic process is still unclear.

Immunolocalization of the Homeless protein will aid in determining how it acts to affect segregation within the nucleus. Nuclear localization will leave open the possibility of direct effects on meiotic processes, but would also be consistent with a role in mRNA splicing, while cytoplasmic localization suggests an indirect mechanism of action. The fusion construct used in rescue experiments contains a c-myc tag which can be visualized with fluorescent antibodies against the tag. However, since this construct does not rescue the nondisjunction phenotype, its localization cannot be accepted as conclusive. Insertion lines containing the full-length construct used in the Berg lab for rescue were not maintained and, therefore, it is necessary for new lines to be produced with this insertion so that it can be used in rescue experiments. If rescue is complete, antibodies made against this construct would be meaningful in identifying the cellular location of Homeless.

Whether Stellate is the only or one of many genes whose regulation is dependent on Homeless is unclear at this point. In order to easily determine the effects of *hls* in addition to *Stellate* regulation, it would be helpful to have a line which lacked the *Ste* repeats. However, attempts to obtain viable lines with no *Ste* copies have been unsuccessful, suggesting an as yet unknown necessary function of Stellate or close linkage of an essential gene (Palumbo et al. 1994). Although it is not possible to look at *hls* phenotype in the complete absence of *Stellate* copies, there are lines with very low copy numbers of this locus. Determination of lines with the lowest possible copy number and analysis of these lines in a *hls* background may sufficiently answer the question of whether Homeless plays roles in meiosis other that in the repression of Stellate.

A new factor involved in *Drosophila* meiosis

Accidental discoveries often lead to major advances in the understanding of biological mechanisms. This may be the case with a newly identified factor that is necessary for proper meiosis in both *Drosophila* males and females. Its involvement in first division segregation of homologs makes *nondisjunction in males and females (nmf)* one of a small group of genes known to function in meiosis I rather than meiosis II segregation in both sexes. Although *nmf* has been localized to the X chromosome, its exact genetic location is not yet known leaving open the possibility that there are actually two affected genes, one male-specific and one responsible for the phenotypes observed in females.

Hemizygous *nmf* males exhibit high levels of sex chromosome nondisjunction as well as 4th chromosome segregation defects. The 2nd chromosome is unaffected. Females homozygous for *nmf* also show sex chromosome nondisjunction. The rate of nondisjunction is even higher in females heterozygous for *nmf* and an X chromosome balancer. The 2nd and 4th chromosomes also show segregation defects in females in the presence of a second chromosome balancer or an X chromosome balancer respectively. The 4th autosome pair disjoins normally when no X chromosome balancer is present suggesting that *nmf* may be involved in the female distributive system which is responsible for segregating noncrossover bivalents (Grell 1962). Although the 4th chromosome pair which does not recombine is an obligate member of the distributive pool, it is often unaffected by mutations of the distributive system unless X chromosome nondisjunction is occurring (Hawley 1989). Presumably this is because the mechanism of partner choice within the distributive system has been disrupted, but with only two chromosomes present in the pool they are forced to act as segregation partners. However, when the X chromosome pair is also within the pool due to lack of exchange, the X and 4th chromosomes are allowed to segregate from each other in the absence of proper mechanisms to control partner choice. The presence of noncrossover X chromosomes has been demonstrated to increase the nondisjunction of the 4th chromosome pair in backgrounds mutant in the distributive system (Zitron and Hawley 1989).

The fact that the distributive system segregates noncrossover bivalents and the absence of recombination in males suggests that there could be some overlap in the proteins used in the two systems, although none have been discovered yet. A finding supporting this hypothesis is that the 4th chromosome and sex chromosome nondisjunction seen in *nmf* males is correlated. Scoring of progeny from genetic crosses indicates that all nullo-4 sperm contain both the X and Y chromosomes suggesting that the sex and 4th chromosomes are segregating away from each other yielding nullo-4, XY and diplo-4, nullo-XY sperm. This is reminiscent of what occurs in the distributive system of females where nondisjunction of the 4th autosomes often results from their disjunction from the X pair. If *nmf* is shown to be a single locus operating in the female distributive system and male meiosis, it would be the first of such proteins.

Much work is still lacking in the studies of the *nmf* factor. Most importantly, the factor must be precisely mapped in both males and females. A chromosome with additional X chromosomal genetic markers will be used in a further attempt to map *nmf* in males by recombination studies. A deficiency kit for the X chromosome has been obtained from the Bloomington Stock Center (Indiana University) to use for mapping *nmf* in females. This kit contains stocks with deficiencies covering the entire X chromosome. Each deficiency line will be tested in trans with *nmf* or an X chromosome balancer to determine which lines show the phenotypes observed in *nmf*

females. The location of the deficiency that shows *nmf* phenotype will give a general location of *nmf* in females.

Cytological defects in the spermatocytes of *nmf* males give some insight into how the gene product could function in male meiosis. While homolog pairing appears normal, chromatin bridging and incomplete chromatin separation are seen in primary spermatocytes. Thus, the defect occurs at some point between pairing and actual segregation of the chromosomes during anaphase. Aneuploid secondary spermatocytes are also observed which are presumably the result of the anomalies occurring during the reductional division. A failure to release homolog attachment is suggested which implicates the *nmf* gene product as a component of the system which regulates release of this adhesion. However, the defect could be in the mechanism that controls timing of release (a component of the cell cycle) or it could be involved in the attachment machinery itself. Once the gene is cloned and protein product identified, determining if the protein localizes to chromosomes should be helpful in ruling out one of these possibilities.

Comparison of *homeless* and *nmf*

Meiotic abnormalities seen in *nmf* males differ from those previously described in *hls* males. Sex chromosome nondisjunction is seen in both but the 4th chromosome nondisjoins in *nmf* males while 2nd chromosome segregation is normal. As mentioned in the discussion of Part II, if the meiotic effects of *homeless* are due to Stellate interference with the regulation of Topo II, the 4th chromosome may be minimally

affected due to its small size. Topo II functions to disentangle chromatin and the small size of the 4th autosome may leave it less vulnerable to entanglement during replication and/or pairing. Therefore, Homeless may not function in a chromosome specific pathway, but instead in a pathway that differs in its necessity depending on the likelihood of interconnections (e.g. the chromosome size). On the other hand, *nmf* causes nondisjunction of the 4th and sex chromosomes with no effect on the 2nd autosomes, suggesting that size is not the factor of importance in an *nmf*-dependent process. The distributive system appears to be affected in *nmf* females. Balancer chromosomes which prevent crossing over increase the incidence of chromosome nondisjunction presumably because these achiasmate bivalents are now a part of the distributive pool. Although the 4th chromosome segregates normally in *nmf* homozygous females, the presence of an X chromosome balancer leads to increased levels of 4th chromosome nondisjunction. Placement of the X chromosomes within the distributive pool seems to interfere with proper disjunction of the 4th autosomes. Hawley (1989) has suggested that there are numerous factors involved in distributive segregation with the first being availability (i.e., the presence of only two chromosomes within the pool results in their segregation from one another). However, if more than two chromosomes are present, homology and/or size affects partner choice. Therefore, *nmf* may affect only one component of the distributive disjunction. The 4th chromosomes disjoin normally if choice is based on availability. However, when other chromosomes are present, *nmf* results in a defect in partner choice so that the X

chromosomes often disjoin from the 4th autosomes. Similar effects may occur in males so that 4th chromosome nondisjunction is increased in the presence of sex chromosome nondisjunction. The observation that all observed progeny resulting from nullo-4 sperm are XXY females supports this theory. Abnormal 4th chromosome segregation appears to only occur in the presence of XY nondisjunction and, furthermore, the XY pair and the 4th autosome pair segregate from each other giving rise to either nullo-4, XY or nullo-XY, diplo- 4 sperm without any nullo-4,nullo-XY or diplo-4,diplo-XY sperm. These observations may lead to findings of similarities in mechanism between the female distributive system and male achiasmate segregation.

