The Drosophila ord gene, sister-chromatid cohesion, and chromosome segregation

by

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ABSTRACT

Sister-chromatid cohesion is a fundamental attribute of chromosomes that allows them to be properly segregated during the meiotic and mitotic cell divisions. Presently, however, the full spectrum of the roles that cohesion might perform can only be guessed at. I defined the functions of the *ord* gene of *Drosophila melanogaster* by genetic and cytological means. The isolation of new EMS alleles and the use of deficiencies that uncover *ord* were pivotal in demonstrating that *ord* function is required at an earlier time in meiosis than a mutant with a similar phenotype, *mei-S332*. The early manifestations of the *ord* phenotype include premature doubling of kinetochores, reciprocal recombination defects and failure of crossovers to ensure meiosis I disjunction, phenotypes that implicate *ord* in being required for sister-chromatid arm cohesion as well as for cohesion at the centromere. Strikingly, defects in the gonial mitotic divisions that lead up to meiosis are also aberrant, although analysis of mitosis in the brain showed no defects associated with *ord*. The differences found between *ord* and *mei-S332* argue that the control of sister-chromatid cohesion may be complex, being regulated both temporally and in a tissue-specific manner. A highly unusual allele of *ord*, *ord*4, suggests that *ord* function is mediated through protein-protein interactions, and that the *ord* recombination and segregation phenotypes are genetically separable. A molecular analysis could link the protein structure with the functions delineated here. The cloning of *ord* was initiated by a chromosome walk starting from the closely linked *brown* locus.
Because at the moment you are most in awe of all there is about life that you don’t understand, you are closer to understanding it all than at any other time.

-Trudy

*The Search for Signs of Intelligent Life in the Universe*
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Sister-chromatid cohesion in mitosis and meiosis

**CYTOLOGICAL OVERVIEW**

Faithful chromosome segregation in cell division is of fundamental importance to the continued viability of a cell, the organism, and its offspring. Chromosome segregation has evolved into a highly accurate process, for example a chromosome segregates aberrantly only once every $10^5$ divisions in yeast (Hartwell *et al.*, 1982). Much attention has been focused on the obvious key players of mitotic chromosome movements: spindle structure (see Mitchison, 1988), kinetochore and centromere functioning (see Earnshaw and Tomkiel, 1992), and cell cycle control (reviewed in Nurse, 1990). A generally overlooked property of chromosome segregation yet essential for its proper execution is that of sister-chromatid cohesion. Sister-chromatid cohesion has been defined classically as a cytological phenomenon. This cohesion is obvious as replicated chromatids enter mitosis and condense: the replicated sister chromatids lie side-by-side, closely apposed not only at the centromere but along the entire length of their arms, the classic "textbook" example of a chromosome. Such juxtaposition of the sister chromatids is thought to result from an underlying physical attachment.

This textbook obviousness of sister-chromatid cohesion may have helped exalt it to unquestioned acceptance, and relative obscurity. Nevertheless, the presence or absence of this cohesion marks important cell cycle events. It is the dissolution of the elements holding sister chromatids
together that marks the metaphase/anaphase boundary as defined cytologically in both mitosis and meiosis II. Moreover, the metaphase/anaphase transition in meiosis I is correlated with a loss of sister-chromatid arm cohesion (John, 1990; e.g., see Suja et al., 1992). The sequential release of cohesion in meiotic chromosomes, first between the chromosome arms at meiosis I, and then between sister centromeres at meiosis II, hints at the possible underlying complexity of the mechanisms and regulation of such cohesion. The loss of cohesion in mitotic chromosomes at anaphase has been described in differing accounts, one suggesting sequential (although almost contemporaneous) separation (Mole-Bajer, 1958), and another arguing synchronous release of all cohesive forces (Bajer and Mole-Bajer, 1972).

**ROLES OF SISTER-CHROMATID COHESION**

Mechanistically, it is the pairing of sister kinetochores that allows for the proper movements of sister chromatids in both mitosis and meiosis II (Ostergren, 1951; reviewed in Rieder, 1991). The tension between the two linked yet oppositely oriented kinetochores is thought to allow the attachment of the paired chromatids to both spindle poles, followed by their oscillatory movements to the metaphase plate, a process known as congression. The balance of forces on each sister kinetochore would then maintain the chromatids at the metaphase plate until anaphase. If one of the sister kinetochores is ablated with laser irradiation, congression and stable metaphase orientation does not occur (McNeill and Berns, 1981). In addition, no metaphase II plates are observed in spermatocytes mutant for *ord* or *mei-S332*, in which precocious separation of sister chromatids occurs during meiosis I (Goldstein, 1980; Kerrebrock *et al.*, 1992; Miyazaki and Orr-Weaver, 1992). However, single mammalian kinetochores detached from their
chromosomal DNA can undergo congression as do paired kinetochores, but are forcibly split in an anaphase-like separation (Brinkley et al., 1988). Obviously, such a scenario would have disastrous consequences on an unpaired chromosome. The kinetochores in these studies may act aberrantly since they can only be obtained from cells after treatment with hydroxyurea and caffeine: they may be assembled abnormally or perhaps flanking centromeric DNA is needed for monopolar orientation of a single kinetochore.

In meiosis, the roles proposed for sister-chromatid cohesion are not well defined. Meiosis I prophase is unique in that homologous chromosomes pair and recombine in most organisms. Reciprocal recombination takes place in the context of the synaptonemal complex (SC), a structure that brings into alignment the replicated homologous chromosomes. The possibility that sister-chromatid cohesion may be necessary for proper recombination is suggested by several mutations in Drosophila and yeast that decrease recombination and also exhibit precocious sister-chromatid disjunction in the meiotic divisions (ord, Mason, 1976, Miyazaki and Orr-Weaver, 1992; red1, Rockmill and Roeder, 1988, 1990; med1, Rockmill and Roeder, 1994). The SC phenotype of the spo76 mutation in Sordaria is interesting in that the lateral elements consisting of the replicated sister chromatids are often split (Moreau et al., 1985). On the other hand, it has been proposed that proper SC formation is necessary for subsequent sister-chromatid cohesion (Maguire, 1990), so the interpretation of cause and effect may be uncertain in these mutants. That is, a defect in SC structure could be the proximal cause of failure to recombine and thus indirectly affect cohesion, or a defect in cohesion could indirectly affect recombination but be directly required for proper chromosome segregation.
Reciprocal recombination leads to the formation of chiasmata that cytologically act to link the two homologous chromosomes of the bivalent. Chiasmata are stable physical links that enable the bivalent to orient and form stable attachments to both meiosis I poles, much as sister kinetochore cohesion functions in mitosis and meiosis II. Micromanipulation experiments in grasshopper spermatocytes revealed the importance of chiasmata in creating the tension necessary for stable meiosis I orientation: maloriented bivalents could be stabilized by external forces supplied by a glass needle (Nicklas and Koch, 1969; also see Hawley, 1988). Dissolution of the chiasmate linkage allows homologs to begin anaphase movement. Darlington (1932) first considered the notion that chiasmata can be stabilized by the association of sister-chromatid arm segments distal to the crossover. The loss of arm cohesion at the beginning of anaphase I, as is observed cytologically, would be sufficient to resolve the chiasma. A premature release of the chiasmate linkage might result in the nondisjunction of chromosomes already recombined. Consistent with this model, the three proposed cohesion- and recombination-defective mutations mentioned earlier, ord, red1, and med1, exhibit meiosis I nondisjunction of recombined chromosomes. Alternatively, it has also been proposed that functional chiasmata (i.e., those able to ensure meiosis I disjunction) only arise if a crossover occurs in the proper chromosomal context, for example in the presence of SC (Engebrecht et al., 1990; Ross et al., 1992). These two possibilities are best resolved at the cytological level, as the chiasma is a cytological phenomenon; unfortunately chiasmata cannot be seen in either Drosophila or yeast meiosis.

Finally, as alluded to earlier, sister-chromatid cohesion must act between sister kinetochores so that proper congression to the metaphase II plate can occur. However, this entails maintaining the linkage through the
metaphase/anaphase transition in meiosis I. The mei-S332 gene of
Drosophila is thought to mediate the cohesion between the time when sister
kinetochores differentiate in meiosis I (Goldstein, 1981) until the metaphase/
anaphase II transition (Kerrebrock et al., 1992).

MECHANISMS OF ESTABLISHMENT

The models suggested to account for sister-chromatid cohesion fall into
two categories: those in which sister chromatids are linked through virtue of
DNA structure or chromatin topology, and those in which linkage is directly
conferred by chromatid-linking proteins. These models are not mutually
exclusive, and indeed they may act in concert to fulfill the predicted functions
of sister-chromatid cohesion. Meiotic chromosomes, moreover, may acquire
some cohesiveness as a result of synaptonemal complex formation.

DNA Linkage Models

It has been suggested that sister chromatids remain linked until
anaphase by a stretch of DNA that remains unreplicated (Tschumper and
Carbon, 1983; Clarke and Carbon, 1985). This hypothesis is attractive
because the heterochromatin that flanks the centromere of higher eukaryotic
chromosomes is known to be late-replicating (Lima-de-Faria and Jaworska,
1968). In this model of mitosis, the unreplicated stretches flank the
replicated centomere; the replication of the centromeric DNA would assure
that two kinetochore structures were assembled. In meiotic cells, however,
the entire centromeric region remains unreplicated, guaranteeing that the
sister chromatids share a common kinetochore and thus segregate to the
same meiosis I pole (Clarke and Carbon, 1985). This model was disproven by
the finding that S. cerevisiae centromeres actually replicate early in mitotic S
phase (McCarroll and Fangman, 1988). In addition, human fibroblasts that were pulse-labelled with tritiated thymidine, then fixed and examined for those cells in metaphase and early anaphase, revealed no DNA replication (Comings, 1966). However, the issue of late-replicating centromeres in meiosis remains an open question.

Alternatively, sister-chromatid strands could be intertwined prior to anaphase. Sundin and Varshavsky (1980, 1981) had shown that the final stages of SV40 viral replication resulted in the formation of catenated dimers when replication forks from opposite directions met. Topoisomerase II (top II) is an enzyme that catalyzes the double-strand passing of DNA in an ATP-dependent manner, and thus should be involved in the resolution of these catenated DNA strands (for review see Wang, 1985). By controlling the activity or access of topoisomerase II to these catenates, a temporary but releasable linkage between replicated molecules could be established, sufficient to account for the cohesion seen in mitotic prophase and metaphase (Murray and Szostak, 1985).

In addition, Murray and Szostak reasoned that DNA catenation could account for the chromosome behavior required in meiosis. Thus, the catenation of sister-chromatid arms distal to a reciprocal recombination event would be sufficient to account for chiasma maintenance in meiosis I, consistent with Darlington's (1932) hypothesis. Activity of topo II at the metaphase/anaphase I boundary would decatenate sister chromatids and allow recombined homologs to segregate. Centromeric DNA replication was postulated to be delayed until after meiosis I, which explained why sister chromatids of a homolog travel to the same anaphase I pole -- they share a common centromere. Delayed replication also allows the regeneration of
catenation between these sister chromatids for proper meiosis II segregation (Murray and Szostak, 1985).

Overall, there is much evidence favoring catenation as a means of achieving cohesion. The catenation hypothesis has been tested by examining the localization and roles of topo II using cytological, genetic, and biochemical means. Topo II has been localized to the chromosome scaffold in mitotic metaphase chromosomes, suggesting that it plays a structural role as well as a catalytic one (Earnshaw et al., 1985; Gasser et al., 1986). In addition, topo II is found in the axial cores of chicken and yeast meiotic pachytene chromosomes (i.e., those with full SC formation) (Moens and Earnshaw, 1989; Klein et al., 1992). It should be noted that the presence of topo II in these chromosome scaffolds is merely circumstantial evidence for the catenation model, as topo II is also required for chromosome condensation in mitotic chromosomes (Wood and Earnshaw, 1990; Adachi et al., 1991). Topo II function (or a topo II-like function) has also been proposed for the resolution of meiotic chromosome interlocks formed during SC zippering (von Wettstein et al., 1984), and diplotene/diakinesis condensation later during meiosis (Moens and Earnshaw, 1989).

Topoisomerase II mutants give a clearer insight into the functioning of this protein in chromosome segregation. Temperature-sensitive and cold-sensitive mutations have been studied in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe; mitotic cells at the restrictive temperature undergo high levels of nondisjunction and chromosome breakage (Holm et al., 1989; Uemura et al., 1987). Both of these phenotypes are consistent with a failure to resolve catenated DNA molecules. With respect to the meiotic role of topo II in S. cerevisiae, Rose et al. (1990) found that mutant top2 cells appeared normal but failed to enter anaphase I. The introduction of a rad50
mutation that blocked reciprocal recombination allowed top2 cells to progress though meiosis I but subsequently they performed an aberrant meiosis II division. These results were interpreted to suggest that topo II activity is required to allow the separation of recombined homologs as a prerequisite for anaphase I movement, namely by decatenating sister chromatid arms distal to the chiasma. These data are also consistent with a failure to resolve interlocked chromosomes during SC formation. Since a spreading technique has made feasible the visualization of yeast pachytene chromosomes (Dresser and Giroux, 1988), it would be of interest to observe such chromosomes in top2 cells to determine whether interlocks could partly account for the failure to segregate in meiosis I.

The role of topo II has also been examined biochemically in genetically intractable organisms or in in vitro assays by employing specific topo II inhibitors. The addition of the topo II inhibitor teniposide prior to fertilization of clam (Spisula) oocytes had no effect on germinal vesicle breakdown but oocytes were blocked at the first meiotic metaphase (Wright and Schatten, 1990). As unfertilized oocytes are arrested in late meiotic prophase, this finding suggests that topo II activity is needed for the resolution of the bivalent itself and that SC interlocks have already been resolved and are thus not a factor in this system. Teniposide added after meiosis I caused a metaphase II arrest, consistent with a topo II requirement for the mitosis-like meiosis II division. Similarly, in a cell-free extract made from Xenopus oocytes, various topo II inhibitors prevented mitotic anaphase separation of replicated chromosomes (Shamu and Murray, 1992).

Taken together, these results strongly implicate topo II as being involved in sister chromatid separation in yeast and higher eukaryotes, a mechanism consistent with the catenation model for cohesion. However,
topological interlocking of circular minichromosomes was not detected before mitotic anaphase in *S. cerevisiae*, and the sister chromosomes went on to segregate with fidelity (Koshland and Hartwell, 1987). In other studies, topo II activity appears to be necessary but not sufficient for anaphase segregation. In the yeast genetic studies, topo II was unable to separate sister chromatids during a period in which spindle function was temporarily disrupted using nocodazole or a cold-sensitive tubulin mutant (Holm *et al.*, 1989; Uemura *et al.*, 1987). When the spindle was allowed to re-form, chromosomes still did not segregate without topo II function. Holm *et al.* (1989) proposed that topo II-mediated separation requires a functional spindle, perhaps to generate directed forces on the chromosomes that facilitate topo II-catalyzed strand passing. Another explanation, not mutually exclusive, is that proper spindle function is a prerequisite for other cellular events, some of which may be permissive for topo II action. Cytological studies on the regulation of the metaphase/anaphase transition suggest a feedback mechanism in which separation occurs only if all the chromosomes are aligned on the metaphase plate. Irradiation of newt cell cytoplasm during mitosis is able to cause a delay in anaphase separation after all chromosomes have congressed to the metaphase plate (Zirkle, 1970). Such a feedback system would be dependent on the proper functioning of the spindle.

*Cohesion Proteins*

A class of proteins, by virtue of their chromosomal localization, have become candidates for promoting cohesion between sister chromatids. The INCENPs (inner centromere proteins) were detected using monoclonal antibodies raised against the metaphase chromosome scaffold fraction (Cooke
et al., 1987). At metaphase the antigens are located just above and below the centromeric constriction and more intriguingly between the sister chromatids along the length of their arms. In colcemid-blocked cells the proteins remain tightly bound to the inner centromere but are lost along the arms, consistent with the relaxation of chromatid arm associations in these treated cells. As anaphase commences, however, the INCENPs move rapidly from the chromosome onto the spindle interzone, and are also later found at telophase associated with the cell membrane of the cleavage furrow (Cooke et al., 1987; Earnshaw and Cooke, 1991). Although the localization of the INCENPs on the metaphase chromosome is highly suggestive of a role in sister-chromatid cohesion, the dynamic redistribution of these proteins (among others) has led to the hypothesis of "chromosomal passenger" proteins (Earnshaw and Bernat, 1991). These passenger proteins would associate with metaphase chromosomes simply for the purposes of transport to their required site of action. Thus other possible functions for the INCENPs, such as spindle elongation in anaphase B movement or cleavage furrow stabilization, cannot be excluded (Cooke et al., 1987).

The CLiP (centromere-linking protein) antigens are detected using autoimmune sera from CREST patients, and like the INCENPs, are found at points of contact between sister chromatids (Rattner et al., 1988). Colcemid-treated cells similarly only show staining at the primary constriction, with chromatid arm staining lost. The CLiP antigens do not undergo a relocalization during the cell cycle -- anaphase chromosomes stain only at the kinetochore while antigen along the length of the chromosome is no longer observed. It would be of great interest to examine the spatial and temporal patterns of the CLiP and INCENP antigens in meiotic chromosomes if cross-reactivity in an amenable meiotic system could be found.
Although one of the functions put forward for the INCENPs and other passenger proteins has been to regulate sister-chromatid disjunction (Earnshaw and Bernat, 1991), the pattern of localization is only consistent with such a role and unfortunately little direct functional evidence exists. The product of the \( l(1)zw10 \) gene (\( zw10 \)) of Drosophila, although not a passenger protein, does undergo dynamic redistribution during the cell cycle, moving from the spindle at metaphase to the leading edge of the chromosomes in early anaphase (Williams et al., 1992). Genetic lesions in \( zw10 \) are available, moreover, to assess the function of the gene product. Interestingly, animals mutant for \( zw10 \) die as late larvae or pupae, and neuroblasts from such larvae exhibit frequent lagging anaphases (Williams et al., 1992). Thus \( zw10 \) exhibits genetic and cytological phenotypes consistent with a role in the separation or segregation of sister chromatids.

Spatial Differences in Chromosome Associations

Many lines of evidence suggest that there are regional differences in sister-chromatid cohesion along the chromosome. The cytology of higher eukaryotic chromosomes distinguishes two regions of interest: the centromere (or primary constriction), and the chromosome arms. In chromosome studies performed in the presence of the spindle-disrupting drug colchicine and its derivatives, the centromere region remains paired in most organisms whereas the sister-chromatid arms lose their associations. This relationship holds true for chromosomes in organisms as diverse as Drosophila (Gatti and Goldberg, 1991; Gonzalez et al., 1991), chicken (Cooke et al., 1987), muntjac (Rattner et al., 1988), and human and mouse (Sumner, 1991). In addition, hypotonic treatment produces similar results in Drosophila cells (Gatti and Goldberg, 1991). However, studies in plant
endosperm by Mole-Bajer (1958) indicate that sister chromatids can completely separate even in the presence of colchicine. The discrepancy may indicate species-specific differences in centromeric pairing strengths.

The region of colchicine-resistant pairing at the centromere coincides with large blocks of highly repeated 'satellite' DNA sequences in both Drosophila and mammalian chromosomes (Ashburner, 1989; for review see Tyler-Smith and Willard, 1993). Moreover, the pairing seen at the centromeric constriction appears to be an intrinsic property of the DNA sequence and not due to kinetochore proximity, for instance. Drosophila chromosomes with translocations of heterochromatin away from the centromere exhibit paired heterochromatic blocks interspersed with separated euchromatic DNA in colchicine-arrested metaphase cells (Pimpinelli and Ripoll, 1986; and references therein). Analogous results are observed in a mouse chromosome containing secondary constrictions composed of satellite DNA blocks (Lica et al., 1986). Interestingly, in Roberts' syndrome, a human condition resulting in abnormal puffing of the heterochromatic region of the centromere, the aberrant chromatin morphology is associated with its premature separation (German, 1979; Tomkins et al., 1979).

These observations made using treated cells closely parallel what was observed in some studies of untreated cells. In cine-micrographic analysis, the centromere split after the chromatid arms had already separated (Mole-Bajer, 1958). Such sequential loss of cohesion has already been noted for the two meiotic divisions. It is unclear whether these properties in different regions of the chromosome reflect the utilization of different mechanisms of sister-chromatid cohesion, or reflect an altered spatial control of a single mechanism.
Meiotic Chromosome Cohesion

A priori there is no necessity for different mechanisms promoting sister-chromatid cohesion between mitotic and meiotic chromosomes. The release of meiotic cohesion in the stepwise fashion observed can be envisaged as the spatial and temporal regulation of mitotic cohesion mechanisms. A meiotic role proposed for sister-chromatid cohesion is the maintenance of chiasmata that hold homologs together until the metaphase/anaphase I transition (Darlington, 1932). Using the desynaptic strain of maize with an apparent chiasma maintenance defect, Maguire has postulated that the elaboration of the synaptonemal complex along the length of the bivalent is instrumental in subsequent sister-chromatid cohesion (Maguire, 1978; Maguire et al., 1991). In other studies using trisomic maize, the univalent chromosome excluded from exchange with the other two homologs frequently underwent equational separation of sister centromeres at anaphase I (Maguire, 1978). Interpreted with regard to this model, the lack of SC formation resulted in subsequent precocious centromere separation. However, it may also be the case that physical constraints conferred by chiasmata in the bivalent are critical in orienting sister centromeres to the same meiosis I pole.

The Maguire hypothesis cannot be generalized to other organisms, however. In D. melanogaster males, no SC is formed (Meyer, 1960), yet sister-chromatid cohesion is present. Also, in recombination-defective Drosophila females in which SC is absent, precocious sister-chromatid separation does not occur (Baker et al., 1976). In two grasshopper species sister-chromatid associations were normal in sex chromosome univalents and B chromosome univalents (Suja et al., 1992). In the latter cases it cannot be excluded that nonhomologous synapsis in the unpaired chromosomes
occurred in a “fold-back” fashion as is observed in meiosis in haploid yeast (Loidl et al., 1991), but these results do indicate that centromeres of univalents behave differently in different species.

**MAINTENANCE AND RELEASE**

*Maintenance of the Paired State*

Once established, sister-chromatid cohesion must be preserved until the (proper) metaphase/anaphase transition. Because the commencement of anaphase segregation needs to be a highly synchronous event, this suggests global mechanisms that either actively maintain cohesion or inhibit its premature release. Such maintenance functions would exist to ensure that all chromosomes or bivalents had congressed properly to the metaphase plate before the anaphase trigger for chromatid or homolog separation was given. Cell cycle checkpoints of this sort have been postulated (Hartwell and Weinert, 1989). Recently the kinetochore has been implicated in the detection of proper metaphase alignment since chromosomes assembled in the presence of anti-kinetochore antibodies can perform all the prometaphase movements necessary for congression to the metaphase plate, yet do not undergo anaphase separation (Bernat et al., 1991).

In addition, some developmental events might require precise cell cycle regulation, perhaps exerted at the level of sister-chromatid separation. Oocytes of many organisms are arrested prior to fertilization; Drosophila oocytes are arrested at metaphase I (Doane, 1960). Metaphase I arrest in Drosophila eggs does not occur in recombination-defective oocytes; instead these oocytes can proceed through to anaphase II (McKim et al., 1993). These observations led McKim et al. (1993) to hypothesize that the presence of chiasmata is necessary and sufficient for metaphase I arrest. If Darlington's
model of sister-chromatid arm cohesion as chiasma binder is correct, then this result would imply that maintenance of cohesive forces subsequent to chiasma formation is in itself necessary and sufficient for metaphase I arrest.

**Are Spindle Forces Required for Sister-Chromatid Separation?**

Anaphase segregation of chromatids clearly requires the forces exerted by the spindle, but it appears the trigger for separation does not reside there. A model in which anaphase separation is initiated by a sudden increase in spindle poleward forces is precluded by the fact that forces acting on kinetochores do not increase between metaphase and anaphase (Nicklas, 1988).

The anaphase signal may be necessary but not sufficient for chromatid separation, perhaps requiring a functional spindle for realization of complete separation. Evidence that the blocks of heterochromatic DNA require active forces on them in order to separate is provided by observing anaphase figures in Drosophila cells carrying translocation chromosomes. These chromosomes have heterochromatin translocated distally from the centromere, and in larval metaphase neuroblasts these translocated regions provide an extra site of sister-chromatid association in addition to the centromere (Pimpinelli and Ripoll, 1986; Gonzalez et al., 1991). In anaphase neuroblasts carrying these translocation chromosomes, a pair of lagging chromatids joined distally is seen after other chromosomes have segregated, suggesting that separation of heterochromatic regions requires spindle forces (Gonzalez et al., 1991). It is interesting to note that yeast chromosomes also require a functional spindle in order to separate, in that prolonged arrest in the presence of topo II activity in the absence of a functional spindle is insufficient for subsequent segregation (Holm et al., 1989; Uemura et al., 1987). However, while S.
*pombe* centromeres are large (30-100 kb) and contain large regions of non-transcribed, heterochromatin-like DNA flanking the functional core sequences (Clarke *et al.*, 1986; Fishel *et al.*, 1988; Nakaseko *et al.*, 1987), *S. cerevisiae* centromeres have only 220-250 bp cores that may be flanked by active genes (reviewed in Clarke and Carbon, 1985).

