# The Drosophila *mei-S332* Gene Promotes Sister-Chromatid Cohesion in Meiosis Following Kinetochore Differentiation

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ABSTRACT

The Drosophila *mei-S332* gene acts to maintain sister-chromatid cohesion before anaphase II of meiosis in both males and females. By isolating and analyzing seven new alleles and a deficiency uncovering the *mei-S332* gene we have demonstrated that the onset of the requirement for *mei-S332* is not until late anaphase I. All of our alleles result primarily in equational (meiosis II) nondisjunction with low amounts of reductional (meiosis I) nondisjunction. Cytological analysis revealed that sister chromatids frequently separate in late anaphase I in these mutants. Since the sister chromatids remain associated until late in the first division, chromosomes segregate normally during meiosis I, and the genetic consequences of premature sister-chromatid dissociation are seen as nondisjunction in meiosis II. The late onset of *mei-S332* action demonstrated by the mutations was not a consequence of residual gene function because two strong, and possibly null, alleles give predominantly equational nondisjunction both as homozygotes and in *trans* to a deficiency. *mei-S332* is not required until after metaphase I, when the kinetochore differentiates from a single hemispherical kinetochore jointly organized by the sister chromatids into two distinct sister kinetochores. Therefore, we propose that the *mei-S322* product acts to hold the doubled kinetochore together until anaphase II. All of the alleles are fully viable when in *trans* to a deficiency, thus *mei-S332* is not essential for mitosis. Four of the alleles show an unexpected sex specificity.

IN meiosis haploid gametes are produced by two successive rounds of chromosome segregation that are not separated by DNA replication. In the first, or reductional, meiotic division the homologs pair and segregate from each other. The second, or equational, meiotic division resembles mitosis in that the sister chromatids segregate. Since the sister chromatids move as a unit to the poles in the reductional division, functions promoting cohesion of the sister chromatids must exist in meiosis I. Such functions might also act in meiosis II or mitosis to maintain the association of the sister chromatids until anaphase.

The mechanisms that promote sister-chromatid cohesion are currently not well understood. Sister-chromatid cohesion is likely to require both structural proteins that hold the sisters together and timing mechanisms that delay separation until the appropriate anaphase. Cytological studies in maize suggest that sister-chromatid cohesion in early meiosis I may involve the synaptonemal complex (SC) found between homologous chromosomes during the pachytene stage (reviewed in MAGUIRE 1990). Unpaired homologs (univalents) in trisomic strains of maize undergo sister-chromatid separation during meiosis I rather than meiosis II (MAGUIRE 1978b, 1979). Since these univalents can still organize the axial (or lateral) elements of the SC between the two sister chromatids, it is likely that complete SC is required to prevent the premature separation of sister chromatids in meiosis I (MAGUIRE 1990). Another mechanism that could ensure sister-chromatid cohesion would be the catenation of sister chromatids that arises as a consequence of DNA replication (MURRAY and SZOSTAK 1985). However, analysis of plasmids in yeast failed to demonstrate extensive interlocking prior to anaphase of mitosis (KOSHLAND and HARTWELL 1987). Candidate regulatory or structural proteins that act to hold the sister chromatids together have yet to be identified.

Functions required for meiotic chromosome segregation can be identified by the isolation of mutants, an approach employed in several organisms. However, only a few mutations have been isolated that potentially affect sister-chromatid cohesion. In the desynaptic (*dy*) mutant of maize, homologs pair and undergo recombination, but the chiasmata are not maintained (MAGUIRE 1978a). The resulting univalents often undergo sister-chromatid separation during meiosis I. The *dy* gene is proposed to have a role in promoting sister-chromatid cohesion, which in turn is required for chiasma maintenance. Cytological analysis of the *pc* mutant in tomato reveals premature sister-chro-

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matid separation in late anaphase I (CLAYBERG 1959). In yeast, one interpretation of the mutations in the *RED1* (ROCKMILL and ROEDER 1988) and *DIS1* (ROCKMILL and FOGEL 1988) genes is that they result in defects in sister-chromatid cohesion during meiosis I. The *red1* mutant fails to assemble synaptonemal complex (ROCKMILL and ROEDER 1990); this could lead to premature separation of the sister chromatids.

Two genes have been identified in *Drosophila melanogaster*, *mei-S332* and *ord*, which have been proposed to maintain sister-chromatid cohesion until anaphase II of meiosis (DAVIS 1971; GOLDSTEIN 1980; MASON 1976; SANDLER *et al.* 1968). Mutations in *mei-S332* and *ord* are unusual in that they affect chromosome segregation during meiosis I in both males and females. The majority of mutations that affect meiotic chromosome segregation in Drosophila show sex specific defects in meiosis I (BAKER and HALL 1976), indicating that meiosis I differs profoundly in Drosophila males and females. In females, the homologs undergo recombination and form synaptonemal complex, and mutants defective in recombination show high levels of nondisjunction (BAKER and HALL 1976). Thus, as has been observed in a number of organisms, recombination is linked to proper chromosome segregation in Drosophila females. In addition, a backup system for the segregation of nonrecombinant chromosomes (the distributive system) has been demonstrated genetically in females (GRELL 1976). In males the synaptonemal complex is not formed and recombination does not occur. Homologs appear to pair via specialized pairing sites or collochores (MCKEE and KARPEN 1990). Despite the differences between males and females the phenotypes of *mei-S332* and *ord* imply that some aspects of meiosis I such as sister-chromatid cohesion are under common genetic control in both sexes.

The previously characterized phenotype resulting from a mutation in the *mei-S332* locus indicated that this gene promotes sister-chromatid cohesion in meiosis. Cytological analysis of male meiotic segregation in the *mei-S332* mutant demonstrated that the sister chromatids prematurely dissociate in meiosis I (DAVIS 1971; GOLDSTEIN 1980). In this mutant nondisjunction does not occur until meiosis II because the sister chromatids do not precociously disjoin until late anaphase I, thus meiosis I segregation is unaffected. The onset of *mei-S332* activity during anaphase I was in contrast to observations obtained with the single allele of *ord*. *ord*, like *mei-S332*, appears to promote sister-chromatid cohesion. However, in flies mutant for the *ord* locus mostly reductional (meiosis I) nondisjunction occurs (MASON 1976), and cytologically, the sister chromatids were observed to be aberrantly associated as early as prophase I (GOLDSTEIN 1980). Thus the previous analysis raised the possibility that *mei-S332* and *ord* acted at different times in meiosis I to promote sister cohesion. Previous data also suggested that these genes might play a role in mitotic chromosome segregation (BAKER, CARPENTER and RIPOLL 1978).

Only a single allele existed for *mei-S332*, and no deficiencies were identified that uncovered this locus. Consequently, it was not known whether the observed phenotype corresponded to loss of gene function, and whether this phenotype accurately reflected the true biological role of the gene. Therefore it was essential to isolate additional alleles to determine whether the wild-type *mei-S332* function was to promote sister-chromatid cohesion in meiosis. New alleles would also permit the role of *mei-S332* in mitosis to be examined. Moreover, the apparent difference in time of action of *mei-S332* and *ord* in meiosis I could be explained as a result of the initial allele of *mei-S332* being leaky, and this hypothesis could be tested with additional alleles. We have isolated seven new alleles of *mei-S332* as well as deficiencies uncovering the locus. In this paper we present a genetic analysis of these new alleles which demonstrates that the *mei-S332* gene product promotes sister-chromatid cohesion in meiosis I. The onset of the requirement for *mei-S332* action is after metaphase I, following differentiation of the kinetochore.

## MATERIALS AND METHODS

**Stocks:** All Drosophila stocks and crosses were raised at 25° on standard cornmeal-brewer's yeast-molasses-agar food. Unless noted, all stocks were received from the Bloomington Stock Center at the University of Indiana. The phenotypes of the original meiotic mutations *mei-s332* and *ord* are fully described in (DAVIS 1971; GOLDSTEIN 1980; MASON 1976; SANDLER *et al.* 1968). In our experiments, we utilized a deficiency uncovering *mei-S332* named *Df(2R)X58-6*. We isolated this deficiency in a screen for deficiencies in cytological interval 58 by screening for X-ray-induced loss of a *P* element containing the *white* gene inserted into 58D (the P[(w)$A^R$]4-043 transformant, obtained from R. LEVIS at the Fred Hutchinson Cancer Research Center) (LEVIS, HAZELRIGG and RUBIN 1985). The breakpoints of *Df(2R)X58-6* are approximately 58A3-B2; 58E3-10. Other deficiencies isolated in this screen will be described in a separate report.

All other mutations used in these experiments are described in (LINDSLEY and GRELL 1968). The *cn bw sp* chromosome used for ethyl methane sulfonate (EMS) mutagenesis was derived from a stock obtained from J. TAMKUN (University of California at Santa Cruz) via R. LEHMANN (Whitehead Institute). Stocks used in the recombination mapping experiments included *al dp b pr c px sp/CyO*, *al dp b pr Bl c px sp/SM1* and *In(2R) $mam^{N2G}$*, *S Sp Tft $mam^{N2G}$ $Pu^2$/CyO* (from R. LEHMANN) and *a px sp* (from the Mid-America Stock Center at Bowling Green State University). The latter two stocks were crossed to obtain the recombinant *In(2R) $mam^{N2G}$*, *Tft $mam^{N2G}$ $Pu^2$ a px sp* chromosome used in the second mapping experiment. The isogenized *X* and *Y* chromosomes, which were crossed into the *mei-S332* mutant stocks (see below), came from a *y/$B^S$Y* stock and a *y $sn^{ts}$*/

*FM7a/y⁺Y; spa^pol* stock, respectively. A *y cv v f car* stock (from R. S. HAWLEY, University of California at Davis) was crossed to Canton-S to isolate the *cv v f car* recombinant chromosome used in the female nondisjunction tests. Compound chromosomes used in the nondisjunction tests included: *C(1)RM, y² su(w^a)w^a; Y^S X·Y^L y⁺, In(1)EN, y v f B; C(2)EN, b pr; C(3)EN; C(4)RM, ci ey^R*. For the rest of this report, the *C(1)RM* chromosome will be symbolized as "$\widehat{XX}$," and the *Y^S X·Y^L y⁺, In(1)EN* chromosome will be symbolized as "$\widehat{XY}$."