Another phenotypic difference between *nmf* and *homeless* males is the absence of meiotic drive in *nmf* males which is observed in a *homeless* mutant background. The meiotic drive parameters discussed in Part II suggested a moderate drive against the Y chromosome with a mild effect on the X in *homeless* males. The calculated disjunctional frequency (P), R_x, and R_Y values for *nmf* males are 0.93, 0.63, and 0.57 respectively. For comparison the corresponding calculated values for *homeless* from Part II Table 3 were 0.98, 0.85, and 0.55. These parameters do not take into account the occurrence of chromosome loss. Therefore, for *nmf* the excess of XO males in comparison to XXY females (Part III, Table 1) must be accounted for and that is why the R_x and R_Y values are low making it appear at first that meiotic drive is occurring. However, the amount of drive against the X and Y is essentially equal with values of 0.63 and 0.57. The factor that confirms the presence of drive instead of chromosome

loss in *homeless* males is the greater than 50% excess recovery of XX females over XY males (Part II, Table 3). This discrepancy could not be due to chromosome loss and thus must result from drive against sperm carrying the Y chromosome. On the other hand, progeny counts from *nmf* males do not show the greater than 50% excess recovery of XX over XY progeny supporting the hypothesis that chromosome loss and not drive causes the genotypic recovery differences.

Although *nmf* and *homeless* have different effects on particular chromosomes, the cytological abnormalities suggest they may be involved in a common function. Both give rise to defects in segregation of chromosomes, thus their timing of action is similar. They also both exhibit bridging chromosomes and incomplete chromatin separation at telophase further linking them in a potential pathway to release chromosome attachment. Further studies of these proteins may show them both to function in a common pathway or at least two pathways with a common goal.

Two *Drosophila mutL* homologs

Homologs of proteins originally identified as components of a mismatch repair system have been shown to be involved in meiotic events in yeast (Ross-Macdonald and Roeder 1994; Hollingsworth et al. 1995; Paquis-Flucklinger et al. 1997; Hunter and Borts 1997), and mice (Baker et al. 1996; Baker et al. 1995; Edelmann et al. 1999). In order to determine if these proteins might be conserved in *Drosophila melanogaster* and function in male meiosis, two *Drosophila* homologs, *dmlh1* and *dpms2*, of the bacterial *mutL* gene were identified, cloned and sequenced. The putative

protein sequences reveal a strong similarity and identity to human and mouse Mlh1 and Pms2, respectively, and to the corresponding yeast homologs of Mlh1 and Pms1 as well. Mice deficient in Mlh1 are male- and female- sterile (Baker et al. 1996). The sterility appears to result from a defect in meiotic crossing-over. Lack of Pms2 in mice results in male sterility due to the inability of homologs to synapse properly while females remain fertile (Baker et al. 1995).

Initial characterization of *dmlh1* and *dpms2* indicates the possibility of meiotic functions in *Drosophila*. The expression pattern of *dmlh1* shows high levels of transcript in females and ovaries with only low levels present in males and testis. These differential levels of expression in the sexes is consistent with a role in meiotic crossing-over since *Drosophila* males do not recombine. The low levels present in males would be expected if the protein also functions in mismatch repair. The *dpms2* transcript is seen in all tissues examined including testes and ovaries. High levels in these meioitc tissues lend support to a meiotic function of this protein as well. Additionally, differential transcription start sites in males and females bring raise the possibility of sex specific functions of the dPms2 protein. Production and studies of *Drosophila* lines mutant in these two proteins are necessary to confirm meiotic functions.

Obtaining mutants for *dmlh1* and *dpms2* is the most important future work for this portion of the project. Two methods are being used to accomplish this goal. First, a screen of EMS-treated lines is being performed as described in the body of this work. Two potential *dmlh1* mutants obtained in this screen were described. Additionally,

four lines for each of the genes were found to be female-sterile when placed in trans with a deficiency for the respective gene. Further analysis of these lines is necessary to determine if they are mutations within *dmlh1* or *dpms2*. The second method used to determine the phenotypic characteristics of lines lacking these two proteins is the injection of double-stranded RNA. The effectiveness of this method has been demonstrated in *Drosophila* and, therefore, we anticipate its usefulness in determination of dMlh1 and dPms2 protein function. The RNA has been prepared and and is ready for injection into embryos. Our final method currently in use to obtain *dmlh1* and *dpms2* mutants is a collaborative effort with the lab of Bill Engels. The Engels lab has initiated a large scale P element screening to identify insertions into specific genes of interest. P element mobilization will be followed by probing with large P1 or cosmid clones containing the gene of interest. *dmlh1* and *dpms2* are only two of many genes that they have included in their screen. Once insertions are obtained that are in or near the location of the gene, the stocks will be sent to us for phenotype examination as well as P element excision to induce deletions if necessary. The collaborative effort will offer the Engels lab an opportunity to test a large number of mutants for defects in double-strand break repair in mitosis and is an additional route of obtaining mutants for meiotic studies in the McKee lab.

Steps toward an understanding of meiotic mechanisms

In hopes of revealing the mechanism by which *Drosophila* males pair and segregate chromosomes in the absence of synaptonemal complexes and chiasma, we have identified and began characterization of four proteins that may act in this process. The continuation of these studies should reveal whether they are indeed involved in the processes of interest and, if so, how they function to insure proper function of this system. Our studies may reveal new ways to identify additional mutants for further analysis.

Our studies of *nmf* in females is also of great interest especially considering the possibility of overlapping mechanisms between male segregation and the female distributive system. *dmlh1* and *dpms2* may also function outside of male meiosis. The phenotypes observed in other organisms are strongly suggestive of roles in meiosis, but whether they will be male-specific, female-specific or function in both is not clear. Even though our initial goal was to study factors involved in male meiosis, our studies may lead into more general areas of the meiotic process.

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APPENDICES

APPENDIX

Part II

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

w/B^{s}Yy^{+}; hls$^{\Delta 125}$,e/TM3,Sb,Ser,e ♂ X *yw* ♀

⇓

Score for nondisjunction

Vial #	*B^{s}Yy^{+}* ♂	*y^{+}* ♂	*B^{s}* ♂	*yw* ♂	*B^{s}* ♀	*yw/w* ♀
1	15	0	0	0	0	32
2	14	0	0	0	0	18
3	16	0	0	0	0	28
4	46	0	0	0	0	56
5	57	0	0	0	0	61
6	45	1	0	0	0	34
7	65	0	0	0	0	57
8	42	0	0	0	0	59
9	27	0	0	0	0	54
10	44	0	0	0	0	69
11	18	0	0	0	0	31
12	51	0	0	1	0	43
13	52	0	0	0	0	60
14	31	0	0	0	0	52
15	76	0	0	0	0	57
16	36	0	0	0	0	37
17	60	0	0	0	0	76
18	33	0	0	0	0	33
19	54	0	0	0	0	39

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$w/w;\ hls^{\Delta 125}, e/TM3, Sb, Ser, e$ ♀ X $+/B^{s}Yy^{+};\ hls^{DE3}/TM3,\ Sb,\ e$ ♂