Chromosome arm association in colchicine-treated cells lapses. This result might indicate that chromosome arms can respond to the trigger for release in the absence of spindle forces, or perhaps such release is an artifact of colchicine on the cell. In investigations of mitotic cells with an intact spindle, chromatid arm separation occurred normally even without spindle forces. After liberation of chromosome arms by irradiation, these acentric fragments separated synchronously with their kinetochore-containing neighbors (Carlson, 1938; Liang *et al.*, 1993).

Similarly, the loss of meiotic cohesion between sister-chromatid arms at the metaphase/anaphase I boundary appears not to require transverse forces acting between chromatids. Suja *et al.* (1991) point out that when a chiasma is present, the two sister arm segments distal to the crossover are connected to a different kinetochore; thus as the homologs separate, spindle forces are sufficient to account for the loss of cohesion distal to the crossover. On the other hand, the sister arm segments proximal to the chiasma are connected to the same kinetochore, yet linkage is lost here as well. These observations suggest that loss of sister-chromatid cohesion at meiosis I may not be dependent on spindle pulling forces.

*Cell Cycle Regulation of Chromatid Separation*

Recently there has been an explosion in our understanding of the molecular mechanisms governing the cell division cycle. As the
metaphase/anaphase transition is, on the cytological level, solely defined by the sudden separation of sister chromatids, the signal triggering anaphase would be expected to be under tight cell cycle control.

Cyclins were first discovered in sea urchin eggs as proteins whose abundance varied cyclically with the cell cycle (Evans et al., 1983). They associate with the p34cdc2 catalytic subunit to form an active protein kinase complex, variously termed mitosis- or maturation promoting factor (MPF; for review see Nurse, 1990). The striking degradation of the mitosis-specific cyclin B at the metaphase/anaphase transition, concomitant with MPF inactivation, was proposed to be the trigger for anaphase separation (Murray and Kirschner, 1989).

This paradigm was radically overturned when several studies indicated that cyclin B degradation is not a prerequisite for anaphase. In S. cerevisiae the overproduction of wild-type cyclin B or expression of a nondegradable mutant cyclin B did not inhibit chromosome separation, but cells thereafter could not exit from mitosis (Surana et al., 1993). In their Xenopus in vitro system, Holloway et al. (1993) also found that a nondegradable form of cyclin B could allow anaphase separation. However, the addition of the N-terminal cyclin B fragment delayed the onset of anaphase, even in the presence of high MPF levels. This portion of the cyclin B molecule contains the ‘cyclin destruction box’ responsible for recognition by the ubiquitin-mediated pathway (Glotzer et al., 1991), and thus should act as a competitor for proteolysis. The anaphase delay strongly suggests that the trigger for anaphase requires the proteolysis of proteins other than cyclin B (Holloway et al., 1993). This group favors the idea that these proteins function to occlude potential topo II substrates from topo II activity. The control of topo II activity at the level of steric hindrance is consistent with
results indicating there is no increase of topo II activity at the metaphase/anaphase boundary (Shamu and Murray, 1992). Possible proteins downstream of the proposed proteolysis trigger would include the INCENPs and CLiP antigens.

The analysis of mutants defective in chromosome separation may be fruitful in identifying the functions required for anaphase activation and sister-chromatid cohesion. In light of the cyclin B findings, mutants specifically defective in chromatid separation might be expected to exhibit a metaphase arrest of their chromosomes, but without chromosomal overcondensation or a block in cytokinesis or exit from mitosis. That is, cyclin B degradation would commence as usual, allowing normal events downstream of MPF inactivation to occur. In fact, top2 mutants in S. pombe and topo-II inhibited mammalian cells fail to separate chromosomes but nevertheless undergo cytokinesis (Uemura et al., 1987; Downes et al., 1991). A mitotic mutation in Drosophila, three rows (thr), is intriguing in that chromosomes fail to separate, eventually decondensing, yet cyclin B is degraded normally (Philp et al., 1993).

Mutations in the dis genes of S. pombe result in the failure of mitotic cells to segregate their chromosomes (Ohkura et al., 1988). Subsequent analysis revealed that both dis2 and the functionally redundant sds21 encode type 1 protein phosphatases (PP1s) (Ohkura et al., 1989). Mutations in the homologous PP1 87B gene of Drosophila allow chromosomes to segregate, but thereafter result in larval neuroblast phenotypes that are consistent with an exit-from-mitosis function (Axton et al., 1990). One of the roles ascribed to PP1s is to undo the effects of the various mitotic cdc2 kinases, and return the cell to the interphase state. It is therefore surprising that dis2 mutant cells exhibit an apparent failure of chromosomes to disjoin. Perhaps dis2+ action
is needed to counteract the consequences of a protein kinase functioning earlier in the cell cycle.

**ord AND DROSOPHILA AS A MODEL SYSTEM**

Defects in sister-chromatid cohesion in Drosophila have been documented best for the meiotic genes *ord* and *mei-S332* (Davis, 1971; Mason, 1976; Goldstein, 1980). These two genes are especially interesting in that among all of the Drosophila meiotic mutations examined to date, they represent two of the three loci that are required in both males and females (the third is *Dub*, see Appendix I). *ord* acts earlier than *mei-S332* in both genetic assays and cytological observations, affecting processes as early as recombination (Kerrebrock *et al.*, 1992; Miyazaki and Orr-Weaver, 1992). Thus, the analysis of the *ord* locus should yield clues regarding the hypothesized roles of sister-chromatid cohesion in early meiotic events as well as the roles of cohesion needed for proper segregation.

The ability to exploit Drosophila as a powerful genetic and cytological tool to infer gene function, coupled with advancing techniques for gene isolation and molecular analysis, holds promise of the identification of the genes and proteins required for sister-chromatid cohesion. To that end, this thesis describes the genetic and cytological analyses of *ord* function in both meiosis and mitosis, along with an account of the chromosome walk prefacing the cloning of *ord*. The relationship of *ord* function to that of the *mei-S332* and *Dub* loci is presented as well.
BIBLIOGRAPHY


Chapter II.

Sister-chromatid misbehavior in Drosophila *ord* mutants

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Sister-Chromatid Misbehavior in Drosophila ord Mutants

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ABSTRACT

In Drosophila males and females mutant for the ord gene, sister chromatids prematurely disjoin in meiosis. We have isolated five new alleles of ord and analyzed them both as homozygotes and in trans to deficiencies for the locus, and we show that ord function is necessary early in meiosis of both sexes. Strong ord alleles result in chromosome nondisjunction in meiosis I that appears to be the consequence of precocious separation of the sister chromatids followed by their random segregation. Cytological analysis in males confirmed that precocious disjunction of the sister chromatids occurs in prometaphase I. This is in contrast to Drosophila mei-5332 mutants, in which precocious sister-chromatid separation also occurs, but not until late in anaphase I. All three of the new female fertile ord alleles reduce recombination, suggesting they affect homolog association as well as sister-chromatid cohesion. In addition to the effect of ord mutations on meiosis, we find that in ord mutants chromosome segregation is aberrant in the mitotic divisions that produce the spermatocytes. The strongest ord alleles, ord1 and ord2, appear to cause defects in germline divisions in the female. These alleles are female sterile and produce egg chambers with altered nurse cell number, size, and nuclear morphology. In contrast to the effects of ord mutations on germline mitosis, all of the alleles are fully viable even when in trans to a deficiency, and thus exhibit no essential role in somatic mitosis. The ord gene product may prevent premature sister-chromatid separation by promoting cohesion of the sister chromatids in a structural or regulatory manner.

MEIOSIS is a specialized cell division that accomplishes haploidization of cells by two successive rounds of division without an intervening S phase. In order to accomplish this halving of the chromosome number during meiosis I, unique features have evolved that differ from the archetypal mitotic cell division (Hawley 1988). First, in most organisms replicated homologous chromosomes pair and undergo reciprocal recombination during prophase I. Second, as a result of reciprocal exchange events, chiasmata linking homologous chromosomes form and are required to achieve balanced orientation of homologs to opposite poles in metaphase I. Lastly, for each homolog to move as a unit to either pole at anaphase I, controls must exist to prevent sister chromatids from separating equationally as they do in meiosis II.

Proper sister-chromatid cohesion has been postulated to be necessary for many of the unique events in meiosis I, but little is known about the regulation of sister-chromatid cohesion. Sister chromatids remain cohesive along the lengths of their arms until the metaphase I/anaphase I transition, at which time the forces holding the chromatid arms relax and sister chromatids are held together only at the centromere. (John 1990). Darlington (1932) originally proposed that this cohesion along the length of the sister chromatids is responsible for preventing premature dissolution of the chiasmata that link homologs. A precocious release of chiasmatic linkage would result in genetic exchange being insufficient for proper meiosis I disjunction. When sister chromatids are held together only at the centromere, cohesive functions must be acting until anaphase II to prevent premature separation of the chromatids, especially once kinetochores have doubled and each sister chromatid is associated with its own kinetochore.

Several mechanisms have been proposed to account for the regulation of sister-chromatid cohesion during meiosis. Maguire (1978) postulated a role for the synaptonemal complex (SC) in promoting cohesion of sister chromatids in the kinetochore region and along the length of the arms. Univalents which had presumably failed to synapse or recombine with either partner in trisomic maize strains precociously separated into single sister chromatids in metaphase I or by prophase II (Maguire 1978). In addition, defects in pachytene SC in the desynaptic strain were argued to result in abnormal cohesion along sister-chromatid arms, thus not allowing for proper chiasma maintenance (Maguire, PareDES and RIES 1991).

Murray and Szostak (1985) proposed that catenation of sister chromatids is responsible both for the chromatid-arm cohesion needed for chiasma maintenance in meiosis I and for the cohesion of sister chromatids after centromeric DNA replication in pro-
phase II (Murray and Szostak 1985). They reasoned that the action of topoisomerase II would release DNA catenation at the metaphase/anaphase boundary of both meiotic divisions, resulting in resolution of the chiasma linkage in meiosis I or separation of sister chromatids in meiosis II. Topoisomerase II has been found in the meiotic chromosome core (Moens and Earnshaw 1989; Klein et al. 1992), and it is required to avoid chromosome breakage of recombined chromosomes in yeast meiosis (Rose, Thomas and Holm 1990). However, plasmids in yeast failed to show catenation prior to mitotic anaphase (Koshland and Hartwell 1987).

Sister chromatids also may be directly linked together by structural proteins. Proteins have been localized on mitotic chromosomes to regions thought to be important for chromatid cohesion. Autoantibodies to CLIP antigens recognize the centromere pairing domain and also bind to the chromosomal domain where sister chromatid arms are in close apposition (Rattner, Kingwell and Fritzler 1988). The INCENP antigens localize as well to both the centromere and between the chromatid arms; however at anaphase they dissociate from the chromosomes and remain at the metaphase plate (Cooke, Heck and Earnshaw 1987), thus their function(s) remains unclear.

The isolation of mutations provides a powerful tool for identifying and characterizing functions that control sister-chromatid cohesion. In D. melanogaster, the ord and mei-S332 genes have been proposed to be necessary for sister-chromatid cohesion until anaphase II since sister chromatids precociously separate in anaphase of male meiosis (Goldstein and Earnshaw 1988). In contrast, recombination is greatly reduced in ord' females (Mason 1976). In addition, though reciprocal recombination is usually necessary and sufficient for meiosis I disjunction in most organisms (reviewed in Hawley, 1988), meiosis I nondisjunction in ord' females is not restricted to nonexchange chromosomes: chromosomes nondisjoin whether they have undergone an exchange event or not (Mason 1976).

Only one mutant allele of ord was previously known and no deficiencies were available for the previous studies. Thus it was unclear whether the phenotypes ascribed to ord' represented the ord loss-of-function or null phenotype. Additional alleles were needed to determine if the timing differences between ord' and mei-S332' accurately reflect their biological roles during meiosis. Furthermore, new alleles could elucidate the possible function(s) of ord in the processes of recombination and segregation in females by genetically separating these phenotypes. Finally, reports of ord' being involved in somatic (Baker, Carpenter and Ripoll 1978) or germine (Lin and Church 1982) mitoses could be addressed with additional ord alleles. In this paper we describe the isolation of five new ord alleles with varying strengths. We find that all fertile alleles have an earlier manifestation of the mutant phenotype in both males and females than alleles of mei-S332, and that the recombination and segregation phenotypes in females can be differentially affected by mutations in ord. Though ord does not appear to be essential for somatic mitosis, it may be required for mitosis in the germine.

MATERIALS AND METHODS

Stocks: All Drosophila stocks and crosses were raised at 25 °C on standard cornmeal-brewers' yeast-molasses-agar food. The deficiency Df(2R)J-7O was isolated in this laboratory by Daniel Moore by recovering chromosomes having undergone X-ray-induced loss of a A element carrying the white gene inserted into 59C (the P[w, y]A3-1 transformant, obtained from R. Lewis) (Hazeldrigg, Lewis and Rubin 1984). All other mutations used in these experiments are described in (Lindsley and Zimm 1992). The cn bw sp, iso-X,Y, cv v f car, compound-X, compound-Y, and compound autosomes were described in Kerrebrock et al. (1982). The a px or stock used in the mapping experiments was received from the Mid-America Stock Center at Bowling Green State University. The deficiency Df[2R]bucH44 (Simpson 1985) which uncovers ord was obtained from R. Lehmann.

Screen for new ord alleles: EMS mutagenesis and screen design were as described previously (Figure 1) (Kerrebrock et al. 1992).

Recombination mapping of alleles: We mapped the new alleles of ord in two experiments. In the first mapping test, we crossed the cn bw sp noncomplementers or cn ord' males to a px or females, allowed recombination to occur in heterozygous F1 daughters, and selected for F2 sons which carried second chromosomes recombinant in the px (2-100.5) to 0r (2-107.0) interval. These males were individually crossed to ord'/SM1 females to stock the recombinant chromosome and to generate sons carrying the px- or ord' or ord- or SM1 or ord'/SM1 chromosomes.
Drosophila ord gene

A. Cross: \( y^+y^+O \) \( \times \) \( X^+X, y^+su(we) \) \( \text{male} \)

<table>
<thead>
<tr>
<th>Gameses</th>
<th>Ova</th>
<th>Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( x^+x^+y^+su(we) )</td>
<td>yellow male</td>
<td>yellow, lethal</td>
</tr>
<tr>
<td>( Y ) or ( YY )</td>
<td>male</td>
<td></td>
</tr>
<tr>
<td>( y^+ ) or ( YY )</td>
<td>white-apricot female</td>
<td></td>
</tr>
<tr>
<td>( XX )</td>
<td>lethal</td>
<td></td>
</tr>
<tr>
<td>( X^+XYY ) or ( XXYY )</td>
<td>yellow, male</td>
<td></td>
</tr>
</tbody>
</table>

B. Cross: \( ev \text{ } f \text{ } car \text{ } ly \) \( \times \) \( X^+Y, v \text{ } f \text{ } b \text{ } c \)

<table>
<thead>
<tr>
<th>Gameses</th>
<th>Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( x^+y^+ )</td>
<td>Bar female</td>
</tr>
<tr>
<td>( X^+Y )</td>
<td>Bar+ male</td>
</tr>
</tbody>
</table>

C. Cross: \( c^+ \) \( \times \) \( C(2)\text{EN} \)

<table>
<thead>
<tr>
<th>Gameses</th>
<th>C(2)\text{EN} parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y^+ )</td>
<td>( \text{male} )</td>
</tr>
</tbody>
</table>

**Score for sex chromosome nondisjunction**

Regular progeny: \( y^+y^+ \) (yellow+ females) \( y^+y^+ \) (yellow+ males)

Exceptional progeny: \( y^+O \) (yellow males)

**FIGURE 1**—Screen for new alleles of ord and mei-S332. Males with a \( y^+ \) and a second chromosome marked with \( cn \) \( bw \) \( sp \) were mutagenized with EMS and crossed to a stock with a tester chromosome mutagenized for both \( ord \) and \( mei-S332 \). Single males were scored for mutations failing to complement either \( ord \) or \( mei-S332 \) 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 chromosome and restested.

**FIGURE 2**—Crossovers 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 ovum 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 \( C(1)\text{EN} \) stocks.

For \( ord \) closely linked lethal mutations did not allow viable \( ord^d \) \( bw \) \( sp \) \( cn \) \( ord \) \( bw \) \( sp \) males to be selected in the scheme above. Therefore, \( px \) \( ord^d \) \( bw \) \( sp \) and \( ord^d \) \( bw \) or stocks generated in the recombination mapping above were crossed to yield viable and fertile progeny.

As found for the chromosomes carrying the new alleles of \( mei-S332 \) (Kerrebrock et al. 1992), a recessive male-sterile mutation elsewhere on the second chromosome resulted in sterility in association with \( ord^d \) and \( ord^d \) flies. This mutation was most likely present on the originally mutagenized \( cn \) \( bw \) \( sp \) chromosome and only became a factor when fertility was reduced in the presence of meiotic mutants such as \( mei-S332 \) and strong alleles of \( ord \). A dominant suppressor present on the original \( cn \) \( bw \) \( sp \) chromosome was able to suppress the sterility of the male-sterile mutation (unpublished observations), and was most likely recombined off along with the lethal mutations in the above crosses. Since A. Kerrebrock had determined that the male-sterile mutation was located on 2R distal to \( px \), we recombinated the marker \( sp \) on the \( ord^d \) \( bw \) \( sp \) and \( ord^d \) \( bw \) sp...
chromosomes and found that the resulting recombinant chromosomes were now fertile when homoygous.

To control for possible recovery differences among heterogeneous X and Y chromosomes that could affect male nondisjunction frequencies, isogenic X and Y chromosomes (the iso-X chromosome; KERREBROCK et al. 1992) were incorporated as a common background into all ord and control stocks.

Nondisjunction tests: Male nondisjunction tests, female recombination and nondisjunction tests, and assays for autosomal nondisjunction were performed as described in Figure 2 and in KERREBROCK et al. (1992).

To calculate theoretical frequencies for random segregation, combinatorial analysis of four chromatids (n) taken four, three, two, one, or none at a time (m) was used to "segregate" chromatids to two poles; the number of combinations of a particular segregation pattern is given by n! / m!(n - m)!. Frequencies were calculated for two successive rounds to simulate the two meiotic divisions. The assumptions made for this analysis are that chromatids segregate independently of each other and that there is no chromatid loss. To correct for inviable progeny due to aneuploidy, we assumed that diplo-Y and diplo-X, diplo-Y sperm are nonrecoverable in the male test, and that triplo-X and tetra-X ova are nonrecoverable in the female test.

Cytology of meiosis in males: Preparation of testes for squashing and staging of cells were performed as described (GOLDSTEIN 1980). Aberrant behavior of chromatids was scored for the sex chromosomes and major autosomes, but not for the easily obscured dotlike fourth chromosomes. Aberrant behavior of at least one bivalent/dyad/chromatid in a cell was the criterion for scoring a cell as aberrant; thus the question of penetrance (i.e., how many bivalents/dyads per cell exhibit the phenotype) cannot be addressed by the data in Table 1 except in the special instance of the presence or absence of metaphase II plates.

Preparation of testes by the colchicine-hypotonic treatment was initially performed as described (LIN and CHURCH 1982). However, we found that a 3-5-min incubation in a solution of 3 mM CaCl2 in Ringer's solution (EPHRUSI and BEADLE 1936) instead of the colchicine incubation, or simply the 5-10-min hypotonic swelling alone, provided similar results. Cells from all three treatments were scored and are included in Table 6. Only the sex chromosomes and major autosomes were scored in this analysis. In addition, to avoid trivial scoring errors all chromatids must have been resolvable and the sex bivalent identifiable. The last requirement was included because a pair of acrocentric X chromatids might be mistaken for a single metacentric autosomal chromatid.

Phase contrast microscopy was done using a Zeiss Axioskop equipped with Plan Neofluar 20x objective; all scoring was done using the 100x objective.

Definition of cytological terms: The term bivalent is used to denote a pair of replicated homologous chromosomes (i.e., four chromatids) associated by chiasmata from prophase I until metaphase I. A univalent is an unpaired member of a homologous pair (i.e., two sister chromatids); a trivalent is a homologous "triplet" of chromatids. At anaphase I, a bivalent is separated into its two constituent dyads, each being a pair of joined sister chromatids. The dyad remains joined until anaphase II, when sister chromatids proceed to opposite poles. The term "nondisjunction" as used in this paper refers not to the classically defined segregation of both homologs or sister chromatids to the same pole either during anaphase I or II, respectively, but, owing to the precarious separation of sister chromatids observed in mutant alleles of this gene, to any aberrant segregation event of single chromatids.

Female cytology: Ovaries were dissected in 1 x phosphate-buffered saline (PBS) and transferred to a solution of 1 x PBS containing 1 µg/ml of 4',6-diamidino-2-phenylindole (DAPI). Ovaries were stained for 5-10 min and washed twice in 1 x PBS for 5 min each. After transfer to a slide containing a drop of 70% glycerol/30% 1 x PBS, the individual ovarioles were teased apart with forceps and layered with a coverslip. Egg chambers were viewed under fluorescence microscopy on a Zeiss Axiophot or a Zeiss Axioskop equipped with a Plan Neofluar 20x objective.

A caveat should be made concerning the observed defects in the nurse cell number. Specifically, the sickled nucleus phenotype might have given rise to counting artifacts: if two sickled nuclei had become closely apposed and nested together then they may have been scored as a single nucleus.

RESULTS

Isolation of new ord alleles: We required additional ord alleles in order to dissect genetically the array of ord phenotypes that were observed previously. We therefore performed an EMS screen designed to recover second chromosome noncomplementers of both ord' (2-103.5) and mei-3322 (2-99.5) (Figure 1; also previously described in KERREBROCK et al. 1992). Mutagenized en bw sp males were crossed to females carrying the mei-3322 ord' double mutant chromosome, and progeny mei-3322 ord'/en bk sp* sons were tested by singly crossing them to yellow females. Since these males carried the wild-type copy of the yellow gene on both the X and Y chromosomes, all progeny resulting from regular disjunction in the male were wild type in body color. However, if nondisjunction occurred in the tester male to produce nullo-XY sperm, the resulting flies were yellow X/O males, easily distinguished among wild-type siblings.

We screened 9906 second chromosomes and found six that failed to complement ord'. By assaying the male nondisjunction phenotype, five of these noncomplementing mutations were mapped between px (2-100.5) and or (2-106.7), and in a separate experiment between px and bw (2-104.5), giving map positions from 103.1 to 103.5 cm (see MATERIALS AND METHODS). ord' was previously mapped to 103.5 cm (MASON 1976). We conclude that these five noncomplementers represent new alleles of the ord locus. We could not determine allelism of the sixth noncomplementer by recombination mapping because it only gave weak nondisjunction of the sex chromosomes: 6-7% in males and 3-4% in females.

ord results in early defects in cytological analysis of males: ord' was previously shown to affect sister chromatid associations as early as prophase I in male testis squashes (GOLDSTEIN 1980). Since we had obtained new ord alleles of varying strengths as judged by genetic nondisjunction assays (see below), we wanted to determine if allele strength correlated with the cytologically observed timing of the ord defect.
Thus, we chose to focus on the strong allele ord^2, the moderate allele ord^3, and the very weak allele ord^4. These cytological studies revealed that for all three alleles, the primary defect is an increased frequency of premature sister-chromatid disjunction.

In prophase of meiosis I, homologous chromosomes pair and condense into bivalents (Figure 3A). (See Definition of cytological terms in MATERIALS AND METHODS). No pairing defects were evident at this stage in ord cells, as the bivalents were normal in number (n = 4) and no univalents were observed. However, the morphology of prophase bivalents was abnormal in the three ord alleles studied because of the presence of protrusions from the bivalent, which may have been either chromatid arms or the kinetochore region of a chromatid (e.g., Figure 3B). In ord^2 cells we observed the additional defect of bivalents with a more loosely packed appearance (Figure 3C). Protrusions and loosely packed bivalents were previously observed in ord^1 mutants (GOLDSTEIN 1980). Additionally, in the strong mutant ord^2 clear instances of single kinetochore regions could be seen being pulled from the prometaphase bivalent mass (Figure 3C, arrows). It should be noted that the loose configuration of ord^2 chromosomes was distinctive from chromosomes which were in the process of condensing during early prophase. Those chromosomes had the appearance of fine threadlike netting, whereas the ord^2 chromosomes were thick ropyl structures.

