Morewright (mwr), a New Meiotic Mutant of Drosophila melanogaster Affecting Nonexchange Chromosome Segregation

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ABSTRACT

A new meiotic mutation, morewright (mwr) was identified by screening for new mutations that act as dominant enhancers of the dosage-sensitive Drosophila melanogaster female meiotic mutant, nod<sup>DF75</sup>. mwr is a recessive meiotic mutant, specifically impairing the segregation of nonexchange chromosomes. Cytological evidence suggests that the meiotic defect in mwr/mwr females is in homologue recognition because the chromosomes appear to be misaligned on an intact spindle. The mwr mutation was recovered during a screen of random P-element insertions on a chromosome with a single insertion located at 50C. The P-element insertion is a recessive female-sterile mutation. While excision of the P element from the mwr-bearing chromosome partially relieves the female sterility, the excisions retain the dominant nod<sup>DF75</sup>-enhancing activity. The mwr meiotic phenotype maps very close to the female-sterile P insertion. Thus the mwr locus appears to encode a function required for partner recognition in meiosis, although its relationship to the neighboring female-sterile mutation remains to be elucidated.

The purpose of meiosis is to allow the parent to supply exactly one copy of each chromosome pair to the offspring. This process must be precise because aneuploidy has severe consequences for developing embryos and is often lethal. In most organisms, the two disjoining homologues undergo exchange (physically manifested as chiasmata), forming a physical connection between them. The bivalents are correctly positioned by balancing the poleward forces exerted by the spindle and spindle-based motor proteins of the dynein and kinesin families with the polar ejection force (mainly chiasmata) (Nicklas 1974; Murray and Szostak 1985). Although exchange is generally sufficient to ensure proper alignment and disjunction of homologues (reviewed by Hawley 1989), Drosophila females and other organisms have evolved mechanisms for segregating nonexchange (achiasmate) chromosomes, referred to historically in Drosophila as the “distributive system” (Grell 1962).

Nonexchange chromosomes first choose partners. Recent evidence suggests that homologues will generally pair with each other, while nonhomologues will disjoin from each other based on similarities in size and shape (Hawley et al. 1993a). While nonhomologue pairs are physically paired before segregation in Saccharomyces cerevisiae (LoiDl et al. 1994), in Drosophila females nonhomologues are not paired but homologues are associated throughout prophase I whether or not they have an exchange (Dernburg et al. 1996). Heterochromatin appears to mediate the pairing between chromosomes. In the case of exchange chromosomes, discrete sites of intercalary heterochromatin distributed along the length of the chromosome appear to act as pairing sites (Hawley 1980). For nonexchange homologues, the centromeric heterochromatin appears to be the site of pairing (Dernburg et al. 1996; Karpent et al. 1996).

One approach to identifying the components of the meiotic machinery has been to identify meiotic mutations that impair chromosome segregation. While such mutants have been invaluable tools for dissecting meiosis (Baker and Hall 1976), these mutations do not identify genes that encode proteins with redundant or minor functions, or genes that encode products that are essential at some other time during development. Even when mutations with a subtle meiotic phenotype have been recovered, such as mei-251 (Baker and Carpenter 1972) and mei-1029 (Szauter 1984), it has been difficult even to map them because the low frequency of nondisjunction makes it impossible to determine if an individual female is mutant.

For this reason a screen for transposon-induced mutations that interact with an existing meiotic mutant was undertaken. The screen looked for cases in which the double heterozygote has a more severe phenotype than either of the individual heterozygotes, similar to the approach of Kennison and Tamkun (1988) who found regulators of homeotic genes by looking for dominant modifiers of Pc and Antp.

The rationale behind the screen was that if the meiotic machinery is slightly impaired by an existing mutation, a second mutation in another component can have a synergistic effect, causing a more severe pheno-
type. Such an interaction was seen in double heterozygotes for two recessive meiotic mutations, *ned* and *nod* (Knowles and Hawley 1992). Thus the present screen used an existing mutant, *nod* "w" as a "magnifying glass" to examine the role of mutations whose phenotype alone might be too subtle to study. The screen identified a new meiotic mutation that specifically affects the segregation of nonexchange homologues.

MATERIALS AND METHODS

**Genetic stocks:** *Df(2R)wxα2* with breakpoints 49F;50C6 was generously provided by S. Dougan and S. Dinardo (Rockefeller University), who also provided a stock of *Df(2R)87F98* with breakpoints 49B11-C1;2;50A12-B1.2, which was generated and characterized by I. D. Alexandrov (Moscow State University). *Df(2R)CX1* has breakpoints at 49D1;50C2-D1 (N. Baker, personal communication). *Df(1)kad* is described by Zhang and Hawley (1990). All other mutations and chromosomes that are not described in this report are described in Lindsey and Zimm (1999). Throughout this report the fourth chromosome marker *sped"* will be abbreviated as *pol*. The *X5Y·YL(In)EN3 v f B·y* chromosome will be denoted simply as *XY·f B*.