⇓

$w/B^{s}Yy^{+};\ hls^{\Delta 125}, e/\ hls^{DE3}$ ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	$B^{s}Yy^{+}$ ♂	y^{+} ♂	B^{s} ♂	yw ♂	B^{s} ♀	yw/w ♀
1	1	0	0	3	1	14
2	9	0	0	3	2	35
3	11	0	0	12	0	35
4	14	0	0	5	0	19
5	13	0	0	5	0	26
6	17	0	0	10	0	33
7	11	0	0	7	0	35
8	3	0	0	4	0	14
9	21	0	0	23	1	49
10	7	0	0	9	0	30
11	19	0	0	15	3	50
12	20	0	0	10	0	40
13	10	1	0	12	0	38
14	15	0	0	6	0	31
15	8	0	0	10	1	28
16	3	0	0	8	0	22
17	19	0	0	10	0	40
18	8	0	0	5	0	38
19	12	1	0	3	1	41
20	11	0	0	3	1	36
21	14	0	0	8	3	37
22	8	0	0	13	0	39
23	3	0	0	8	1	24

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^{s}Yy^{+}$; hls^{DE3}/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^{s}Yy^{+}$; hls^{DE3}/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	$B^{s}Yy^{+}$ ♂	y^{+} ♂	B^{s} ♂	yw ♂	B^{s} ♀	yw/+ ♀
1	19	0	0	0	0	13
2	16	1	0	1	0	20
3	40	0	0	2	0	38
4	17	0	0	0	0	26
5	13	0	0	0	0	15
6	19	0	0	0	0	20
7	4	0	0	0	0	6
8	6	0	0	1	0	11
9	36	0	0	0	1	45
10	29	0	0	0	0	31

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

w/B^sYy^+; $hls^{\Delta 125}$,e/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^sYy^+$; $hls^{\Delta 125}$,e/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	yw ♂	B^s ♀	yw/+ ♀
1	26	0	0	0	0	60
2	39	1	0	0	0	67
3	48	0	0	2	2	73
4	37	0	0	2	2	77
5	31	0	0	0	1	57
6	23	1	0	0	0	34
7	62	0	0	3	1	55
8	36	3	0	3	1	72
9	38	0	0	2	2	63
10	34	0	0	1	2	52
11	37	1	0	1	1	72
12	41	2	0	1	1	75
13	28	0	0	2	0	43
14	51	0	0	0	0	82
15	48	0	0	4	1	62
16	53	0	0	3	0	70
17	51	0	1	0	1	89
18	43	0	0	1	0	51
19	38	1	0	1	0	56
20	33	2	0	7	0	43
21	7	0	0	0	0	12
22	14	0	0	1	0	21
23	30	0	0	0	1	39
24	17	0	0	0	0	28
25	27	0	0	0	0	67

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^sYy^+$; hls^{DE8}/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^sYy^+$; hls^{DE8}/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	yw ♂	B^s ♀	yw/+ ♀
1	25	0	0	0	0	41
2	14	1	0	3	0	13
3	28	0	0	1	0	46
4	32	3	0	5	2	38
5	26	0	0	0	0	33
6	38	0	0	0	0	26
7	22	0	0	2	1	47
8	34	0	0	1	0	69
9	21	0	0	2	1	25
10	49	0	0	0	0	66
11	22	0	0	0	0	36

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^{s}Yy^{+}$; hls^{E1}/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^{s}Yy^{+}$; hls^{E1}/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	$B^{s}Yy^{+}$ ♂	y^{+} ♂	B^{s} ♂	yw ♂	B^{s} ♀	yw/+ ♀
1	59	0	0	1	1	79
2	36	0	0	2	2	65
3	5	1	0	0	0	10
4	18	0	0	0	2	18
5	16	1	0	2	0	34
6	12	0	0	0	1	21
7	4	0	0	0	0	5
8	19	0	0	1	0	40
9	27	0	0	3	0	44

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^{s}Yy^{+}$; hls^{E616},e/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^{s}Yy^{+}$; hls^{E616},e/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	$B^{s}Yy^{+}$ ♂	y^{+} ♂	B^{s} ♂	yw ♂	B^{s} ♀	yw/+ ♀
1	39	1	0	5	0	67
2	27	1	0	2	0	55
3	9	0	0	1	0	43
4	15	0	0	0	0	31
5	34	1	1	5	0	42
6	37	2	0	0	0	57
7	15	1	0	3	1	49
8	70	2	0	1	0	70
9	19	0	0	1	1	20
10	36	2	0	2	1	52
11	14	0	0	1	0	26
12	19	0	0	1	1	36
13	18	0	0	3	0	46
14	17	0	0	2	1	43
15	35	0	0	0	0	46
16	37	0	0	0	0	58
17	27	0	0	7	1	58
18	19	0	0	1	0	32
19	45	1	1	2	1	64
20	15	0	0	0	0	31
21	10	0	1	1	0	12
22	25	2	0	2	0	29
23	19	0	0	4	1	33
24	12	0	0	3	0	32
25	20	1	1	3	0	31
26	37	0	1	0	0	42
27	23	3	0	1	0	70
28	8	2	0	0	0	15

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^sYy^+$; hls^{W10}/*TM3,Sb,Ser,e* ♂ X +/+; hls^{E616},*e*/*TM3, Sb, e* ♀

⇓

$+/B^sYy^+$; hls^{W10}/ hls^{E616},*e* ♂ X *yw* ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	*yw* ♂	B^s ♀	*yw*/+ ♀
1	11	0	0	3	0	23
2	21	0	0	0	0	28
3	13	0	0	1	0	23
4	16	1	1	2	1	34
5	30	0	0	0	0	45
6	21	0	0	1	0	33
7	2	1	0	2	0	23
8	16	0	0	2	0	23
9	10	0	0	0	0	20
10	2	0	0	0	0	5
11	2	0	0	1	0	0

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^sYy^+$; hls^{CT5}/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^sYy^+$; hls^{CT5}/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	yw ♂	B^s ♀	yw/+ ♀
1	21	0	0	2	0	26
2	22	0	0	3	0	36
3	20	0	1	4	0	60
4	20	0	0	4	0	38
5	36	0	0	1	0	61
6	31	0	0	8	0	38
7	20	0	0	1	0	56
8	21	0	0	3	0	55
9	26	0	0	1	0	40
10	7	0	0	2	0	24
11	31	0	1	4	0	58
12	31	0	0	0	0	54
13	28	0	0	1	1	54
14	11	0	0	0	0	28

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

w/B^sYy^+; hls^{CT4}/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^sYy^+$; hls^{CT4}/hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	yw ♂	B^s ♀	yw/+ ♀
1	36	0	0	7	0	51
2	37	0	0	1	0	59
3	58	1	1	3	0	78
4	23	0	0	3	0	39
5	19	0	0	3	0	50
6	16	0	0	0	0	39

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^{s}Yy^{+}$; hls^{3987},e/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^{s}Yy^{+}$; hls^{3987},e/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	$B^{s}Yy^{+}$ ♂	y^{+} ♂	B^{s} ♂	yw ♂	B^{s} ♀	yw/+ ♀
1	41	0	0	9	0	96
2	16	0	0	7	0	53
3	40	0	0	9	0	82
4	31	0	0	3	2	48
5	44	0	0	1	0	82
6	24	0	0	3	0	82
7	29	0	0	11	0	68
8	37	0	0	2	0	58
9	36	1	0	11	0	67
10	20	0	0	6	0	38
11	33	1	0	4	0	45
12	23	0	0	4	1	51
13	60	0	0	12	1	87
14	34	0	0	5	0	73
15	30	0	0	2	0	60
16	26	0	0	2	0	59
17	2	0	0	1	0	4
18	20	0	0	2	0	31
19	27	0	0	5	1	53
20	24	0	0	2	0	60
21	16	0	0	3	0	41
22	32	0	0	0	0	50
23	32	0	0	5	0	62
24	19	0	0	2	0	53
25	12	0	0	0	0	10
26	25	0	0	3	0	54
27	22	0	0	1	0	64
28	20	0	0	3	0	26
29	27	0	0	0	0	56
30	42	0	0	5	0	63