In the ord alleles examined, bivalents congressed normally to the metaphase plate. However, it was frequently seen in ord^2 and ord^3 cells that sister kinetochores, instead of pulling sister chromatids as an intact dyad to each pole, were behaving as separate entities and pulling their respective chromatids independently from the bivalent mass (e.g., Figure 3C, arrows). Precocious separation of sister chromatids was unambiguous as anaphase I progressed in all three alleles (e.g., Figure 3D). ord^4 exhibited low frequencies of aberrant phenotypes in metaphase I and anaphase I cells (Table 1).

When we examined the frequency of precocious separation in early to mid anaphase I ord^2 cells, only 28% appeared normal (Table 1). However, this same reduction in the frequency of normal-appearing cells was not seen in ord^3 cells (24%) until a later stage, mid to late anaphase I (Table 1). This indicates that sister-chromatid cohesion is maintained on average longer in ord^2 mutants.

The second meiotic division revealed abnormalities as well, resulting from the premature separation of sister chromatids in the earlier division. In prophase II, unpaired chromatids were always seen in ord^2 cells, and metaphase figures were never observed (Table 1). This suggests that by the end of prophase II all chromosome dyads had separated (i.e., penetrance is complete in this allele). In ord^4, however, about half of the prophase II cells appeared normal, and metaphase II figures were seen. Of these metaphase cells, 37% appeared normal, while the remainder had dyads lying in the position where a metaphase plate would be expected, with single chromatids proceeding to or already at the poles. As anaphase II commenced, anomalous events included the unequal segregation
of chromosomes to the poles and chromosome laggards. The frequency of anaphase irregularities was high in both ord\(^2\) and ord\(^6\), and very low in ord\(^4\).

In summary, we observed precocious sister-chromatid disjunction in the ord mutants. In all alleles tested abnormalities were visible as early as prophase I. Premature sister-chromatid separation occurred early enough in both ord\(^2\) and ord\(^6\) cells to disrupt chromosome segregation in anaphase I.

**ord acts early in meiosis in genetic nondisjunction assays:** By following marked chromosomes in appropriate crosses, we were able to infer their behavior through the two meiotic divisions in both males and females (see Figure 2, A and B) (Kerrebrock et al. 1992). In the male test employed here, the presence of XY-carrying sperm is diagnostic of meiosis I nondisjunction, the presence of XX-carrying sperm of meiosis II nondisjunction, and nullo-XY sperm of meiosis I or meiosis II nondisjunction or chromosome loss (Figure 2A). Meiosis II nondisjunction was underestimated in this assay because equational diplo-\(Y\) gametes were not expected to be recovered efficiently (Goldstein 1980; Lindsley and Grell 1968). The levels of sex chromosome nondisjunction observed in males are presented in Table 2. A range of strengths was seen among the various alleles. Of the new ord alleles, three (ord\(^2\), ord\(^4\), ord\(^6\)) exhibited levels of nondisjunction of 49–51\% which were stronger than the 42\% observed in the original allele ord\(^1\). Two alleles (ord\(^2\), ord\(^4\)) were weaker than ord\(^1\), giving levels of 1\% and 28\%, respectively. The weak nondisjunction seen in ord\(^2\) will be addressed in a separate paper (W. Y. Miyazaki and T. L. Orr-Weaver, manuscript in preparation).

The relative allele strengths with respect to sex chromosome misbehavior observed in these genetic tests paralleled the order found when we scored all chromosomes in the cytological analysis (ord\(^2\) > ord\(^4\) > ord\(^6\)). The strong alleles ord\(^2\) and ord\(^4\) were statistically alike by a \(6 \times 2\) \(\chi^2\) contingency analysis (d.f. = 5, 0.1 > \(P > 0.05\)) (Lindren, McClarath and Berry 1978). Moreover, the frequencies of aneuploid gametes recovered from ord\(^2\) and ord\(^4\) males agreed well with the theoretical frequencies expected from random segregation of the four sex bivalent chromatids through both meiotic divisions (Table 2 and materials and methods).

All alleles resulted in high levels of reductional XY (meiosis I) nondisjunction and lower levels of equational XX (meiosis II) nondisjunction. This is in contrast to alleles of mei-S332, all of which showed predominantly equational exceptional progeny and few reductional exceptions. The ratio of the percent reductional exceptions to the percent equational exceptions increased with increasing strength of the allele. For example the strong allele ord\(^2\) exhibited a ratio of 4.6:1, while the weaker ord\(^6\) allele was only 1.8:1. This lower level of meiosis I nondisjunction for ord\(^6\) as compared to the stronger ord alleles is consistent with the conclusion reached in the cytological analysis: sister chromatids in ord\(^6\) cells precociously disjoined on average later than those in ord\(^2\) cells, after meiosis I orientation toward opposite poles had been achieved.

Several of the alleles were tested in trans to a deficiency for the ord locus to determine if any allele behaved as a null allele, and if a shift toward earlier meiosis I nondisjunction could be induced by increasing the severity of the phenotype of weaker alleles. The results for ord\(^2\), ord\(^4\), and ord\(^6\) are shown in Table 2. The surprising finding is that for all three alleles, the amount of nondisjunction seen when the alleles were homozygous was identical to that seen when hemizygous, by \(\chi^2\) analysis (ord\(^2\), 0.5 > \(P > 0.1\); ord\(^4\), 0.9 > \(P > 0.5\); ord\(^6\), 0.5 > \(P > 0.1\)). The functional equivalence of the hemizygous vs. the homozygous condition normally argues for the null state of a particular allele, but is not meaningful when exhibited by strong, moderate and weak alleles of ord. We

**TABLE 1**

Cytological analysis of ord spermatocytes of the indicated genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Prophase I</th>
<th>Metaphase I</th>
<th>Early-mid anaphase I</th>
<th>Mid-late anaphase I</th>
<th>Prophase II</th>
<th>Metaphase II</th>
<th>Anaphase II</th>
<th>Total number of cells scored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
<td>Abnormal</td>
<td>Normal</td>
<td>PSSC(^5)</td>
<td>Normal</td>
<td>PSSC</td>
</tr>
<tr>
<td>+/+</td>
<td>14</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>50</td>
<td>1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>ord(^2)/Df(^6)</td>
<td>(100)</td>
<td>(0)</td>
<td>(100)</td>
<td>(0)</td>
<td>(98)</td>
<td>(2)</td>
<td>(100)</td>
<td>(0)</td>
</tr>
<tr>
<td>ord(^4)/ord(^6)</td>
<td>17</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>16</td>
<td>4</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>ord(^6)/Df(^6)</td>
<td>(39)</td>
<td>(61)</td>
<td>(23)</td>
<td>(77)</td>
<td>(28)</td>
<td>(72)</td>
<td>(15)</td>
<td>(85)</td>
</tr>
</tbody>
</table>

\(^5\) PSSC, precocious separation of sister chromatids.

\(^6\) Values in parentheses are percent.

\(^7\) The deficiency used in this study was Df(2R)3-70.
TABLE 2
Sex chromosome nondisjunction in males

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>ord°</th>
<th>ord'</th>
<th>ord°</th>
<th>ord'</th>
<th>ord°</th>
<th>Df(2)R+</th>
<th>Df(2)R-</th>
<th>ord°</th>
<th>Df(2)R+</th>
<th>Df(2)R-</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular sperm</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>1312</td>
<td>808</td>
<td>288</td>
<td>454</td>
<td>1255</td>
<td>293</td>
<td>919</td>
<td>156</td>
<td>1206</td>
<td>1232</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y(Y)</td>
<td>1504</td>
<td>927</td>
<td>514</td>
<td>478</td>
<td>1115</td>
<td>594</td>
<td>896</td>
<td>160</td>
<td>1102</td>
<td>1205</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exceptional sperm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>787</td>
<td>558</td>
<td>708</td>
<td>21</td>
<td>427</td>
<td>442</td>
<td>230</td>
<td>26</td>
<td>666</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X(Y)</td>
<td>3</td>
<td>327</td>
<td>157</td>
<td>186</td>
<td>12</td>
<td>233</td>
<td>160</td>
<td>76</td>
<td>12</td>
<td>265</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XX</td>
<td>0</td>
<td>150</td>
<td>43</td>
<td>53</td>
<td>1</td>
<td>51</td>
<td>89</td>
<td>19</td>
<td>4</td>
<td>156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XX(Y)</td>
<td>0</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total progeny</td>
<td>2823</td>
<td>5015</td>
<td>1168</td>
<td>1865</td>
<td>2402</td>
<td>1403</td>
<td>2512</td>
<td>645</td>
<td>2350</td>
<td>3531</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Nullo-X(Y)</td>
<td>0.1</td>
<td>26.1</td>
<td>50.7</td>
<td>58.0</td>
<td>0.9</td>
<td>50.4</td>
<td>17.6</td>
<td>35.7</td>
<td>1.1</td>
<td>18.9</td>
<td>32.9</td>
<td></td>
</tr>
<tr>
<td>% X(Y)</td>
<td>0.1</td>
<td>10.8</td>
<td>15.4</td>
<td>10.0</td>
<td>0.5</td>
<td>16.6</td>
<td>6.4</td>
<td>11.8</td>
<td>0.5</td>
<td>7.4</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>% XX</td>
<td>0.0</td>
<td>5.0</td>
<td>5.7</td>
<td>2.8</td>
<td>0.04</td>
<td>3.6</td>
<td>3.5</td>
<td>2.9</td>
<td>0.2</td>
<td>4.4</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>% XX(Y)</td>
<td>0.0</td>
<td>0.5</td>
<td>0.7</td>
<td>0.2</td>
<td>0.0</td>
<td>0.4</td>
<td>0.2</td>
<td>0.6</td>
<td>0.0</td>
<td>0.3</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Total observed nondisjunction</td>
<td>0.2</td>
<td>42.4</td>
<td>48.5</td>
<td>51.0</td>
<td>1.4</td>
<td>51.0</td>
<td>27.7</td>
<td>51.0</td>
<td>1.8</td>
<td>31.0</td>
<td>56.0</td>
<td></td>
</tr>
</tbody>
</table>

a See MATERIALS AND METHODS for calculations of theoretical values.

TABLE 3
Sex chromosome nondisjunction in females

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>ord°</th>
<th>ord'</th>
<th>ord°</th>
<th>ord'</th>
<th>ord°</th>
<th>Df(2)R+</th>
<th>Df(2)R-</th>
<th>Theoretical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular ova</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>3558</td>
<td>1373</td>
<td>554</td>
<td>2324</td>
<td>1990</td>
<td>424</td>
<td>741</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exceptional ova</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>480</td>
<td>238</td>
<td>45</td>
<td>403</td>
<td>154</td>
<td>203</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>1</td>
<td>368</td>
<td>181</td>
<td>53</td>
<td>319</td>
<td>117</td>
<td>225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total progeny</td>
<td>3559</td>
<td>2221</td>
<td>973</td>
<td>2422</td>
<td>2712</td>
<td>675</td>
<td>1169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted totalb</td>
<td>3540</td>
<td>5069</td>
<td>1592</td>
<td>2520</td>
<td>5454</td>
<td>926</td>
<td>1597</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Nullo-X</td>
<td>0.00</td>
<td>31.3</td>
<td>54.2</td>
<td>5.6</td>
<td>23.5</td>
<td>28.9</td>
<td>25.4</td>
<td>53.3</td>
<td></td>
</tr>
<tr>
<td>% diplo-X</td>
<td>0.03</td>
<td>24.0</td>
<td>26.0</td>
<td>4.2</td>
<td>18.6</td>
<td>25.3</td>
<td>28.2</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Total X nondisjunction</td>
<td>0.03</td>
<td>55.3</td>
<td>60.2</td>
<td>7.8</td>
<td>42.1</td>
<td>54.2</td>
<td>53.6</td>
<td>55.5</td>
<td></td>
</tr>
</tbody>
</table>

b The progeny total is adjusted to correct for recovery of only half of the exceptional progeny.

da See MATERIALS AND METHODS for calculations of theoretical values.

postulate that ord action in males is not sensitive to the 50% reduction in gene product when alleles are placed in trans to a deficiency. This behavior of ord in males prevents us from using deficiencies to identify conclusively a null allele among the new ord alleles.

X chromosome nondisjunction was tested in females mutant for the various ord alleles (Figure 2B; Tables 3 and 4). In this assay we could score for regular mono-X ova, and exceptional nullo-X and diplo-X ova. In addition, since the females were heterozygous for the centromere-linked marker carnation (car), diplo-X progeny could be tested to determine if nondisjunction had occurred in meiosis I (two non-sister centromeres) or meiosis II (two sister centromeres). As seen in the male tests, the new ord alleles exhibited varying strengths. We did not encounter any sex-predominant alleles as were found in the mei-S332 analysis (KEREBOCK et al. 1992): the relative strengths were similar in both males and females. However, two of the alleles that were among the strongest in males, ord2 and ord3, were highly infertile in females and sufficient progeny could not be obtained for analysis. Of the remaining alleles, both ord1 and ord2 showed high levels of total nondisjunction, between 55% and 60%, ord6 showed a slightly lower level, 42%, and ord4 showed weak nondisjunction, 8%. Similar to the male analysis, we found that the levels of nondisjunction of the strongest alleles, in this case ord1 and ord2, agreed closely with the theoretical frequencies expected of four randomly segregating X chromatids through two meiotic divisions (Table 3 and MATERIALS AND METHODS).

To ascertain which meiotic division was affected in ord females, the progeny derived from diplo-X ova
were tested to determine which combination of centromere-linked car alleles they carried: car\(^*\)/car flies were indicative of meiosis I nondisjunction, and car/car or car\(^*\)/car\(^*\) flies were evidence of meiosis II nondisjunction (Table 4). In agreement with the male results, all alleles tested gave primarily reductive nondisjunction. However, ord\(^*\) in females, as in males, gave weaker reductive nondisjunction than did the stronger alleles ord\(^*\) and ord\(^{4}\) (0.564 × 18.6\% = 10.5\% of total gametes vs. 16.4\% and 21.1\%, respectively, from Tables 3 and 4).

ord\(^*\) and ord\(^{4}\) were tested in trans to ord deficiencies (Table 3). In the X chromosome nondisjunction assay, ord\(^*\) showed a slight decrease in total levels when hemizygous (from 60.2\% to 54.2\%), whereas nondisjunction in hemizygous ord\(^{4}\) females increased from the level observed in the homozygote (from 42.1\% to 53.6\%) and was similar to hemizygous ord\(^{4}\). Moreover, the percentage of exceptional diplo-X progeny increased in hemizygous ord\(^*\) (28.2\%) as compared to homozygous ord\(^{4}\) (18.6\%, Table 3). Of the 18.6\% diplo-X progeny scored in the homozygous ord\(^{4}\) test, the fractions representing reductive and equational exceptions were 10.5\% and 8.1\% respectively; these values in the hemizygous ord\(^*\) test were 19.8\% and 8.4\%, respectively (Tables 3 and 4). Thus the increase seen in the number of diplo-X progeny was due to an almost twofold increase in reductive nondisjunction, suggesting that the timing of the ord\(^*\) defect was shifted earlier in meiosis I with the 50\% reduction of gene product. The increased severity of the ord phenotype when ord\(^*\) is in trans to a deficiency suggests that ord\(^*\) is not a null allele.

Nondisjunction of chromosomes 2 and 3 was assayed in males and females homozygous for the new ord alleles by crossing them to strains carrying the compound chromosomes C(2)EN or C(3)EN (Figure 2C) (Kerrebrock et al. 1992). Progeny derived from regular mono-2 or mono-3 gametes are aneuploid (either monosomic or trisomic) and do not survive. However, aneuploid nullosomic or disomic gametes from ord parents can be complemented by the appropriate compound autosome-carrying or compound autosome-lacking gamete, respectively, from the other parent. Thus the presence of viable progeny in these crosses is an indication of autosomal nondisjunction events. All ord alleles resulted in nondisjunction of chromosomes 2 and 3 in both males and females, albeit weakly for ord\(^*\) (data not shown). Chromosome 4 nondisjunction was tested in small numbers by crossing ord; spa\(^{10}\) flies to a tester stains carrying the compound-4 chromosome C(4)RM, ci or\(^{8}\) (Kerrebrock et al. 1992). All alleles of ord exhibited chromosome 4 nondisjunction, although the levels for ord\(^*\) were lower than for the stronger alleles (data not shown).

In summary, all alleles of ord resulted in nondisjunction of all chromosome pairs. Frequencies of sex chromosome nondisjunction of the strongest alleles in both males and females were consistent with frequencies expected for randomized segregation of sister chromatids through the two meiotic divisions. Nondisjunction occurred primarily at the first meiotic division in all alleles tested; our interpretation is that the function of ord in sister-chromatid cohesion is required at an earlier time than mei-5332 function.

ord acts early in females to perturb recombination: The original allele ord\(^{1}\) was found to dramatically decrease recombination in females. However, the segregation defect in ord\(^{1}\) females was shown to be independent of the recombination defect (MASON 1976). Therefore, part of our interest in obtaining new alleles of ord was to determine if we could mutationally separate the ord functions that contribute to proper recombination and segregation in females. The same test in which nondisjunction was assayed also served to monitor reciprocal recombination on the X chromosome, since the females tested were heterozygous for five recessive markers that divided the X chromosome into four genetic intervals (y-cv; co-v; v-f; f-car).

All four fertile alleles decreased recombination to varying degrees (Table 5). ord\(^{1}\), ord\(^{4}\) and ord\(^{9}\) exhibited recombination at 10-13\% of the wild-type control.

**TABLE 4**

Frequencies of reductive and equational exceptions among the diplo-X progeny of females

<table>
<thead>
<tr>
<th>Reductional</th>
<th>Equational</th>
</tr>
</thead>
<tbody>
<tr>
<td>ord(^*)</td>
<td>ord(^{4})</td>
</tr>
<tr>
<td>(car(^*)/car)</td>
<td>(car/car)</td>
</tr>
<tr>
<td>251</td>
<td>147</td>
</tr>
<tr>
<td>76</td>
<td>19</td>
</tr>
<tr>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>368</td>
<td>181</td>
</tr>
<tr>
<td>% reductional</td>
<td>% equational</td>
</tr>
<tr>
<td>68.2</td>
<td>81.2</td>
</tr>
<tr>
<td>51.8</td>
<td>18.8</td>
</tr>
</tbody>
</table>

| ord\(^{4}\) |
| Df(2R)h66 |
| 180 | 90 |
| 81 | 20 |
| 58 | 7 |
| 519 | 117 |
| 56.4 | 76.9 |
| 45.6 | 70.2 |
| 25.1 | 29.8 |
level along the X chromosome. Using the binomial distribution test (LINDREN, MCCLRATH and BERRY 1978), the average total map values observed in these alleles were not significantly different from each other. Since these alleles perturbed recombination to the same extent, yet ord gave significantly better segregation than either ord or ord (Table 5), it appears that certain mutations in ord differentially affected the ability of the ord product to function in recombination and segregation. Consistent with the observations of MASON (1976), ord was semidominant for the effect on recombination. However, none of the five new alleles was semidominant (data not shown).

The nondisjunctionally weak allele ord also showed a less severe defect in the recombination assay; recombination was 59% of the wild-type control. Furthermore, the reduction in recombination along the length of the X chromosome was not uniform: the telomere-proximal interval from y to cv showed the greatest reduction (to 21% of the control), whereas the centromere-proximal interval f to car actually exhibited an increase (to 147% of control levels). This phenomenon of a nonuniform effect on recombination along the length of a chromosome has been exhibited by other Drosophila meiotic mutations and has been used to argue that a gene product is required as a precondition for a crossover event rather than for the crossover event per se (CARPENTER and SANDLER 1974). We presume that polar decreases were not observed for the other alleles because the recombination defects were too strong, and thus not enough recombinant progeny were scored for a statistically valid sampling.

When the ord and ord alleles were tested in trans to deficiencies, total map distances were reduced still further to one-third of the values observed in the respective homozygous condition (Table 5). These decreases were significantly different by the binomial distribution test. Since the recombination phenotype of both alleles became more severe when in trans to a deficiency, these results suggest that the ord and ord mutations are not null alleles of ord.

A crossover event leading to a chiasma normally is sufficient for proper meiosis I disjunction of homologs to opposite poles (HAWLEY 1988). In the course of progeny testing the diplo-X exceptional daughters arising from ord females, we noticed the presence of reductional diplo-X daughters homozygous for one or more X-linked markers, indicative of X chromosomes derived from a tetrad which had undergone a recombination event. Among the reductional exceptions from homozygous ord, ord and ord females, 2–4% exhibited homozygosis of X-linked markers (data not shown). These values are an underestimate of the actual number of El (single crossover) tetrads undergoing meiosis I nondisjunction, since 50% of possible segregation products from such tetrads would have been two nonechange chromatids or two complementary crossover chromatids. Both of these segregation products would be heterozygous for all markers and indistinguishable (at our level of observation) from diplo-X daughters resulting from nonechange tetrads. Thus similar to ord (MASON 1976), we observed that the presence of a crossover was not always sufficient for normal reductional disjunction of tetrads in females carrying the new alleles of ord.

ord affects mitosis in the male germ line but has no effect on overall viability: LIN and CHURCH (1982) had previously reported mitotic misbehavior in the germline gonial cells of ord males (LIN and CHURCH 1982). However, mitotic nondisjunction was not seen by GOLDSTEIN (1980). LIN and CHURCH observed univalents and trivalents of the large autosomes in ord primary spermatocytes, suggesting that a nondisjunction event had occurred in some preced-
chromosomes present. However, treatment of intact
genetic nondisjunction tests.
forces that held bivalents together, and thus we
MATERIALS AND METHODS) resulted in a relaxation of
testes with a hypotonic solution prior to fixation (see
it was extremely difficult to determine the number of
type and
ure 3), prophase I to metaphase I bivalents in wild
cytological techniques presented earlier
was a special allele that affected the gonial mitotic
movement of each sister chromatid of the univalent. The other
univalent is observed in an
preparation. (B) Early anaphase I segregation of an autosomal
bivalents are labeled
sex chromosome bivalent is labeled
A wild-type prophase I cell displays a normal diploid complement:
of chromosomes present (compare Figure 4A with Figure 3A). (A)
associations and consequently allow determination of the number
matocytes subjected to a brief hypotonic treatment lose their tight
spermatocytes to determine ploidy. Chromosomes in primary sper-
mits to generate aneuploid primary spermatocytes.
found that ord/Df(2R)3-70 cells exhibited univalent (Figure 4B) and trivalent figures in hypotonically
treated primary spermatocytes (Table 6) in similar fashion to ord (LIN and CHURCH 1982). In contrast,
trivalent figures were observed in wild-type control cells (Figure 4A). The large autosomes were
aneuploid more frequently than were the sex chromosomes: 15% as compared to about 3%, respectively,
yet the preponderant aneuploidy seen for the autosomes were univalents (Table 6). According to the
scoring criteria that we used in this analysis (see MATERIALS AND METHODS), we did not score figures in
which all chromatids could not be individually resolved and counted. Therefore, we believe that a
systematic scoring bias was introduced against trivalents since the extra chromatids would have made a
confusing figure more likely; likewise there would have been a bias toward the simpler configurations of
univalents. Alternatively, there may have been a greater incidence of mitotic chromosome loss rather
than nondisjunction which would lead to the generation of monosomes without accompanying trisome
formation. We conclude that ord, like ord', is not specific for meiosis but is also able to perturb germline
mitoses to generate aneuploid primary spermatocytes.
To determine if the ord gene product was required
for somatic mitotic divisions, we crossed the six ord alleles to a stock with a deficiency for the locus and
scored the relative viability of the ord/Df trans-heterozygotes. If ord were essential for mitosis in a large
number of the somatic cells, we would expect to see a decrease in the viability of mutant flies as compared
to their +/Df siblings. As shown in Table 7, none of the alleles exhibited any decrease in viability over the
deficiency, including the strongest alleles as determined by male nondisjunction tests, ord and ord'.