**EMS mutagenesis:** Adult *y/y⁺Y; cn bw sp* males were mutagenized with 0.035 M EMS as described (LEWIS and BACHER 1968), and mated to *y⁺; cn mei-S332¹ ord¹/SM1* females (see Figure 1). From the progeny of this cross, males with a mutagenized *cn bw sp* chromosome over the *cn mei-S332¹ ord¹* tester chromosome were selected and individually tested for nondisjunction in matings to yellow females. Exceptional nullo-*XY* sperm produced by these males resulted in yellow (*y*/O) males in a background of yellow⁺ males (*y*/*y⁺Y*) and females (*y*/*y⁺*). Vials with more than two yellow males were scored as positive for nondisjunction; most vials had 20–30 progeny. Positives were retested over the single *ord* and *mei-S332* mutations.

Lacto-aceto orcein squashes of salivary gland chromosomes (ASHBURNER 1989) from the eight *mei-S332* noncomplementers showed that only one of them contained a visible chromosomal rearrangement, a deficiency in region 58 that we have named *Df(2R)R1-8*.

**Recombination mapping:** We mapped the eight *mei-S332* alleles in two separate experiments using standard techniques. In the first experiment, six of the eight *mei-S332* noncomplementers from the EMS screen (*mei-S332^2,4,6,7,8*, *Df(2R)R1-8*) were mapped to the *c* (75.5)-*px* (100.5) interval on chromosome 2. Recombination took place in females which had the second chromosome containing the noncomplementer over a *al dp b pr c px sp* chromosome. These females were crossed to *y/y+Y; cn mei-S332¹ px/SM1* males, and their male progeny with recombinant or nonrecombinant second chromosomes over the original *mei-S332¹* allele were isolated. These males, which were also *y/y⁺Y* or *y⁺/y⁺Y*, were mated singly to *y; al dp b pr Bl c px sp/CyO,bw* virgin females. The progeny of this cross were scored for the visible mutations and the presence of yellow (*X*/O) males arising from nondisjunction events in the male parent giving rise to nullo-*XY* sperm. Only vials with at least 20 progeny were scored: those with three or more yellow males were scored as mei-S332⁻, those with one or no yellow males were scored as mei-S332⁺, and those with two yellow males were retested. The number of recombinants in the *c-px* interval scored for each allele was: *mei-S332²*, 42; *mei-S332⁴*, 60; *mei-S332⁶*, 59; *mei-S332⁷*, 53; *Df(2R)R1-8*, 58. In all cases, the meiotic nondisjunction phenotype was found to map close to *px* (99.1–100.0 cM). In the *mei-S332⁸* mapping cross, we recovered one *mei-S332-px* recombinant, placing this allele at 99.5 cM.

We performed a second experiment to map the original allele and seven of the EMS-induced noncomplementers (excluding *Df(2R)R1-8*) more precisely. These mutations were mapped within the *Pu* (97 cM)-*px* (100.5) interval using an *In(2R)mam^N2G, Tft mam^N2G Pu² a px sp* chromosome. Recombinants in this interval were selected over the *Df(2R)X58-6* chromosome, which uncovers both *mei-S332* and *px*. Single males that were recombinant in the *Pu-px* interval were mated to *y; Sco/SM1* females to score for nondisjunction as described above. For the weak *mei-S332⁵* allele, recombinant males were mated to $\widehat{XX}$, *y² su(w^a)w^a* females to score for nullo-*XY* and diplo-*X* exceptions. The number of fertile recombinants in the *Pu-px* interval scored for each allele was: *mei-S332¹*, 35; *mei-S332²*, 49; *mei-S332³*, 85; *mei-S332⁴*, 67; *mei-S332⁵*, 48; *mei-S332⁶*, 63; *mei-S332⁷* 62; *mei-S332⁸*, 61.

**Construction of isogenic stocks:** In order to minimize differences between the *mei-S332* alleles due to genetic background, and to remove lethals and steriles from the EMS-mutagenized chromosomes, we constructed stocks which were isogenic for the sex chromosomes and which had most of the original mutagenized second chromosome replaced. We first made a stock of the genotype *y/y⁺Y; +/SM1; spa^pol* in which the *X* and *Y* chromosomes had been isogenized; this stock will be referred to as the iso-*X,Y* stock. Recombinants for all eight *mei-S332* alleles and the nonrecombinant *Df(2R)X58–6* chromosome were crossed into the iso-*X,Y* background.

Recombinants of the eight *mei-S332* alleles were isolated as follows. The left arm of chromosome 2 was replaced by recombining a *pr cn bw* chromosome with either a *al dp b pr c mei-S332 bw sp* recombinant chromosome from the first mapping experiment (*mei-S332^2,3,4,6*), the original mutagenized *cn bw sp* chromosome (*mei-S332^5,7,8*) or a recombinant chromosome with the distal right arm of chromosome 2 replaced with *px* and *sp* (*mei-S332¹*), and then selecting for recombinant chromosomes which were marked with either *pr cn bw sp* (*mei-S332^2-8*) or *pr cn px sp* (*mei-S332¹*). Approximately 10 recombinant chromosomes were stocked for each allele, and these stocks were scored for homozygote viability and fertility and the nondisjunction phenotype. A single line for each allele was selected, and crossed into the iso-*X,Y* background (the "A" stock).

The homozygous *mei-S332^2-8* alleles were male sterile in the iso-*X,Y* background, presumably due to mutations elsewhere on the second chromosome that interacted with iso-*X,Y*. We therefore crossed recombinants from the second mapping experiment, which had the distal right arm of the chromosome 2 replaced by *px* and *sp*, into the iso-*X,Y* background (the "B" stock). Fertile homozygous males were obtained by crossing the A and B stocks together. For the *mei-S332⁴*, *mei-S332⁷*, and *mei-S332⁸* alleles, we also crossed the A and B stocks to obtain fertile homozygous females. Otherwise, we used homozygous females from the A stock for nondisjunction tests.

To avoid the accumulation of modifiers which decrease the frequencies of nondisjunction in *mei-S332* stocks (DAVIS 1971; HALL 1972), we maintained the *mei-S332¹* stock by crossing heterozygous males and virgin females each generation. This was not necessary for the other alleles, since homozygotes in those stocks were either male sterile or inviable.

**Nondisjunction tests:** All experiments were performed at 25°, using the iso-*X,Y* stock (*y/y⁺Y; +/SM1; spa^pol*) as the wild-type control. Haplo-4 Minute flies, triploids, triploid intersexes and metafemales appeared rarely among the progeny of the nondisjunction tests and were not included in the final progeny totals. Tests to measure sex chromosome nondisjunction were set up as described (ZITRON and HAWLEY 1989): crosses were set up on day 0, parents were discarded on day 5, and progeny were scored until day 18.

*Sex chromosome nondisjunction in males:* Nondisjunction was measured in crosses of *y/y⁺Y* males to $\widehat{XX}$, *y² su(w^a) w^a* females. The frequencies of six out of the nine possible types of sperm could be deduced from the phenotypes of the progeny of this cross (see Figure 2A). Nondisjunction at either meiotic division or chromosome loss events resulted in nullo-*XY* sperm, nondisjunction at the first meiotic division resulted in *XY* sperm, nondisjunction at the second meiotic division resulted in *XX* sperm, and nondisjunction at both divisions resulted in *XXY* sperm.

Percentages for each exceptional class were calculated by

dividing the number of progeny in that class by the total number of progeny; these percentages were summed to give the value of "Total observed nondisjunction". However, exceptional diplo-*Y* sperm were not scored in this cross because they were phenotypically indistinguishable from regular mono-*Y* sperm. Moreover, since diplo-*Y* sperm are not efficiently recovered in *XX* females (GOLDSTEIN 1980; LINDSLEY and GRELL 1968), the equational diplo-*Y* exceptional class was greatly underrepresented among the progeny. Consequently, the "Total observed nondisjunction" values in these tests (see Tables 1 and 2) are underestimates of the actual levels of nondisjunction in males. Since we could not determine the frequency of diplo-*Y* sperm in these tests, we were unable to calculate the frequencies of chromosome loss in males.

*Sex chromosome nondisjunction in females:* Nondisjunction was measured in crosses of *y/cv v f car* or *y/y* females to $\widehat{XY}$, *v f B* males. All four types of ova gave rise to phenotypically distinguishable progeny in this cross (see Figure 2B). In this cross, only half of the total number of exceptions, but all of the regular *X* gametes, were recovered (Figure 2B). Therefore, percentages for the exceptional classes were determined by doubling the number of exceptional progeny and dividing by the "Adjusted total," which is the number of progeny in the regular *X* classes plus twice the number of progeny in the exceptional classes.

Females in the diplo-*X* exceptional class that arose from first (reductional) or second (equational) division nondisjunction were distinguished using the centromere-linked mutation *carnation*. Carnation females were immediately scored as equational exceptions. Carnation$^+$ females were mated to $\widehat{XY}$ males, and their male progeny were scored for the presence of the *car* mutation to determine whether the mother was a reductional (*car$^+$/car*) or equational (*car$^+$/car$^+$*) exception. Recombination in four intervals spanning the *X* chromosome was also assayed in the female tests by scoring the regular *X* class (Bar$^+$ males) for the recessive *X*-linked mutations *y, cv, v, f* and *car*.

*Autosomal nondisjunction:* To test nondisjunction of chromosomes *2* and *3* in males, 10 males which were homozygous, heterozygous or wild type for the eight *mei-S332* alleles were mated to 15 *C(2)EN* or *C(3)EN* virgin females. Reciprocal crosses were performed to test autosomal nondisjunction in females, using the same number of parents per vial. For both sets of crosses, parents were discarded on day 7, and the total number of progeny were counted until day 18. When flies with compound *C(2)EN* or *C(3)EN* autosomes are crossed to flies with unattached second and third chromosomes, virtually no progeny are obtained due to lethal zygotic aneuploidy (Figure 2C). The only surviving progeny in these crosses arise from nondisjunction events in the mei-S332$^+$ or mei-S332$^-$ parents.