31	25	0	0	0	1	47
32	15	0	0	3	0	53
33	21	1	0	10	0	52
34	15	1	0	5	1	40
35	22	0	0	5	0	41
36	26	0	0	1	0	38
37	18	0	0	2	0	24
38	18	0	1	3	0	65
39	14	0	0	1	0	23
40	22	0	0	7	1	59
41	8	0	0	3	0	9

Counts from individual cross vials for Part II Table 2. Data is from the following cross:

$+/B^{s}Yy^{+}$; hls^{3987},e/ hls^{3987},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	$B^{s}Yy^{+}$ ♂	y^{+} ♂	B^{s} ♂	yw ♂	B^{s} ♀	yw/+ ♀
1	30	0	0	8	1	39
2	18	0	0	6	0	51
3	33	1	0	4	0	58
4	35	0	0	6	0	76
5	4	0	0	0	0	15
6	18	0	0	6	0	47
7	13	0	0	5	0	41
8	34	0	0	3	0	54
9	28	0	0	1	0	64
10	25	0	0	3	0	48
11	10	0	0	4	0	18
12	22	0	0	5	0	29
13	1	0	0	2	1	8

Counts from individual cross vials for Part II Table 4. Data is from the following cross:

$C(2)EN, b, pr$ ♀ X $+/B^{s}Yy^{+}; hls^{E616}, e/hls^{E616}, e$ ♂

⇓

At day 20 count number of progeny

Vial #	Number of progeny on day 20
1	7
2	6
3	17
4	5
5	14
6	5
7	1
8	1
9	0
10	6
11	8
12	4
13	7
14	9
15	7
16	3

Counts from individual cross vials for Part II Table 4. Data is from the following cross:

C(2)EN, b, pr ♀ X *Oregon R* control ♂

⇓

At day 20 count number of progeny

Vial #	Number of progeny on day 20
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	3
10	2

Counts from individual cross vials for Part II Table 6. Data is from the following cross:

w/B^sYy^+; $hls^{\Delta 125}$,e/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^sYy^+$; $hls^{\Delta 125}$,e/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	yw ♂	B^s ♀	yw/+ ♀
1	26	0	0	0	0	60
2	39	1	0	0	0	67
3	48	0	0	2	2	73
4	37	0	0	2	2	77
5	31	0	0	0	1	57
6	23	1	0	0	0	34
7	62	0	0	3	1	55
8	36	3	0	3	1	72
9	38	0	0	2	2	63
10	34	0	0	1	2	52
11	37	1	0	1	1	72
12	41	2	0	1	1	75
13	28	0	0	2	0	43
14	51	0	0	0	0	82
15	48	0	0	4	1	62
16	53	0	0	3	0	70
17	51	0	1	0	1	89
18	43	0	0	1	0	51
19	38	1	0	1	0	56
20	33	2	0	7	0	43
21	7	0	0	0	0	12
22	14	0	0	1	0	21
23	30	0	0	0	1	39
24	17	0	0	0	0	28
25	27	0	0	0	0	67

Counts from individual cross vials for Part II Table 6. Data is from the following cross:

$+/B^sYy^+$; hls^{E616},e/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3, Sb, e ♀

⇓

$+/B^sYy^+$; hls^{E616},e/ hls^{E616},e ♂ X yw ♀

⇓

Score for nondisjunction

Vial #	B^sYy^+ ♂	y^+ ♂	B^s ♂	yw ♂	B^s ♀	yw/+ ♀
1	39	1	0	5	0	67
2	27	1	0	2	0	55
3	9	0	0	1	0	43
4	15	0	0	0	0	31
5	34	1	1	5	0	42
6	37	2	0	0	0	57
7	15	1	0	3	1	49
8	70	2	0	1	0	70
9	19	0	0	1	1	20
10	36	2	0	2	1	52
11	14	0	0	1	0	26
12	19	0	0	1	1	36
13	18	0	0	3	0	46
14	17	0	0	2	1	43
15	35	0	0	0	0	46
16	37	0	0	0	0	58
17	27	0	0	7	1	58
18	19	0	0	1	0	32
19	45	1	1	2	1	64
20	15	0	0	0	0	31
21	10	0	1	1	0	12
22	25	2	0	2	0	29
23	19	0	0	4	1	33
24	12	0	0	3	0	32
25	20	1	1	3	0	31
26	37	0	1	0	0	42
27	23	3	0	1	0	70
28	8	2	0	0	0	15

Counts from individual cross vials for Part II Table 7. Data is from the following cross:

$+/B^sYy^+$; hls^{E616},e/hls^{E616},e ♂ X yw ♀

⇓

Score sex chromosome nondisjunction and Y breakage

Vial #	B^sYy^+ ♂	yw ♂	y^+ ♂	B^s ♂	B^s ♀	$yw/+$ ♀
1	39	5	1	0	0	67
2	27	2	1	0	0	55
3	9	1	0	0	0	43
4	15	0	0	0	0	31
5	34	5	1	1	0	42
6	37	0	2	0	0	57
7	15	3	1	0	1	49
8	70	1	2	0	0	70
9	19	1	0	0	1	20
10	36	2	2	0	1	52
11	14	1	0	0	0	26
12	19	1	0	0	1	36
13	18	3	0	0	0	46
14	17	2	0	0	1	43
15	35	0	0	0	0	46
16	37	0	0	0	0	58
17	27	7	0	0	1	58
18	19	1	0	0	0	32
19	45	2	1	1	1	64
20	15	0	0	0	0	31
21	10	1	0	1	0	12
22	25	2	2	0	0	29
23	19	4	0	0	1	33
24	12	3	0	0	0	32
25	20	3	1	1	0	31
26	37	0	0	1	0	42
27	23	1	3	0	0	70
28	8	0	2	0	0	15

Counts from individual cross vials for Part II Table 7. Data is from the following cross:

w/B^sYy^+; $hls^{\Delta 125}$,e/TM3,Sb,Ser,e ♂ X +/+; hls^{E616},e/TM3,Sb,e ♀

⇓

$+/B^sYy^+$; $hls^{\Delta 125}$,e/ hls^{E616},e ♂ X yw ♀

⇓

Score for sex chromosome nondisjunction and Y breakage

Vial #	B^sYy^+ ♂	yw ♂	y^+ ♂	B^s ♂	B^s ♀	yw/+ ♀
1	26	0	0	0	0	60
2	39	0	1	0	0	67
3	48	2	0	0	2	73
4	37	2	0	0	2	77
5	31	0	0	0	1	57
6	23	0	1	0	0	34
7	62	3	0	0	1	55
8	36	3	3	0	1	72
9	38	2	0	0	2	63
10	34	1	0	0	2	52
11	37	1	1	0	1	72
12	41	1	2	0	1	75
13	28	2	0	0	0	43
14	51	0	0	0	0	82
15	48	4	0	0	1	62
16	53	3	0	0	0	70
17	51	0	0	1	1	89
18	43	1	0	0	0	51
19	38	1	1	0	0	56
20	33	7	2	0	0	43
21	7	0	0	0	0	12
22	14	1	0	0	0	21
23	30	0	0	0	1	39
24	17	0	0	0	0	28
25	27	0	0	0	0	67