**Crosses:** Crosses were performed on standard medium at 23.5°. Bottles with 15 pairs of parents or vials with a single female and three males were set up on day 0 and transferred on day 6. Parents were discarded on day 13. The original vial was scored until day 21, and the transfers were scored until day 27.

**Measurement of primary nondisjunction:** The frequency of *X* and fourth chromosome nondisjunction was measured by mating females to *XY·f B/0; C(4)RM, cy9/0* males as described in Rasooly et al. (1991). In most crosses the number of *X* chromosome exceptional progeny is doubled before calculation, and this number is used as the adjusted total in the tables. This correction accounts for the viability of diplo-X ova fertilized by *XY*-bearing sperm and nullo-X ova fertilized by sperm not carrying a sex chromosome. Sex chromosome nondisjunction is calculated as the sum of two times the exceptional progeny classes divided by the adjusted total. Special cases where one or more classes of regular progeny were inviable or had very low viability are noted in the tables.

Fourth chromosome nondisjunction was calculated by doubling simultaneous *X*·4 exceptional progeny, adding fourth chromosome exceptions and dividing the sum by the adjusted total. In some crosses, the females were heterozygous for *pol*. In those cases, diplo-4 exceptions could not be scored. Although the frequency of fourth chromosome nondisjunction was taken directly as the frequency of nullo-4 exceptions, these frequencies are actually an underestimate of the actual rate of fourth chromosome nondisjunction.

**Calculations:** A chi-squared homogeneity test was used to compare the rate of nondisjunction in two or more experiments. When carrying out the test, the number of *X* exceptional progeny and the number of *X* regular female progeny in each experiment are compared to avoid having to include inviable progeny in the calculation. Tetrad frequencies for diplo-X ova were calculated as described by Merriam and Frost (1964).

**Identification of the *mur* mutation:** Pelement mutagenesis was carried out by generating dsygenic males with the *P[cy - lar]WP* "enhancer-trap" element on the *X* chromosome (which will be referred to as *P[cy - ]* (Bier et al. 1989) and a constitutive source of transposase on the third chromosome, *P[y·Δ2,3]99B* (which will be referred to as *Δ2,3*). In the next generation, males bearing new inserts were mated individually to females heterozygous for *nod" w*/*nod" w* females. The *m"* allele (at 36.1 map units) was used as a marker for the presence of *nod" w* (at 35.3) because *nod" w*/*Balancer* and *nod" w* homozygous females have very high rates of nondisjunction. The insert was then screened for the ability to enhance the *nod" w* meiotic phenotype in the resulting *w*/*nod" w* females, by looking for ~20% *X* nondisjunction. Complete progenies were not counted. Instead, the *P* insertions were discarded when there were enough regular progeny to indicate that the data were outside the 95% (cumulative binomial) confidence limit for a 20% rate of *X* nondisjunction. Putative enhancers were retested twice. The single enhancer identified was given the name *mur* (more wildtype) and was outcrossed to a *C(1)RM,w/Y;C(4)RM, cy9/0* stock. The resulting *w*/*pol" daughters were mated to *w/m/Y; CyO/Sco; pol/pol* males yielding *w/m/Y; CyO/mur; pol/ pol* sons that were crossed to *w/m/w; CyO/Sco; pol/pol* females to generate a *w m; mur/CyO; pol* stock without *XY* females or individuals with four chromosomal segments.

**Exclusion of *Pelement*:** Exclusion of the *Pelement* was induced by introducing a constitutive source of transposase, *Δ2,3*. The resulting *w/m/Y; mur/CyO; Δ2,3/+; pol/+* males were then mated to *w/m/w; CyO/Sco; pol/pol* females, and exclusion events were identified by the loss of the *w"* allele contained within the *Pinsertion* (white-eyed *Cy Sco"* or white-eyed *Sco"* flies).