Nondisjunction of the *X* and fourth chromosomes in females was assayed by crossing *y; spa$^{pol}$* females to $\widehat{XY}$, *v f B; C(4)RM ci ey$^R$* males. Regular mono-*4* ova resulted in either triplo-*4* progeny, which were wild type for fourth chromosome markers, or haplo-*4* sparkling-poliert Minute progeny (not included in the final totals). Nullo-*4* exceptional ova gave rise to cubitus interruptus eyeless-Russian progeny, and diplo-*4* exceptional ova gave rise to sparkling-poliert progeny that were not Minute. The types of regular and exceptional gametes with respect to the *X* chromosomes in this test have already been described (Figure 2B). Frequencies of the exceptional nullo-*X* and diplo-*X* ova were corrected as previously described; no such correction was necessary for chromosome *4* exceptional ova. Independent segregation of the *X* and *4* chromosomes could be monitored in this cross by comparing the observed numbers for each

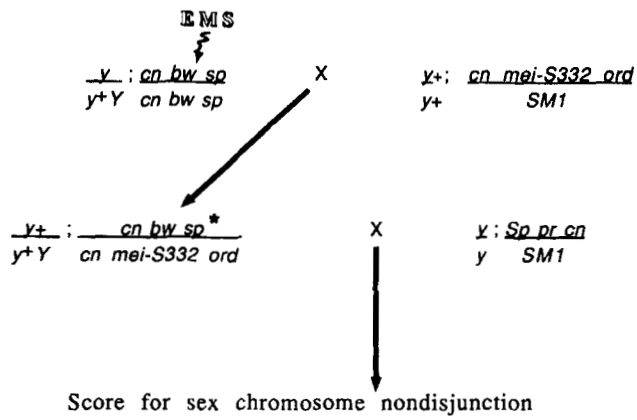


FIGURE 1.—Screen for new alleles of *mei-S332* and *ord*. Males with a *y$^+$Y* and a second chromosome marked with *cn bw sp* were mutagenized with EMS and crossed to a stock with a tester chromosome mutated for both *mei-S332* and *ord*. Single males were scored for mutations failing to complement either *mei-S332* or *ord* by crossing them to yellow mutant females and scoring for sex chromosome nondisjunction. Progeny from vials in which exceptional *XO* male progeny were observed were stocked over the balancer and retested.

gamete class to the expected numbers, determined by multiplying the product of the frequencies of the individual classes of *X* and fourth chromosome exceptions by the total number of progeny.

Nondisjunction of the fourth chromosomes in males was assayed by crossing *y/y$^+$Y; spa$^{pol}$* males to *y; C(4)RM ci ey$^R$* females. The types of progeny resulting from regular and exceptional gametes for the fourth chromosomes are described above. Independence of the sex and fourth chromosomes were not monitored in this cross, because diplo-*X* exceptional sperm were not recovered.

**Cytology of meiosis in males:** Testis squashes to analyze meiotic chromosome behavior were prepared, and staging was determined as described (GOLDSTEIN 1980). Precocious sister-chromatid segregation was scored only for the sex chromosomes and the autosomes, not for the fourth chromosome. Phase contrast microscopy was done using a Zeiss Axioskop or Axiophot equipped with Plan Neofluar 40X and 100X and Plan Apochromat 63X objectives.

**Viability tests:** Males that were *cn mei-S332$^{1\text{-}8}$ sp/SM1* were crossed to *Df(2R)X58-6, pr cn/SM1* females in bottles. The parents were discarded on day 5, and progeny were counted until day 18. The three surviving classes of progeny could be distinguished phenotypically: *mei-S332/+* flies were Curly speck, *Df/+* flies were Curly speck$^+$, and *mei-S332/Df* flies were Curly$^+$ speck$^+$ (the *mei-S332$^+$* allele was on the balancer). Percentages for each class were determined by dividing the number of flies in each class by the total.

## RESULTS

**Isolation of new alleles of *mei-S332*:** We conducted an EMS screen for mutations that failed to complement the initial *mei-S332* allele (Figure 1). Noncomplementers of both *ord$^1$* and *mei-S332$^1$* were recovered over a second chromosome carrying both mutations

by screening for sex chromosome nondisjunction in males (MATERIALS AND METHODS). A total of eight *mei-S332* noncomplementers and five *ord* noncomplementers were found out of 9900 chromosomes screened. All pairwise combinations of the 13 noncomplementers were tested in both males and females for nondisjunction of the sex chromosomes. The eight *mei-S332* noncomplementers and five *ord* noncomplementers fell into two separate complementation groups (data not shown). The five noncomplementers of *ord* will be described further in a separate report (W. MIYAZAKI and T. ORR-WEAVER, manuscript in preparation).

We mapped the *mei-S332* noncomplementers to demonstrate that they were true alleles of *mei-S332* (MATERIALS AND METHODS). The strongest noncomplementers were first mapped to the *c-px* interval on distal *2R*. One of these noncomplementers was found to be a deficiency in cytological interval 58 (*Df(2R)R1-8*); unfortunately, high sterility associated with this deficiency precluded its use in subsequent experiments. To map the remaining seven noncomplementers and the original *mei-S332*$^1$ allele more precisely, recombinants in the *Pu-px* interval were recovered over the deficiency *Df(2R)X58-6* (MATERIALS AND METHODS) and tested for nondisjunction in males. *mei-S332*$^1$ was found to map to position 99.2 cM, a position which is more consistent with its cytological location of 58B-D (DAVIS 1977; A. KERREBROCK, unpublished data) than was the previously reported map position of 95 cM (DAVIS 1971). The map positions of the seven noncomplementers in this experiment were: *mei-S332*$^2$, 99.3 cM; *mei-S332*$^3$, 99.5 cM; *mei-S332*$^4$, 99.3 cM; *mei-S332*$^5$, 99.1 cM; *mei-S332*$^6$, 99.3 cM; *mei-S332*$^7$, 99.5 cM; *mei-S332*$^8$, 99.5 cM. Thus we conclude that all seven newly isolated noncomplementers map to the *mei-S332* locus and are authentic alleles of *mei-S332*.

**Sex chromosome nondisjunction in males:** We assayed nondisjunction of the sex chromosomes in males in order to determine whether the new *mei-S332* alleles affected chromosome segregation in the first or second meiotic division (Figure 2A). In this cross, *XY* sperm were diagnostic of nondisjunction in the first meiotic division and *XX* sperm were diagnostic of meiosis II nondisjunction. The other major class of exceptional gametes, nullo-*XY* sperm, was produced from nondisjunction at either meiotic division or by chromosome loss. The six strongest *mei-S332* alleles resulted primarily in meiosis II nondisjunction when homozygous in males (Table 1). For most of the alleles, the frequency of observed equational exceptions (*XX* sperm) ranged from five to 15-fold higher than the frequency of reductional exceptions (*XY* sperm). The low levels of reductional exceptions that we observed for each of the homozygous *mei-S332*

**A. Cross:** *y/y+Y* ♂ X *X^X, y² su(w^a) w^a* ♀

| Gametes | Ova: *X^X, y² su(w^a) w^a* | Ova: *O* |
|---|---|---|
| **Regular Sperm** | | |
| *X* | lethal | yellow male |
| *Y or YY* | yellow+, suppressed white-apricot female | lethal |
| **Exceptional Sperm** | | |
| *O* | yellow², suppressed white-apricot female | lethal |
| *XY or XYY* | lethal | yellow+ male |
| *XX* | lethal | yellow female |
| *XXY or XXYY* | lethal | yellow+ female |

**B. Cross:** *cv v f car/y* ♀ X *X^Y, v f B* ♂

| Gametes | Sperm: *X^Y* | Sperm: *O* |
|---|---|---|
| **Regular Ova** | | |
| *X* | Bar female | Bar+ male |
| **Exceptional Ova** | | |
| *O* | Bar male | lethal |
| *XX* | lethal | Bar+ female |

**C. Cross:** *+/+* X *C(2)EN*

| Gametes | *C(2)EN* parent: *2^2* | *C(2)EN* parent: *O* |
|---|---|---|
| **Regular** | | |
| *2* | lethal | lethal |
| **Exceptional** | | |
| *O* | viable | lethal |
| *2,2* | lethal | viable |

FIGURE 2.—Crosses to test for nondisjunction. A, Sex chromosome nondisjunction in males. Progeny arising from regular or exceptional sperm are distinguishable by their sex and their eye-color and body-color phenotypes. Unless otherwise noted, all progeny have wild type eye color. B, Sex chromosome nondisjunction in females. Progeny arising from regular or exceptional ova are distinguishable by their sex and their phenotype with respect to *Bar*. C, Chromosome *2* nondisjunction. Only progeny arising from exceptional gametes survive. Progeny arising from regular mono-*2* gametes die due to lethal zygotic aneuploidy. Similar results are obtained in crosses to *C(3)EN* stocks.

alleles in males did not correlate with the strength of the allele, and they may have been due to background mutations in the stocks. Although the frequencies of exceptional sperm that we observed for the *mei-S332*$^1$ allele were slightly lower than previously reported frequencies (GOLDSTEIN 1980), the relative frequencies of exceptional gametes were essentially the same (nullo-*XY* > diplo-*X* ≫ *XY*).