Counts from individual cross vials for Part II Table 7. Data is from the following cross:

$wP(w^+;$ *hls-myc*$)/B^sYy^+;$ hls^{E616},*e*/hls^{E616},*e* ♂ X *yw* ♀

⇓

Score for sex chromosome nondisjunction and Y breakage

Vial #	B^sYy^+ ♂	*yw* ♂	y^+ ♂	B^s ♂	B^sYy^+ ♀	$wP(w^+;$ *hls-myc*$)/yw$ ♀
1	23	1	1	0	0	26
2	23	1	0	0	0	30
3	20	8	0	0	0	66
4	12	6	0	0	0	26
5	21	11	0	0	1	49
6	27	10	2	0	2	66
7	25	5	0	0	1	54
8	7	0	0	0	0	8
9	21	8	1	0	0	51

Counts from individual cross vials for Part II Table 7. Data is from the following cross:

$wP(w^+; hls\text{-}myc)/B^sYy^+; hls^{E616}, e/hls^{\Delta 125},e$ ♂ X yw ♀

⇓

Score for sex chromosome nondisjuntion and Y breakage

Vial #	B^sYy^+ ♂	yw ♂	y^+ ♂	B^s ♂	B^sYy^+ ♀	$wP(w^+;hls\text{-}myc)/yw$ ♀
1	9	5	0	0	0	20
2	20	5	0	0	0	48
3	20	4	1	0	1	57
4	5	4	0	0	0	26
5	52	0	0	0	0	69
6	11	3	0	0	0	19
7	37	2	1	0	0	32
8	29	16	0	0	1	48
9	17	6	0	0	0	39
10	16	12	1	0	1	55
11	40	0	0	0	0	42
12	45	0	0	0	0	33
13	19	7	0	0	0	37
14	34	0	0	0	0	39
15	39	0	0	0	0	26
16	40	0	0	0	0	45
17	26	2	0	0	0	47

APPENDIX

Part III

Counts from individual cross vials for Part III Table 1A. Data is from the following cross:

yw nmf/y^{+}*Y; Df(2R)XTE-18/Cy* ♂ X *yw* ♀

⇓

Score for nondisjunction

Vial #	y^{+}♂	*y w* ♂	y^{+} ♀	*y w* ♀
1	54	9	2	73
2	52	10	2	42
3	36	0	0	5
4	40	11	2	42
5	30	9	0	42
6	27	7	0	33
7	16	0	0	3
8	39	8	1	48
9	28	0	0	20
10	40	0	0	57
11	35	6	1	44
12	30	0	0	27
13	25	9	3	50
14	37	0	0	46

* Not all vials counted for Table 1A are listed.

Counts from individual cross vials for Part III Table 1B. Data is from the following cross:

yw nmf/yw nmf; Df(2R)XTE-18/Cy ♀ X *+/B^{s}Y^{+}* ♂

⇓

Score for nondisjunction

Vial #	B^{s}Yy^{+} ♂	*y w* or *+/O* ♂	B^{s}Yy^{+} ♀	wild-type ♀
1	25	0	0	30
2	23	0	0	22
3	7	0	0	20
4	29	1	1	31
5	11	1	0	13
6	21	2	0	18
7	4	0	0	6
8	28	1	0	24
9	6	0	0	6
10	15	0	1	16
11	7	0	0	19
12	6	0	1	4
13	16	0	1	14
14	13	2	0	10
15	14	0	0	18
16	22	0	0	35
17	21	0	0	22
18	3	0	0	5
19	10	0	0	10
20	8	2	0	25
21	4	0	0	0
22	6	0	0	3

* Not all vials counted for Table 1B are listed.

Counts from individual cross vials for Part III Table 3. Data is from the following cross:

yw nmf/+; Df(2R)XTE-18/+ ♀ X *+/B^{s}Y^{+}* ♂

⇓

Score for nondisjunction

Vial #	B^{s}Y^{+} ♂	*y w* or *+/O* ♂	B^{s}Y^{+} ♀	wild-type ♀
1	26	0	0	29
2	28	0	0	27
3	20	0	0	30
4	16	0	0	21
5	42	0	1	31
6	33	0	0	26
7	17	0	0	20
8	10	0	0	24
9	12	0	0	23
10	19	0	0	23
11	33	0	1	45
12	24	0	0	23
13	24	0	0	25
14	26	0	0	33
15	21	0	0	27
16	34	0	0	32
17	14	0	0	22
18	25	0	0	22
19	42	0	1	30
20	22	0	0	27
21	28	0	0	29
22	30	0	0	29
23	13	0	0	21
24	15	2	0	19

Counts from individual cross vials for Part III Table 3. Data is from the following cross:

yw nmf/+; Cy/+ ♀ X *+/B^{s}Yy^{+}* ♂

⇓

Score for nondisjunction

Vial #	B^{s}Yy^{+} ♂	*y w* or *+/O* ♂	B^{s}Yy^{+} ♀	wild-type ♀
1	6	0	0	21
2	29	1	0	40
3	21	0	0	13
4	9	0	0	19
5	29	0	0	24
6	14	0	0	25
7	9	0	0	3
8	37	0	1	47
9	16	0	0	22
10	25	0	0	30
11	25	0	2	36
12	24	0	0	20
13	23	0	0	34
14	29	0	0	29
15	20	0	0	32
16	22	0	0	44
17	8	0	0	11
18	21	1	0	28
19	22	1	0	39
20	27	0	0	32
21	12	0	1	7

Counts from individual cross vials for Part III Table 4. Data is from the following cross:

$w/y^{+}Y;\ 3*/3*$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for nondisjunction

Vial #	$y^{+}Y$ ♂	$y\ w$ ♂	w ♀
1	55	0	55
2	24	0	22
3	23	0	41
4	42	0	48
5	44	0	64
6	19	0	24
7	34	0	33
8	32	0	42
9	45	0	43
10	20	0	25
11	24	0	24
12	27	0	52
13	62	0	60
14	51	0	54
15	11	0	8
16	73	1	47
17	71	0	66
18	52	0	51
19	20	0	32
20	36	0	33
21	52	0	71
22	62	0	67
23	49	0	72
24	26	0	38
25	33	0	50

Counts from individual cross vials for Part III Table 4. Data is from the following cross:

w/w; 3/3** ♀ X *y w/B^{s}Yy$^+$; Cy/Sp* ♂

⇓

Score for nondisjunction

Vial #	*B^{s}Yy$^+$* ♂	*y w* ♂	*B^{s}Yy$^+$* ♀	*w* ♀
1	52	0	0	59
2	34	0	0	36
3	29	0	0	25
4	39	0	0	35
5	31	0	0	29
6	9	1	0	33
7	32	0	0	34
8	21	0	0	10
9	20	0	0	25
10	28	1	0	40
11	21	0	0	33
12	33	0	0	41
13	31	0	0	36
14	23	0	0	27
15	3	0	0	5
16	24	0	0	31
17	20	0	0	30
18	17	0	0	25
19	2	0	0	1