**Mapping of *mur*:** To recover recombinants between *w"* and the *w"* allele contained within the *P* element, *C(1)RM,w/Y; w/+*/*w/+* females were mated to *w/Y; b ug/Cy* males. Two thousand eighty-three non-*Cy* progeny were scored, of which 26 had *w"* and 20 had *w"* recombinant chromosomes. Males with recombinant chromosomes were used to make stocks by mating to *w/w; In(2L)Cy In(2R)Cy, al2* CY pr Bl c2·v *cg*·sF/Sco females. To recover recombinants between *c* and *w* and *w", *w/m/w; mur/ab/c sp; pol/+* females were mated to *w/Y; al dp b cx px sp/ CyO* males. Two thousand three hundred ninety-four non-*Cy* progeny were scored, of which 75 had *w"* and 63 had *w"* recombinant chromosomes. Males with recombinant chromosomes were used to make stocks by mating to *w/w; In(2L)Cy In(2R)Cy, al2* C7 pr Bl c2·v *cg*·sF/Sco females.

**Molecular techniques:** Genomic DNA was prepared by the method of Bender et al. (1983), and further purified using Elu-Tips (Schleicher and Schuell) or phenol/chloroform extraction. Southern blotting and other molecular techniques were carried out using standard procedures (Sambrook et al. 1989). The pCASPHER plasmid, which was used as a probe, is described by Ashburner (1989).

**Fluorescent cytology of meiosis:** Meiosis was examined in stage 13 oocytes prepared and stained as described by Theurkauf (1995). Anti-tubulin monoclonal antibody was purchased from Amersham (N.35 m and Sigma (DM10). Rhodamine-conjugated sheep anti-mouse (Boehringer) and FITC-conjugated rabbit anti-mouse (Cappel) secondary antibody was preabsorbed with *D. melanogaster* embryos (Karr and Alberts 1986) before use.

RESULTS

**Screen for novel meiotic mutations:** A screen for new meiotic mutations was designed based on the variable meiotic phenotype of *nod" w* (no distributive disjunction, Dominant Ted Wright allele), a dominant mutation affecting a kinesin-like protein involved in meiotic and mitotic chromosome segregation isolated by Wright (1974). The dominant meiotic phenotype of *nod" w*
varies with dosage and temperature (RASOOLY et al. 1991). At 25°, heterozygous females have a meiotic phenotype that is indistinguishable from that of females homozygous for loss-of-function nod alleles: only nonexchange chromosomes undergo nondisjunction and loss. Therefore, at 25°, the achiasmate fourth chromosomes segregate nearly at random, while there is little X chromosome nondisjunction, because in most meioses the X chromosomes undergo exchange. However, in heterozygous females at lower temperatures, or in homozygous females at 25°, the segregation of both exchange and nonexchange chromosomes is affected (Table 1).

The goal of the screen was to identify other genes involved in meiosis by looking for mutations that made nod"+; mutant/+ heterozygous females exhibit the more severe meiotic phenotype of nod"/nod" homozygous females (see MATERIALS AND METHODS). Random autosomal transposon inserts were generated and screened for a dominant enhancer phenotype, reasoning that having half as much of another meiotic protein would exacerbate the defect in nod" females.

**Initial characterization of mwr:** Out of 732 inserts screened, one second chromosome insert acted as a dominant enhancer of nod" and was named mwr (more Wright). The meiotic phenotype of mwr is shown in Table 1; it increases the rate of X chromosome nondisjunction sevenfold in nod"/+ females. However, mwr has no dominant phenotype of its own, even when X chromosome exchange is nearly eliminated by introducing a balancer chromosome (FM7a) (Table 1). mwr also does not enhance Df(1)nod, a loss-of-function nod mutant (Table 1).

Since nod" specifically impairs the segregation of nonexchange chromosomes under the conditions of these experiments (heterozygous females at 25°), it was important to determine if mwr acted as a dominant suppressor of recombination, thereby creating a larger pool of nonexchange chromosomes. Second chromosome recombination was measured in C(1)RM, w/Y; adp b pr c px sp/mwr females, and in control C(1)RM, w/Y; adp b pr c px sp/+ sisters from the same mating. The measured distances for mwr/+ and control females were as follows: adp 14.7 mwr/+; 10.9 control; adp 37.0, 31.5; b c 30.7, 27.2; c px 24.7, 20.6; px-sp 6.9, 7.0 (n = 570, 690). There are small differences in the rates of recombination that are significant for the two regions of the left arm, adp (x² = 4.23, 0.025 < P < 0.05) and dp-b (x² = 4.09, 0.025 < P < 0.05). However, in each case there is more recombination in mwr females than in control females. Thus the effect of mwr on meiotic nondisjunction cannot be attributed to an overall decline in the rates of recombination, and the high rate of nondisjunction in nod"/+; mwr/+ females must result from the failure of both exchange and nonexchange chromosomes to segregate properly.