All eight *mei-S332* alleles over a deficiency also resulted in much higher frequencies of meiosis II nondisjunction relative to meiosis I nondisjunction in males (Table 2). Most alleles gave from 10- to 20-fold higher frequencies of equational exceptions relative to reductional exceptions. If these values are corrected to include diplo-*Y* equational exceptions (MATERIALS AND METHODS), then most alleles over a deficiency probably gave 20–30-fold more equational

**TABLE 1**

**Sex chromosome nondisjunction in males homozygous for the indicated allele**

| Sample | + | *mei-S332¹* | *mei-S332²* | *mei-S332³* | *mei-S332⁴* | *mei-S332⁵* | *mei-S332⁶* | *mei-S332⁷* | *mei-S332⁸* |
|---|---|---|---|---|---|---|---|---|---|
| Regular sperm | | | | | | | | | |
| *X* | 1357 | 1815 | 2990 | 1026 | 1934 | 1766 | 1947 | 2076 | 1915 |
| *Y(Y)* | 1412 | 1630 | 2303 | 1081 | 1781 | 1490 | 1569 | 2300 | 1718 |
| Exceptional sperm | | | | | | | | | |
| O | 3 | 1015 | 400 | 267 | 713 | 28 | 51 | 1332 | 895 |
| *XY(Y)* | 0 | 30 | 18 | 3 | 31 | 23 | 10 | 37 | 74 |
| *XX* | 0 | 431 | 130 | 97 | 264 | 11 | 13 | 580 | 385 |
| *XXY(Y)* | 0 | 5 | 0 | 0 | 3 | 0 | 0 | 1 | 6 |
| Total progeny | 2772 | 4926 | 5841 | 2474 | 4726 | 3318 | 3590 | 6326 | 4993 |
| % nullo-*XY* | 0.1 | 20.6 | 6.8 | 10.8 | 15.1 | 0.8 | 1.4 | 21.1 | 17.9 |
| % *XY(Y)* | 0.0 | 0.6 | 0.3 | 0.1 | 0.7 | 0.7 | 0.3 | 0.6 | 1.5 |
| % *XX* | 0.0 | 8.7 | 2.2 | 3.9 | 5.6 | 0.3 | 0.4 | 9.2 | 7.7 |
| % *XXY(Y)* | 0.0 | 0.1 | 0.0 | 0.0 | 0.06 | 0.0 | 0.0 | 0.02 | 0.1 |
| Total observed nondisjunction | **0.1** | **30.0** | **9.3** | **14.8** | **21.5** | **1.8** | **2.1** | **30.9** | **27.2** |

**TABLE 2**

**Sex chromosome nondisjunction in males with the indicated alleles over *Df(2R)X58-6***

| Sample | *mei-S332¹* | *mei-S332²* | *mei-S332³* | *mei-S332⁴* | *mei-S332⁵* | *mei-S332⁶* | *mei-S332⁷* | *mei-S332⁸* |
|---|---|---|---|---|---|---|---|---|
| Regular sperm | | | | | | | | |
| *X* | 767 | 2069 | 899 | 792 | 1597 | 1901 | 1478 | 1470 |
| *Y(Y)* | 709 | 1821 | 932 | 723 | 1539 | 1489 | 1753 | 1488 |
| Exceptional sperm | | | | | | | | |
| O | 609 | 849 | 303 | 446 | 103 | 311 | 1108 | 831 |
| *XY(Y)* | 12 | 38 | 8 | 25 | 12 | 28 | 41 | 37 |
| *XX* | 203 | 399 | 148 | 215 | 28 | 122 | 638 | 452 |
| *XXY(Y)* | 4 | 0 | 1 | 2 | 0 | 0 | 5 | 5 |
| Total progeny | 2304 | 5176 | 2291 | 2203 | 3279 | 3851 | 5023 | 4283 |
| % nullo-*XY* | 26.4 | 16.4 | 13.2 | 20.2 | 3.1 | 8.1 | 22.1 | 19.4 |
| % *XY(Y)* | 0.5 | 0.7 | 0.3 | 1.1 | 0.4 | 0.7 | 0.8 | 0.9 |
| % *XX* | 8.8 | 7.7 | 6.5 | 9.8 | 0.9 | 3.2 | 12.7 | 10.6 |
| % *XXY(Y)* | 0.2 | 0.0 | 0.04 | 0.09 | 0.0 | 0.0 | 0.1 | 0.1 |
| Total observed nondisjunction | **35.9** | **24.8** | **20.0** | **31.2** | **4.4** | **12.0** | **35.7** | **31.0** |

than reductional exceptions. This assumes similar frequencies of *X* and *Y* chromosome nondisjunction, as was observed by Goldstein (GOLDSTEIN 1980) for the homozygous *mei-S332¹* allele. Therefore, the high numbers of equational exceptions produced by males homozygous or hemizygous for the eight *mei-S332* alleles indicate that most nondisjunction in these males occurred at the second meiotic division. Since the strong alleles did not show a shift to higher frequencies of reductional exceptions when placed over a deficiency, the preponderance of meiosis II nondisjunction did not result from residual activity of hypomorphic alleles.

The alleles can be placed into three classes, based on the observed frequency of nondisjunction. *mei-S332¹*, *mei-S332⁴*, *mei-S332⁷* and *mei-S332⁸* were classified as strong alleles (20–30% total nondisjunction), *mei-S332²* and *mei-S332³* were moderate alleles (10–15% total nondisjunction), and *mei-S332⁵* and *mei-S332⁶* were weak alleles (2% total nondisjunction) (Table 1). When placed over a deficiency, all alleles showed an increase in total nondisjunction that was due primarily to increases in the nullo-*XY* and diplo-*X* exceptional gamete classes. These increases were less dramatic for the strong alleles *mei-S332¹*, *mei-S332⁷* and *mei-S332⁸* (1.1–1.2-fold) than for the weak *mei-S332⁶* allele (6-fold).

Two of the four strongest alleles (*mei-S332¹* and *mei-S332⁷*) are likely candidates for nulls, since they showed only a slight increase in total nondisjunction when placed over a deficiency in both males and in females (see below). For both alleles, however, the increase observed in males was significant by the $\chi^2$ contingency test (LINDREN, MCELRATH and BERRY 1978). If these alleles are indeed nulls, then this slight increase in nondisjunction may reflect variability in the strength of the mutant phenotype due to different genetic backgrounds. It is also possible that the

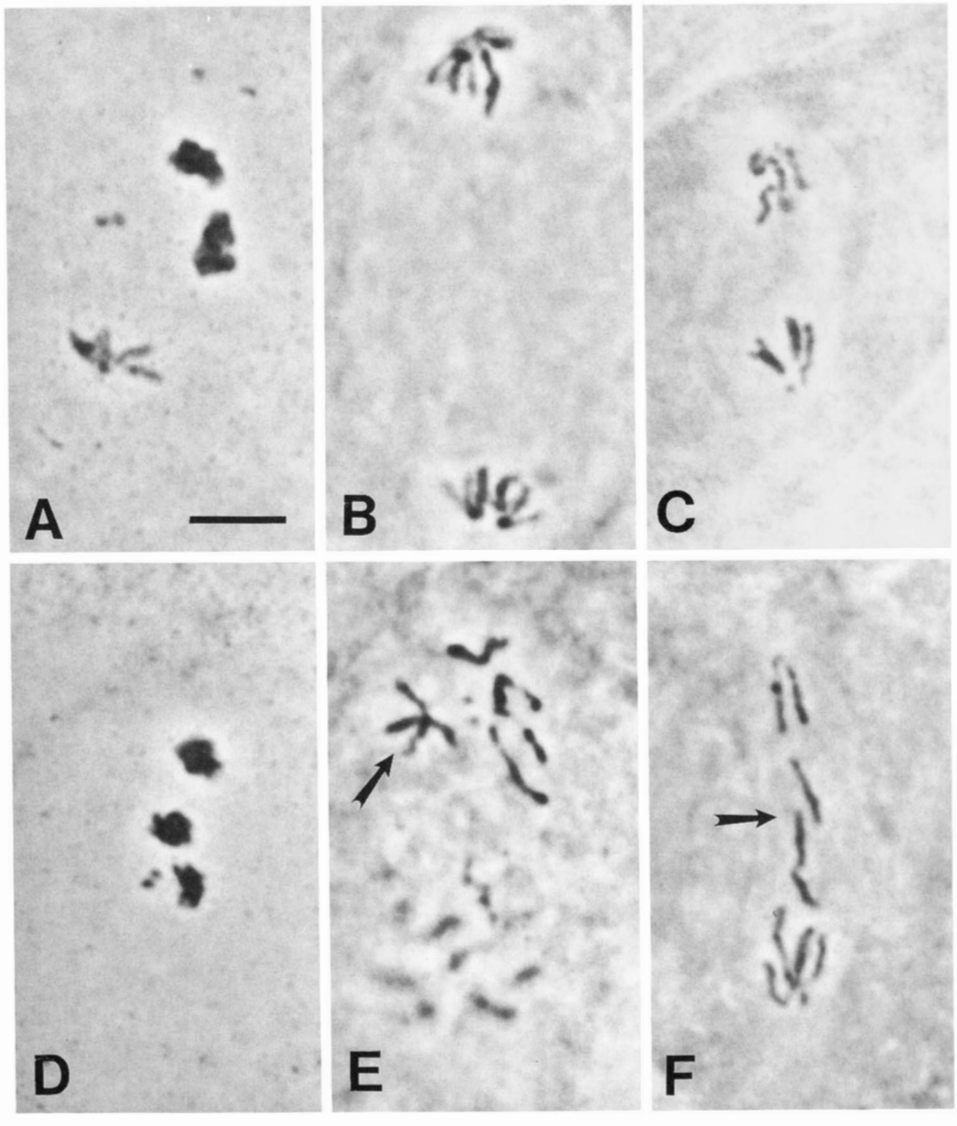


FIGURE 3.—Cytological analysis of meiosis in a *mei-S332*$^{7}$ homozygous male. Meiotic chromosomes were analyzed in testis squashes from homozygous wild type or *mei-S332*$^{7}$ mutants. A–C, wild type; D–F, *mei-S332*$^{7}$. In both wild type and mutant the sister chromatids are associated in prophase I (A, D); however, by late anaphase I (B, E) the sister chromatids have precociously disjoined in the *mei-S332* mutant. In (E) all of the sisters have disjoined except for the one dyad indicated by the arrow. In anaphase II of wild type equal numbers of chromosomes can be seen at each pole (C). Nondisjunction and laggards are seen in anaphase II in the mutant (F). Arrow points to laggards.

*Df(2R)X58-6* deficiency uncovers a second gene that enhances the mei-S332$^{-}$ phenotype.