TABLE 6
Primary spermatocyte aneuploidy in ord testes

<table>
<thead>
<tr>
<th>ord/Df(2R)3-70</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex chromosomes</td>
<td></td>
</tr>
<tr>
<td>Bivalent</td>
<td>62</td>
</tr>
<tr>
<td>Univalent</td>
<td>0</td>
</tr>
<tr>
<td>Trivalent</td>
<td>0</td>
</tr>
<tr>
<td>Large autosome</td>
<td></td>
</tr>
<tr>
<td>Bivalent</td>
<td>62</td>
</tr>
<tr>
<td>Univalent</td>
<td>0</td>
</tr>
<tr>
<td>Trivalent</td>
<td>0</td>
</tr>
<tr>
<td>Number of cells scored</td>
<td>62</td>
</tr>
<tr>
<td>Sex chromosome aneuploidy (%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Large autosome aneuploidy (%)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

For scoring criteria see MATERIALS AND METHODS and RESULTS.
Drosophila ord gene

**TABLE 7**
Numbers of progeny from the cross ord/+; Df(2R)ord'/CyO, bw 8

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ord</th>
<th>ord</th>
<th>ord</th>
<th>ord</th>
<th>ord</th>
<th>ord</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(2R)ord'/+</td>
<td>422</td>
<td>136</td>
<td>209</td>
<td>60</td>
<td>140</td>
<td>144</td>
</tr>
<tr>
<td>Df(2R)ord'/ord</td>
<td>425</td>
<td>149</td>
<td>185</td>
<td>65</td>
<td>141</td>
<td>162</td>
</tr>
<tr>
<td>CyO, bw +</td>
<td>518</td>
<td>164</td>
<td>215</td>
<td>111</td>
<td>175</td>
<td>176</td>
</tr>
<tr>
<td>CyO, bw ord</td>
<td>564</td>
<td>184</td>
<td>202</td>
<td>65</td>
<td>155</td>
<td>158</td>
</tr>
<tr>
<td>Total</td>
<td>1929</td>
<td>653</td>
<td>809</td>
<td>299</td>
<td>611</td>
<td>640</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate values normalized to the recovery of Df(2R)ord'/+ siblings.

Although ord/Δ flies were recovered at only 89% of the frequency of +/Δ flies (Table 7), χ² analysis indicates that these values are statistically similar (d.f. = 1, 0.5 > P > 0.1). Also, since ord was completely viable over an ord deficiency, this result indicates that null alleles should have been recoverable from the EMS screen. In the remote case that ord mutations only acted to disrupt mitosis in males, the recovery of males and females was scored in these tests for the ord, ord, ord and ord alleles; there was no statistical difference in the recovery of male vs. female flies (data not shown). The absence of gynandromorphs among progeny from ord females in the genetic nondisjunction assays above suggests that maternally contributed ord product was not required for early mitotic divisions in the embryo. Thus, although we failed to detect an essential role for any of our ord alleles for mitosis in somatic tissues of either sex, our results and those of Lin and Church (1982) indicate that at least two alleles of ord affect both mitosis and meiosis of the germline tissue in males.

Two alleles result in female sterility and alter nurse cell nuclear morphology: Due to extreme sterility, we found that we were unable to generate enough progeny from homozygous ord and ord females for a meaningful analysis of the nondisjunction and recombination phenotypes. Though the female sterility phenotype was not rigorously mapped to the ord locus, the sterility remained associated with the ord and ord mutations after two consecutive rounds of free recombination of the second chromosome and also when in trans to deficiencies that uncovered the ord locus. This suggests that the lesion resulting in female sterility maps very near to or colocalizes with the ord and ord mutations.

Because the female nondisjunction frequencies of strong yet fertile alleles such as ord and ord were already indicative of chromatids randomly segregating through two divisions (Table 3), we at first were puzzled how a more "severe" ord or ord defect could affect meiosis such that near sterility resulted. By analogy to the gonial mitotic defect seen in ord males, we reasoned that a female premeiotic defect might exacerbate the expected decrease in fertility due to meiotic missegregation by producing oocytes that entered meiosis with aneuploid complements. It might therefore be possible to observe the consequences of abnormal gonial divisions in the female, as observed earlier in the male. The four gonial mitotic divisions leading to the 16-cell cyst of primary spermatocytes in males has as its female counterpart the production of the 16-cell egg chamber. Fifteen of these cells polyploidize their nuclei and are termed nurse cells; the remaining nucleus becomes the oocyte nucleus. We dissected out ovaries, stained them with the DNA-specific dye DAPI, and observed them using fluorescence microscopy. The nurse cell nuclei were scored based on number, size and shape (Table 8).

ord egg chambers revealed striking phenotypes when analyzed in this manner. First, although ord heterozygotes only gave egg chambers containing 15 nurse cell nuclei, 30% of homozygous or hemizygous ord egg chambers had abnormal numbers of nurse cell nuclei (Table 8). The morphology of these polyploid nuclei was also altered in ord mutant females. Whereas heterozygous ord egg chambers gave uniformly spherical nuclei (Figure 5A), 80–90% of nuclei from homozygous or hemizygous ord egg chambers were "sickled" in appearance: they tended to be slightly elongate and had a concave edge (Table 8 and Figure 5B). The last abnormality evident in ord egg chambers was an alteration in the size of the nuclei. In normal egg chambers there is a size gradient between the more highly polyploid posterior nurse cells and the less polyploid anterior nurse cells (Figure 5A). However, in ord egg chambers we could often see nuclei which were conspicuously smaller than their surrounding neighbors (Figure 5C, open arrow).

We tested other ord alleles for the presence of these phenotypes (Table 8). Surprisingly, the other female
TABLE 8
Nurse cell phenotypes in ord females

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Stage</th>
<th>Percent egg chambers with indicated numbers of nurse cell nuclei</th>
<th>Percent egg chambers with &quot;sickled&quot; nuclei</th>
<th>Percent egg chambers with nuclei of irregular size</th>
<th>Total egg chambers scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>ordS8</td>
<td>9-10</td>
<td>0 0 100 0</td>
<td>9</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>ordS8</td>
<td>9-10</td>
<td>0 0 100 0</td>
<td>9</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>SM1</td>
<td>9-10</td>
<td>0 0 100 0</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>ordS8</td>
<td>9-10</td>
<td>22 8 85 5</td>
<td>96</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>DfS8</td>
<td>9-10</td>
<td>11 19 70 0</td>
<td>89</td>
<td>65</td>
<td>27</td>
</tr>
<tr>
<td>ordS8</td>
<td>9-10</td>
<td>0 0 100 0</td>
<td>2</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>SM1</td>
<td>9-10</td>
<td>0 0 100 0</td>
<td>9</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>ordS8</td>
<td>9-10</td>
<td>2 6 88 4</td>
<td>32</td>
<td>3</td>
<td>118</td>
</tr>
<tr>
<td>DfS8</td>
<td>9-10</td>
<td>0 2 98 0</td>
<td>11</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>ordS8</td>
<td>9-10</td>
<td>0 1 96 3</td>
<td>16</td>
<td>0</td>
<td>74</td>
</tr>
</tbody>
</table>

* The deficiency used in these studies was Df2Ry5.44.

sterile allele, ordS8, did not show as strong an effect as did ordS8. In stage 9–10 egg chambers, only 12% had abnormal numbers of nurse cell nuclei, and only 32% exhibited the sickled nucleus phenotype. Similar values to ordS8 were obtained for ordS8; and the weaker allele ordS8 showed low frequencies of the abnormal phenotypes.

We observed morphological abnormalities in the nurse cell nuclei that are the products of the four gonial mitotic divisions in the female. The phenotypes observed are consistent with nondisjunction during the gonial divisions to generate aneuploid nurse cell nuclei. However, other possibilities such as aberrant polyploidization of nurse cell nuclei or a defect in chromatin condensation may have given rise to these nuclear phenotypes.

DISCUSSION

ord mutants fail to maintain sister-chromatid associations through meiosis I: The cytological observations of male meiosis in ord mutants demonstrate that sister chromatids precociously separate and move independently to the poles in meiosis I. Moreover, the genetic nondisjunction frequencies in both ord mutant males and females are consistent with premature separation of all four chromatids of the sex chromosome bivalent, followed by their random segregation through two divisions.

One explanation for the premature separation of sister chromatids observed in meiosis I of ord mutants is that there is loss of sister-chromatid cohesion. The ord gene could encode a product that acts as a structural glue to hold sister chromatids together. Classical cytological observations on meiosis indicate that cohesion between sister chromatids is first lost between the chromatid arms at anaphase I, and is only later released between sister centromeres at anaphase II (John 1990). Since we observed single sister chromatids orienting to the poles in prometaphase I in ord mutants, the wild-type ord product would need to ensure cohesion at least at the centromere. It is possible that ord maintains cohesion along the sister-chromatid arms as well. Loss of this cohesion would explain the protrusions and loosely packed appearance of bivalents in male meiosis. As described below, it could also account for the recombination defect in females.

A second explanation for the ord phenotype is that the ord gene regulates the time at which sister chromatids can behave independently. Sister chromatids may be constrained to segregate as a unit in meiosis I because they share a single hemispherical kinetochore structure prior to spindle attachment (Goldstein 1981). Later in meiosis I the kinetochore doubles, presumably permitting the sister chromatids of the dyad to orient to opposite poles of the spindle in meiosis II. The ord gene could regulate the timing of kinetochore differentiation, since in ordS8 mutants the kinetochore was observed to have prematurely doubled (Lin and Church 1982). However, it is difficult to exclude that the premature doubling might be a consequence of loss of sister-chromatid cohesion. It is possible that if sister centromeres precociously separate, each chromatid is capable of organizing its own kinetochore structure (Goldstein 1981). Another potential regulatory role for ord would be in timing the
activity of topoisomerase II in decatenating sister chromatids.

**ord mutants affect homolog association and recombination**: All fertile alleles of *ord* reduce recombination. This suggests that *ord* is required for proper homolog association in females, although the effect of the mutations may be direct or indirect. *ord* may indirectly affect recombination by altering sister-chromatid behavior (by either of the two models presented above): if sister chromatids were not properly aligned, synaptonemal complex might not form, and recombination levels would be reduced.

Alternatively, *ord* could be directly involved in synaptonemal complex formation and recombination. It has been proposed that proper formation of the synaptonemal complex is necessary for sister-chromatid cohesion later in meiosis (Maguire 1990); this would also explain the nondisjunction observed in the female genetic tests. However, since *ord* affects sister-chromatid separation in males in which no synaptonemal complex is formed (Meyer 1960), this cohesion model would require that the *ord* gene have different functions in the two sexes. Furthermore, there is no evidence in Drosophila that proper homolog association is needed for sister-chromatid cohesion. On the contrary, deletions that disrupt homolog association in males (McKee and Karpen 1990) or mutations that abolish the synaptonemal complex in females (Baker et al. 1976) do not lead to precocious sister-chromatid separation.

In the new *ord* alleles the nondisjunctional gametes contain both nonrecombinant and recombinant chromosomes, as was observed for *ord* (Mason 1976). Since reciprocal recombination is usually necessary and sufficient for proper meiosis I disjunction (reviewed in Hawley 1988), this result indicates that the presence of a crossover does not insure correct meiosis I disjunction and that nondisjunction is not solely a consequence of decreased recombination. The observation that crossovers are not sufficient for meiosis I disjunction is consistent with the hypothesis that chiasmata are not maintained in *ord* mutants because of precocious sister-chromatid arm separation. However, it is also possible that premature separation of sister kinetochores, analogous to that observed in *ord* male meioses, disrupts the normal bivalent orientation conferred by chiasmata.

One of the reasons we isolated new alleles of *ord* was to determine whether the effects of *ord* on chromosome segregation and recombination could be separated. The *ord* mutation demonstrates this separation of function, since recombination levels are reduced in *ord* to the same extent as in *ord* or *ord* but nondisjunction is less frequent. Mutations in *ord* may have different consequences on recombination and segregation, and *ord* could have a more severe effect on the recombination than the segregation function. One simple way to envision how this might be achieved is to postulate that recombination indirectly requires cohesiveness of the sister-chromatid arms whereas proper disjunction is mediated through cohesion at the centromere, and that cohesive functions in these domains can be differentially altered by mutation to *ord*.

**Role of ord in mitosis?** The strongest *ord* alleles appear to affect mitosis in the male germline and...
cause abnormalities in the female germline. In ord2 male testis squashes we found that prophase I figures were frequently aneuploid as a consequence of chromosome nondisjunction or loss in the mitotic divisions of the spermatogonia. This is in agreement with previous observations on ord1 (LIN and CHURCH 1982). The two strongest ord alleles, ord2 and ord6, are female sterile, and we have been unable to separate this sterility from the ord mutations. Both as homozygotes and in trans to a deficiency, these mutations result in nurse cell nuclei that are altered in size and morphology, and ord2 may cause a reduction in nurse cell number. The effect of these mutations on nurse cell phenotype is consistent with defects in the cytosolic mitotic divisions that give rise to the 15 nurse cell nuclei cluster. Chromosome nondisjunction in these mitotic divisions could produce aneuploid nurse cells that would vary in the size of the nucleus observed after polyploidization. However, it is also possible that the ord mutations affect the process of polyploidization, resulting in aberrant shape and size of the nurse cell nuclei.

Despite the effects on germline mitosis, none of the ord alleles is essential for somatic mitosis, since they do not reduce viability even when in trans to a deficiency. An increase in somatic clones arising either from increased nondisjunction or mitotic recombination was previously reported for ord2 mutants (BAKER, CARPENTER and RIPOLL 1978). Somatic clones may be a more sensitive assay for accurate mitosis. However, since in the previous study the mutation responsible for the mitotic phenotype was not mapped, it is possible that the effects were due to a second mutation on the ord2 chromosome. The eventual determination of null alleles of ord will be essential to define the role of ord in germline mitotic divisions and to determine whether it is required in somatic cells.

Comparison of ord and mei-S332: The results of both the genetic nondisjunction tests and the male cytological analysis show that ord acts early in meiosis I. Sister chromatids can be seen in male testis squashes that have separated precociously in prometaphase I, in contrast to mei-S332. Even in apparent null mutants of mei-S332, predominantly meiosis II nondisjunction occurs, and the sister chromatids do not precociously disjoin until late in anaphase I (KERREBROCK et al. 1992).

The similarity of the precocious disjunction phenotypes in mei-S332 and ord mutants, save for timing, is striking. The simplest interpretation of our results is that the onset of ord function precedes that of mei-S332. It is possible that functional ord product is a prerequisite for mei-S332 to function, either because ord directly activates mei-S332 or indirectly sets up a precondition necessary for mei-S332 function. However, our data do not address whether ord function persists later in meiosis to overlap with the time of action of mei-S332. One interpretation of the delayed separation of the sister chromatids in the ord6 mutant compared to the stronger ord alleles is that ord function is required after anaphase I. Alternatively, ord may be required only early in meiosis, and the ord phenotype could be a delayed manifestation of an earlier defect.

While the ord phenotypes are suggestive of the primary role of the gene being the maintenance of sister-chromatid cohesion, further cytological investigation of the mutant phenotypes in female meiosis will be informative. It will be interesting to examine the structure of the synaptonephal complex in ord mutants. Ultimately, identifying the protein product encoded by the ord locus and examining its expression and location in meiotic and mitotic cells will provide definitive information concerning the role of this intriguing gene in chromosome segregation.

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LITERATURE CITED


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Chapter III.

The Drosophila mei-S332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation

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My contribution to this study was carrying out the EMS screen for new mei-S332 alleles (Fig. 1).
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 reductive (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-S332 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 reductive, 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 reductive 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-
Thus the previous analysis raised the possibility that the sister chromatids were observed to be aberrantly disjuncted (MASON 1976), and cytologically, disjunction occurs (MASON 1976), and cytologically, disjunction occurs. The redl 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. 1966). 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 males, 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 colochores (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 meiotic segregation in the mei-S332 mutant demonstrated that the sister chromatids prematurely dissociate in meiosis I (DAVIS 1971; GOLDSTEIN 1980). In this mutant non-disjunction 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) non-disjunction 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 RICOLL 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. 1966). 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]Ac4-045 transformatant, 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 cv 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/SIM1 and Im[2R] mam+26, S Sp Tfi mam+26 Pu+/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 Im[2R] mam+26, Tfl mam+26 Pu+/px sp chromosome used in the second mapping experiment. The isogonized X and Y chromosomes, which were crossed into the mei-S332 mutant stocks (see below), came from a y/bv stock and a y wv/
The Drosophila mei-S332 Gene

The Drosophila mei-S332 Gene

FMTay/y'Y; spd" stock, respectively. A ye v f aer stock (from R. S. HAWLEY, University of California at Davis) was crossed to Canton-S to isolate the ev v f aer recombinant chromosome used in the female nondisjunction tests. Compound chromosomes used in the nondisjunction tests included: C(1)RM, y' su(wu)'; YX-Y', y', ln(1)EN, y if B; C(2)EN, b pr; C(3)EN, C(4)RM, a y'. For the rest of this report, the C(1)RM chromosome will be symbolized as "XX," and the YX-Y', y', ln(1)EN chromosome will be symbolized as "XY."

EMS mutagenesis: Adult y/y'; cn bw sp males were mutagenized with 0.035 M EMS as described (LEWIS and BAHNER, 1968), and mated to y'; cn mei-S332' ord/SU1 females (see Figure 1). From the progeny of this cross, males with a mutagenized cn bw sp chromosome over the cn mei-S332' ord' 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') 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 (ASHBURNE, 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/2R1R1-8.

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-S332'63-24, mei-S332'63-25, mei-S332'63-26, mei-S332'63-27, mei-S332'63-28, mei-S332'63-29) were mapped to the c (75.5)-ps (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 ps sp chromosome. These females were crossed to y/y'; cn mei-S332' ps/SU1 males, and their male progeny with recombinant or nonrecombinant second chromosomes over the original mei-S332' allele were isolated. These males, which were also y/y'Y or y/y', were mated singly to y, al dp b pr zl c ps 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-ps interval scored for each allele was: mei-S332', 2; mei-S332', 60; mei-S332', 59; mei-S332', 55; DF/2R1R1-8, 58. In all cases, the meiotic nondisjunction phenotype was found to map close to ps (99.1-100.0 cm). In the mei-S332' mapping cross, we recovered one mei-S332'-ps 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/2R1R1-8) more precisely. These mutations were mapped within the Pu (97 cm)-ps (100.5) interval using an rIn(2R)map20, Tfl map20, Pu2 a ps sp chromosome. Recombinants in this interval were selected over the DF/2R1R1-8 chromosome, which uncovers both mei-S332 and ps. Single males that were recombinant in the Pu-ps interval were mated to y; Sio/SU1 females to score for nondisjunction as described above. For the weak mei-S332' allele, recombinant males were mated to XX, y' su(wu)y' females to score for nullo-XY and diplo-X exceptions. The number of fertile recombinants in the Pu-ps interval scored for each allele was: mei-S332', 35; mei-S332', 49; mei-S332', 85; mei-S332', 67; mei-S332', 48; mei-S332', 63; mei-S332', 62; mei-S332', 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'; +/SMI; spd" 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/2R1R1-8 chromosome were crossed into the iso-X,Y background.

Recombinants for the eight mei-S332 alleles were isolated as follows. The left arm of chromosome 2 was replaced by recombinating a pr cn bw chromosome with either a al dp b ps mei-S332 bw sp recombinant chromosome from the first mapping experiment (mei-S332') or a recombinant chromosome with the distal right arm of chromosome 2 replaced by px and sp (mei-S332'), and then selecting for recombinant chromosomes which were marked with either pr cn bw sp (mei-S332') or pr cn px sp (mei-S332'). Approximately 10 recombinant chromosomes were scored for each allele, and these stocks were scored for homoygous 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-S332a 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-S332', mei-S332', and mei-S332' 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-S332' 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'; +/SMI; spd") as the wild-type control. Hoplo-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 XX, y' su(wu)y' 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 XY 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; Lindsey 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/eve f car or y/ females to X7, y/F 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 scored as the absence 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, 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 discared 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 x, spe males to X7, y/F females. Regular mono-4 ova resulted in either triplo-4 progeny, which were wild type for fourth chromosome markers, or haplo-4 sparkling-polliot 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-polliot 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
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' allele more precisely, recombinants in the Pux-px interval were recovered over the deficiency Df(2R)X58-6 (MATERIALS AND METHODS) and tested for nondisjunction in males. mei-S332' 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', 99.3 cM; mei-S332', 99.5 cM; mei-S332', 99.3 cM; mei-S332', 99.1 cM; mei-S332', 99.3 cM; mei-S332', 99.5 cM; mei-S332', 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 assessed 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 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' 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-X equational exceptions (MATERIALS AND METHODS), then most alleles over a deficiency probably gave 20-30-fold more equational exceptions.
TABLE 1
Sex chromosome nondisjunction in males homozygous for the indicated allele

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<thead>
<tr>
<th>Sample</th>
<th>+</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
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<td>2503</td>
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<tr>
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<td>400</td>
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<td>51</td>
</tr>
<tr>
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<td>18</td>
<td>9</td>
<td>51</td>
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<td>4726</td>
<td>3318</td>
<td>3590</td>
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<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
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<tbody>
<tr>
<td>Exceptional sperm</td>
<td>O</td>
<td>609</td>
<td>849</td>
<td>303</td>
<td>446</td>
<td>103</td>
<td>511</td>
<td>1108</td>
</tr>
<tr>
<td>X(Y)</td>
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<td>8</td>
<td>25</td>
<td>12</td>
<td>28</td>
<td>41</td>
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<td>XX</td>
<td>203</td>
<td>399</td>
<td>148</td>
<td>215</td>
<td>29</td>
<td>122</td>
<td>658</td>
<td>452</td>
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<tr>
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<td>2</td>
<td>0</td>
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<td>5</td>
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<tr>
<td>Total progeny</td>
<td>2504</td>
<td>5176</td>
<td>2291</td>
<td>2205</td>
<td>5279</td>
<td>5851</td>
<td>5023</td>
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<table>
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<tr>
<th>Sample</th>
<th>mei-S332'</th>
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<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
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<tr>
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<td>511</td>
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<tr>
<td>XY(Y)</td>
<td>4</td>
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<td>1</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Total progeny</td>
<td>2504</td>
<td>5176</td>
<td>2291</td>
<td>2205</td>
<td>5279</td>
<td>5851</td>
<td>5023</td>
<td>4285</td>
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</table>

than reductional exceptions. This assumes similar frequencies of X and Y chromosome nondisjunction, as was observed by Goldstein (GOLDSTEIN 1980) for the homozygous mei-S332' 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-S332', mei-S332' and mei-S332' were classified as strong alleles (20-30% total nondisjunction), mei-S332' and mei-S332' were moderate alleles (10-15% total nondisjunction), and mei-S332' and mei-S332' 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-S332', mei-S332' and mei-S332' (1.1-1.2-fold) than for the weak mei-S332' allele (6-fold).

Two of the four strongest alleles (mei-S332' and mei-S332') 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 $x^2$ 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
The Drosophila mei-S332 Gene

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, mei-S332, mei-S332 and mei-S332). Previous analysis of meiotic chromosome behavior in homozygous mei-S332 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, mei-S332, mei-S332, mei-S332), the frequency of premature separation of sister chromatids (PSSC) observed in prophase II and prometaphase II cells was high, with mei-S332 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, 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 homozygotes (data not shown).
Cytological analysis of males homozygous for the indicated alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Prophase I to metaphase I</th>
<th>Early to mid anaphase I</th>
<th>Mid to late anaphase I</th>
<th>Prophase II and prometaphase I</th>
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<tbody>
<tr>
<td></td>
<td>No PSSC</td>
<td>PSSC</td>
<td>No PSSC</td>
<td>PSSC</td>
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<td>mei-S332'</td>
<td>39</td>
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<td>24</td>
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</table>

* Aberrant anaphases are those in which nondisjunction or lagging chromosomes were observed.

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.