Counts from individual cross vials for Part III Table 5. Data is from the following cross:

y w nmf/B^{s}Yy$^+$; Df(2R)XTE-18/+ ♂ X *yw* ♀

⇓

Score for nondisjunctional progeny

Vial #	*B^{s}Yy$^+$* ♂	*y w* ♂	*B^{s}Yy$^+$* ♀	*y w* ♀
1	23	1	0	25
2	29	2	4	40
3	26	6	1	23
4	40	0	0	37
5	45	4	2	67
6	34	0	0	37
7	21	6	1	32
8	15	4	0	20
9	4	3	0	21
10	36	0	0	54
11	30	6	4	30
12	16	0	0	18
13	34	0	0	19
14	25	9	3	42
15	35	1	0	29
16	11	0	0	7
17	3	0	1	9
18	42	4	2	54
19	26	3	1	22
20	37	4	1	41
21	41	4	0	51
22	21	0	0	26
23	52	4	5	39
24	10	2	1	13
25	24	2	0	32
26	36	0	0	46
27	28	1	2	26
28	9	1	1	12

29	27	0	0	22
30	46	0	0	38
31	7	0	0	10

Counts from individual cross vials for Part III Table 5. Data is from the following cross:

$$y\ w\ nmf/B^sYy^+;\ Cy/+\ \male\ \mathbf{X}\ yw\ \female$$

$$\Downarrow$$

Score for nondisjunctional progeny

Vial #	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	$y\ w$ ♀
1	14	0	0	15
2	22	4	3	19
3	25	0	0	19
4	27	8	2	61
5	5	0	0	10
6	29	3	0	25
7	12	3	0	15
8	12	4	0	15
9	19	2	1	38
10	4	0	0	4
11	24	0	2	25
12	10	0	0	20
13	16	1	2	11
14	6	1	0	10
15	7	2	0	10
16	11	2	0	11
17	1	1	0	4
18	12	2	1	13
19	47	1	0	46
20	35	3	5	42
21	35	0	0	33
22	34	11	8	50
23	60	0	0	47
24	54	1	0	37
25	48	2	2	58
26	14	0	1	13
27	2	0	0	7

Counts from individual cross vials for Part III Table 5. Data is from the following cross:

$y\ w/B^sYy^+;\ +/+\ ♂\ \mathrm{X}\ y\ w/y\ w\ ♀$

⇓

Score for nondisjunctional progeny

Vial #	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	$y\ w$ ♀
1	18	2	2	35
2	19	0	0	16
3	45	0	0	29
4	28	4	1	26
5	54	4	1	42
6	20	1	1	31
7	22	4	1	29
8	35	0	0	43
9	51	0	0	39
10	37	2	3	35
11	50	5	3	44
12	33	2	0	33
13	24	4	0	28
14	43	0	0	46
15	30	0	0	18
16	15	2	0	7
17	21	0	0	27
18	11	0	0	30
19	47	0	0	44
20	2	0	0	8
21	31	1	0	27
22	13	0	0	13
23	9	0	0	18
24	22	4	3	34
25	35	2	0	55
26	53	0	0	64
27	49	14	18	53
28	35	0	0	28
29	60	0	0	83

30	67	19	6	39
31	51	0	0	47
32	65	0	0	54

Counts from individual cross vials for Part III Table 7. Data is from the following cross:

10 *y w/y*$^{+}$*Y; Df(2R)XTE-18/Cy* ♂ X 10 *C(2)EN b, pr* ♀

⇓

Score for number of progeny after 20 days

Vial #	Number of Progeny
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	1
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
21	0
22	0
23	0
24	0
25	1
26	1
27	0
28	1
29	0
30	0
31	0
32	0
33	0
34	2
35	0
36	0
37	0
38	0

Counts from individual cross vials for Part III Table 6. Data is from the following cross:

10 *y w/y*$^{+}$*Y; Df(2R)XTE-18/Cy* ♂ X 10 *C(2)EN b, pr* ♀

⇓

Score for number of progeny after 20 days

Vial #	Number of Progeny
1	28
2	18
3	26
4	18
5	17
6	31
7	7
8	11
9	28
10	23
11	15
12	17
13	7
14	15
15	7
16	10
17	10
18	11
19	14
20	21
21	16
22	12
23	18
24	11
25	15
26	24
27	1
28	20

Counts from individual cross vials for Part III Table 8. Data is from the following cross:

y w/ y⁺Y; Df(2R)XTE-18/Cy ♂ X *C(4) ci ey* ♀

⇓

Score for 4th chromosome nondisjunction

Vial #	wild-type progeny	*ci ey* progeny
1	19	0
2	60	2
3	48	2
4	60	1
5	57	2
6	30	3
7	48	4
8	44	0
9	49	5
10	51	0
11	5	1
12	6	0
13	41	3
14	45	1
15	15	0
16	74	4
17	61	3
18	41	0
19	45	3
20	49	2
21	29	0
22	10	0
23	18	2
24	29	2
25	45	2
26	45	1
27	84	2
28	59	3
29	18	0
30	31	7
31	8	1

32	9	2
33	32	0
34	6	0
35	20	0
36	5	1
37	6	0
38	47	3
39	33	1
40	21	1
41	85	1
42	16	1
43	46	0
44	21	5
45	16	1
46	34	5

Counts from individual cross vials for Part III Table 7. Data is from the following cross:

y w/ y w; Df(2R)XTE-18/Cy ♀ X *C(4) ci ey* ♂

⇓

Score for 4th chromosome nondisjunction

Vial #	wild-type progeny	*ci ey* progeny
1	5	0
2	14	0
3	17	0
4	23	0
5	6	0
6	11	0
7	2	0
8	12	0
9	7	0
10	8	0
11	8	0
12	15	0
13	6	0
14	2	0
15	5	0
16	6	0
17	3	1
18	3	0
19	27	0
20	45	0
21	26	0

Counts from individual cross vials for Part III Table 7. Data is from the following cross:

y w/FM6; Df(2R)XTE-18/+ ♀ X *C(4) ci ey* ♂

⇓

Score for 4th chromosome nondisjunction

Vial #	wild-type progeny	*FM6* progeny	*ci ey* progeny	XY nondisjunctional progeny
1	19	13	2	3
2	3	3	0	0
3	14	0	0	0
4	7	2	0	0
5	16	6	0	0
6	5	2	1	1
7	4	0	0	0
8	2	0	0	0
9	12	3	1	0
10	7	0	0	2
11	6	1	0	0
12	5	4	0	5
13	2	0	0	2
14	10	5	0	1
15	4	2	0	0
16	4	0	0	2
17	3	0	0	0
18	3	0	0	0
19	4	5	1	1
20	3	4	1	3
21	5	3	0	0
22	8	2	0	0
23	3	1	0	1
24	3	2	0	0
25	6	2	0	1
26	9	5	1	2
27	3	0	0	1
28	8	4	0	1
29	4	2	0	1
30	5	2	2	1
31	8	4	0	0
32	4	2	0	1
33	5	1	0	1
34	2	5	0	2
35	8	4	1	1
36	1	3	0	1

Counts from individual cross vials for Part III Table 10. Data is from the following cross:

C(1)RM/O ♀ X *y w nmf/y⁺Y; Df(2R)XTE-18/Cy* ♂

⇓

Score progeny

Vial #	*y w/O* ♂	*y w/y⁺Y* ♂	*C(1)RM/O* ♀	*y w/y w* ♀	*C(1)RM/y⁺Y* ♀
1	4	1	0	0	18
2	2	0	0	0	8
3	0	0	0	0	3
4	4	0	0	0	2
5	2	0	0	0	5
6	2	0	0	0	4
7	2	0	0	0	4
8	8	0	3	0	23
9	9	1	2	0	17
10	0	0	0	0	14
11	4	0	0	0	16
12	13	0	0	0	23
13	9	0	0	0	29
14	9	1	1	0	24
15	4	1	0	0	6
16	15	1	7	0	29
17	2	1	1	0	12
18	11	0	1	0	3
19	4	0	0	0	15
20	3	0	0	0	7
21	5	1	5	0	18
22	2	1	0	0	4
23	0	0	0	0	9
24	4	0	0	0	18
25	10	0	0	0	19
26	10	0	3	0	11
27	0	0	0	0	3
28	30	5	6	0	45
29	6	1	0	0	22