**Cytology of male meiosis:** We performed testis squashes in order to examine the behavior of chromosomes during meiosis in males homozygous for the new *mei-S332* alleles. Early meiosis I stages (prophase I to early anaphase I) appeared to be normal for all of the alleles (Figure 3D and Table 3). In later meiotic stages (mid anaphase I to prometaphase II), the primary defect observed in mutant males was a failure to maintain sister-chromatid cohesion (Figure 3E and Table 3). Although it was difficult to obtain scorable cells for the early stages, premature sister-chromatid separation was readily seen in mid-late anaphase I in the alleles which were strong in males in the genetic tests (*mei-S332*$^{3}$, *mei-S332*$^{4}$, *mei-S332*$^{7}$ and *mei-S332*$^{8}$). Previous analysis of meiotic chromosome behavior in homozygous *mei-S332*$^{1}$ males revealed that sister-chromatid separation frequently occurred in mid-late anaphase I, but was only rarely seen earlier (GOLDSTEIN 1980). Our results and those of GOLDSTEIN (1980) demonstrate that the earliest manifestation of the requirement for *mei-S332* product that can be observed cytologically is in mid-late anaphase I.

In meiosis II all of the alleles showed precocious sister-chromatid separation and aberrant anaphase II figures (Table 3 and data not shown). For the strong alleles (*mei-S332*$^{3}$, *mei-S332*$^{4}$, *mei-S332*$^{7}$, *mei-S332*$^{8}$), the frequency of premature separation of sister chromatids (PSSC) observed in prophase II and prometaphase II cells was high, with *mei-S332*$^{8}$ showing the highest levels of precocious sister-chromatid separation. The levels of aberrant segregation events quantified in Table 3 are an underestimate, because ambiguous chromosomes were not scored. This is particularly true for *mei-S332*$^{7}$, since a high number of prophase II cells in this mutant contained clumped chromatin that may have obscured any separated sister chromatids. Rare prophase cells with PSSC were also observed in *mei-S332*$^{5}$ homozygotes (data not

TABLE 3

**Cytological analysis of males homozygous for the indicated alleles**

| Allele | Prophase I to metaphase I | | Early to mid anaphase I | | Mid to late anaphase I | | Prophase II and prometaphase II | | Anaphase II | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No PSSC | PSSC | No PSSC | PSSC | No PSSC | PSSC | No PSSC | PSSC | Normal | Aberrant[a] |
| *mei-S332²* | 90[b] | 0 | 17 | 0 | 17 | 6 | 127 | 11 | 72 | 12 |
| | | | | | (74%) | (26%) | (92%) | (8%) | (86%) | (14%) |
| *mei-S332³* | 45 | 0 | 2 | 0 | 31 | 6 | 45 | 28 | 44 | 24 |
| | | | | | (84%) | (16%) | (62%) | (38%) | (65%) | (35%) |
| *mei-S332⁴* | 23 | 1 | 21 | 0 | 7 | 3 | 20 | 21 | 26 | 24 |
| | | | | | (70%) | (30%) | (49%) | (51%) | (52%) | (48%) |
| *mei-S332⁶* | 37 | 0 | 2 | 0 | 12 | 0 | 108 | 4 | 56 | 1 |
| | | | | | | | (96%) | (4%) | (98%) | (2%) |
| *mei-S332⁷* | 65 | 0 | 14 | 0 | 17 | 12 | 74 | 37 | 4 | 35 |
| | | | | | (59%) | (41%) | (67%) | (33%) | (10%) | (90%) |
| *mei-S332⁸* | 39 | 0 | 24 | 0 | 1 | 15 | 21 | 97 | 3 | 36 |
| | | | | | (6%) | (94%) | (18%) | (82%) | (8%) | (92%) |

[a] Aberrant anaphases are those in which nondisjunction or lagging chromosomes were observed.
[b] Numbers indicate the number of cells observed. Only unambiguous cases of cells with precocious sister-chromatid cohesion of at least one dyad were scored as such, thus the number of cells with PSSC is underestimated.

shown). No metaphase II cells were observed in males homozygous for the strongest alleles (data not shown; GOLDSTEIN 1980). However, some metaphase II plates were observed in males homozygous for the weaker alleles.

For all of the alleles, we observed aberrant anaphase II figures in which sister chromatids had segregated to the same pole or which had lagging chromosomes that had not segregated to either pole (Figure 3F, Table 3, and data not shown). The frequencies of anaphase II cells showing missegregation of sister chromatids of the major autosomes and sex chromosomes paralleled the strengths of the alleles in males based on the genetic tests. The frequency of normal anaphase II cells seen in homozygous *mei-S332⁷* and *mei-S332⁸* mutants (8–10%) is consistent with the expected number of cells showing normal segregation of the major chromosomes if sister chromatids are segregating randomly ($0.5^3 = 12\%$).

The cytological phenotypes of the new alleles, namely separation of sister chromatids in late anaphase I and nondisjunction or lagging chromosomes in anaphase II, appeared to be identical to the defects observed for the original *mei-S332¹* allele (DAVIS 1971; GOLDSTEIN 1980). Thus these alleles also result in defects in sister-chromatid cohesion.

**Sex chromosome nondisjunction in females:** We also assayed nondisjunction of the sex chromosomes in females (Figure 2B) in order to determine whether the seven new *mei-S332* alleles produced similar effects in both sexes, as had been found for the *mei-S332¹* allele (DAVIS 1971; GOLDSTEIN 1980). Meiosis I and II nondisjunction events were distinguished among the diplo-*X* exceptional females by determining their genotypes with respect to the centromere-linked mutation *carnation*. Chromosome loss events in these tests were indicated by an excess of nullo-*X* relative to diplo-*X* exceptions.

As had been observed in males, the alleles which had the highest nondisjunction frequencies when homozygous in females (Table 4) resulted primarily in meiosis II nondisjunction (Table 5). The total nondisjunction frequency of 43.3% that we observed for the *mei-S332¹* allele in females was very similar to the value of 44.6% reported by DAVIS (1971), except that we observed less chromosome loss. Three of the strong alleles in males (*mei-S332¹*, *mei-S332⁴* and *mei-S332⁷*; Table 1) were strong in females, with total nondisjunction frequencies of 36–40%. The *mei-S332²* and *mei-S332⁶* alleles were also strong in females, with total nondisjunction frequencies of 33–34%. These five alleles when homozygous in females, and the three strongest alleles (*mei-S332¹*, *mei-S332⁴* and *mei-S332⁷*) over a deficiency, gave 10–20-fold greater equational exceptional ova relative to reductional exceptional ova (Table 5). Similar patterns were observed for the weaker *mei-S332⁸* allele in females (data not shown). The frequency of reductional exceptions in *mei-S332* homozygotes were consistently higher in females (5–14%) than in males (≤1%). However, the high numbers of equational relative to reductional exceptions demonstrate that these alleles result primarily in nondisjunction during the second meiotic division in females.

The frequencies of total sex chromosome nondisjunction obtained for the *mei-S332¹* and *mei-S332⁷* alleles over a deficiency in females (Table 6) were very similar to the frequencies obtained for these alleles when homozygous (Table 4). These results are consistent with the hypothesis that these alleles, which are strong in both sexes and which show little change in strength over a deficiency, may be nulls of the *mei-*

**TABLE 4**

**Sex chromosome nondisjunction in females homozygous for the indicated allele**

| Sample | + | *mei-S332*$^{1}$ | *mei-S332*$^{2}$ | *mei-S332*$^{3}$ | *mei-S332*$^{4}$ | *mei-S332*$^{5}$ | *mei-S332*$^{6}$ | *mei-S332*$^{7}$ | *mei-S332*$^{8}$ |
|---|---|---|---|---|---|---|---|---|---|
| Regular ova | | | | | | | | | |
| *X* | 3821 | 477 | 1749 | 2948 | 1280 | 3405 | 3188 | 2272 | 2329 |
| Exceptional ova | | | | | | | | | |
| O | 1 | 101 | 213 | 13 | 291 | 5 | 391 | 420 | 27 |
| *XX* | 0 | 81 | 236 | 12 | 175 | 5 | 394 | 225 | 14 |
| Total progeny | 3822 | 659 | 2198 | 2973 | 1746 | 3415 | 3973 | 2917 | 2370 |
| Adjusted total[a] | 3823 | 841 | 2647 | 2998 | 2212 | 3425 | 4758 | 3562 | 2411 |
| % nullo-*X* | 0.05 | 24.0 | 16.1 | 0.4 | 26.3 | 0.3 | 16.4 | 23.6 | 2.2 |
| % diplo-*X* | 0.00 | 19.3 | 17.8 | 0.4 | 15.8 | 0.3 | 16.6 | 12.6 | 1.2 |
| Total *X* nondisjunction | **0.05** | **43.3** | **33.9** | **0.8** | **42.1** | **0.6** | **33.0** | **36.2** | **3.4** |

[a] The progeny total is adjusted to correct for recovery of only half of the exceptional progeny.