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-S332' and mei-S332' mutants (8–10%) is consistent with the expected number of cells showing normal segregation of the major chromosomes if sister chromatids are segregating randomly (0.5³ = 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-S332' 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-S332' 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-S332' 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-S332', mei-S332' and mei-S332'; Table 1) were strong in females, with total nondisjunction frequencies of 36–40%. The mei-S332' and mei-S332' 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-S332', mei-S332' and mei-S332') over a deficiency, gave 10–20-fold greater equational exceptional ova relative to reductive exceptional ova (Table 5). Similar patterns were observed for the weaker mei-S332' allele in females (data not shown). The frequency of reductive exceptions in mei-S332 homozygotes were consistently higher in females (5–14%) than in males (≤1%). However, the high numbers of equational relative to reductive 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-S332' and mei-S332' 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-
The Drosophila mei-S332 Gene

TABLE 4
Sex chromosome nondisjunction in females homozygous for the indicated allele

<table>
<thead>
<tr>
<th>Sample</th>
<th>+</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular ova X</td>
<td>5821</td>
<td>477</td>
<td>1749</td>
<td>2948</td>
<td>1280</td>
<td>3405</td>
<td>3188</td>
<td>2272</td>
</tr>
<tr>
<td>Exceptional ova O</td>
<td>1</td>
<td>101</td>
<td>213</td>
<td>13</td>
<td>291</td>
<td>5</td>
<td>591</td>
<td>420</td>
</tr>
<tr>
<td>XX</td>
<td>0</td>
<td>81</td>
<td>236</td>
<td>12</td>
<td>175</td>
<td>5</td>
<td>594</td>
<td>225</td>
</tr>
<tr>
<td>Total progeny</td>
<td>5822</td>
<td>659</td>
<td>2158</td>
<td>2975</td>
<td>1746</td>
<td>3415</td>
<td>3973</td>
<td>2917</td>
</tr>
<tr>
<td>Adjusted total</td>
<td>5823</td>
<td>841</td>
<td>2647</td>
<td>2098</td>
<td>2212</td>
<td>3425</td>
<td>4758</td>
<td>3542</td>
</tr>
<tr>
<td>% nullo-X</td>
<td>0.05</td>
<td>2.4</td>
<td>16.1</td>
<td>0.4</td>
<td>21.3</td>
<td>0.3</td>
<td>16.4</td>
<td>21.6</td>
</tr>
<tr>
<td>% diplo-X</td>
<td>0.00</td>
<td>19.3</td>
<td>17.8</td>
<td>0.4</td>
<td>15.8</td>
<td>0.3</td>
<td>16.6</td>
<td>12.6</td>
</tr>
<tr>
<td>Total X nondisjunction</td>
<td>0.05</td>
<td>43.3</td>
<td>33.9</td>
<td>0.8</td>
<td>42.1</td>
<td>0.6</td>
<td>35.0</td>
<td>36.2</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reductional (car/*car)</td>
<td>8</td>
<td>11</td>
<td>8</td>
<td>32</td>
<td>29</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Equational (car/*car)</td>
<td>23</td>
<td>83</td>
<td>75</td>
<td>157</td>
<td>84</td>
<td>94</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>198</td>
<td>159</td>
<td>353</td>
<td>211</td>
<td>230</td>
<td>197</td>
</tr>
<tr>
<td>% Reductional</td>
<td>10.7</td>
<td>5.6</td>
<td>5.0</td>
<td>9.1</td>
<td>13.7</td>
<td>5.2</td>
<td>6.1</td>
</tr>
<tr>
<td>% Equational</td>
<td>89.3</td>
<td>94.4</td>
<td>95.0</td>
<td>90.9</td>
<td>86.3</td>
<td>94.8</td>
<td>93.9</td>
</tr>
</tbody>
</table>

TABLE 6
Sex chromosome nondisjunction in females with the indicated allele over Df2R/X58-6

<table>
<thead>
<tr>
<th>Sample</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
<th>mei-S332</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular ova X</td>
<td>1117</td>
<td>2637</td>
<td>714</td>
<td>677</td>
<td>1171</td>
<td>191</td>
<td>1956</td>
</tr>
<tr>
<td>Exceptional ova O</td>
<td>245</td>
<td>35</td>
<td>130</td>
<td>152</td>
<td>211</td>
<td>49</td>
<td>130</td>
</tr>
<tr>
<td>XX</td>
<td>254</td>
<td>42</td>
<td>153</td>
<td>96</td>
<td>187</td>
<td>25</td>
<td>151</td>
</tr>
<tr>
<td>Total progeny</td>
<td>1616</td>
<td>2712</td>
<td>977</td>
<td>925</td>
<td>1569</td>
<td>256</td>
<td>2237</td>
</tr>
<tr>
<td>Adjusted total</td>
<td>2115</td>
<td>2787</td>
<td>1240</td>
<td>1175</td>
<td>1967</td>
<td>339</td>
<td>2518</td>
</tr>
<tr>
<td>% nullo-X</td>
<td>25.2</td>
<td>2.4</td>
<td>21.0</td>
<td>21.5</td>
<td>29.9</td>
<td>21.5</td>
<td>28.9</td>
</tr>
<tr>
<td>% diplo-X</td>
<td>24.0</td>
<td>5.0</td>
<td>21.5</td>
<td>16.4</td>
<td>19.0</td>
<td>14.7</td>
<td>12.0</td>
</tr>
<tr>
<td>Total X nondisjunction</td>
<td>47.2</td>
<td>5.4</td>
<td>42.5</td>
<td>42.3</td>
<td>40.5</td>
<td>45.6</td>
<td>32.3</td>
</tr>
</tbody>
</table>

* Tests 1 and 2 were performed at different times using the same chromosomes.

S332 locus. Although the levels of chromosome loss seen for the mei-S332 (and mei-S332') 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' 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' 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' allele, which was moderate in males (15% total nondisjunction) was very weak in females (only 0.8% total nondisjunction). Conversely, the mei-S332' and mei-S332' alleles, which were moderate (9% total nondisjunction) and weak (2% total nondisjunction) in males, respectively, were strong when homozygous in females (both gave about
alleles distinguishable with practice. For three of the strong bleed recombinants in the intersex progeny (70.2 cM) was due to the inclusion of triploid mally high map distance seen in alleles as homozygotes (data not shown). The abnor-
distances were found for the remaining control map distance when homozygous. Similar map
tions levels in females. In the nondisjunction test
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 exam-
ined whether the seven new mei-S332 alleles affected segregation of the autosomes, as did the mei-S332' allele (DAVIS 1971; GOLDBERG 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-
S332', 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-S332', mei-S332', mei-S332', mei-S332', mei-S332', mei-S332' Table 4) also showed nondisjunction of chromosome 3 (Table 8), whereas alleles that were weak in females in the sex chromosome nondisjunction tests (mei-S332', mei-
S332', mei-S332') did not. All alleles, except the weak mei-S332' allele, resulted in chromosome 2 nondis-
junction (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>
<thead>
<tr>
<th>Sample</th>
<th>+</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map distances (cM)</td>
<td>Number scored</td>
<td>1555</td>
<td>229</td>
<td>771</td>
<td>565</td>
<td>1425</td>
<td>1052</td>
</tr>
<tr>
<td>1 y-cv</td>
<td>12.0</td>
<td>11.4</td>
<td>26.8</td>
<td>14.0</td>
<td>9.9</td>
<td>10.8</td>
<td>15.1</td>
</tr>
<tr>
<td>2 cv-v</td>
<td>21.9</td>
<td>17.9</td>
<td>18.4</td>
<td>21.2</td>
<td>18.0</td>
<td>15.7</td>
<td>18.8</td>
</tr>
<tr>
<td>3 v-f</td>
<td>19.1</td>
<td>16.2</td>
<td>20.6</td>
<td>17.7</td>
<td>18.8</td>
<td>16.2</td>
<td>21.2</td>
</tr>
<tr>
<td>4 f-car</td>
<td>5.7</td>
<td>5.1</td>
<td>4.4</td>
<td>4.6</td>
<td>4.5</td>
<td>5.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Total map distance (cM)</td>
<td>(1.00)</td>
<td>(0.83)</td>
<td>(1.20)</td>
<td>(0.98)</td>
<td>(0.87)</td>
<td>(0.83)</td>
<td>(1.00)</td>
</tr>
</tbody>
</table>

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 (LINDBERG, McELRATH and BERRY 1978).
results demonstrate that mutations in mei-332 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-332 alleles in order to determine whether this chromosome was affected by mutations in mei-332. We also examined whether chromosomes were segregating independently in mei-332 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-332 mutants were mated to flies with a compound fourth chromosome (C(4)RM ci e(3)l). 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-332 wild type (37.9%) than for the other strong mei-332, mei-332' and mei-332' alleles (9-17%). Although these strong alleles gave low levels of chromosome 4 nondisjunction relative to mei-332' 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-332 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-332', mei-332', mei-332', mei-332' and mei-332' gave fairly high levels of total fourth chromosome nondisjunction (34-54%; Table 9), whereas the male-predominant alleles mei-332' and mei-332' and the weak allele mei-332' 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 \( x^2 \) test (mei-332': P > 0.95; mei-332': 0.5 > P > 0.3) (LINDREN, MCELRAHY 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-332 mutants.

Absence of semidominance in mei-332 mutants: The original mei-332' 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-332 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-332 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-332' 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-332 over SM1 result from a second-site noncomplementer on the SM1 chromosome.

Viability of mei-332 mutants: We reasoned that if the mei-332 gene product was required for proper
TABLE 9

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

<table>
<thead>
<tr>
<th>Ova</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
</tr>
</thead>
<tbody>
<tr>
<td>X; 4</td>
<td>370</td>
<td>98</td>
<td>60</td>
<td>34</td>
<td>166</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>(100.0)</td>
<td>(57.1)</td>
<td>(56.0)</td>
<td>(165.1)</td>
<td>(66.2)</td>
<td></td>
</tr>
<tr>
<td>X; O</td>
<td>2</td>
<td>57</td>
<td>21</td>
<td>24</td>
<td>52</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(57.0)</td>
<td>(22.4)</td>
<td>(20.8)</td>
<td>(51.2)</td>
<td>(24.2)</td>
</tr>
<tr>
<td>X; 4'</td>
<td>0</td>
<td>61</td>
<td>11</td>
<td>18</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(59.0)</td>
<td>(12.5)</td>
<td>(19.3)</td>
<td>(55.8)</td>
<td>(18.5)</td>
</tr>
<tr>
<td>O; 4</td>
<td>0</td>
<td>20</td>
<td>10</td>
<td>14</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(23.1)</td>
<td>(11.8)</td>
<td>(9.9)</td>
<td>(20.9)</td>
<td>(12.2)</td>
</tr>
<tr>
<td>O; O</td>
<td>0</td>
<td>15</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13.2)</td>
<td>(4.6)</td>
<td>(3.7)</td>
<td>(6.5)</td>
<td>(4.5)</td>
</tr>
<tr>
<td>O; 4'</td>
<td>0</td>
<td>15</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(13.6)</td>
<td>(2.6)</td>
<td>(3.3)</td>
<td>(4.5)</td>
<td>(3.4)</td>
</tr>
<tr>
<td>XX; 4</td>
<td>0</td>
<td>38</td>
<td>12</td>
<td>4</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(32.9)</td>
<td>(22.4)</td>
<td>(12.5)</td>
<td>(17.0)</td>
<td>(14.6)</td>
</tr>
<tr>
<td>XX; O</td>
<td>0</td>
<td>17</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18.8)</td>
<td>(5.1)</td>
<td>(3.5)</td>
<td>(5.3)</td>
<td>(5.3)</td>
</tr>
<tr>
<td>XX; 4'</td>
<td>0</td>
<td>16</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19.4)</td>
<td>(2.9)</td>
<td>(3.3)</td>
<td>(3.7)</td>
<td>(4.1)</td>
</tr>
<tr>
<td>Adjusted total</td>
<td>372</td>
<td>458</td>
<td>172</td>
<td>144</td>
<td>568</td>
<td>197</td>
</tr>
<tr>
<td>% X exceptions</td>
<td>0.0</td>
<td>52.8</td>
<td>46.5</td>
<td>47.2</td>
<td>31.5</td>
<td>44.7</td>
</tr>
<tr>
<td>% 4 exceptions</td>
<td>0.5</td>
<td>33.7</td>
<td>37.9</td>
<td>52.7</td>
<td>34.5</td>
<td>39.2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Ova</th>
<th>+</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td>SMI</td>
<td>SM1</td>
</tr>
<tr>
<td>Regular ova</td>
<td>X</td>
<td>2948</td>
<td>4220</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>XX</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total progeny</td>
<td></td>
<td>2952</td>
<td>4222</td>
</tr>
<tr>
<td>Adjusted total</td>
<td></td>
<td>2956</td>
<td>4224</td>
</tr>
<tr>
<td>% nullo-X</td>
<td>0.14</td>
<td>0.05</td>
<td>8.2</td>
</tr>
<tr>
<td>% diplo-X</td>
<td>0.14</td>
<td>0.05</td>
<td>6.0</td>
</tr>
<tr>
<td>Total X nondisjunction</td>
<td>0.28</td>
<td>0.10</td>
<td>14.2</td>
</tr>
</tbody>
</table>

* The progeny total is adjusted to correct for recovery of only half of the exceptional progeny.

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 dispensable for meiosis.

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).
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 seven EMS-induced alleles, as well as in all later tests of 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' 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' 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 1958). 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 

### TABLE 11

Numbers of progeny from the cross mei-S332(SM1 Δ × Df(2R)X58-6/SM1 9

<table>
<thead>
<tr>
<th>Genotype</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
<th>mei-S332'</th>
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<td>552</td>
<td>178</td>
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<td>37.9</td>
<td>38.1</td>
<td>39.4</td>
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<td>28.7</td>
<td>28.5</td>
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<tr>
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<td>269</td>
<td>155</td>
<td>529</td>
<td>157</td>
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<td>159</td>
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<td>35.4</td>
<td>32.4</td>
</tr>
<tr>
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<td>859</td>
<td>490</td>
<td>998</td>
<td>470</td>
<td>565</td>
<td>490</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the percentage of the total.
Kinetochore Differentiation

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; CUMBERLIDGE 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<sup>2</sup> and mei-S332<sup>6</sup> alleles when homozygous were stronger in females than in males, whereas the mei-S332<sup>1</sup> and mei-S332<sup>2</sup> 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<sup>1</sup> and female-predominant mei-S332<sup>6</sup> 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<sup>1</sup> 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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LITERATURE CITED


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Chapter IV.

The $ord^4$ mutation results in chromosome-specific recombination reductions and exhibits negative complementation with other $ord$ alleles

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ABSTRACT

The hypomorphic allele ord⁴ exhibits unusual genetic properties. Our findings indicate that although chromosomes segregated with nearly normal efficiency in ord⁴ homozygous males and females, heterozygotes of ord⁴ with other ord alleles resulted in nondisjunction in an allele-specific manner. Deficiencies for ord had no such effect in ord⁴ transheterozygotes, suggesting that interference of ord⁴ function occurs at the level of protein-protein interactions rather than a decrease in ord function per se. This negative complementation also affected recombination in females, but using a combination of genetic and cytological assays we found that reductions in exchange in ord⁴-carrying flies were mostly restricted to the X chromosome. By observing meiotic figures in mature oocytes, we obtained results consistent with a differential recombination defect among the chromosomes. Precocious separation of sister chromatids (PSSC) was observed in ord¹ oocytes, consistent with genetic predictions that ord-induced nondisjunction is largely a result of PSSC and not strictly a result of low exchange.
INTRODUCTION

Sister chromatids separate and segregate from each other in anaphase of mitosis and anaphase II of meiosis. However, they remain paired throughout anaphase I of meiosis. These precise chromosome movements suggest mechanisms that keep sister chromatids paired and those that ensure their timely release. The Drosophila genes ord and mei-S332 are excellent candidates for genes whose products promote sister-chromatid cohesion, since mutations in both genes result in precocious separation of sister chromatids (PSSC) during meiosis (Davis, 1971; Mason, 1976; Goldstein, 1980; Kerrebrock et al., 1992; Miyazaki and Orr-Weaver, 1992). Such premature release of sister chromatids leads to aberrant segregation patterns and allows abnormal numbers of sister- and/or homologous chromosomes to become incorporated into the same gamete. ord function appears to be required earlier than mei-S332, both because the premature separation is earlier in ord males, and because ord females are deficient in reciprocal recombination whereas mei-S332 females are not (Miyazaki and Orr-Weaver, 1992; Kerrebrock et al., 1992).

Recombination and homolog disjunction in meiosis I are correlated in most organisms that undergo crossing over: a crossover is normally necessary and sufficient for proper meiosis I disjunction of homologs (Hawley, 1988). In recombination-deficient Drosophila females the frequency of nondisjunction is inversely correlated with the recombination frequency, but not in a linear fashion (Baker and Hall, 1976). This is because in Drosophila there exists a mechanism for segregating nonexchange (or otherwise achiasmate) homologous chromosomes. Originally termed the distributive system (Grell, 1962a), it has recently been renamed the achiasmate segregation system (Hawley and Theurkauf,
The achiasmate segregation system can only reliably segregate a limited number of achiasmate homologs, and this limit is frequently reached in females defective in recombination. Strong ord alleles exhibit both great reductions in crossing over and high levels of nondisjunction in females (Miyazaki and Orr-Weaver, 1992).

We present in this report our analysis of the weak allele ord⁴, which demonstrates unusual genetic behavior. Contrary to the disjunctional paradigm above, ord⁴ at first appeared to be defective in recombination yet exhibited faithful segregation -- in examining the anomaly we found an interesting chromosome-specific defect in recombination. Moreover, ord⁴ interacts with other ord alleles in a negative complementing manner, suggestive of a model in which Ord cohesive function is mediated through protein-protein interactions.
MATERIALS AND METHODS

Stocks All Drosophila stocks and crosses were raised at 25°C on standard cornmeal-brewer's yeast-molasses-agar medium. The isolation and genetic mapping of the ord^{2-6} alleles were described in Miyazaki and Orr-Weaver (1992). The deficiency Df(2R)3-70 was isolated by Daniel Moore in this laboratory; the deficiency Df(2R)bw^{S46} and the ru h th st • cu sr e^8 ca ("rucuca") chromosome were obtained from R. Lehmann (Whitehead Institute). The cv v f car and compound-X/compound-XY stocks were described in Kerrebrock et al. (1992). The mei-9 alleles and the a px sp stock were obtained from the Mid-America Drosophila Stock Center at Bowling Green State University. All genes are described in Lindsley and Zimm (1992).

Nondisjunction and recombination tests Nondisjunction tests in females and males were performed as diagrammed in Figure 2 of Chapter 2. Briefly, by mating mutant y/y^+Y males to compound-X females, or mutant females to compound-XY males, gametes bearing all normal and most abnormal sex chromosome constitutions are recoverable and distinguishable. Females in addition were y/cv v f car on the X chromosome: this allowed recombination in four intervals to be assayed, and the centromere-linked marker car allowed determination of the meiotic division at which nondisjunction occurred. Tetrad analysis was performed by the method of Weinstein (1936).

Crossing over in the px-sp interval on chromosome 2 was assayed by mating px ord^4 bw sp/ord^4 bw females or px ord^4 bw sp/+ controls to a px sp males and scoring progeny for the recombinant classes. Crossing over on chromosome 3 was measured by mating hetero-, homo-, and hemizygous
ord⁴ females simultaneously heterozygous for rucuca, to rucuca males and scoring for recombination in the ru-h, h-st, and st-cu intervals.

Oocyte cytology Oocytes for immunolabelling were prepared by the method of Theurkauf and Hawley (1992). The salient points in their protocol are: blender disruption of fattened females into hypertonic modified Robb's medium, differential gravity settling of mature oocytes, formaldehyde (8%) fixation in a hypertonic solution, manual (by rolling) dechorionation, and Triton-X100 extraction of dechorionated oocytes. The resulting oocytes were BSA-blocked, and incubated with a 1:500 dilution of anti-histone mouse monoclonal antibodies (Chemicon, Temecula, CA), followed by a rhodamine-conjugated goat anti-mouse secondary antibody (Jackson ImmunoResearch, West Grove, PA). A short 15-minute incubation again with the mouse monoclonal antibody bound up excess goat anti-mouse epitope binding sites. This prevented cross-reactivity with the anti-tubulin rat monoclonal antibodies YL1/2 and/or YOL1/34 (1:5 dilution, Accurate Chemical, Westbury, NY), which were followed with fluorescein-conjugated goat anti-rat secondary antibodies with low cross-reactivity to mouse (Cappel, Durham, NC).

Scoring of oocyte figures was performed using epifluorescence on a Zeiss Axioskop equipped with a Plan Neofluar 40x objective lens. Laser scanning confocal microscopy was performed using a MRC 600 confocal scanning head (Bio-Rad Laboratories) mounted on a Zeiss Axioskop equipped with a Plan Neofluar 40x objective. Staging of oocyte figures was as described in McKim et al. (1992), except that we found the presence or absence of the 4th chromosomes to be a poor indicator of meiosis I or meiosis II, respectively. That is, even in clear metaphase I or anaphase I
oocytes the 4ths were oftentimes not visible. We therefore assumed colinear spindle structures were two widely-spaced meiosis I half-spindles, and scored non-colinear ones as meiosis II spindles.
RESULTS

\textit{ord}^4 is nearly wild-type when homozygous but fails to complement \textit{ord}^1

A recent screen for new noncomplementers of \textit{ord}^1 resulted in five new alleles of \textit{ord} (Miyazaki and Orr-Weaver, 1992). The mutation that was eventually designated \textit{ord}^4 failed to complement \textit{ord}^1 in both males and females for the nondisjunction phenotype. The mutation on this chromosome was subsequently mapped to 103.6 cM on the second chromosome by following its noncomplementation with \textit{ord}^1 (103.5 cM), thereby confirming \textit{ord} allelism. However, \textit{ord}^4 has several intriguing features.

We were surprised to find that \textit{ord}^4 exhibited nearly wild-type segregational behavior (1.4%) when homozygous in males (Table 1). The nearly wild-type segregation pattern normally conferred by \textit{ord}^4 was disrupted when in \textit{trans} to \textit{ord}^1, as \textit{ord}^1/\textit{ord}^4 males exhibited 13.9% observed nondisjunction. The transheterozygous nondisjunction level was intermediate between the \textit{ord}^4 homozygous value and the \textit{ord}^1 homozygous value (42%). This increase in nondisjunction from homozygous \textit{ord}^4 levels was not simply in response to an overall reduced \textit{ord} dosage, because when \textit{ord}^4 was placed in \textit{trans} to an \textit{ord} deficiency, segregation was again nearly wild-type (1.8%, and statistically identical to homozygous \textit{ord}^4 levels by $\chi^2$ analysis, 0.9>p>0.5). Nor was this due to semidominance of the \textit{ord}^1 mutation, as all alleles \textit{ord}^{1-6} were completely recessive to \textit{ord}^+ in male nondisjunction tests (Table 1 and data not shown). Genetic interactions possessing properties such as these exhibited between \textit{ord}^1 and \textit{ord}^4 are examples of negative complementation (see Discussion).

Negative complementation was observed in females as well. As in males, \textit{ord}^1/\textit{ord}^4 transheterozygotes exhibited intermediate nondisjunction
<table>
<thead>
<tr>
<th>genotype</th>
<th>males, percent nondisjunction</th>
<th>females, percent nondisjunction</th>
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<td></td>
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</tr>
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</tr>
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<tr>
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<td>0.0</td>
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</tr>
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<td></td>
<td>(84)</td>
<td>(81)</td>
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</table>

$y^{ly+}Y$ males were crossed to attached-$X$, $y^2 su(w^a)$ $w^a$ females; $y/cv v f car$ females were crossed to attached-$XY$, $v f B$ males. Numbers in parentheses are progeny scored.

$Dl(2R)bw^{sa}$
(17.6%) between that produced by homozygous ord\textsuperscript{1} and ord\textsuperscript{4} females (Table 1). The ord\textsuperscript{1}/ord\textsuperscript{4} nondisjunction levels were at least two-fold higher than in Df\textsuperscript{ord4} females, again indicating that the decrease of ord\textsuperscript{+} function per se did not result in the phenotype. (The background nondisjunction found in the +/ord\textsuperscript{4}, ord\textsuperscript{4}/ord\textsuperscript{4}, and Df\textsuperscript{ord4} tests was due to a semidominant (dose-dependent), female-specific locus somewhere else on the ord\textsuperscript{4} chromosome used for the tests in Table 1. This locus was separable from the ord\textsuperscript{4} mutation, which then exhibited a much lower level of nondisjunction by itself (e.g., compare to +/ord\textsuperscript{4} and ord\textsuperscript{4}/ord\textsuperscript{4} in Table 3)).

Thus, ord\textsuperscript{4} has nearly normal segregation by itself in both females and males when in one or two copies, but this ord\textsuperscript{+}-like function is disrupted by the presence of the ord\textsuperscript{1} allele. These results suggest two interesting conclusions: 1) ord\textsuperscript{4}, although it shows near wild-type function, is fundamentally different from ord\textsuperscript{+} in its sensitivity to the presence of ord\textsuperscript{1}; and 2) the differential responses of ord\textsuperscript{1} and an ord deficiency in the ord\textsuperscript{4} background suggest that the ord\textsuperscript{1} allele may not code for a protein null.