To confirm that exchange chromosomes are nondisjoining, progeny tests of diplo-X exceptional daughters from γ nod" f car/u m; mwr/+; pol/+ females were carried out. More than 80% of the 42 tested daughters result from nondisjunction of exchange tetrads (tetrad distribution: E₀ = 0.03; E₁ = 0.77; E₂ = 0.21). In the 32

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**Table 1**

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>T°</th>
<th>% X nondisjunction</th>
<th>%Y nondisjunction</th>
<th>%nullo-X</th>
<th>%nullo-Y</th>
<th>Adjusted total progeny*</th>
</tr>
</thead>
<tbody>
<tr>
<td>w nod&quot; f/w m&lt;sup&gt;-&lt;/sup&gt;</td>
<td>23.5</td>
<td>1.7</td>
<td>59</td>
<td>72</td>
<td>88</td>
<td>3,394</td>
</tr>
<tr>
<td>w nod&quot; f/nod&lt;sup&gt;+&lt;/sup&gt; f</td>
<td>23.5</td>
<td>25.9</td>
<td>68</td>
<td>80</td>
<td>93</td>
<td>764</td>
</tr>
<tr>
<td>y nod&quot;/y</td>
<td>18</td>
<td>16.8</td>
<td>56</td>
<td>68</td>
<td>81</td>
<td>5,826</td>
</tr>
</tbody>
</table>

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*Adjusted total = total regular X progeny / (2 × X nondisjunctonal progeny).

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**Phenotype of mwr**

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>T°</th>
<th>% X nondisjunction</th>
<th>%Y nondisjunction</th>
<th>%nullo-X</th>
<th>%nullo-Y</th>
<th>Adjusted total progeny*</th>
</tr>
</thead>
<tbody>
<tr>
<td>w nod&quot; f/w m; mwr/+</td>
<td>23.5</td>
<td>12.2</td>
<td>65</td>
<td>86</td>
<td>81</td>
<td>2,946</td>
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<tr>
<td>FM7a/w m; mwr/+</td>
<td>23.5</td>
<td>0.6</td>
<td>0.4</td>
<td>40</td>
<td>71</td>
<td>1,703</td>
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<td>FM7a/y</td>
<td>23.5</td>
<td>0.5</td>
<td>0.1</td>
<td>57</td>
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<tr>
<td>nod&quot;/FM7a</td>
<td>23.5</td>
<td>31.2</td>
<td>51.7</td>
<td>58</td>
<td>71</td>
<td>5,402</td>
</tr>
<tr>
<td>FM7a, Df(1)nod&quot;/nod&lt;sup&gt;+&lt;/sup&gt;</td>
<td>23.5</td>
<td>54.2</td>
<td>83.8</td>
<td>66</td>
<td>96</td>
<td>7,464</td>
</tr>
<tr>
<td>FM7a, Df(1)nod&quot;/γ cv v f car&lt;sup&gt;-&lt;/sup&gt;</td>
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<td>0.5</td>
<td>0.4</td>
<td>56</td>
<td>88</td>
<td>8,661</td>
</tr>
<tr>
<td>FM7a, Df(1)nod&quot;/w m; mwr/+</td>
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<td>0.9</td>
<td>1.3</td>
<td>60</td>
<td>33</td>
<td>1,911</td>
</tr>
</tbody>
</table>

* Data from RASOOLY et al. (1991).
* Data from ZHANG et al. (1990).
cases where the genotype for the proximal marker car could be determined (in w' females), only two were homozgous. Therefore, the large majority of these nondisjunctional events occur at meiosis I, based on heterozygosity for car. Given that car is ~3.5 map units from the centromere, 2.2 car-centromere crossovers would be expected among 64 chromosomes where car could be scored. Therefore, the two females homozygous for car may well have resulted from meiosis I nondisjunction of a tetrad with a very proximal exchange.

I conclude that mwr acts to enhance nodPROW so that heterozygous females exhibit the phenotype of homozygous nodPROW/nodPROW females who give nondisjunction of both exchange and nonexchange chromosomes at meiosis I.

The mwr chromosome contains a female-sterile allele. Thus, mwr/mwr females are completely sterile, although they lay eggs that appear grossly normal but fail to hatch. In addition, mwr/mwr males and females show 57% reduced viability compared to heterozygous siblings.

Mapping of mwr: In situ hybridization using P-element sequences as a probe revealed the presence of a single element at 50C (data not shown). The element was mapped genetically, using the w' allele within the P element as a genetic marker (see MATERIALS AND METHODS) to a map position at 69.5, 2.2 map units from vg (67; 49F) and 5.8 map units from c (75.5; 52D3-9). The female-sterility was not allelic to any of the female sterile loci previously isolated by SCUDBACH and WIESCHAS (1991) in this region.