**TABLE 5**

**Frequencies of reductional and equational exceptions among the diplo-*X* progeny of females homozygous or hemizygous for the indicated allele**

| Sample | *mei-S332*$^{1}$ | *mei-S332*$^{2}$ | *mei-S332*$^{4}$ | *mei-S332*$^{6}$ | *mei-S332*$^{7}$ | *mei-S332*$^{1}$/*Df(2R)X58-6* | *mei-S332*$^{4}$/*Df(2R)X58-6* | *mei-S332*$^{7}$/*Df(2R)X58-6* |
|---|---|---|---|---|---|---|---|---|
| Reductional | | | | | | | | |
| (*car*$^{+}$/*car*) | 8 | 11 | 8 | 32 | 29 | 12 | 12 | 16 |
| Equational | | | | | | | | |
| (*car*$^{+}$/*car*$^{+}$) | 23 | 83 | 75 | 157 | 84 | 94 | 70 | 70 |
| (*car*/*car*) | 44 | 104 | 76 | 164 | 98 | 124 | 115 | 93 |
| Total | 75 | 198 | 159 | 353 | 211 | 230 | 197 | 179 |
| % Reductional | **10.7** | **5.6** | **5.0** | **9.1** | **13.7** | **5.2** | **6.1** | **8.9** |
| % Equational | **89.3** | **94.4** | **95.0** | **90.9** | **86.3** | **94.8** | **93.9** | **91.1** |

**TABLE 6**

**Sex chromosome nondisjunction in females with the indicated allele over *Df(2R)X58-6***

| Sample | *mei-S332*$^{1}$ | *mei-S332*$^{3}$ | *mei-S332*$^{4}$ Test 1[a] | *mei-S332*$^{4}$ Test 2[a] | *mei-S332*$^{7}$ Test 1[b] | *mei-S332*$^{7}$ Test 2[b] | *mei-S332*$^{8}$ |
|---|---|---|---|---|---|---|---|
| Regular ova | | | | | | | |
| *X* | 1117 | 2637 | 714 | 677 | 1171 | 191 | 1956 |
| Exceptional ova | | | | | | | |
| O | 245 | 33 | 130 | 152 | 211 | 49 | 130 |
| *XX* | 254 | 42 | 133 | 96 | 187 | 25 | 151 |
| Total progeny | 1616 | 2712 | 977 | 925 | 1569 | 265 | 2237 |
| Adjusted total | 2115 | 2787 | 1240 | 1173 | 1967 | 339 | 2518 |
| % nullo-*X* | 23.2 | 2.4 | 21.0 | 25.9 | 21.5 | 28.9 | 10.3 |
| % diplo-*X* | 24.0 | 3.0 | 21.5 | 16.4 | 19.0 | 14.7 | 12.0 |
| Total *X* nondisjunction | **47.2** | **5.4** | **42.5** | **42.3** | **40.5** | **43.6** | **22.3** |

[a] Tests 1 and 2 were performed at different times using the same chromosomes.
[b] Tests 1 and 2 were performed at different times using different chromosomes.

*S332* locus. Although the levels of chromosome loss seen for the *mei-S332*$^{7}$ (and *mei-S332*$^{4}$) allele over a deficiency varied somewhat from test to test, the frequencies of total nondisjunction did not change (Table 6). We believe that the differences in levels of chromosome loss in females are due to the presence of modifiers, which may also explain why we observed less chromosome loss in *mei-S332*$^{1}$ homozygotes (Table 4) than DAVIS (1971).

Surprisingly, results from the female tests revealed that some of the new *mei-S332* alleles exhibited sex specific differences (compare Tables 1 and 4). The *mei-S332*$^{8}$ allele, which was comparable in strength to the three other strong alleles in males (27% total nondisjunction), was weak in females (3.4% total nondisjunction). Similarly, the *mei-S332*$^{3}$ allele, which was moderate in males (15% total nondisjunction) was very weak in females (only 0.8% total nondisjunction). Conversely, the *mei-S332*$^{2}$ and *mei-S332*$^{6}$ alleles, which were moderate (9% total nondisjunction) and weak (2% total nondisjunction) in males, respectively, were strong when homozygous in females (both gave about

TABLE 7

**Recombination in females homozygous or hemizygous for the indicated allele**

| Sample | + | *mei-S332¹* | *mei-S332²* | *mei-S332⁴* | *mei-S332⁶* | *mei-S332⁷* | *mei-S332¹ / Df(2R)X58-6* | *mei-S332⁴ / Df(2R)X58-6* | *mei-S332⁷ / Df(2R)X58-6* |
|---|---|---|---|---|---|---|---|---|---|
| Number scored | 1555 | 229 | 771 | 565 | 1425 | 1052 | 542 | 569 | 673 |
| Map distances (cM) | | | | | | | | | |
| 1 *y-cv* | 12.0 | 11.4 | 26.8 | 14.0 | 9.9 | 10.8 | 15.1 | 15.6 | 10.8 |
| 2 *cv-v* | 21.9 | 17.9 | 18.4 | 21.2 | 18.0 | 15.7 | 18.8 | 20.7 | 19.5 |
| 3 *v-f* | 19.1 | 16.2 | 20.6 | 17.7 | 18.8 | 16.2 | 21.2 | 19.5 | 21.5 |
| 4 *f-car* | 5.7 | 3.1 | 4.4 | 4.8 | 4.5 | 5.8 | 3.5 | 5.3 | 3.7 |
| Total map distance (cM) | **58.7** | 48.6 | 70.2 | **57.7** | 51.2 | 48.5 | **58.6** | **61.1** | **55.5** |
| | (1.00) | (0.83) | (1.20) | (0.98) | (0.87) | (0.83) | (1.00) | (1.04) | (0.95) |

Numbers in parentheses indicate values for the map distances normalized to the control map distance. Total map distances in bold print indicate those that are identical to the control map distance by the binomial distribution test (LINDREN, MCELRATH and BERRY 1978).

33% total nondisjunction). The reasons for the sex specificity exhibited by these alleles will be further addressed in the DISCUSSION. We will refer to these alleles in subsequent sections as "male-predominant" (*mei-S332³*, *mei-S332⁸*) or "female-predominant" (*mei-S332²*, *mei-S332⁶*), since their effects are not restricted to one sex.

**Mutations in *mei-S332* do not affect recombination:** Since few reductional exceptions were recovered among the progeny of females homozygous or hemizygous for any of the *mei-S332* mutant alleles, it seemed unlikely that the *mei-S332* gene product would be required to maintain homolog pairing in females. However, we looked for more subtle effects on homolog pairing in these mutants by measuring recombination levels in females. In the nondisjunction test crosses, the mei-S332⁻ female parents were heterozygous for five recessive *X*-linked mutations in order to measure recombination within four intervals spanning the *X* chromosome (*y-cv; cv-v; v-f; f-car*). We anticipated that defects in homolog pairing in these mutants would result in decreased recombination, which we could observe as a decrease in the map length of the *X* chromosome.

In general, the eight *mei-S332* alleles had little or no effect on recombination between *X* homologs (Table 7). The *mei-S332¹* allele was originally reported to have no effect on recombination (DAVIS 1971), but the stock that we used had a slight recombination defect (80% of wild type). The other alleles showed from 80% (*mei-S332⁷*) to 100% (*mei-S332⁴*) of the control map distance when homozygous. Similar map distances were found for the remaining *mei-S332* alleles as homozygotes (data not shown). The abnormally high map distance seen in *mei-S332²* homozygotes (70.2 cM) was due to the inclusion of triploid intersex progeny (2X3A), which phenotypically resembled recombinants in the *y-cv* interval, but which were distinguishable with practice. For three of the strong alleles (*mei-S332¹*, *mei-S332⁴*, *mei-S332⁷*) over a deficiency, the *X* chromosome map distance was statistically identical to that of the wild type control (Table 7). These results argue that the slight decreases in recombination seen for the homozygous alleles are due to other mutations on the second chromosome. We conclude that since mutations in *mei-S332* have virtually no effect on recombination, the product of the *mei-S332* locus is not required for homolog pairing.

**Nondisjunction of the autosomes:** We also examined whether the seven new *mei-S332* alleles affected segregation of the autosomes, as did the *mei-S332¹* allele (DAVIS 1971; GOLDSTEIN 1980). For the two major autosomes *2* and *3*, nondisjunction was assayed by mating males or females homozygous for the new *mei-S332* alleles to stocks carrying the compound autosomes *C(2)EN* or *C(3)EN*. In these crosses, only progeny arising from nullo or diplo exceptional gametes can survive (Figure 2C). Since the regular gamete classes were not recovered, the nondisjunction frequencies for either major autosome were unknown. Therefore, we scored these tests solely for the presence or absence of nondisjunction by comparing the numbers of progeny in the mei-S332⁻ test crosses to the numbers of progeny in the mei-S332⁺ control cross.

All *mei-S332* alleles, except the weak allele *mei-S332⁵*, resulted in nondisjunction of chromosomes *2* and *3* when homozygous in males (Table 8). In females, the alleles that gave high frequencies of sex chromosome nondisjunction in females (*mei-S332¹*, *mei-S332²*, *mei-S332⁴*, *mei-S332⁶*, *mei-S332⁷*; Table 4) also showed nondisjunction of chromosome *3* (Table 8), whereas alleles that were weak in females in the sex chromosome nondisjunction tests (*mei-S332³*, *mei-S332⁵*, *mei-S332⁸*) did not. All alleles, except the weak *mei-S332⁵* allele, resulted in chromosome 2 nondisjunction (Table 8), possibly because chromosome *2* is more sensitive to alterations in the *mei-S332* product in females than are the *X* or third chromosome. These

**TABLE 8**

**Autosomal nondisjunction in *mei-S332* mutants homozygous for the indicated allele**

| Test cross | + | *mei-S332*$^1$ | *mei-S332*$^2$ | *mei-S332*$^3$ | *mei-S332*$^4$ | *mei-S332*$^5$ | *mei-S332*$^6$ | *mei-S332*$^7$ | *mei-S332*$^8$ |
|---|---|---|---|---|---|---|---|---|---|
| *mei-S332* ♂ | − | + | + | + | + | + | + | + | + |
| ×*C(2)EN* ♀ | (4) | (72) | (12) | (65) | (38) | (16) | (9) | (100) | (56) |
| *mei-S332* ♂ | − | + | + | + | + | − | + | + | + |
| ×*C(3)EN* ♀ | (1) | (53) | (21) | (41) | (39) | (2) | (13) | (47) | (40) |
| *mei-S332* ♀ | − | + | + | + | + | − | + | + | + |
| ×*C(2)EN* ♂ | (2) | (21) | (63) | (34) | (23) | (3) | (40) | (40) | (20) |
| *mei-S332* ♀ | − | + | + | − | + | − | + | + | − |
| ×*C(3)EN* ♂ | (0) | (53) | (38) | (0) | (30) | (0) | (34) | (34) | (4) |

"+" and "−" indicate the presence and absence of nondisjunction, respectively. Numbers in parentheses indicate the number of progeny obtained in the test cross. Vials with less than five progeny were considered negative.

results demonstrate that mutations in *mei-S332* affect segregation of the major autosomes in both males and females.