**ord\textsuperscript{4} negative complementation is allele specific**

We tested the ability of ord\textsuperscript{4} to complement other ord alleles in males (Table 2). ord\textsuperscript{4} complements ord\textsuperscript{2}, ord\textsuperscript{3}, and ord\textsuperscript{5}, but weak levels of nondisjunction (3.0%) were observed in the ord\textsuperscript{6}/ord\textsuperscript{4} combination. This finding was unexpected since ord\textsuperscript{6} homozygous males show more faithful segregation than homozygotes of all other ord alleles except ord\textsuperscript{4} (Miyazaki and Orr-Weaver, 1992). Thus, the ability of other ord alleles to disrupt ord\textsuperscript{4} function in males was not correlated with the severity of the homozygous phenotype but was rather an allele-specific interaction.
<table>
<thead>
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<th>$Y$</th>
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<td>8.4</td>
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<td>0.3</td>
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</tbody>
</table>

$y/y^{+}$ males were crossed to attached-$X$, $y^2$ su($w^a$) $w^a$ females. Numbers in parentheses are progeny scored.
The female nondisjunction levels in \textit{ord}^4\textsuperscript{h} heteroallelic combinations also showed allele specificity that was not correlated with homozygous allele strength (Table 3). For example, as in the male test, the highest nondisjunction value was obtained from \textit{ord}^1/\textit{ord}^4\textsuperscript{f} females (17.6\%), but \textit{ord}^4 in combination with a presumed stronger allele, \textit{ord}^2, only exhibited about one-third as much missegregation (6.3\%). Similarly, females with \textit{ord}^4\textsuperscript{f} transheterozygous to \textit{ord}^6 gave three-fold as much nondisjunction as those in combination with \textit{ord}^3, even though the \textit{ord}^3 phenotype was 1.5 times more severe than the \textit{ord}^6 phenotype in homozygous tests (Miyazaki and Orr-Weaver, 1992).

The presence of meiosis I and meiosis II nondisjunction events could be readily examined in both male and female tests: in males by virtue of the differentially marked X and Y chromosomes employed, and in females by the X centromere-linked marker \textit{carnation}. Both meiosis I and II nondisjunctional progeny were recovered from \textit{ord}^4\textsuperscript{h} homozygous, hemizygous, and certain transheterozygous males and females (Tables 1, 2, and 3). These results suggest that PSSC was contributing to the observed nondisjunction phenotypes. Also, because the ratios of meiosis I to meiosis II nondisjunction were similar among strong \textit{ord} combinations (\textit{e.g.}, \textit{ord}^1\textsuperscript{h} homozygotes) and weaker combinations in females (Table 3), PSSC may account for all the nondisjunction observed in the weaker combinations, as is proposed for strong \textit{ord} combinations (Miyazaki and Orr-Weaver, 1992).

We observed that the absolute nondisjunction levels seen in \textit{ord}^4\textsuperscript{f} transheterozygous flies were enhanced by a modifier that was present on the originally isolated \textit{ord}^4\textsuperscript{c} chromosome but was not present on certain recombinant \textit{ord}^4\textsuperscript{c} chromosomes. In the presence of this modifier, \textit{ord}^4 failed to complement all other \textit{ord} alleles. Interestingly, the enhanced
### TABLE 3. RECOMBINATION AND NONDISJUNCTION PHENOTYPES OF *ord*<sup>4</sup> TRANSHETEROZYGOUS FEMALES

<table>
<thead>
<tr>
<th></th>
<th>±</th>
<th>+</th>
<th><em>ord</em>&lt;sup&gt;1&lt;/sup&gt;</th>
<th><em>ord</em>&lt;sup&gt;2&lt;/sup&gt;</th>
<th><em>ord</em>&lt;sup&gt;3&lt;/sup&gt;</th>
<th><em>ord</em>&lt;sup&gt;4&lt;/sup&gt;</th>
<th><em>ord</em>&lt;sup&gt;5&lt;/sup&gt;</th>
<th><em>ord</em>&lt;sup&gt;6&lt;/sup&gt;</th>
<th>D&lt;sup&gt;4&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Number scored</td>
<td>1536</td>
<td>1176</td>
<td>609</td>
<td>1203</td>
<td>757</td>
<td>705</td>
<td>864</td>
<td>944</td>
<td>713</td>
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<td>Map distance (cM)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>y-cv</em></td>
<td>10.4</td>
<td>7.9</td>
<td>1.8</td>
<td>0.9</td>
<td>0.4</td>
<td>1.3</td>
<td>0.5</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(0.76)</td>
<td>(0.17)</td>
<td>(0.09)</td>
<td>(0.04)</td>
<td>(0.13)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.11)</td>
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<tr>
<td><em>cv-v</em></td>
<td>19.2</td>
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<td>0.9</td>
<td>0.3</td>
<td>2.3</td>
<td>3.6</td>
<td>1.4</td>
<td>1.4</td>
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<tr>
<td></td>
<td>(1.05)</td>
<td>(0.10)</td>
<td>(0.05)</td>
<td>(0.02)</td>
<td>(0.12)</td>
<td>(0.19)</td>
<td>(0.07)</td>
<td>(0.07)</td>
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<td><em>v-f</em></td>
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<td>21.9</td>
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<td>1.6</td>
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<td>9.8</td>
<td>9.5</td>
<td>5.5</td>
<td>6.2</td>
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<tr>
<td></td>
<td>(1.12)</td>
<td>(0.06)</td>
<td>(0.08)</td>
<td>(0.13)</td>
<td>(0.50)</td>
<td>(0.49)</td>
<td>(0.28)</td>
<td>(0.32)</td>
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<td><em>f-car</em></td>
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<td>7.6</td>
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<td>0.9</td>
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<td>4.3</td>
<td>3.2</td>
<td>4.9</td>
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<tr>
<td></td>
<td>(1.49)</td>
<td>(0.14)</td>
<td>(0.22)</td>
<td>(0.18)</td>
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<td>(0.84)</td>
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<td>(0.96)</td>
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<td>17.8</td>
<td>10.6</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>(1.06)</td>
<td>(0.10)</td>
<td>(0.08)</td>
<td>(0.08)</td>
<td>(0.33)</td>
<td>(0.33)</td>
<td>(0.20)</td>
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<tr>
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<td>0.168</td>
<td>0.888</td>
<td>0.920</td>
<td>0.929</td>
<td>0.674</td>
<td>0.653</td>
<td>0.797</td>
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<tr>
<td>E&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>0.515</td>
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<td>0.298</td>
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<td>E&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>0.013</td>
<td>0.011</td>
<td>0.028</td>
<td>0.093</td>
<td>0.085</td>
<td>0.000</td>
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<td></td>
<td></td>
<td></td>
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<td>gametes, %</td>
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<td></td>
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<tr>
<td><em>XX</em></td>
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<td>1.5</td>
<td>1.2</td>
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<tr>
<td>O</td>
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<td>0.1</td>
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<td>0.4</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td>total ndj</td>
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<td>0.3</td>
<td>55.3</td>
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<td>1.1</td>
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<td>[2446]</td>
<td>[3069]</td>
<td>[3422]</td>
<td>[1868]</td>
<td>[2076]</td>
<td>[1956]</td>
<td>[2030]</td>
<td>[1937]</td>
</tr>
</tbody>
</table>

*y/cv v f-car* females were crossed to attached-X, v f-B males.
Values in parentheses are map distances normalized to +/+ control.
Values in square brackets are adjusted totals (see Materials and Methods).

*a Df(2R) bw<sup>S46</sup>
nondisjunction only occurred in conjunction with other EMS-induced \textit{ord} alleles; there was no enhancement of \textit{ord} deficiencies (data not shown). Although the modifier affected the quantitative amount of nondisjunction observed, it did not alter the relative ability of an allele to disrupt \textit{ord} function: \textit{ord}^1, \textit{ord}^6 and \textit{ord}^2 were strong interacters with \textit{ord}^4 (21\%, 14\%, and 11\%, respectively), while \textit{ord}^3 was weak (3\%). None of the \textit{ord}^4 chromosomes used in the nondisjunction assays reported here carried the modifier.

\textit{ord}^4 reduces \textit{X} recombination but has little effect on \textit{X} segregation

We were interested to determine whether \textit{ord}^4 would show similar allele-specific, negative interactions in female meiosis. Strong mutations in \textit{ord} affect both reciprocal recombination and chromatid segregation, phenotypes that might be differentially affected by \textit{ord}^4. The levels of \textit{X} chromosome recombination and subsequent disjunction were assayed in various \textit{ord}^4 transheterozygous combinations (Table 3).

Recombination values along the \textit{X} chromosome were reduced dramatically in all genotypes, ranging from 8\% of control levels in \textit{ord}^1/\textit{ord}^4 and \textit{ord}^2/\textit{ord}^4 females to 30\% of the control in \textit{ord}^3/\textit{ord}^4 females. In addition, the distribution of residual crossovers revealed polarity, defined as a gradient of decreasing recombination from the centromere to the distal tip of the \textit{X} chromosome. Recombination in the most distal interval \textit{y-cv} was highly depressed, to 5-10\% of the control, whereas recombination in the centromere-proximal interval \textit{f-car} was much higher, 60-95\% of control in most genotypes. Polarity of residual crossovers has been interpreted to indicate that the mutated gene affects the preconditions for exchange and not the exchange process itself (Carpenter and Sandler, 1974). In general,
the strengths of the various genotypes as assayed recombinationally were consistent with the strengths inferred from the segregation phenotype.

We found that the qualitative relationship between the strength of the recombination defect and the strength of the segregation defect were consistent within a given heteroallelic combination. However, the absolute values of the observed nondisjunction appeared incongruously low when compared with expected values based on other recombination-defective mutations. Since recombination is usually necessary and sufficient for proper meiosis I segregation of homologs in recombination-proficient organisms, the amount of observed nondisjunction increases as the fraction of nonexchange tetrads (the $E_0$ value) increases. In Drosophila this is not a simple linear relationship as the homologous achiasmate segregation system can faithfully segregate a limited number of nonexchange homologs. Nevertheless, the anomaly of $ord^4$-containing females can be demonstrated by comparing the $E_0$ and nondisjunction values of various genotypes.

Two well-studied recombination-deficient mutations in Drosophila, $mei-9$ and $mei-218$, have calculated $E_0$s for the $X$ chromosome of 0.89 and 0.95, and $X$ nondisjunction values of 28% and 30%, respectively (Baker and Hall, 1976). However, whereas $ord^1/ord^4$ and $ord^2/ord^4$ females exhibited $E_0$s of 0.92-0.93, $X$ nondisjunction rates in these females were only 17.6% and 6.3%, respectively. So even in the absence of $X$ recombination, these $ord^4$ transheterozygous females were for the most part properly segregating their $X$ chromosomes.

Three models could explain these results. In the first, the recombination defect is restricted to the $X$ chromosome; with only this homologous pair added to the “distributive pool,” the achiasmate
segregation system reliably segregates the nonexchange Xs in meiosis I. In the second model, even in the absence of a reciprocal recombination event, the homologous chromosomes are now associated by some mechanism. One can hypothesize that since ord+ is postulated to promote sister-chromatid associations, ord4 is a neomorphic (gain-of-function) mutation that can promote associations between nonexchange homologs, or perhaps can cause chiasmata to form in the absence of a crossover event. The third model assumes that crossovers are present to regularize X segregation, but occur outside of the genetically defined y-car interval. For the distal end, the position of y at 0.0 cM and the severe polar defect here would seem to exclude the region distal to y from having undetected crossovers. car lies 3.5 cM distal to bb, a locus within the heterochromatin that should define the proximal limit for euchromatic crossovers on the X. Taking into account the estimated polarity effect in the proximal car-bb interval, the inclusion of these recombination events would only increase the total X map distance by about 1 cM in ord1/ord4 or ord2/ord4 females, an amount not sufficient to alter the E0 values by much. Therefore, tests for only the first two models are considered below.

ord4 has little effect on overall recombination levels on the autosomes

By assaying recombination levels on the major autosomes, we could test whether the ord4 recombination defect was indeed X-specific as proposed in the first model. For chromosome 2, the far distal interval px-sp was chosen since ord4 exhibited a strong polar reduction in distal X intervals; likewise the greatest reduction in recombination would be expected to occur on distal 2R. To test recombination on chromosome 3, we
employed the multiply-marked chromosome "ruca," and scored three intervals from ru to cu.

The px-sp interval on 2R showed no decrease in recombination between ord⁴ homozygotes and their +/ord⁴ heterozygous control siblings (Table 4). This result was in striking contrast to the reduction seen in the distal y-cu interval on the X for ord⁴/ord⁴ females (Table 3, 6% of +/ord⁴ values). Because the px-sp interval constitutes but a small window of recombination events on 2R, it is possible that this specific interval was uniquely refractory to ord⁴-induced alterations in recombination.

The third chromosome recombination data were more comprehensive since they covered all of 3L and included the centromere-proximal region of 3R. Overall, total recombination levels of the entire ru-cu interval were similar in all genotypes, being slightly higher in ord⁴/ord⁴ females (117%) and slightly lower in Df/ord⁴ females (93%) when compared to +/ord⁴ controls (Table 4). However, an examination of the smaller intervals revealed polarity in the distribution of residual crossovers, as in the X chromosome. Oocytes from homozygous and hemizygous ord⁴ females underwent recombination in the distal ru-h interval at only 55-60% of the rate of heterozygous controls. Recombination in the pericentromeric interval st-cu, on the other hand, was increased almost five-fold over controls. As this region spans the centromere, the enhancement was probably exaggerated with respect to other intervals on 3L since proximal events from both 3L and 3R were included.

E₀ values for the ru-cu interval were calculated to assess the effect that these changes in recombination would have on the nonexchange chromosome population (Table 4). An approximation of the E₀ value for the entire third chromosome can be made by taking the square of the 3L E₀.
TABLE 4. RECOMBINATION ON THE MAJOR AUTOSOMES

<table>
<thead>
<tr>
<th>genetic interval</th>
<th>Redbook value</th>
<th>+/ord⁴</th>
<th>ord⁴</th>
<th>Df⁴</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>ord⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2nd chromosome

Number scored 1224 1196
Map distance (cM)

- px-sp
  - Df(2R)3-70

3rd chromosome

Number scored 1273 1267 1171
Map distance (cM)

- ru-h
- h-st
- st-cu

Total map distance 47.4 46.0 53.9 42.6

<table>
<thead>
<tr>
<th></th>
<th>E₀</th>
<th>E₁</th>
<th>E₂</th>
<th>E₃</th>
<th>E₀'</th>
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<td></td>
<td>0.210</td>
<td>0.143</td>
<td>0.332</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.668</td>
<td>0.655</td>
<td>0.483</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.116</td>
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<td>0.185</td>
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</tr>
<tr>
<td></td>
<td>0.006</td>
<td>0.019</td>
<td>0.000</td>
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<tr>
<td></td>
<td>0.215</td>
<td>0.210</td>
<td>0.420</td>
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<td></td>
</tr>
</tbody>
</table>

Values in parentheses are map distances normalized to +/ord⁴ controls.

\(^a\) Df(2R)3-70

N.D. Not determined since the deficiency is included within this interval.
since the third chromosome is metacentric and 3L and 3R are of similar genetic lengths. However, a correction must first be made to the ru-cu $E_0$ values to exclude the recombinants on 3R between the centromere and $cu$, giving a "3L-only" $E_0$ value (referred to as $E_0'$ in Table 4). This was accomplished by halving the observed number of recombinants in the $st-cu$ interval, since $st$ and $cu$ lie at similar distances from the centromere. By squaring the $E_0'$ values for $+/ord^4$ and $ord^4/ord^4$ females, we approximate that only about 4-5% of third chromosomes in these genotypes were nonexchange. By this calculation, only 18% of third chromosomes were nonexchange for the $ord^4/Df$ genotype, whereas 73% of X chromosomes were nonexchange (Table 3).

These results, along with the second chromosome recombination data, suggest that the first model is correct, and that the recombination defect in $ord^4$ backgrounds has the greatest effect on the X chromosome. Consistent with this interpretation are tests of the second model: namely, that a neomorphic $ord^4$ mutation might be able to rescue the nondisjunction phenotype of a recombination-defective mutation such as mei-9 by associating nonexchange X homologs. We found that $ord^4$ was unable to regularize mei-9-induced nondisjunction in either a dominant ($+/ord^4$) or a recessive ($ord^4/ord^4$) fashion (Appendix A).

**Cytological analysis of ord oocytes**

Direct cytological examination of spermatocytes was key in demonstrating that nondisjunction in ord males is the result of PSSC (e.g., see Goldstein, 1980). Similarly, observations of oocyte meiotic figures can yield clues as to the proper functioning of genes involved in female meiosis (Theurkauf and Hawley, 1992; Hatsumi and Endow, 1992; McKim et al.,
Mature stage 14 oocytes are arrested at metaphase I, with chromosomes arrayed on the spindle based on their chiasmate status: homologous chromosomes that have undergone reciprocal recombination resulting in chiasmata are found at the metaphase plate, while nonexchange chromosomes have precociously disjoined polewards under the aegis of the distributive system (Theurkauf and Hawley, 1992). In the absence of any chiasmata, the oocyte appears to traverse the metaphase I block and proceeds through the second meiotic division (McKim et al., 1993).

We stained fixed stage 14 oocytes with monoclonal antibodies specific to histones and to tubulin to visualize the position of the chromosomes with respect to the spindle apparatus (see Materials and Methods for techniques and subsequent scoring criteria). Wild-type (yellow) control oocytes for the most part showed expected metaphase I-arrested figures, with a large chiasmate mass at the metaphase plate and precociously disjoining 4th chromosomes (Figure 1A). However, our preparations also included some oocytes that had undergone premature activation, for example, with
numerous achiasmate chromosomes moving off the plate towards the poles (Figure 1B). The use of modified Robb's medium and a hypertonic fixation solution (see Materials and Methods) were employed to prevent such hypotonic activation, but in our preparations some background activation occurred in most genotypes (see below).

In the recombination-deficient mei-9 and ord^1 oocytes (and the presumed recombination-defective oocytes from sterile ord^2/Df females), many anaphase I figures were apparent (e.g., Figure 1D and Table 5). However, consistent with the residual levels of crossing over present in these genotypes, a minority of oocytes exhibited a chromatin mass where a metaphase plate might be expected and were scored as metaphase arrested (e.g., Figure 1C and Table 5). In ord^2/ord^4 and ord^4/ord^4 oocytes these metaphase figures were two- to three-fold more common than in the mei-9, ord^1/ord^1, and ord^2/Df oocytes (Table 5).

A small fraction of oocytes displayed meiosis II spindles (Figure 1E and Table 5). Interestingly, metaphase II-like figures were observed in both ord^1/ord^1 and ord^2/Df genotypes. As genetic nondisjunction data suggest that randomized segregation of chromatids is occurring at least in ord^1 females (Miyazaki and Orr-Weaver, 1992), we would not expect to see any oocytes in metaphase II, because dyads would not be able to orient to form a metaphase plate; indeed, no metaphase II cells were observed in ord^2/Df spermatocytes (Miyazaki and Orr-Weaver, 1992). Single chromatids may act differently on the female spindle than on the male spindle.

Since ord mutations in males result in single sister chromatids acting independently throughout the meiotic divisions, we were interested to determine whether the same were true in females. If premature separation were complete, one might expect a maximum of 16
TABLE 5. OOCYTE CYTOLOGY OF ord GENOTYPES

<table>
<thead>
<tr>
<th>genotype</th>
<th>metaphase I</th>
<th>anaphase I</th>
<th>metaphase II</th>
<th>anaphase II</th>
<th>n</th>
<th>metaphase I observed</th>
<th>metaphase I expected&lt;sup&gt;b&lt;/sup&gt;</th>
<th>E&lt;sub&gt;0&lt;/sub&gt;</th>
<th>X ndj. (%)</th>
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</thead>
<tbody>
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<td>+/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52</td>
<td>14</td>
<td>0</td>
<td>0</td>
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<td>0.78</td>
<td>1.00</td>
<td>0.15 (X)</td>
<td>0.03</td>
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<td>mei-g&lt;sup&gt;L1&lt;/sup&gt;/mei-g&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
<td>30</td>
<td>6</td>
<td>7</td>
<td>58</td>
<td>0.26</td>
<td>0.39</td>
<td>0.91 (X)</td>
<td>27.2</td>
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<td>ord&lt;sup&gt;1&lt;/sup&gt;/ord&lt;sup&gt;1&lt;/sup&gt;</td>
<td>77</td>
<td>90</td>
<td>15</td>
<td>3</td>
<td>185</td>
<td>0.42</td>
<td>0.45</td>
<td>0.88 (X)</td>
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<td>ord&lt;sup&gt;2&lt;/sup&gt;/Df&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>2</td>
<td>30</td>
<td>0.23</td>
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<td>56</td>
<td>0.63</td>
<td>0.31</td>
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<td>ord&lt;sup&gt;4&lt;/sup&gt;/ord&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>56</td>
<td>0.70</td>
<td>0.99</td>
<td>0.65 (X)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<sup>b</sup> Expected fraction of metaphase I oocytes is estimated by \([1-(E_0)^5]\) if only X value is given, and \([1-(E_0,X)(E_0,3L)^4]\) if both X and 3L values are available.

<sup>c</sup> y/y oocytes were used for the cytology; and y/cv v f car females were used for the genetic tests.

<sup>d</sup> Df(2R)3-70

<sup>e</sup> This value is E<sub>0</sub>' from Table 4.

N.D. Not determined due to female sterility.
chromosomes arrayed on the spindle, corresponding to all chromatids of the four bivalents. Surprisingly, we never observed more than about five chromatin masses in homozygous ord\textsuperscript{1} or hemizygous ord\textsuperscript{2} oocytes. One preparation of ord\textsuperscript{1} oocytes, however, contained some broader-than-normal spindles, and some examples of PSSC were then observed (Figure 1F). We speculate that the unique nature of the female meiotic spindle (Theurkauf and Hawley, 1992) normally obscures any PSSC that may occur.

It is possible to correlate the genetic recombination results with the recombination levels inferred from cytological analysis. The frequency of oocytes that have proceeded past the metaphase I arrest point are presumably those that have not recombined on any chromosome arm, and should be equivalent to the genetically determined value of $E_0X \cdot E_{0,2L} \cdot E_{0,2R} \cdot E_{0,3L} \cdot E_{0,3R}$. Since the large chromosome arms of the Drosophila genome are close in genetic map distance, a good approximation of the above is given by $E_0^5$, where $E_0$ is the value for a single arm. In Table 5 are shown the fraction of oocytes that were observed to be arrested in metaphase I, and the expected fraction of arrested oocytes based on genetic recombination assays (i.e., $[1-E_0^5]$).

In wild-type yellow controls we would expect all oocytes to be arrested in metaphase I, but as mentioned earlier, our preparations displayed some premature activation. In the recombination-defective mei-9 control oocytes and in homozygous ord\textsuperscript{4} oocytes, there was a good correspondence between the observed and expected frequencies of metaphase I-arrested oocytes, after taking into account this background level of activation (Table 5). ord\textsuperscript{1} homozygous oocytes, however, showed good correlation between the observed (0.42) and expected (0.45) levels of arrested oocytes, even without allowing for premature activation. This particular ord\textsuperscript{1} stock may have
been resistant to premature activation. The $\text{ord}^2/\text{ord}^4$ heteroallelic combination was the only genotype in which the number of observed metaphase I oocytes was actually greater than that expected (Table 5). The most likely explanation is that the estimate of $[1-(E_{0,X})^5]$ was erroneously low. As demonstrated genetically in the homo- and hemizygous $\text{ord}^4$ cases, recombination was much greater on the autosomes than assumed by simply testing the X alone. Similarly, our cytological observations of $\text{ord}^2/\text{ord}^4$ oocytes are inconsistent with an equal crossover reduction on all chromosomes, instead suggesting that the X chromosome was the worst affected.
DISCUSSION

We have demonstrated that the hypomorphic allele ord⁴ displays intriguing genetic behavior: allele-specific noncomplementation reveals that some ord alleles but not ord deficiencies attenuate the normal function of ord⁴; and a reduction in recombination mostly restricted to the X chromosome in ord⁴-bearing flies results in a high frequency of nonexchange X tetrads that nonetheless disjoin properly. Both of these seeming anomalies can give us insights into the normal functioning of the Ord⁺ product.

Negative complementation suggests models for Ord action

The allele-specific noncomplementation observed in certain ord⁴ transheterozygotes is an example of a class of genetic interactions termed negative complementation (Fincham, 1966). In negative complementation, two alleles of a locus in trans to one another exhibit a more severe phenotype than that expected from classic allelic noncomplementation (i.e., the opposite of what is observed in interallelic complementation). A subset of negative complementing interactions is exhibited by mutations known as antimorphs (Muller, 1932), also referred to as dominant negatives (Herskowitz, 1987). Antimorphs are special cases in that they can antagonize the function of the wild-type allele, thus giving a dominant phenotype. More rare are cases in which two recessive alleles show negative complementation with each other. Certain recessive viable Abruptex alleles of the Notch locus in Drosophila combine to result in lethality (Foster, 1975; Portin, 1975), and specific alleles of the flb locus (Drosophila EGF receptor) demonstrate negative complementation (Raz et al., 1991). In C. elegans, the body morphology loci sqrt-1, sqrt-3, and rol-8 all
display negatively complementing heteroallelic combinations (Kusch and Edgar, 1986).