Twenty of the recombinants between vg and the w' allele within the P element and 12 recombinants between c and the P element were scored and tested. All w' recombinant chromosomes (vg w' or w' c) were homozygous female sterile. The reciprocal w vg' and w c' recombinants lacking the P element were homozygous female fertile. Since the female sterile phenotype cosegregates with the P element, it cannot map more than 0.44 map units proximal or 1.9 map units distal to the w' allele carried by the transposable element.

Several deficiencies in the region of the P insertion were also tested (Figure 1). The two deficiencies that include the 50C region are sterile over mwr. Thus conventional mapping and deficiency mapping experiments place the female sterile phenotype of mwr at approximately 50C on the right arm of the second chromosome, and the female sterility appears to result from a loss-of-function mutation.

Mapping the nodPROW enhancing activity: Mapping the enhancement of nodPROW proved to be much more difficult. All w vg chromosomes enhanced nodPROW and none of the w vg showed any enhancement, placing the enhancing activity at least 1.8 map units distal to vg (data not shown). However, the mapping with respect to c was confounded by the existence of an unmapped nodPROW-enhancing activity on the original parental al b c sp chromosome (data not shown).

Reversion analysis of mwr: An alternative approach to mapping the meiotic phenotype of mwr was to generate excisions of the P element and measure the effect of the resulting excision chromosomes on meiotic chromosome nondisjunction in nodPROW/nodPROW females. P-element excision was induced by introducing a constitutive source of transposase, [Δ2,3] (see MATERIALS AND METHODS). Among 1020 flies scored, 54 excision events were observed, giving a w'-reversion rate of 5.3%. Seven individual excision chromosomes were put into stock for further study.

Two of the excision lines appear to have suffered additional damage as a result of the excision. One, mwr1, is recessive female sterile. Another, mwr2, is a recessive lethal, although it is fully complemented for lethality by the original mwr insertion. mwr2 is most likely a deletion that includes a neighboring locus or loci.

Southern analysis of mwr and three excision lines (mwr2, mwr3, and mwr4) show only a single insertion in the original mwr stock, and a complete absence of P-sequences in the excisions (data not shown).

In no case did the excision completely relieve any of the phenotypes. While five of the seven lines were homozygous female fertile, all seven lines retained the dominant meiotic enhancer phenotype (for example see Table 2), and all also appear to retain the effect on viability. While the females are fertile as homozygotes, they fail to complement the original female fertility defect of the mwr chromosome; mwr/excision females are nearly or completely sterile.

The excision lines may be weaker alleles of mwr. While

![Figure 1](image-url)
**TABLE 2**
Meiotic phenotype of nod<sup>mm</sup>; mwr females

<table>
<thead>
<tr>
<th>Gametes</th>
<th>Mother</th>
<th>Father</th>
<th>mwr&lt;sup&gt;a&lt;/sup&gt;</th>
<th>+&lt;sup&gt;a&lt;/sup&gt;</th>
<th>mwr&lt;sup&gt;1&lt;/sup&gt;</th>
<th>mwr&lt;sup&gt;2&lt;/sup&gt;</th>
<th>mwr&lt;sup&gt;4&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Regular</td>
<td></td>
<td></td>
<td>172</td>
<td>188</td>
<td>220</td>
<td>231</td>
<td>86</td>
</tr>
<tr>
<td>X 4</td>
<td>XY 44</td>
<td>181</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>X nondisjunctional</td>
<td></td>
<td></td>
<td>32</td>
<td>2</td>
<td>22</td>
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<td>7</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>4 nondisjunctional</td>
<td></td>
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<td>45</td>
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<tr>
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<td>0 44</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>X 44</td>
<td>0 0</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>X, 4 nondisjunctual</td>
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<td></td>
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<td>1295</td>
<td>1418</td>
<td>1564</td>
<td>561</td>
</tr>
</tbody>
</table>

- w nod<sup>mm</sup> f/w m; pol/pol females heterozygous for the indicated second chromosomes were mated to tester males and nondisjunction rates were calculated as in MATERIALS AND METHODS.
- 'Sisters from a single mating.

they are homozygous fertile, they fail to complement the original female-sterile allele. One possible hypothesis based on these results is that the mwr locus has two defects: a P element insertion and another sequence change/rearrangement perhaps induced by the original transposon insertion event. Alternatively, two separate, closely linked loci may be affected on the original chromosome, one affecting female fertility (associated with the P element) and one meiotic mutation, mwr.

The recessive meiotic phenotype of mwr: Since the rationale behind the original screen was to identify loci encoding meiotic gene products, the question is whether mwr mutations are themselves recessive meiotic mutations. This question was addressed in two ways.