30	26	3	2	0	36
31	0	0	1	0	16
32	0	0	0	0	39
33	10	1	3	0	14
34	21	3	4	0	33
35	1	0	0	0	4
36	25	3	0	0	33
37	19	0	1	0	19
38	0	0	0	0	25
39	14	4	6	0	14
40	4	1	0	0	11
41	24	1	4	0	44
42	17	2	5	0	17
43	28	1	7	0	36
44	19	0	4	0	12
45	15	0	3	0	25
46	14	3	3	0	23
47	16	2	8	0	21
48	35	1	3	0	35
49	16	2	3	0	34
50	18	3	6	0	56
51	5	0	1	0	8
52	6	0	1	0	13
53	17	0	1	0	15
54	29	0	0	0	42
55	25	0	6	0	25
56	6	0	1	0	11

Counts from individual cross vials for Part III Table 8. Data is from the following cross:

y w nmf/FM6; Df(2R)XTE-18/+ ♀ X *Oregon R* wild-type ♂

⇓

Count Progeny

Vial #	XY ♂	+/O ♂	*y w/FM6/Y* ♀	*y w/y w/Y* ♀	*FM6/FM6* ♀	*y w/+* or *FM6/+* ♀
1	35	4	0	0	0	37
2	9	0	0	0	0	17
3	10	1	1	0	0	16
4	33	0	0	0	0	22
5	12	0	0	0	0	30
6	13	3	0	0	0	16
7	9	0	1	0	0	10
8	14	1	1	0	0	25
9	3	0	0	0	0	5
10	16	0	1	0	0	24
11	5	0	0	0	0	3
12	5	0	0	0	0	25
13	7	1	0	0	0	9
14	24	2	1	0	0	24
15	27	1	1	0	0	20
16	26	0	1	0	0	29
17	20	3	1	0	0	10
18	29	0	1	0	0	30
19	20	0	2	0	0	15
20	12	0	0	0	0	7
21	28	2	2	0	0	25
22	32	0	0	0	0	34
23	19	3	1	0	0	18
24	16	5	5	0	0	29
25	22	0	5	0	0	22
26	15	1	3	0	0	32
27	7	0	0	0	0	6
28	12	2	1	0	0	29
29	16	2	0	0	0	18

30	24	1	2	0	0	38
31	18	0	1	0	0	13
32	16	2	2	0	0	26
33	7	1	0	0	0	7
34	26	0	1	0	0	35
35	16	3	7	0	0	21
36	1	0	0	0	0	7
37	30	1	0	0	0	36
38	12	4	3	0	0	24
39	19	1	1	0	0	23
40	8	1	0	0	0	17
41	13	1	1	0	0	14
42	18	4	2	0	0	22
43	7	0	0	0	0	14
44	16	2	2	0	0	24
45	19	1	1	0	0	11
46	19	2	1	0	0	13
47	31	1	2	0	0	33
48	14	0	0	0	0	13
49	15	0	1	0	0	9
50	17	0	0	0	0	12
51	7	3	0	0	0	5
52	28	2	0	0	0	16
53	12	0	1	0	0	3
54	4	1	1	0	0	4
55	36	3	1	0	0	41
56	20	3	2	0	0	22
57	13	3	1	0	0	14
58	12	0	0	0	0	20
59	23	3	0	0	0	13
60	26	0	1	0	0	18
61	21	4	4	0	0	30
62	22	1	0	0	0	26
63	11	1	0	0	0	12
64	20	4	1	0	0	31
65	22	6	3	0	0	23
66	40	1	0	0	0	32
67	18	0	0	0	0	23
68	15	0	0	0	0	18
69	6	0	0	0	0	8

70	35	0	0	0	0	46
71	8	2	2	0	0	8
72	14	1	0	0	0	18
73	15	0	2	0	0	21
74	15	0	1	0	0	24
75	28	0	2	0	0	28
76	12	2	0	0	0	13
77	19	4	3	0	0	16
78	4	0	0	0	0	3
79	13	2	1	0	0	20
80	2	1	0	0	0	1
81	5	2	2	0	0	2
82	7	1	1	0	0	8
83	28	0	0	0	0	28
84	17	0	0	0	0	20
85	19	2	0	0	0	27
86	14	1	1	0	0	10
87	32	2	0	0	0	40
88	19	1	0	0	0	9
89	18	1	0	0	0	11
90	25	4	0	0	0	26
91	11	1	0	0	0	10
92	16	0	0	0	0	12
93	29	6	1	0	0	30
94	11	0	1	0	0	19
95	18	1	1	0	0	19
96	17	0	0	0	0	23
97	26	4	1	0	0	27
98	11	2	4	0	0	8
99	9	0	0	0	0	16
100	9	0	1	0	0	8
101	5	0	0	0	0	1
102	15	1	2	0	0	29
103	27	0	1	0	0	28
104	13	1	1	0	0	15
105	14	0	0	0	0	26
106	7	0	0	0	0	5
107	18	0	0	0	0	27
108	17	0	2	0	0	17
109	24	1	0	0	0	26

110	25	0	0	0	0	27

Counts from individual cross vials for Part III Table 8. Data is from the following cross:

y w nmf/FM6; Cy/+ ♀ X *Oregon R* wild-type ♂

⇓

Count Progeny

Vial #	XY ♂	+/O ♂	*y w/FM6/Y* ♀	*y w/y w/Y* ♀	*FM6/FM6* ♀	*y w/+* or *FM6/+* ♀
1	25	3	3	0	0	26
2	30	5	3	0	0	15
3	26	3	4	0	0	35
4	33	1	1	0	0	30
5	15	5	3	0	0	33
6	25	3	5	0	0	19
7	14	2	2	0	0	11
8	16	3	2	0	0	22
9	9	1	2	0	0	7
10	14	2	0	0	0	20
11	14	0	2	0	0	16
12	18	4	0	0	0	23
13	17	0	1	0	0	27
14	24	2	0	0	0	17
15	15	5	2	0	0	12
16	32	11	5	0	0	23
17	21	2	0	0	0	21
18	20	0	0	0	0	24
19	13	4	4	0	0	11
20	16	3	1	0	0	26
21	15	2	1	0	0	19
22	9	1	1	0	0	16
23	23	4	0	0	0	20
24	7	2	2	0	0	12
25	8	0	0	0	0	15
26	16	4	3	0	0	17
27	34	3	1	0	0	35
28	10	0	0	0	0	16
29	3	1	0	0	0	4

30	17	0	1	0	0	14
31	35	1	0	0	0	33
32	13	4	3	0	0	27
33	22	1	2	0	0	39
34	2	3	2	0	0	4
35	33	4	3	0	0	30
36	22	0	0	0	0	23
37	16	3	2	0	0	28
38	14	2	2	0	0	26
39	3	0	1	0	0	5
40	23	0	0	0	0	25
41	3	2	1	0	0	10