Nondisjunction of the fourth chromosomes was assayed in males and females homozygous for the eight *mei-S332* alleles in order to determine whether this chromosome was affected by mutations in *mei-S332*. We also examined whether chromosomes were segregating independently in mei-S332$^-$ mutant females in a cross in which nondisjunction of both the *X* and fourth chromosomes could be monitored simultaneously (MATERIALS AND METHODS). For both sets of tests, homozygous *mei-S332* mutants were mated to flies with a compound fourth chromosome (*C(4)RM ci ey*$^R$). Regular fourth chromosome gametes and exceptional nullo-*4* and diplo-*4* gametes were recoverable and distinguishable in this cross; however, we did not assay the frequency of equational and reductional exceptions in the diplo-*4* exceptional class.

As expected, the levels of fourth chromosome nondisjunction observed in either sex roughly paralleled results from the sex chromosome nondisjunction tests. In homozygous males (data not shown), the frequency of total nondisjunction of chromosome *4* was higher for the *mei-S332*$^1$ allele (37.9%) than for the other strong *mei-S332*$^4$, *mei-S332*$^7$ and *mei-S332*$^8$ alleles (9–17%). Although these strong alleles gave low levels of chromosome *4* nondisjunction relative to *mei-S332*$^1$ in this experiment, it should be noted that only a few progeny were scored for each test (80–100 total), so that these values may be inaccurate. Otherwise, it is possible that some of the *mei-S332* alleles show chromosome specificity in males.

In the female tests, frequencies of total chromosome *4* nondisjunction were strikingly similar to frequencies of *X* chromosome nondisjunction (Table 9). The strong alleles *mei-S332*$^1$, *mei-S332*$^2$, *mei-S332*$^4$, *mei-S332*$^6$ and *mei-S332*$^7$ gave fairly high levels of total fourth chromosome nondisjunction (34–54%; Table 9), whereas the male-predominant alleles *mei-S332*$^3$ and *mei-S332*$^8$ and the weak allele *mei-S332*$^5$ gave little or no fourth chromosome nondisjunction (0–0.3%; data not shown). The numbers in parentheses in Table 9 are the expected numbers for each ova class, assuming independent segregation of the *X* and *4* chromosomes (MATERIALS AND METHODS). For all five strong alleles in females, the observed numbers in each ova class were not significantly different from the expected numbers by the contingency $\chi^2$ test (*mei-S332*$^{1,2,6,7}$: $P > 0.95$; *mei-S332*$^4$: $0.5 > P > 0.3$) (LINDREN, MCELRATH and BERRY 1978). Although small numbers of progeny were scored in these tests, the results we obtained strongly indicate that the *X* and *4* chromosomes were segregating independently in *mei-S332* mutants.

**Absence of semidominance in *mei-S332* mutants:** The original *mei-S332*$^1$ mutation was reported to show a slight amount of sex chromosome and fourth chromosome nondisjunction as a heterozygote relative to the wild type control in both sexes (DAVIS 1971). We measured nondisjunction in siblings heterozygous for all eight *mei-S332* alleles as well as the *Df(2R)X58-6* deficiency as additional controls for the sex chromosome and autosome nondisjunction tests. In the male tests, we found that the frequencies of nondisjunction measured in *mei-S332* heterozygotes were essentially identical to the frequency of nondisjunction in the wild-type control, showing that all eight alleles and the deficiency were fully recessive in males (data not shown). All eight alleles and the deficiency were also found to be fully recessive in females, except as transheterozygotes with the second chromosome balancer *SM1* (Table 10 and data not shown); the semidominance that DAVIS observed in females heterozygous for *mei-S332*$^1$ was also in the presence of the *SM1* balancer. We did not see this effect in the presence of the *FM6* balancer (data not shown). It is possible that the slight levels of nondisjunction observed for alleles of *mei-S332* over *SM1* result from a second-site noncomplementer on the *SM1* chromosome.

**Viability of *mei-S332* mutants:** We reasoned that if the *mei-S332* gene product was required for proper

**TABLE 9**

**Nondisjunction of the *X* and fourth chromosomes in females homozygous for the indicated alleles**

| Ova | *mei-S332*$^{+}$ | *mei-S332*$^{1}$ | *mei-S332*$^{2}$ | *mei-S332*$^{4}$ | *mei-S332*$^{6}$ | *mei-S332*$^{7}$ |
|---|---|---|---|---|---|---|
| *X; 4* | 370 | 98 | 60 | 34 | 166 | 68 |
| | | (100.0) | (57.1) | (36.0) | (165.1) | (66.2) |
| *X*; O | 2 | 57 | 21 | 24 | 52 | 23 |
| | | (57.0) | (22.4) | (20.8) | (51.2) | (24.2) |
| *X; 44* | 0 | 61 | 11 | 18 | 34 | 18 |
| | | (59.0) | (12.5) | (19.3) | (35.8) | (18.5) |
| O; *4* | 0 | 20 | 10 | 14 | 21 | 13 |
| | | (23.1) | (11.8) | (9.9) | (20.9) | (12.2) |
| O; O | 0 | 15 | 5 | 4 | 5 | 4 |
| | | (13.2) | (4.6) | (5.7) | (6.5) | (4.5) |
| O; *44* | 0 | 15 | 4 | 3 | 6 | 3 |
| | | (13.6) | (2.6) | (5.3) | (4.5) | (3.4) |
| *XX; 4* | 0 | 38 | 12 | 4 | 16 | 12 |
| | | (32.9) | (13.0) | (6.1) | (17.0) | (14.6) |
| *XX*; O | 0 | 17 | 6 | 2 | 6 | 7 |
| | | (18.8) | (5.1) | (3.6) | (5.3) | (5.3) |
| *XX; 44* | 0 | 16 | 3 | 7 | 4 | 5 |
| | | (19.4) | (2.9) | (3.3) | (3.7) | (4.1) |
| Adjusted total | 372 | 458 | 172 | 144 | 368 | 197 |
| % *X* exceptions | **0.0** | **52.8** | **46.5** | **47.2** | **31.5** | **44.7** |
| % *4* exceptions | **0.5** | **53.7** | **37.9** | **52.7** | **34.5** | **39.2** |

Numbers in parentheses indicate the number of progeny expected if the *X* and fourth chromosomes segregate independently.

**TABLE 10**

**Sex chromosome nondisjunction in heterozygous females**

| Ova | + / *SM1* | *mei-S332*$^{1}$ / + | *mei-S332*$^{1}$ / *SM1* |
|---|---|---|---|
| Regular ova | | | |
| *X* | 2948 | 4220 | 2197 |
| Exceptional ova | | | |
| O | 2 | 1 | 105 |
| *XX* | 2 | 1 | 77 |
| Total progeny | 2952 | 4222 | 2379 |
| Adjusted total[a] | 2956 | 4224 | 2561 |
| % nullo-*X* | 0.14 | 0.05 | 8.2 |
| % diplo-*X* | 0.14 | 0.05 | 6.0 |
| Total *X* nondisjunction | **0.28** | **0.10** | **14.2** |

[a] The progeny total is adjusted to correct for recovery of only half of the exceptional progeny.

chromosome segregation during mitosis as well as meiosis, then strong alleles of this gene would result in a decrease in viability. We therefore performed crosses to compare the number of progeny that had each of the *mei-S332* alleles over the *Df(2R)X58-6* deficiency to the number of their heterozygous siblings that had either the deficiency or the *mei-S332* mutant chromosome over the *mei-S332*$^{+}$ allele. For all eight alleles, including the potential null alleles *mei-S332*$^{1}$ and *mei-S332*$^{7}$, the viability of flies that had the mutant allele over the deficiency was not different from that of their siblings that had the wild-type allele over the deficiency (Table 11). We did not select against lethal alleles of *mei-S332* in our noncomplementation screen, since *mei-S332*$^{1}$ over a deficiency is viable. These results indicate that the *mei-S332* gene product is not absolutely required during mitosis.

## DISCUSSION

**Time of *mei-S332* function:** The *mei-S332* gene is intriguing because it is required for sister-chromatid cohesion in meiosis in both sexes. Our genetic analysis of multiple alleles of *mei-S332* revealed that the gene product encoded by this locus is not needed throughout meiosis. Instead, the earliest onset of the requirement for *mei-S332* function does not occur until late anaphase I. Cytological analysis of homozygous *mei-S332* mutants shows that the primary defect in these mutants is a failure to maintain sister-chromatid cohesion prior to anaphase of the second meiotic division. We have demonstrated also that *mei-S332* is not required for viability and therefore appears dispensible for mitosis.

Loss of function of *mei-S332* results in equational nondisjunction, and the late onset of the action of *mei-S332* we observed is unlikely to result from residual gene function. We identified two alleles of *mei-S332* that are candidates for null alleles of this locus. Both of these alleles, whether homozygous or over a deficiency, give no evidence for an early requirement for *mei-S332* function, as they have little or no effect on either chromosome segregation or recombination during the first meiotic division. This result distinguishes *mei-S332* from the Drosophila meiotic mutation *ord*, which is also defective in sister-chromatid cohesion during meiosis, but has pronounced defects in early meiotic events (MASON 1976).

TABLE 11

**Numbers of progeny from the cross *mei-S332/SM1* ♂ × *Df(2R)X58-6/SM1* ♀**

| Genotype | *mei-S332*$^1$ | *mei-S332*$^2$ | *mei-S332*$^3$ | *mei-S332*$^4$ | *mei-S332*$^5$ | *mei-S332*$^6$ | *mei-S332*$^7$ | *mei-S332*$^8$ |
|---|---|---|---|---|---|---|---|---|
| *mei-S332* / + | 233 (39.1) | 317 (36.7) | 325 (37.8) | 178 (36.3) | 352 (35.3) | 178 (37.9) | 215 (38.1) | 193 (39.4) |
| *Df(2R)X58-6* / + | 182 (30.5) | 296 (34.3) | 265 (30.8) | 157 (32.0) | 317 (31.8) | 135 (28.7) | 161 (28.5) | 138 (28.2) |
| *Df(2R)X58-6* / *mei-S332* | 181 (30.4) | 250 (29.0) | 269 (31.3) | 155 (31.6) | 329 (33.0) | 157 (33.4) | 189 (33.4) | 159 (32.4) |
| Total | 596 | 863 | 859 | 490 | 998 | 470 | 565 | 490 |

Numbers in parentheses indicate the percentage of the total.