First, oocytes from mwr/mwr females, which are sterile, were examined to determine if there were any observable effects on chromosome positioning and orientation in metaphase I (see MATERIALS AND METHODS). In 20/25 w m/w m; mwr/mwr oocytes examined by DAPI staining, the position and orientation of the chromosomes was indistinguishable from wild type (Figure 2A). Of the remaining five figures, one showed X-chromosome nondisjunction and four showed apparent fourth chromosome nondisjunction (Figure 2B). This suggested that mwr/mwr females had a defect in meiosis, particularly affecting the nonexchange fourth chromosomes.

The second approach was to genetically test the female-fertile excision lines for a recessive meiotic phenotype. Homozygous excision females have a slight but significant

![Figure 2](image.jpg) — Chromosome morphology in mwr homozygous females. Female meiosis was examined in oocytes as described in MATERIALS AND METHODS. The chromosomes are stained with DAPI. (A) Normal meiosis with the nonexchange fourth chromosomes closer to the poles. (B) An oocyte with apparent fourth chromosome nondisjunction.
meiotic defect with 2% X and 5% 4 nondisjunction (Table 3). When X-chromosome exchange is reduced sharply by introducing the FM7a balancer, nondisjunction rates go up dramatically, to 12% X and 18% 4 nondisjunction (Table 3). Interestingly, although the fourth chromosomes are always nonexchange, the rate of 4 nondisjunction is low unless the X chromosomes are also nonexchange. This does not appear to be the result of heterologous X,4 segregations (inappropriate partner choice) because when the 4's are attached to (exchange) third chromosomes by a translocation, those FM7a/$y^2$ v f car; $mu^2$/mu$^2$; T(3;4)red/T(3;4)red females still show very high rates of X nondisjunction in the presence of nonexchange X's (Table 3). One possible explanation is that larger chromosomes are more sensitive to the absence of $mu^2$ gene product, and that perhaps the presence of a large nonexchange chromosome titrates some other essential component or mechanically disrupts pairing of the small fourth chromosomes.

### Cytological analysis of nondisjunction in $mu^2$ homozygous females

To determine what the role of $mu^2$ might be in meiosis, the arrangement of chromosomes and spindle structure was examined using fluorescent microscopy. As shown in Figure 3, the chromosomes and spindle appear morphologically normal. However, some ova in homozygous $mu^2$ females have the nonexchange X's or 4's both on the same side of the spindle (Figure 3), and some ova have simultaneous X and 4 nondisjunction. As shown in Figure 4, the rate of nondisjunction observed cytologically in 68 ova (21% X and 24% fourth nondisjunction) was similar to the rates of nondisjunction (12% X and 18% fourth nondisjunction) measured genetically (Table 3). The somewhat higher cytological rates of nondisjunction can probably be attributed to the relatively small sample size compared to genetic experiments. In contrast, only one of 31 ova from control FM7a/w m; $mu^2$/+; pol/pol females showed X nondisjunction, and none appeared to have fourth chromosome nondisjunction.

Importantly, the pattern of nondisjunction measured genetically and observed cytologically in FM7a/w m; $mu^2$/pol/pol females is also consistent in FM7a/
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FIGURE 3.—Chromosome and spindle morphology in mw? homozygous females. Female meiosis was examined in oocytes as described in MATERIALS AND METHODS. The chromosomes are stained purple with DAPI. Tubulin is stained red or green. (A) Wild-type meiosis, with the nonexchange fourth chromosomes closer to the poles. The spindle is shown in B. (C) A normal meiosis from a control Fh47(~/w m; +/mw?;pol/pol oocyte, who have both nonexchange 4’s and nonexchange Xs. As shown, both pairs of nonexchange chromosomes are positioned nearer the poles, consistent with previous observations (Theurkauf and Hawley 1992). (D–H) Representative meiotic figures from FM7a/w m; +/mw?; pom/pom; pol/pol females. (D) A normal chromosome segregation. (E) X nondisjunction, with both X chromosomes on the same side of the spindle, although spindle formation appears normal (F). (G) Fourth chromosome nondisjunction, and H shows another example of X chromosome nondisjunction.

Rather, nondisjunction appears to involve misalignment of the nonexchange chromosomes on an intact spindle apparatus, a conclusion that is supported by the cytological observations.