Counts from individual cross vials for Part III Table 8. Data is from the following cross:

y w /FM6 ♀ X *Oregon R* wild-type ♂

⇓

Count Progeny

Vial #	XY ♂	*+/O* ♂	*y w/FM6/Y* ♀	*y w/y w/Y* ♀	*FM6/FM6* ♀	*y w/+* or *FM6/+* ♀
1	43	0	0	0	0	43
2	24	0	0	0	0	18
3	50	0	0	0	0	57
4	31	0	0	0	0	36
5	36	0	1	0	0	35
6	27	0	0	0	0	26
7	36	0	0	0	0	26
8	58	0	0	0	0	43
9	10	0	0	0	0	14
10	40	0	1	0	0	71
11	27	0	1	0	0	40
12	50	0	0	0	0	63
13	21	0	0	0	0	43
14	21	0	0	0	0	32
15	12	0	0	0	0	18
16	38	0	0	0	0	47
17	38	0	0	0	0	53
18	32	0	0	0	0	47
19	22	0	0	0	0	31
20	32	0	0	0	0	29
21	6	0	0	0	0	2
22	8	0	0	0	0	6
23	10	0	0	0	0	19
24	15	0	0	0	0	22
25	16	0	0	0	0	19
26	6	0	0	0	0	12
27	4	0	0	0	0	5
28	12	0	0	0	0	20
29	17	0	0	0	0	8

30	32	1	0	0	0	40
31	22	1	0	0	0	37
32	14	0	0	0	0	23
33	21	0	0	0	0	27
34	39	0	0	0	0	48
35	39	0	0	0	0	38
36	40	0	0	0	0	44
37	19	0	0	0	0	18
38	32	1	1	0	0	61
39	31	0	0	0	0	39
40	36	0	0	0	0	27
41	40	0	0	0	0	50
42	13	0	0	0	0	21
43	22	0	0	0	0	31
44	15	0	0	0	0	31
45	39	0	0	0	0	36
46	32	0	0	0	0	30

Counts from individual cross vials for Part III Table 8. Data is from the following cross:

y w /FM6; Cy/+ ♀ X *Oregon R* wild-type ♂

⇓

Count Progeny

Vial #	XY ♂	*+/O* ♂	*y w/FM6/Y* ♀	*y w/y w/Y* ♀	*FM6/FM6* ♀	*y w/+* or *FM6/+* ♀
1	11	0	1	0	0	16
2	12	0	2	0	0	32
3	18	0	1	0	0	7
4	8	0	0	0	0	6
5	9	10	6	0	0	9
6	9	0	0	0	0	8
7	27	4	0	0	0	25
8	19	0	0	0	0	13
9	34	1	0	0	0	29
10	22	3	0	0	0	38
11	21	0	0	0	0	18
12	12	0	0	0	0	20
13	21	2	0	0	0	9
14	15	1	0	0	0	18
15	38	0	0	0	0	45
16	18	0	0	0	0	44
17	25	0	0	0	0	23
18	28	1	1	0	0	32
19	4	10	9	0	0	15
20	23	2	1	0	0	20

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y\ w\ cv^{+}\ f^{+}/B^{s}Yy^{+}$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromosome nondisjunction

Vial	$B^{s}Yy^{+}$ ♂	$y\ w$ ♂	$B^{s}Yy^{+}$ ♀	$y\ w$ ♀
1	45	10	1	44
2	41	8	0	38
3	33	6	0	40
4	38	0	0	25
5	40	2	0	37
6	43	0	0	30
7	45	1	0	50
8	31	10	0	40
9	47	5	1	39
10	44	3	4	41
11	64	9	4	45
12	55	0	0	48
13	17	0	0	17
14	38	7	1	29
15	54	9	1	56
16	44	0	0	63
17	32	7	0	35
18	56	0	0	63
19	33	7	1	50
20	22	3	5	28
21	29	1	1	32
22	49	0	0	49
23	24	0	0	42
24	16	4	0	26
25	24	8	1	61
26	57	0	0	51
27	62	0	0	51
28	45	0	0	55
29	30	4	0	31
30	42	5	3	49
31	51	11	1	44
32	64	0	0	63
33	38	0	0	39
34	40	7	4	58
35	34	1	0	38
36	48	0	0	44
37	40	0	0	37
38	45	0	0	50

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y\ w\ cv^{+}\ f/B^{s}Yy^{+}$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	$B^{s}Yy^{+}$ ♂	$y\ w$ ♂	$B^{s}Yy^{+}$ ♀	$y\ w$ ♀
1	41	1	0	53
2	42	0	0	49
3	27	0	0	37
4	43	0	0	46
5	49	0	0	52
6	43	0	0	75
7	56	0	0	73
8	60	0	0	61
9	43	0	0	47
10	43	0	0	43
11	47	1	0	43
12	53	0	0	48
13	36	0	0	37
14	46	0	0	40
15	40	0	0	46
16	58	0	0	64
17	60	0	0	74
18	57	0	0	66
19	60	0	0	48
20	62	0	0	85
21	45	0	0	37
22	56	8	2	59
23	53	0	0	34
24	52	0	0	43
25	27	2	1	32
26	43	0	0	53
27	45	0	0	44
28	61	0	0	72
29	29	0	0	28

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y\ w\ cv\ f/B^sYy^+$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	$y\ w$ ♀
1	50	0	0	58
2	62	0	0	64
3	39	0	0	48
4	36	0	0	39
5	40	0	0	55

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y\ w\ cv\ f^{+}/B^{s}Y y^{+}$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	$B^{s}Y y^{+}$ ♂	$y\ w$ ♂	$B^{s}Y y^{+}$ ♀	$y\ w$ ♀
1	43	4	0	45
2	45	4	0	62
3	53	9	0	71
4	37	4	4	31
5	43	0	0	47

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y^+ w^+ cv\ v\ f/B^sYy^+$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	wild-type ♀
1	39	3	0	30
2	30	1	0	33
3	62	0	0	43
4	42	1	2	22
5	46	1	0	78

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y^+ w^+ cv\ v\ f^+/B^sYy^+$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	wild-type ♀
1	61	0	1	43
2	45	0	0	53
3	65	0	0	63
4	47	0	0	59
5	44	0	0	53
6	29	8	2	32
7	48	0	0	51
8	44	0	0	32

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y^+ w^+ cv^+ v^+ f/B^sYy^+$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	wild-type ♀
1	27	4	1	32
2	50	0	0	31
3	32	1	1	39

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y^+ w^+ cv\ v^+ f^+/B^sYy^+$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	wild-type ♀
1	42	4	3	37
2	36	2	0	40
3	41	2	0	31
4	32	6	1	47
5	38	0	0	46
6	34	1	0	44
7	38	0	0	39

Counts from individual cross vials for Part III Table 13. Data is from the following cross:

$y^+ w^+ cv^+ v^+ f^+/B^sYy^+$ ♂ X $y\ w/y\ w$ ♀

⇓

Score for sex chromsome nondisjunction

Vial	B^sYy^+ ♂	$y\ w$ ♂	B^sYy^+ ♀	wild-type ♀
1	21	0	0	35
2	32	1	0	49
3	34	8	2	50
4	19	0	0	28
5	43	9	2	38
6	41	0	0	26
7	50	0	1	52
8	3	1	0	5
9	43	0	0	50

VITA

Wendy Stapleton grew up in Sweetwater, Tennessee, a small town 50 miles South of Knoxville. Wendy attended the University of Tennessee, Knoxville as a Tennessee Scholar and graduated with a Bachelor of Science in Biology and a Bachelor of Arts in Honors English. She then began graduate studies at the University of Tennessee, Knoxville and received her Ph.D in 1999 as a result of dissertation work completed in the lab of Dr. Bruce McKee in the Department of Biochemistry, Cellular and Molecular Biology Department.

Wendy plans to relocate to Salem, Virginia and hopes to have to opportunity to gain teaching experience and eventually a faculty position at one of the local colleges.