Two defining features common to all these examples are: 1) allele-specific negative complementing interactions, with a mutant allele being more deleterious than a deficiency for the locus; and 2) molecular data for the gene product, when known, predict protein-protein interactions. For example, both Notch and fib encode transmembrane proteins containing motifs in the extracellular domain that are thought to mediate homotypic and heterotypic interactions (Muskavitch and Hoffmann, 1990; Ullrich and Schlessinger, 1990); of seven Abruptex mutations sequenced, all map within six EGF-like repeats in the extracellular domain of Notch (Kelley et al., 1987). The sqt-1 locus encodes a collagen molecule (Kramer et al., 1988); procollagen polypeptides trimerize to form collagen fibrils used in the C. elegans cuticle.

We propose that the genetic properties of the ord allele suggest that Ord+ function requires protein-protein interactions. Ord may be a multidomain protein: one domain required for protein binding (homotypic or heterotypic interactions), and one domain required for promoting sister-chromatid cohesion (the "active site"). The ord mutation can thus be explained as an alteration in the protein binding domain but not in the active site, such that weaker but still sufficient binding occurs. Because the active site is unchanged, Ord is able to give a wild-type phenotype when alone in the cell (i.e., when homo- or hemizygous). A strong non-complementer like ord, however, is postulated to have wild-type binding affinity but a defective active site. In such a heteroallelic combination, Ord either could be incorporated into an inactive complex with the Ord protein, or could have its necessary protein cofactor(s) titrated away by Ord. Allele-
specific negative complementation in this model depends on the ability of the non-Ord⁴ product to have near wild-type binding affinities. Alleles that are complemented by ord⁴ function may encode unstable proteins or proteins that are themselves defective in the postulated binding domain.

Alternatively, Ord⁴ can be outcompeted for protein binding if the ord⁴ mutation simply results in a lower level of wild-type Ord protein. The negative complementation can then be explained as the consequence of the titration of some limiting sites or factor(s) (which may be the ord⁴-encoded Ord⁺ protein itself) by the non-ord⁴ gene product into an inactive complex by the law of mass action. The mechanism of titration is not specified in this model: it may be through protein binding domains as in the previous model, or the mutant protein may disrupt the system through non-specific binding. Since Df/+ is wild-type for recombination in females (Appendix A), whereas exchange in ord⁴/ord⁴ females is reduced to 33% of control levels, we predict a greater than 50% reduction in Ord⁺ protein levels in ord⁴ animals if this model is correct.

**Chromosome specificity displayed by ord⁴ combinations**

Chromosome-specific control of recombination has been previously noted in Drosophila and C. elegans. The fourth chromosome of D. melanogaster, unique among the chromosomes, never undergoes reciprocal exchange under normal circumstances, even though EM ultrastructural analysis of wild-type females revealed that the fourth chromosomes have synaptonemal complex of euchromatic morphology (Carpenter, 1975). The mei-1 mutation on chromosome 3 results in a 50% overall decrease in recombination specifically on the X chromosome (Valentin, 1973). As in homozygous ord⁴ flies, recombination in mei-1 females is most severely depressed in distal regions and least so proximally
on all chromosomes; on the autosomes this results in a redistribution of crossovers along the chromosome but has no effect on overall recombination frequency. In *C. elegans*, mutations in the *him-1*, *him-5*, and *him-8* loci result in X-specific recombination reductions (except for one autosomal interval in *him-5*) (Hodgkin *et al.*, 1979; Broverman and Meneely, 1994).

Does allele strength determine chromosome specificity? *ord*¹ is a strong allele and is thought to affect recombination on all chromosomes to a similar extent (Mason, 1976); whereas *ord*⁴ is a weak allele and predominantly affects only the X chromosome. Because *ord*⁴ and *mei-1* display only moderate reductions (33-50%) in recombination, yet are also X specific, the X may simply be the most susceptible chromosome to perturbations in the recombination process. As only one mutant allele of *mei-1* is extant, it is unclear whether X specificity is intrinsic to the gene, or if this allele is hypomorphic and stronger alleles will display autosomal defects, as in the case of *ord*. Interestingly, akin to *ord*⁴, the semidominant *ord*¹/+ genotype shows 84% of control recombination levels on the X (65% in authors' tests, data not shown), but has no effect on overall 3rd chromosome recombination (Mason, 1976).

Among other *Drosophila* loci involved in recombination, strong mutations in *c(3)G*, *mei-9*, and *mei-218* all reduce recombination on the X and autosomes to a similar degree (*c(3)G*, Gowen and Gowen, 1922; *mei-9* and *mei-218*, Sandler *et al.*, 1968, Carpenter and Sandler, 1974); unfortunately no weaker alleles of these loci are available. However, *mei-S51* and *mei-S282* are mutations that affect recombination levels on X and 2 coordinately, yet give much weaker reductions: about 60% of X and 2 controls for *mei-S51* (Sandler *et al.*, 1968; Robbins, 1971), and about 50% for
mei-S282 (Parry, 1973). These results argue that if allele strength determines chromosome specificity, it does so on a gene-by-gene basis, since mei-1 and ord\(^4\) act differently than mei-S51 and mei-S282 with respect to autosomal recombination. Isolation and analysis of weaker alleles of \(c(3)G\), mei-9, and mei-218, and stronger alleles of mei-1, should greatly clarify the situation.

The X recombination defect in \(ord^1/+\) and \(ord^4\)-containing flies could be the result of interactions with sequences restricted to the X, or even due to the structure of the X chromosome itself. Szauter has suggested that the normal distribution of X crossovers is established by a system of regional constraints on X chromosome exchange, including cis-acting euchromatic responding elements (Szauter, 1984). A similar situation has been reported for the chromosome arm 2L (Valentin, 1982). The chromosome specificity observed for the weak \(ord^4\) allele could be the result of a more pronounced attenuation of the constraints affecting the X chromosome versus those affecting the autosomes. Alternatively, the X differs from the major autosomes in being half their length and acrocentric instead of metacentric. Ord may be sensitive to the presence of sufficient eu- and/or heterochromatin flanking the centromere. Recombination tests employing reverse metacentric compound-X chromosomes could determine if X-specific sequences or X structure were important to the chromosome specificity observed in \(ord^4\) females.

**Cytological considerations of PSSC**

Rare instances of observable PSSC occurred only in \(ord^1\) oocytes in which the spindle had fortuitously undergone a slight broadening. We speculate that PSSC is a more common event than our observations would indicate, but that some property of the spindle obscures its presence. With
respect to the broadening above, the compact nature of the meiotic spindle along its transverse axis (Theurkauf and Hawley, 1992) probably prevents resolution in this direction. Indeed, all chiasmate chromosomes at the metaphase plate appear as one mass. Along the longitudinal axis, there also appears to be a resolution problem in that no more than five chromatin masses were observed when genetic assays would predict 16 (Miyazaki and Orr-Weaver, 1992). This effect occurs at the level of paired sister chromatids as well, since we would predict to see the eight achiasmate dyads in about 60% of homozygous mei-9 oocytes based on $E_0$ values (Table 5).

We were surprised to see what appeared to be metaphase II figures in our mutant ord oocytes. In male cytological studies, no metaphase II plates were detected in homozygous ord$^1$ and hemizygous ord$^2$ spermatocytes (Goldstein, 1980; Miyazaki and Orr-Weaver, 1992). The discrepancy between the male and female cytology might arise if chromosomes were segregating too slowly in the oocytes we observed. The completion of the meiotic divisions in Drosophila oocytes is extremely rapid and coincident with egg-laying and oocyte activation (Doane, 1960; Mahowald et al., 1983). The high frequency of anaphase I figures in our preparations suggests that the progression through the meiotic divisions was not occurring with the same kinetics as in normally laid eggs. Similarly, if chromosomes nucleate their own spindles in meiosis II as they do in meiosis I (Theurkauf and Hawley, 1992), then perhaps single chromatids can be "caught" at a time when the spindle has formed but sluggish segregation has not yet begun.
**Relationship between the recombination and segregation phenotypes**

Strong mutations in *ord* display two phenotypes in female meiosis: a decrease in reciprocal recombination, and an increase in chromosome nondisjunction. Drosophila recombination-defective mutants exhibit these same phenotypes, but nondisjunction is restricted to nonexchange chromosomes at meiosis I, being a direct effect of insufficient recombination (Baker and Hall, 1976). In contrast, these two phenotypes are expressed independently of each other in *ord* females such that nondisjunction of exchange and nonexchange tetrads occurs equally frequently (Mason, 1976). Also, unique among the recombination mutants, *ord* gives equational (meiosis II) nondisjunction events. We believe that the special properties of *ord*-generated nondisjunction are a result of PSSC, based on cytological observations in this study and the agreement of genetic data supporting randomized segregation of chromatids (Miyazaki and Orr-Weaver, 1992).

This exchange-independent segregation defect in *ord* females suggests that it might be possible to separate the recombination and segregation phenotypes by mutation, whereas this would not be expected of recombination mutants in which disjunction is dependent on exchange. One could imagine *ord* mutations with low exchange but no PSSC, resulting in nondisjunction of only nonexchange tetrads, or conversely mutations with normal exchange levels but high amounts of PSSC-induced nondisjunction. Having said this, we believe that defects in the same underlying function lead to both phenotypes in *ord* females, namely a disruption of sister-chromatid cohesion. This paradox can be resolved by postulating that *ord* function (or the consequences thereof) is needed at two
places during meiosis: along the chromosome arms for recombination, and at the centromere for segregation.

In a previous report we hypothesized that the \textit{ord}^6 mutation represented such a separation-of-function allele, since when homozygous it displayed a similar reduction in \(X\) chromosome recombination as did homozygous \textit{ord}^1 and \textit{ord}^3 flies, but only 70-75\% as much nondisjunction (Miyazaki and Orr-Weaver, 1992). In light of our present findings, the previous data can also be interpreted as suggesting that autosomal recombination in \textit{ord}^6 females is better than in \textit{ord}^1 or \textit{ord}^3 females, thus resulting in slightly improved \(X\) disjunction. If chromosome recombination specificity is a function of allele strength, this reinterpretation is consistent with the fact that \textit{ord}^6 is the next strongest allele past \textit{ord}^4 in the \textit{ord} allelic series.

Among the six \textit{ord} alleles analyzed (Miyazaki and Orr-Weaver, 1992; this report), only \textit{ord}^4 exhibits separation of function in that \(X\) recombination is reduced but \(X\) nondisjunction is nearly absent. The twist in this case is that although \(X\) recombination is low, nonexchange \(X\) chromosomes still disjoin since autosomal exchange is sufficiently high to allow the achiasmate segregation system to function properly. The molecular analysis of the lesion associated with the \textit{ord}^4 allele when compared to those of the other \textit{ord} alleles will be of great interest. These data may implicate a certain region of the Ord protein as being necessary for recombination but not segregation, and may also elucidate the molecular mechanism of the negative complementation between \textit{ord} alleles.
ACKNOWLEDGMENTS

We wish to thank Francisco Pelegri and members of the lab for helpful discussions. We also thank members of the Dawson group for enlightening discussion on mechanisms of negative complementation.
**LITERATURE CITED**


## APPENDIX A.

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Chapter V.

Analysis of mitosis in *ord* and *mei-S332* mutants

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My contribution to this study was constructing the *ord* stocks for larval cytology and wing clone analysis, and supervising J. Wu in his UROP stay.
**ABSTRACT**

The mitotic roles of the Drosophila genes *ord* and *mei-S332* required for sister-chromatid cohesion in meiosis have been examined. Mutations in *mei-S332* exhibited few abnormalities when observed cytologically in larval neuroblast squashes or in a genetic assay for *multiple wing hairs* somatic clones in the wing imaginal disc. Similarly, *ord* mutant neuroblasts revealed few aberrations in larval brain, but the incidence of wing clones was elevated two- to six-fold over controls. Surprisingly, *ord* and *mei-S332* neuroblasts both showed high frequencies of separated sister chromatids after incubation in colchicine. Paired with results indicating that *ord* plays a role in gonial mitotic divisions (Lin and Church, 1982; Miyazaki and Orr-Weaver, 1992), our studies argue that *mei-S332* and *ord* have different roles in mitosis. However, the possibility that *mei-S332* functions in a redundant pathway promoting sister-chromatid cohesion cannot be excluded.
INTRODUCTION

The meiotic cycle is distinct from the mitotic cell cycle in that two nuclear divisions follow one round of DNA synthesis, producing haploid endproducts. Despite the different cellular outcomes between these two modes of cell division, mechanistic commonalities would suggest many genetic similarities as well. The first meiotic division differs from mitosis in several important regards: homologous chromosomes must pair and segregate from each other without the separation of sister centromeres. Thus, genes that function in this division may be unique to meiosis. Meiosis II, however, resembles mitosis since sister chromatids of each homolog disjoin to opposite poles. The results of screens of *S. cerevisiae* mitotic mutants looking for meiotic defects (Simchen, 1974) and Drosophila meiotic mutants assaying for mitotic defects (Baker *et al.*, 1978) conclude that the functions of many loci are shared in both mitosis and meiosis.

*ord* and *mei-S332* are two genes required for proper sister-chromatid cohesion in *D. melanogaster* (Davis, 1971; Mason, 1976; Goldstein, 1980; Kerrebrock *et al.*, 1992; Miyazaki and Orr-Weaver, 1992). Sister-chromatid cohesion is an intrinsic feature of both mitotic and meiotic chromosomes in cytological analysis. In mitotic chromosomes, sister chromatids are paired at the centromeres (primary constrictions) and along the arms, and mitotic anaphase commences when arm- and centromere cohesive forces are released together. In meiosis, however, the loss of chromatid cohesiveness occurs in a two-step fashion, first relaxing between chromatid arms at anaphase I, and only releasing between sister-chromatid centromeres at anaphase II. The clear requirements for functions that maintain and trigger release of chromatid cohesion in both mitosis and meiosis lead to speculation that *mei-S332*+ and *ord*+ functions might be required in mitosis as well. Since
homozygous mutant flies are available for meiotic analysis, such an hypothesis would presuppose that the extant alleles are leaky mutations of vital loci (Baker et al., 1978).

Possible mitotic functions of \textit{ord} and \textit{mei-S332} have been investigated but their mitotic roles remain unclear. Sensitive somatic clone analysis suggested that the \textit{mei-S332}^1 and \textit{ord}^1 mutations increased rates of mitotic recombination or nondisjunction in abdominal histoblasts (Baker et al., 1978). The caveat to be considered in this study was that as only one mutant allele and no deficiencies were available for either locus, the observed phenotypes could have arisen from the homozygosis of second-site loci residing on the same chromosome as the meiotic mutations. In a cruder assay, on the other hand, there was no diminished viability of flies hemizygous for new alleles of \textit{mei-S332} and \textit{ord} (including some putative \textit{mei-S332} null alleles) when compared to heterozygous siblings (Kerrebrock et al., 1992; Miyazaki and Orr-Weaver, 1992). Moreover, very few or no gynandromorph progeny were obtained from \textit{ord} and \textit{mei-S332} females; thus these genes appear to have no detectable function in the earliest embryonic cleavage divisions. The \textit{ord} study, however, revealed that the male germline gonial divisions resulted in aneuploidy. Similar gonial aberrations in the male were reported previously (Lin and Church, 1982) Furthermore, abnormalities consistent with mitotic defects were observed in the nurse cell nuclei that are the products of the analogous divisions in the female (Miyazaki and Orr-Weaver, 1992).

In order to define more fully the possible roles of the \textit{ord} and \textit{mei-S332} loci in mitosis, we have undertaken a direct cytological analysis of mitoses in larval neuroblast cells and a genetic assay for somatic clones in the wing epithelium, using the battery of new alleles and deficiencies now available to us. In addition, we have sought to determine if the \textit{ord} female nurse cell
phenotype has its origin in the female gonial divisions by direct cytological observation of such mitoses.
MATERIALS AND METHODS

Stocks  All Drosophila stocks and crosses were raised at 25°C on standard cornmeal-brewer's yeast-molasses-agar medium. The isolation of the ord\textsuperscript{2} and ord\textsuperscript{5} alleles was described in Miyazaki and Orr-Weaver (1992). Genetic analyses of the putative null alleles mei-S332\textsuperscript{1} and mei-S332\textsuperscript{7} were described in Kerrebrock et al. (1992). The deficiency $Df(2R)3-70$ was isolated by Daniel Moore in this laboratory; the deficiency $Df(2R)X58-6$ was isolated by A. Kerrebrock (Kerrebrock et al., 1992). The $T(2;3)TSTL\#14$ stock was obtained from B. Reed; the $mwh e$ chromosome was obtained from J.M. Axton. Gene descriptions can be found in Lindsley and Zimm (1992).

Larval neuroblast cytology  Homo- or hemizygous ord or mei-S332 larvae were distinguished from their heterozygous siblings by employing the dominant larval marker Tubby, carried on the translocation chromosome $T(2;3)TSTL\#14$ (J. Casal and P. Ripoll, cited in Gatti and Goldberg, 1991). Stocks carrying mutant alleles or deficiencies were crossed into the $T(2;3)$ background, then mated together to generate the mutant genotype. Non-Tubby 3rd instar larvae were selected and dissected in isotonic 0.7% NaCl; the sex of the larva was determined prior to dissection by the presence or absence of the larval testes. Colchicine, if used, was applied for approximately 1.5 hours as a 10 μM solution in 0.7% NaCl. After colchicine treatment, brains were incubated for 10 minutes in a hypotonic 0.5% sodium citrate solution. Brains were fixed for 5 sec in 45% acetic acid on a clean coverslip, then stained for 10 minutes in a solution of 2% orcein, 45% acetic acid and 15% lactic acid. A clean microscope slide was layered on top and brains were squashed using vise pressure. Coverslips were ringed with nail polish and slides were scored within seven days.
Slides were viewed using phase microscopy on a Zeiss Axiophot equipped with either a Plan Apochromat 63x (for cells without colchicine treatment) or Plan Neofluar 100x objective lens (for colchicine-treated cells). Two horizontal and two vertical scans were made across an area judged reasonably flat and confluent. A scan consisted of an end-to-end series of fields, each field defined as the area enclosed by the outer frame of the Axiophot’s photographic viewfinder as seen using the respective objective. Photographs of cells were taken using Kodak technical pan film.

Metaphase cells were defined as those containing condensed chromosomes that were not obviously segregating to the poles. Homologous chromosomes generally lay near each other in these metaphase configurations. Ploidy of metaphase cells was only determined if the chromosomes were distinct and separate; these are referred to as “scoreable metaphases” in Tables 1 and 2. Anaphase cells were those exhibiting chromosomes that had been actively moving toward the poles prior to fixation. Lagging chromosomes were defined as those remaining in the vicinity of the metaphase plate after all other chromosomes had reached the poles.

Wing clone analysis  Mutant or heterozygous sibling control flies carrying mwh e/+ third chromosome constitutions were killed and stored in 70% ethanol until ready for dissection. Wings were removed and mounted in 70% glycerol:30% PBS for viewing under phase microscopy using a Plan Neofluar 40x objective. Five male and five female adults (10 wings per sex) were scored for each genotype. No apparent sex-specific differences were observed except in the case of control and mutant genotypes carrying ord1: female
wings exhibited three-fold as many clones as male wings. Data from both sexes were pooled for inclusion in Table 3.

Cytology of female gonial mitoses  The cytology of female germaria was tried using two different staining techniques and females of varying ages. A modification of Zalokar and Erk’s (1976) basic fuchsin protocol was suggested to us by Bruce Reed, who had excellent results in visualizing early nurse cell chromosomes by this method. Ovaries were dissected in 0.7% NaCl and fixed for one hour in 4 ml 95% ethanol:1 ml 50% acetic acid:0.2 ml formaldehyde, after which they were rinsed in 70% ethanol for an hour. Hydrolysis in 2 N HCl was necessary for staining to occur; this was performed at 50°C for exactly 15 minutes. Subsequently, ovaries were briefly rinsed in water, then stained in basic fuchsin (0.5% in 2.5% acetic acid, supplied to us by B. Reed) for an hour. Destaining and mounting solution was 5% acetic acid. Alternatively, ovaries were dissected in 0.7% saline, fixed in 45% acetic acid (the addition of lactic acid to the fixative was found to make the ovaries rubbery and not liable to spread), and stained with the brain orcein stain described above. Ovary preparations were viewed under phase on a Zeiss Axiophot equipped with a Plan Neofluar 40x objective.

Ovaries from females of differing ages were employed in these studies. Although ovaries of yeast-fattened females were easiest to dissect, the apical end containing the gonial divisions had to be sliced away from the rest of the ovary with a razor blade to get a flattened preparation. The best ovaries were obtained from pharate (pupal) females, since any egg chambers were in immature stages and tended not to interfere with gonial observations.
RESULTS

ord and mei-S332 neuroblast chromosomes are normal in the absence of colchicine

The effect of strong ord and mei-S332 mutations on larval brain mitoses was examined in mutant third instar larvae. Gratuitous larval markers were required to distinguish homozygous or hemizygous mutant larvae from their heterozygous siblings. As there are few good larval markers on the second chromosome where ord and mei-S332 are located, we utilized the T(2;3)TSTL#14 stock, which contains a reciprocal translocation between the second chromosome balancer SM5 and the third chromosome balancer TM6B carrying the dominant larval and pupal marker Tubby (Tb). When ord or mei-S332 was crossed into this background, the T(2;3) elegantly combined simultaneous balancing of the meiotic mutant with dominant marking of mutant heterozygotes with Tb. Subsequent interstock matings of T(2;3)-carrying mutant stocks generated the non-Tubby larvae homozygous for ord and mei-S332 used in the study.

In the absence of colchicine treatment, chromosomes in mitotic neuroblasts generally exhibit either a metaphase (Figure 1A) or anaphase (Figure 1B) configuration. Metaphase cells were scored for any apparent aneuploidy (see Materials and Methods); anaphase cells were scored for segregation anomalies. ord\textsuperscript{1}/ord\textsuperscript{1}, ord\textsuperscript{1}/Df, and ord\textsuperscript{2}/Df mutant brains showed no metaphase aneuploidy; there was a single ord\textsuperscript{2}/Df cell with

Figure 1. Neuroblast chromosome cytology from brains untreated with colchicine. A) Wild-type Canton-S cell showing euploid chromosome complement. Homologous chromosomes tend to be adjacent to one another (photo kindly provided by B. Reed). B) Canton-S anaphase. All chromosomes have reached the pole with no chromosomes remaining in the region of the metaphase plate. C) Canton-S aberrant anaphase with disorganized spindle. D) Homozygous ord\textsuperscript{1} anaphase showing X chromosome laggards. This cell was from a genotypically XX animal.
separated sister chromatids (Table 1). Hemizygous *mei-S332* neuroblast metaphase cells had few defects as well, with only 1.7% exhibiting the separated sister chromatid phenotype. However, two of the cells (0.6%) were hyperploid for the X chromosome. In Canton-S control larval brains, all metaphase figures were euploid (Table 1). However, a low percentage of scoreable metaphases, less than 1%, also exhibited the disjoined sister chromatid phenotype observed in the mutant backgrounds.

Anaphase figures in the *ord* and *mei-S332* genotypes were generally normal, but 11-15% of anaphase cells exhibited aberrant figures (Table 1). The abnormal morphologies included broad spindles, chaotic chromosome arrangement, and overall disorganized structure (e.g., Figure 1C). However, 9% of control neuroblasts also contained similar aberrant anaphase figures. Since few instances of aneuploidy were detected in metaphase cells, such anaphase figures probably do not result in an impaired ability of the spindle to segregate chromosomes. A low percentage of spindles with lagging chromosomes was detected in the various *ord* genotypes (0.4-2.2%, Table 1 and Figure 1D). Both the X chromosome and the large autosome(s) were observed to lag. If lagging chromosomes are not incorporated into a daughter nucleus we might then expect to find hypoploidy in the subsequent metaphase, assuming such a chromosome loss event is not lethal. As the observed instances of lagging X chromosomes were in XX cells, viable hypoploid XO cells might have been detected in metaphase figures. The absence of XO cells suggests that the lagging chromosomes eventually reach the poles to be included in a nucleus.