Mapping the recessive meiotic phenotype of mw: To attempt to resolve the question of how many loci are impaired on the the original, female-sterile mw chromosome, mapping experiments (test of location) and complementation tests (tests of function) were carried out. First, the recombinants between w and the P element and between e and the P element that were described above were analyzed. As expected, the w recombinant chromosomes (vg w’ and w’ e) were either completely or nearly sterile in combination with mw? (FM7a/w; w’ recombinant/mw? females), with no more than 8.8 progeny per female. However, in those cases in which a few progeny were recovered, there was often high levels of X and/or fourth chromosome nondisjunction (data not shown). Furthermore, no w recombinants (without the P element; FM7a/w; w recombinant/mw? females) have significantly higher rates of nondisjunction than control FM7a/w; mw?/+ females. Taking the data from the recombinants together, the mw recessive meiotic maps in the same region as the P element, in the 8 map units between e and vg, and is no more than 1.2 map units proximal to the P element.

Both deficiencies that fail to complement mw are also either completely or nearly female sterile in combination with excisions mw? and mw’. Fourteen progeny were recovered from six Df(2R)CX1/mw? females, one was recovered from 50 Df(2R)arr-y2/mw? females and no offspring were recovered from seven Df(2R)CX1/mw? females and five Df(2R)arr-y2/mw? females. In contrast, Df(2R)vg-B, which complements the female-sterility of mw, also fully complements the female steril-ity of mw? and mw’. Df(2R)vg-B also complements the recessive meiotic phenotype of mw excisions (data not shown).

Finally, the excisions were tested for their ability to complement the female sterility defect of the original mutation. As noted above, excision/mw females have few, if any, progeny. Because of the similarity of position and the fact that the excision alleles fail to complement the P-element phenotype, the meiotic and female sterile phenotypes may be due to two mutations affecting a single locus. Alternatively, two closely linked interacting loci, affecting meiosis and female fertility, may be affected.

DISCUSSION

Screening by gene interaction: The purpose of screening random transposon inserts for their ability to enhance an existing meiotic mutant was to identify additional components of the female meiotic machinery in D. melanogaster. The screen was successful in recovering a previously unidentified locus that plays a
role in meiosis, specifically affecting the segregation of nonexchange chromosomes.

Because \textit{mwr} excision alleles have a recessive meiotic phenotype, it is very unlikely that \textit{mwr} acts by altering the expression or stability of the \textit{NOD} protein. This was a concern because the phenotype of \textit{nod} \textit{drw} depends on dosage (Table 1), so any mutation that increases the amount of mutant protein twofold will change the phenotype of the heterozygote to that of the homozygote. However, the recessive effect of \textit{mwr} is seen in a wild-type female, where the only effect would be to alter the expression of the \textit{nod} allele, and extra expression of \textit{NOD} protein does not lead to a meiotic phenotype (\textit{KARPEN} et al. 1996). Furthermore, duplications of the \textit{nod} locus do not render females sterile (for example, \textit{X}/\textit{X} \textit{y} \textit{Y} \textit{v} \textit{B} females are fully fertile). In addition, \textit{mwr} does not appear to enhance the recessive mitotic lethal phenotype of \textit{nod} \textit{drw} (\textit{nod} \textit{drw} \textit{Y}; \textit{mwr}/+ males are fully viable at 23°C; data not shown).

**Role of \textit{mwr} in meiosis:** \textit{mwr}'s recessive meiotic phenotype is distinct from that of other mutations that have been described. \textit{mwr}/ \textit{mwr} females have normal rates of recombination, and apparently normal spindles, but have high rates of nonexchange chromosome nondisjunction during the first meiotic division. \textit{mei-S51} (a synthetic mutation involving two heterochromatic loci, one on the second and one on the third chromosome) also impairs nonexchange chromosome segregation during meiosis I (\textit{ROBBINS} 1971). However, \textit{mei-S51} also reduces exchange rates and appears to affect partner choice; when there is simultaneous nondisjunction of nonexchange \textit{X}'s and \textit{4}'s, most of the ova (91%) are either \textit{XX}-00 or 00-\textit{44}. In contrast, in \textit{mwr}/ \textit{mwr} females the segregation of the pairs is at random: there are as many \textit{XX}-\textit{44} and 00-00 ova (20) as there are \textit{XX}-00 and 00-\textit{44} ova (17) (Table 3, Figure 4). \textit{ald} (located on the third chromosome) females have normal rates of exchange, and high rates of meiosis I nondisjunction, along with apparently normal meiotic spindles (\textit{OTOUSA} 1982). However, \textit{mwr} differs from \textit{ald} in that \textit{ald} causes nondisjunction of exchange (77%) and nonexchange chromosomes (\textit{OTOUSA} 1982) while \textit{mwr} by itself affects primarily nonexchange chromosomes.