The quantitative level of nondisjunction observed in *mei-S332* mutants can be influenced by variability in the genetic background. While we attempted to remove modifiers by recombination, it is possible that some variability remained. However, our major conclusions are not compromised by the possible existence of modifiers. In preliminary nondisjunction tests of the seven EMS-induced alleles, as well as in all later tests of all eight *mei-S332* alleles either as homozygotes or over a deficiency, we consistently observed much higher levels of meiosis II relative to meiosis I nondisjunction. Thus, we think that timing of the defects in *mei-S332* mutants reflects the earliest requirement for *mei-S332* function.

**Model for *mei-S332* action:** The onset of the requirement for *mei-S332* function coincides with the time of differentiation of sister-chromatid kinetochores during the first meiotic division. GOLDSTEIN (1981) has shown by EM analysis that the single kinetochore jointly organized by the two sister chromatids in early prometaphase I undergoes a transformation in structure between late prometaphase I and early anaphase I, giving rise to a "double-disc" kinetochore (Figure 4). This process may involve the reorganization of material comprising the single kinetochore into the double-disc structure (GOLDSTEIN 1981). We propose that the function of the *mei-S332* gene product is to maintain sister-chromatid cohesion after kinetochore differentiation and that this product is not essential for cohesion when the two sister chromatids share a single kinetochore in early meiosis I. Since double-disc kinetochores occasionally were observed to occur as early as prometaphase I (GOLDSTEIN 1981), our model accounts for the low levels of reductional exceptions recovered in the genetic tests. The higher levels of reductional nondisjunction observed in females may reflect an earlier time of kinetochore differentiation in female meiosis. While we think it most probable that the *mei-S332* gene product is localized to the kinetochore and structurally promotes cohesion, it is possible the *mei-S332* product acts in a regulatory manner to prevent dissociation of the kinetochores.

**Regulation of sister-chromatid cohesion in mitosis:** Sister-chromatid cohesion must also be maintained during mitosis prior to separation at anaphase. However, we did not detect a decrease in viability for any of the *mei-S332* alleles over a deficiency, implying that *mei-S332* function is not critical for mitosis. Consistent with this, we observed gynandromorphs only infrequently in the progeny of homozygous *mei-S332* females (data not shown). Our results differ somewhat from those of BAKER, CARPENTER and RIPOLL (1978), who found a slight (approximately sixfold) increase in somatic clones arising either from mitotic recombination or nondisjunction in homozygous *mei-S332*$^1$ mutants. One explanation which would reconcile these results is that *mei-S332* function, although not critical for mitosis, does contribute to proper mitotic segregation, and that the assay for production of somatic clones was more sensitive in detecting a slight mitotic effect. Alternatively, since the mitotic phenotype observed in the earlier experiments was not mapped to the *mei-S332* locus, it could have resulted from a second mutation on the *mei-S332*$^1$ chromosome.

If the *mei-S332* product is indeed meiosis-specific, it is possible that different mechanisms operate to maintain sister-chromatid cohesion during meiosis and mitosis. Cytologically, pairing of sister chromatids appears quite different in the two divisions. In mitosis, sister chromatids remain closely paired along their whole length until anaphase. The kinetochore region does not appear to be essential for sister cohesion in mitosis because acentric fragments remain paired until anaphase in irradiated grasshopper neuroblasts (CARLSON 1938). However, in mammalian cells it has been observed that the centric heterochromatin does not split in two until anaphase (SUMNER 1991). Thus mitotic sister-chromatid cohesion may be mediated both by the components of the mitotic chromosome scaffold found along the length of the entire chromosome and by undivided centric heterochromatin. In contrast, from anaphase I until metaphase II of meiosis in Drosophila males, the sister chromatids are

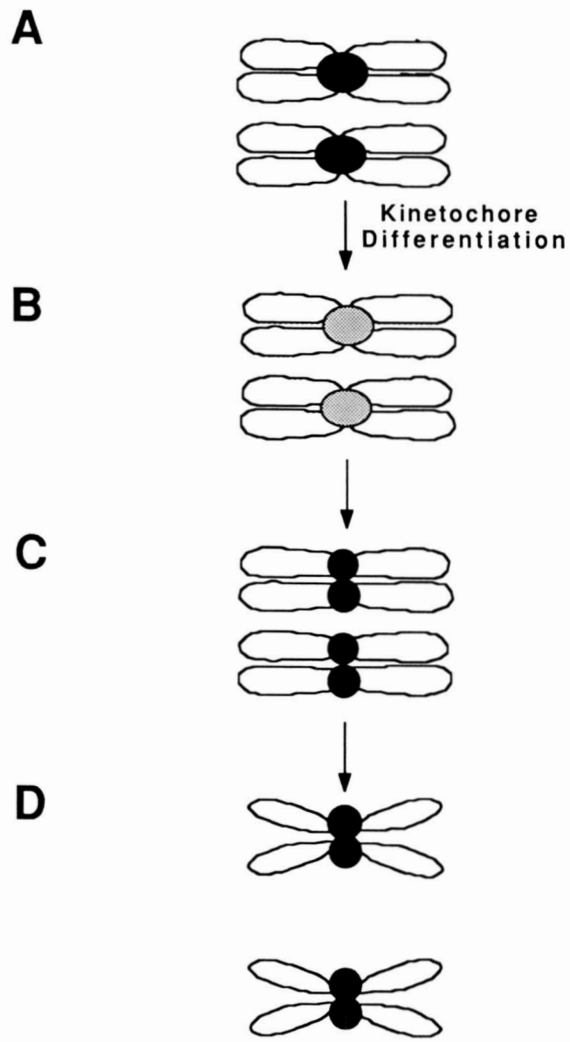


FIGURE 4.—Schematic diagram of meiotic chromosome cytology. Two pairs of homologs are diagrammed for the late prometaphase I through late anaphase I stages of meiosis, with only the chromosomes but no microtubules shown. It has been demonstrated in male meiosis that in late prometaphase to early anaphase I the kinetochore, which is initially a single hemispherical structure shared by the sister chromatids (A), goes through a stage with an amorphous morphology (B), and differentiates into a double-disc structure (C) (GOLDSTEIN 1981). In late anaphase the sister chromatids are joined only at their kinetochores (D). Since the onset of the requirement for *mei-S332* function is late anaphase, following kinetochore differentiation, *mei-S332* may act to promote cohesion of the doubled kinetochore.

paired only at or near the kinetochore regions, and the chromatid arms are completely free of one another (COOPER 1950).

More direct evidence to support the view that sister-chromatid cohesion in meiosis and mitosis is promoted by different mechanisms comes from mutational analyses of centromeres in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In both yeasts, a set of deletions of sequences within the centromere which had little or no effect on mitotic centromere function were found to result in precocious sister-chromatid separation in meiosis (CLARKE and BAUM 1990; CUMBERLEDGE and CARBON 1987; GAUDET and FITZGERALD-HAYES 1989; HAHNENBERGER, CARBON and CLARKE 1991). This suggests that there are meiosis-specific components required to maintain sister-chromatid cohesion. Further studies will reveal whether the product of the *mei-S332* locus is such a meiosis-specific component.

**Sex specificity of the *mei-S332* alleles:** An unexpected result from the analysis of additional *mei-S332* alleles was that four of the hypomorphic alleles had noticeably different strengths in males and females. The *mei-S332*$^{2}$ and *mei-S332*$^{6}$ alleles when homozygous were stronger in females than in males, whereas the *mei-S332*$^{3}$ and *mei-S332*$^{8}$ alleles when homozygous were stronger in males than in females. There are three possible explanations for different effects of these alleles in the two sexes: (1) different dosage requirements for *mei-S332* function in the two sexes; (2) the *mei-S332* gene product interacts with sex-specific proteins or protein complexes, and these male- and female-predominant mutations disrupt such an interaction; or (3) the mutations differentially affect the synthesis or stability of the *mei-S332* transcript or protein product in males and females. Our data make the first possibility very unlikely because it can account for only one of the sex-specific set of alleles. Furthermore, the strengths of the male-predominant *mei-S332*$^{8}$ and female-predominant *mei-S332*$^{6}$ alleles are fairly similar to the strengths of the three strongest alleles in males and females, respectively, but each of these alleles is very weak in the opposite sex. At present, we are unable to distinguish between the latter two possibilities. Molecular analysis of the *mei-S332* locus will help in elucidating the basis for the sex specificity exhibited by these alleles.

A caveat to our observations on the apparent sex specificity of some of the *mei-S332* alleles is that these phenotypes may have been subject to modifiers. Variability in results obtained with different chromosomes suggests that the genetic background may affect the degree of sex specificity observed, but it does not eliminate the female or male predominance. For example, a second test using a different combination of recombinant chromosomes containing the homozygous male-predominant *mei-S332*$^{8}$ allele gave a threefold increase in nondisjunction in females relative to the data presented here (data not shown). Since both of the male-predominant alleles gave consistently higher levels of nondisjunction in males than in females from the time that they were first isolated, we think that the sex specificity is linked to these mutations. The two female-predominant alleles were influenced to a greater extent by genetic background, so their classification as female-predominant alleles should be considered preliminary.

Our genetic and cytological analysis has demon-

strated that *mei-S332* controls sister-chromatid cohesion. The molecular cloning and identification of the *mei-S332* gene product will allow us to determine whether its localization is consistent with a structural role at or near the kinetochore between anaphases I and II of meiosis. The characterization of the *mei-S332* gene product will define further any possible mitotic function of the gene and will clarify the sex specificity observed with some alleles. Importantly, *mei-S332* can be used to identify other genes involved in chromosome segregation, either through genetic screens for second-site noncomplementers, or in biochemical assays for proteins that interact with the *mei-S332* gene product.

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