Several other Drosophila meiotic mutations (\textit{nod}, \textit{Axs}, and \textit{ncd}) affect nonexchange chromosome segregation in meiosis I without affecting exchange. However all except \textit{mwr} cause mechanical defects in spindle formation or chromosome attachment to the spindle, resulting in defective chromosome segregation (\textit{HATSUMI} and \textit{ENDOW} 1992; \textit{HAWLEY} et al. 1993b).

The hypothesis is that \textit{mwr} plays a role in chromosome pairing and in the initial recognition events between homologues. While exchange events can then correct the initial error in \textit{mwr}/ \textit{mwr} females and yield normal chiasmate segregation, nonexchange homologues often fail to form correctly oriented bivalents. However, the \textit{mwr} mutation does not appear to skew partner choice. Unlike \textit{mei-S51}, there is no evidence that \textit{X}'s mistakenly disjoin from \textit{4}'s. The \textit{mwr} gene produc-

![Figure 4](image_url)
uct may play a role in heterochromatic associations that are thought to play a role in pairing of nonexchange homologues (Novitski 1964; Hawley et al. 1993a; Dernburg et al. 1996; Karpen et al. 1996), leading to nondisjunction of nonexchange bivalents in mwr/mwr females.

**A timeline for meiosis:** If nonexchange homologues fail to pair properly why are they not segregated by the "salvage" system that segregates nonhomologues? I suggest that the explanation lies in timing. Initially pairing takes place to form bivalents. Homologues pair with each other, leaving heterologous elements to align such that they segregate from each other. However, once that alignment takes place, the machinery mediating it is inactivated in some way. Thus, if pairing between homologues is weak and breaks down later on, the two chromosomes will now segregate at random. There is no subsequent association of heterologues that would pair up these unequal parts.

This hypothesis is supported two lines of evidence. The first is the existence of mutations such as mei-S51 and old that affect the segregation of homologues, without impairing the segregation of heterologues. These mutations may attenuate homologous pairing, so that it breaks down later on during meiosis. Thus in each mutant there is a high frequency of ova that are nondisjunctional for just the X or just the fourth chromosome; these ova represent the breakdown of pairing of a single nonexchange bivalent later in meiosis.

The second line of evidence comes from the studies of the meiotic behavior of a small duplication consisting primarily of the centric heterochromatin of the X chromosome (Karpen et al. 1996). Deleting portions of the centric heterochromatin weakens pairing, leading to higher rates of nondisjunction. Similarly, duplications inverted with respect to each other that have only a small region of uninterrupted homology also show high rates of nondisjunction. Since the heterologous system of chromosome segregation is presumably intact in these wild-type flies, the increase in the rate of nondisjunction when there is a shortened pairing region must reflect the relative fragility of the initial pairing event that cannot be rescued by subsequent interactions.

Thus, while both Robbins (1971) and Hawley et al. (1993a) hypothesize that homologous associations precede heterologous associations, I suggest a refinement of their model that postulates that there is an endpoint beyond which no further alignment takes place, before spindle formation. One prediction of the model is that mwr, like the other pairing mutants, will have no effect on segregation of nonhomologues from each other, because it is only involved in the heterochromatic associations that lead to pairing of homologous chromosomes. It appears that mwr encodes one of the "special pairing protein or complexes that interact with identi

cal sequences on both homologues" proposed by Karpen et al. (1996).

**mwr as an enhancer of nodDTR:** The proposed role of mwr in heterochromatic associations between homologues might explain the effect of mwr as an enhancer of nodDTR. Wild-type NOD is a kinesin-like protein that attaches chromosomes to the spindle by binding along the length of the chromosome (Afshar et al. 1995). Because nodDTR encodes a defective kinesin-like protein (Rasooly et al. 1991) that also presumably binds along the length of the chromosome, the attachment of all chromosomes (exchange and nonexchange) is impaired. The spindle originates from the chromosome bivalents (Theurkauf and Hawley 1992). My hypothesis is that when a defect in heterochromatic alignment of homologues is superimposed upon a defect in spindle attachment, meiosis becomes more faulty because the exchange and nonexchange bivalents are unable to orient properly to form a functional spindle. This model remains to be tested by cytological and genetic experiments.

**Female sterility and mwr:** mwr acts on meiotic chromosome segregation but is also closely linked to a P-induced recessive female sterile. These two phenotypes are not likely to be related since even females bearing severe mutagenic mutants (such as c(3)G) that dramatically reduce recombination rates and give high rates of nondisjunction of autosomes lay abundant eggs, many of which simply fail to hatch or complete development (see description in Lindsley and Zimm 1992). Furthermore, it has not been possible to determine unequivocally whether one or two loci are impaired. Molecular characterization of the mwr locus should resolve this issue as well as elucidating the role of mwr in meiosis.

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