By averaging the total number of cells in mitosis by the number of fields scored, we calculated a relative mitotic index. As seen in Table 1, this value agrees well in control and mutant larvae, indicating that the *ord*
TABLE 1. *ord* AND *mei-S332* MITOSES ARE GENERALLY NORMAL IN UNTREATED NEUROBLASTS

<table>
<thead>
<tr>
<th></th>
<th>metaphase</th>
<th>anaphase</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>aneuploid</td>
<td>separate</td>
<td>total</td>
<td>abnormal</td>
<td>lagging</td>
<td>total</td>
<td>metaphase</td>
<td>figure/field</td>
<td>total</td>
<td>no. of</td>
<td></td>
</tr>
<tr>
<td></td>
<td>metaphase</td>
<td>sisters (^a)</td>
<td></td>
<td>anaphase</td>
<td>chroms</td>
<td>anaphase</td>
<td>/anaphase ratio</td>
<td>ratio</td>
<td>fields</td>
<td>animals</td>
<td></td>
</tr>
<tr>
<td>Canton-S</td>
<td>0</td>
<td>3 (0.9%)</td>
<td>1006 (344)</td>
<td>24 (9.0%)</td>
<td>0</td>
<td>268</td>
<td>3.75</td>
<td>2.55</td>
<td>499</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>(ord^1/ord^1)</td>
<td>0</td>
<td>nd</td>
<td>769 (317)</td>
<td>35 (13.0%)</td>
<td>6</td>
<td>269</td>
<td>2.86</td>
<td>2.06</td>
<td>503</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>(ord^1/Df^c)</td>
<td>0</td>
<td>nd</td>
<td>778 (315)</td>
<td>23 (12.6%)</td>
<td>4</td>
<td>183</td>
<td>4.25</td>
<td>2.16</td>
<td>444</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>(ord^2/Df^c)</td>
<td>0</td>
<td>1 (0.3%)</td>
<td>847 (311)</td>
<td>26 (11.1%)</td>
<td>1</td>
<td>235</td>
<td>3.60</td>
<td>2.44</td>
<td>443</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>*mei-S332(^7)/ Df(^d)</td>
<td>2</td>
<td>6 (1.7%)</td>
<td>811 (347)</td>
<td>62 (15.1%)</td>
<td>0</td>
<td>412</td>
<td>1.96</td>
<td>2.49</td>
<td>491</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Number is parentheses indicates percent of only scoreable metaphases.

\(^b\) Numbers in parentheses are scoreable metaphases.

\(^c\) Df(2R)3-70

\(^d\) Df(2R)X58-6

nd: not determined
and mei-S332 mutations did not alter a cell’s ability to enter or exit mitosis. Of those cells in mitosis, the metaphase-to-anaphase ratio is an indication of the kinetics with which these cells move from metaphase to anaphase. In ord larvae, the metaphase/anaphase ratio was similar to that seen in control cells, suggesting that ord did not drastically alter the progression of neuroblasts through the cell cycle. In mei-S332/Df neuroblasts, however, more anaphases than expected were observed. This result may indicate that mei-S332 cells spent less time in metaphase and were more likely to enter anaphase prematurely. Subsequent segregation, however, is normal. Our analysis of ord and mei-S332 larval brains indicates that mutations in these genes have little, if any, effect on the proper mitotic segregation of chromosomes in these tissues.

Colchicine treatment of ord and mei-S332 neuroblasts reveals sister-chromatid separation

Larval brains that are incubated in a hypotonic colchicine solution prior to fixation and staining accumulate cells in a metaphase-like state with condensed chromosomes, and an absence of anaphase cells. When Canton-S cells were treated this way, a minority of these cells (8% of total metaphases) exhibited sister chromatids that were disjoined (Figure 2A and Table 2). We presume that these cells were those just commencing anaphase when the colchicine treatment disrupted the spindle. In contrast, ord2/Df and mei-S332/Df cells showed a much greater incidence of such cells, 35% and 21%,
TABLE 2. SISTER-CHROMATID SEPARATION IN THE PRESENCE OF COLCHICINE

<table>
<thead>
<tr>
<th>genotype</th>
<th>cells with separated sister chromatids&lt;sup&gt;a&lt;/sup&gt;</th>
<th>total metaphases&lt;sup&gt;b&lt;/sup&gt;</th>
<th>figure/field ratio</th>
<th>total fields</th>
<th>no. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton-S</td>
<td>58 (7.5%)</td>
<td>772 (262)</td>
<td>1.10</td>
<td>702</td>
<td>6</td>
</tr>
<tr>
<td>ord&lt;sup&gt;2&lt;/sup&gt;I Df(2R)3-70</td>
<td>465 (34.9%)</td>
<td>1331 (568)</td>
<td>2.04</td>
<td>651</td>
<td>6</td>
</tr>
<tr>
<td>mei-S332&lt;sup&gt;71&lt;/sup&gt;</td>
<td>185 (20.8%)</td>
<td>888 (290)</td>
<td>1.37</td>
<td>650</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number in parentheses is percent of total metaphases.

<sup>b</sup> Number in parentheses is all scoreable cells.
respectively (Figure 2B and Table 2). These results suggest that under certain conditions the absence of functional \textit{ord} or \textit{mei-S332} product makes sister chromatids more liable to fall apart. The separation may indicate that wild-type \textit{ord} and \textit{mei-S332} act redundantly to ensure cohesion of sister chromatids in mitotic cells, and the action of colchicine reveals the underlying defect. Alternatively, the mechanism by which colchicine exerts its effects on chromosome structure is unknown, so this phenotype of \textit{ord} and \textit{mei-S332} may be uninformative with respect to their function in mitotic cells.

\textbf{Incidence of wing clones appears to be elevated in \textit{ord} flies}

Although direct cytological observation is possible for organs such as larval brain, the same technique is impractical for other tissues. Indirect genetic assays can be employed in suitable tissues to reveal possible errors in chromosome segregation. In one widely used test, marked homozygous mutant clones that arise in a heterozygous background are scored in the wing epithelium. This clonal assay is sensitive in that it rapidly allows the examination of many cells. Figure 3 diagrams three possible mechanisms for the generation of mutant \textit{multiple wing hairs} (\textit{mwh}) clones in the wing: somatic recombination, double nondisjunction, and mutation or deletion (chromosome breakage). Although it is genetically feasible to demonstrate

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Mechanisms for generation of homozygous \textit{mwh} clones in the wing.}

A) Mitotic recombination. If a somatic recombination event occurs between the centromere and \textit{mwh}, theoretically 50% of the mitotic segregants from such an event will consist of two daughter cells: one homozygous for \textit{mwh}, and the other homozygous for \textit{mwh}+. B) Double nondisjunction. Segregation at mitosis occurs such that both sister chromatids of one homolog are incorporated into the same daughter cell. The mechanism of such an event can occur either by failure of sister chromatids to separate, or by precocious separation followed by random segregation. C) Mutation or deletion. A homozygous \textit{mwh} cell is generated when the normal copy is mutated or lost from one sister chromatid, followed by a normal segregation pattern.
\end{figure}
double nondisjunction conclusively by including a marker on the opposite side of the centromere from \textit{mwh} (arbitrarily designated as "b" in Figure 3), this was not attempted in the present study. (We were only interested in using \textit{mwh} to determine if this approach warranted further study with a more detailed analysis). Clone size, in addition, is an indication of the mitosis at which the clone-generating event occurred: larger clones are derived from earlier events, and smaller clones generally are from more recent mitoses.

The size and frequency of \textit{mwh} mutant patches were scored in \textit{mwh/+} wings from hemizygous \textit{mei-S332\textsuperscript{1}}, \textit{ord\textsuperscript{1}}, and \textit{ord\textsuperscript{5}} flies, along with wings from their heterozygous siblings (Table 3). Hetero- and hemizygous \textit{mei-S332\textsuperscript{1}} wings displayed only a low incidence of clones; the low absolute value of clones even in \textit{mei-S332\textsuperscript{1}} hemizygotes (0.55 clones per wing) suggests that \textit{mei-S332} is not required for somatic mitoses in the wing epithelium. Hemizygous \textit{ord\textsuperscript{1}} and \textit{ord\textsuperscript{5}} wings, on the other hand, displayed an average of two to three clones per wing. For the \textit{ord\textsuperscript{5}} case, the hemizygous clone frequency was almost six times the heterozygous control, whereas there was only about a two-fold increase in \textit{ord\textsuperscript{1}}. These relative figures may be misleading, however, since the \textit{ord\textsuperscript{1}} heterozygous control appeared atypically high when compared to the heterozygous \textit{mei-S332\textsuperscript{1}} and \textit{ord\textsuperscript{5}} controls. The absolute clone frequency was actually higher in \textit{ord\textsuperscript{1}} hemizygotes (3.2 per wing) than for \textit{ord\textsuperscript{5}} hemizygotes (2.3 per wing). We note that the presence of the \textit{Df(2R)3-70} chromosome is correlated with a constant increase of 1.4 to 1.9 clones per wing in either an \textit{ord\textsuperscript{1}} or \textit{ord\textsuperscript{5}} background (Table 3); unfortunately the \textit{Df/+} control was not done.

Most genotypes displayed clones consisting of one or two cells, suggesting that the event that generated the homozygous \textit{mwh} genotype occurred in the preceding one or two mitoses. The distribution of clone sizes
<table>
<thead>
<tr>
<th>genotype</th>
<th>clone size (number of cells)</th>
<th>Total clones</th>
<th>Number of wings scored</th>
<th>Clones/wing</th>
<th>Clone freq. normalized to heterozygous controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3-4</td>
<td>5-8</td>
<td>9-16</td>
</tr>
<tr>
<td>Dfα/+</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>mei-S3321/Dfα</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ord1/+</td>
<td>24</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ord1/Dfβ</td>
<td>40</td>
<td>22</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ord5/+</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>ord5/Dfβ</td>
<td>25</td>
<td>16</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Flies with the indicated second chromosome constitutions were marked with mwh/+ on the third chromosome.

*a Df(2R)X58-6
*b Df(2R)3-70
in the mutant ord genotypes versus the heterozygous controls may show a slight skewing towards smaller clone sizes (Table 3). In nearly all the genotypes, the geometric progression in the clone frequency as a function of clone size (i.e., single cell clones were twice as frequent as 2-cell clones, which were roughly twice as frequent as 4-cell clones, etc.) indicates that during the time that clones were being produced, the probability of a clone-generating event was proportional only to cell number. Thus each cell had an equal probability for experiencing such an event regardless of which mitosis in the development of the wing was occurring.

As 3R was unmarked in these tests, the origin of the clones in ord wings (if indeed they were ord-induced) is unclear. However, one prediction of clone generation by the mechanism of deletion or chromosome breakage is that other phenotypes might be uncovered by such a deletion. Specifically, there is a Minute locus very close to mwh on the cytogenetic map, and the dominant Minute phenotype superimposed on mwh clones would result in small, slow-growing patches with thin hairs. This phenotype was not observed in our clones.

Cytology of female gonial divisions

The abnormal nurse cell phenotype found in ord2 and ord5 females is consistent with the hypothesis of aberrant gonial mitotic divisions (Miyazaki and Orr-Weaver, 1992), similar to those seen in males (Lin and Church, 1982; Miyazaki and Orr-Weaver, 1992). The four successive gonial divisions that result in the interconnected 16-cell oocyte:nurse cell egg chamber occur in the region of the ovary known as the germarium. The germarium can be subdivided into three distinct domains; the gonial divisions have been observed in the most apical domain (Koch and King, 1966).
We attempted to observe these divisions by two different staining protocols and by using females of different ages (see Materials and Methods). The clearest cytological preparations were obtained by the basic fuchsin method applied to ovaries obtained from pharate pupal females. These ovaries consisted almost solely of germaria with very little material of the later vitellarium stages, which made observations on ovaries from older females difficult. However, after many repeated attempts no mitotic figures were ever observed in the germaria from females of different ages. Ovaries stained with orcein were also examined, but the background staining of the cytoplasm made observations much more difficult than ovaries stained by the fuchsin technique. Although the ovaries from pupal females have been claimed to be a rich source of gonial divisions (Gatti and Goldberg, 1991), by other accounts the frequency of these divisions is quite rare (A.T.C. Carpenter, pers. comm.). The rarity of these divisions may partly account for our failure to detect them.
DISCUSSION

The studies presented here extend those of Baker et al. (1978) in three ways: by employing the somatic clone analysis in a tissue (wing epithelium) other than the abdominal cuticle; by assaying directly for abnormal metaphase and anaphase figures in larval brain; and by minimizing the effects of unrelated loci by utilizing various strong ord and mei-S332 alleles when hemizygous with the appropriate deficiency chromosomes.

Functions of ord and mei-S332 in somatic mitosis

Neuroblast cytology of mutant ord and mei-S332 larval brains in the absence of colchicine indicated few abnormalities in this tissue in either genotype. This finding suggests that these genes play no essential role in neuroblast mitosis; if they do have a role, such a function must be assumed by the normal functioning of a redundant gene in mutant larvae. Mitotic abnormalities would become evident only when two or more redundant genes were mutated, of interest might be the mei-S332 ord double mutant.

The somatic wing clone assay as well yielded negative results for mei-S332. Only background levels of clones were observed even for the putative null genotype of mei-S332\(^7\)/Df. Hemizygous ord\(^1\) and ord\(^5\) wings exhibited roughly two- and six times the numbers of clones as their respective controls, although the extent to which the deficiency chromosome was contributing to these values was untested. If these clones were truly reflective of an ord-dependent anomaly, the origin of the clones could not be traced with the one cell marker used: double nondisjunction, mitotic recombination, and mutation are all possibilities, with only chromosome breakage being ruled out. Since we were treating this clonal assay as a preliminary test of mitotic function, the next step would be to demonstrate double nondisjunction by including a cell marker on the opposite side of the centromere as mwh. For
example, in *mwh flr/+ +* or *mwh +/+ flr* genotypes the presence of *mwh- flr-* single spots or *mwh*/flr- twin spots, respectively, would be a unique indication of double nondisjunction events.

**The behavior of sister chromatids in the presence of colchicine**

Both *ord* and *mei-S332* mutant neuroblasts showed an unexpected separated sister chromatid phenotype when cells were pre-incubated in colchicine. The most conservative conclusion that can be drawn from this result is that these genes are expressed in larval brain tissue and the lack of functional gene product results in this striking phenotype. This may indicate that *ord* and *mei-S332* are genes involved in a redundant pathway necessary for sister-chromatid cohesion in mitosis, and the colchicine treatment uncovers the inherent weakness of centromere connections in the single mutants. However, it would be premature to attempt to conclude any function from this phenotype alone, because work on *l(1)zw10*, a gene with a similar phenotype in colchicine, suggests another interpretation.

The function of the late larval lethal gene *l(1)zw10* was originally proposed to be required to hold sister centromeres together, since larval brain preparations performed in the presence of colchicine revealed separated sister chromatids similar to those observed here for *ord* and *mei-S332* (Smith *et al.*, 1985). However, *l(1)zw10* neuroblasts without the addition of colchicine exhibited another phenotype, that of frequent lagging chromatids (Williams *et al.*, 1992), leading to the alternate hypothesis that *l(1)zw10*+ is required for the proper separation of sister chromatids at anaphase. This difference in phenotypes may reflect the possibility that colchicine-induced arrest of cells may have metabolic consequences beyond simply affecting spindle structure, as cyclin B levels remain high (Whitfield *et al.*, 1990) and the metaphase-like chromosomes are condensed beyond their normal non-colchicine-arrested
state (Gatti and Baker, 1989). Another complication may arise from the use of hypotonic solution in the colchicine squashing protocol: incubation in hypotonic medium disrupts cohesion between the chromatid arms (Gatti and Goldberg, 1991) and thus may also affect centromere cohesion in subtle ways. Because of the unknown effects that colchicine and hypotonic treatment may indirectly have on chromosome integrity, along with the call for cautious interpretation as evidenced by the l(1)zw10 case, we do not conclude any function attributable to ord or mei-S332 based solely on this separated sister chromatid phenotype.

**Genetic overlap of mitotic and meiotic functions**

Many Drosophila genes have been found to function in a similar or identical fashion in mitosis and meiosis, presumably reflecting the considerable similarities between these cell cycles. Functions carried out by these genes span the temporal progression of events of the cell division cycle. Thus, shared between meiosis and mitosis are genes involved in DNA metabolism (mei-9 and mei-41, Baker, et al., 1978), proper spindle function (mgr, Gonzales et al., 1988; polo, Sunkel and Glover, 1988, Llamazares et al., 1991, Fenton and Glover, 1993; asp, Ripoll, et al., 1985), chromosome segregation (l(1)zw10, Williams et al., 1992; rod, Karess and Glover, 1989), and cytokinesis (l(1)d.deg-4 and l(3)7m62, cited in Gatti and Goldberg, 1991). On the other hand, there are genes that fulfill a strictly meiotic role with no detectable role in mitosis. In some cases the functions so defined have no obvious mitotic counterpart (e.g., the distributive system kinesin-like motor nod, Zhang et al., 1990), whereas in other cases the meiotic specificity is the result of a meiosis-restricted isoform or homolog of a mitotic gene (e.g., the testis-specific β-tubulin TubB85D (B2t), Kemphues et al., 1982, 1983; twine,

ord and mei-S332 appear to have different roles in mitosis, despite the apparent similarities in their proposed meiotic functions. We conclude that mei-S332+ function is only required for meiosis, based on failure to find mitotic phenotypes in the early mitotic cleavage divisions, larval neuroblasts, wing imaginal disc, and overall viability. However, the existence of a redundant gene for mitotic cohesion cannot be excluded. The small effect of mei-S332 on somatic clones produced in the abdominal cuticle (Baker et al., 1978) we attribute to second-site loci present on the mei-S332 chromosome employed in that study. In contrast, ord has exhibited mitotic defects in the male germline gonial divisions (Lin and Church, 1982; Miyazaki and Orr-Weaver, 1992). Although the ord female nurse cell phenotype also hints at a gonial division defect, we were unable to find adequate material that would permit observation of such a defect. In somatic cells, we found that tests for ord function were negative in neuroblasts and inconclusive in the wing clone assay. The requirement for ord function may be restricted to germline cells as opposed to somatic cells. If so, ord would be unique among the genes with both mitotic and meiotic roles examined to date. This specificity may indicate spatial and temporal control of ord expression, or alternatively the restricted expression of a gene with redundant function to the soma and not the germline. Ultimately, the cloning and expression patterns of ord and mei-S332, now well underway in the lab, should clarify the roles of these genes in mitosis.
ACKNOWLEDGMENTS

We gratefully acknowledge the kind assistance of Bruce Reed in demonstrating brain squashes and in suggesting the basic fuchsin staining of ovaries. We thank J. Myles Axton for engaging discussions in which it was suggested that colchicine treatment of neuroblasts would be informative. Finally, we thank Adelaide Carpenter for encouragement and helpful tips on the gonial cytology.
LITERATURE CITED


Appendix I.

Double or nothing: A Drosophila mutation affecting meiotic chromosome segregation in both females and males

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Whitehead Institute and Dept. of Biology, MIT

This work is in press:

Genetics (March 1994)

My contribution to this work was the female nondisjunction assays demonstrating temperature-sensitivity and X;4 cosegregation (Table 1).
ABSTRACT

We describe a novel Drosophila mutation, Double or nothing (Dub), that causes meiotic nondisjunction in a conditional, dominant manner. Previously isolated mutations in Drosophila specifically affect meiosis either in females or males, with the exception of the mei-S332 and ord genes which are required for proper sister-chromatid cohesion. Dub is unusual in that it causes aberrant chromosome segregation almost exclusively in meiosis I in both sexes. In Dub mutant females both nonexchange and exchange chromosomes undergo nondisjunction, but the effect of Dub on nonexchange chromosomes is more pronounced. Dub reduces recombination levels slightly. Multiple nondisjoined chromosomes frequently cosegregate to the same pole. Dub results in nondisjunction of all chromosomes in meiosis I of males, although the levels are lower than in females. When homozygous, Dub is a conditional lethal allele and exhibits phenotypes consistent with cell death.
**INTRODUCTION**

Meiosis is a specialized cell division that produces haploid gametes, permitting a diploid genome to be restored in the zygote after fertilization. The reduction of the chromosomes to a haploid number during meiosis is accomplished by two rounds of chromosome segregation that follow a single duplication of the DNA. The first meiotic division (meiosis I) differs from mitosis in that the two homologs pair and segregate. In both meiosis II and mitosis the replicated copies of each chromosome, the sister chromatids, segregate.

Organisms utilize several strategies to carry out the specialized aspects of meiosis I (Baker *et al.* 1976). The most common mechanism of homolog pairing and segregation involves the formation of synaptonemal complex and requires recombination for proper segregation (John 1990). Recombination is proposed to lead to the formation of chiasmata that serve as stable attachments between the homologs, persisting after the dissolution of the synaptonemal complex in diplotene until the metaphase I-anaphase I transition. The stable homolog attachments are thought to constrain the kinetochores so that they are oriented in opposite directions and attach to different spindle poles (Nicklas 1974). Mutations that reduce recombination result in nondisjunction in meiosis I.

Although recombination is a widely adopted solution to homolog segregation, alternatives exist. These have been best characterized in *Drosophila melanogaster*, where at least three mechanisms are postulated for segregating chromosomes in the absence of recombination.

Recombination normally occurs in Drosophila females, however the tiny fourth chromosome virtually never recombines yet segregates faithfully. Furthermore, recombination can be reduced or eliminated on the other
chromosomes by the presence of multiple inversions (Baker and Hall 1976). Nevertheless, these chromosomes segregate with high fidelity (Grell 1976). Mutations have been isolated that define a pathway for this segregation of nonexchange chromosomes. This pathway, called distributive segregation or more recently achiasmate segregation (Hawley and Theurkauf 1993), is used to segregate heterologous chromosomes as well as achiasmate homologous chromosomes. Separate mechanisms for these two types of events have been proposed based on the behavior of chromosomal rearrangements (Hawley et al. 1993). Nonexchange homologs appear to pair and segregate by a homology based mechanism, while the heterologous system segregates chromosomes based on size, shape, and availability (Grell 1976). Nonexchange chromosomes have been shown to disjoin correctly in the yeast S. cerevisiae, implying that this organism also has a mechanism for achiasmate segregation (Dawson, Murray, and Szostak 1986; Guacci and Kaback 1991; Sears, Hegemann, and Hieter 1992).

In Drosophila males there is no detectable recombination, and synaptonemal complex is not formed (Baker and Hall 1976; Meyer 1960; Rasmussen 1973). Mutations affecting distributive segregation in the female have no effect on meiosis I in the male, thus a distinct pathway must exist for homolog segregation in males. This mechanism has been most fully investigated for the sex chromosomes in which specific pairing sites are responsible for pairing and proper segregation (Cooper 1964). The cis-acting pairing site for the X and Y chromosomes has been localized to part of the rDNA repeat (McKee and Karpen 1990; McKee, Habera, and Vrana 1992). It appears that pairing sites also mediate autosomal segregation (McKee, Lumsden, and Das 1993, Yamamoto 1979).
The specificity of meiotic mutations isolated in Drosophila provides strong evidence for multiple pathways of chromosome segregation in meiosis I. For example, with two exceptions, all of the mutations affect meiosis only in the female or only in the male. The majority of mutations affecting chromosome segregation in the female reduce recombination (Baker and Hall 1976). Other mutations, also female specific, almost exclusively cause nondisjunction of nonexchange chromosomes. Mutations in the nod, Axs, ald, and mei-S51 genes belong to this class (Carpenter 1973; O'Tousa 1982; Robbins 1971; Zhang and Hawley 1990; Zitron and Hawley 1989). The ncd gene is unusual in that mutations in this gene result in aberrant segregation of both exchange and nonexchange chromosomes (Davis 1969). Trans-acting mutations affecting homolog segregation specifically in the male are not well defined.

Mutations in the mei-S332 and ord genes are unique because they result in nondisjunction in both sexes. They also differ from other mutations in exhibiting larger amounts of meiosis II nondisjunction (Davis 1971; Kerrebrock et al. 1992; Mason 1976; Miyazaki and Orr-Weaver 1992). mei-S332 and ord mutants show premature sister-chromatid separation in meiosis I, and therefore the products of these genes appear to maintain sister-chromatid cohesion in meiosis.

We describe a novel mutation in Drosophila, Double or nothing (Dub), that affects meiosis I in both females and males. This conditional dominant mutation causes nondisjunction predominantly of nonexchange chromosomes in female meiosis, but it also significantly disturbs the segregation of exchange chromosomes. When homozygous, Dub is a conditional lethal allele.
MATERIALS AND METHODS

Stocks: All Drosophila stocks and crosses were grown at 25°C (unless otherwise noted) on a standard mix of cornmeal, brewer's yeast, molasses and agar. All balancer chromosomes and all mutations other than Dub are described in Lindsley and Zimm (1992). C(1)RM, y^2 s u(w^a) w^a will be referred to in this paper as compound-X or \(\hat{X}\). \(Y^{SX}\) • \(Y^{L}\) y\(^+\), \(In(1)EN, y v f B\) was used as the compound-XY chromosome and is referred to as \(\hat{X}\hat{Y}\) in this paper. \(C(4)EN, ci eyR\) is referred to as \(44\). These compound chromosomes, the \(cv v f car\) and the compound autosome stocks are described in Kerrebrock et al. (1992). The FM7c balancer has the markers \(y^{31d} sc^{8} w^a sn^{X2} vof g^4 B\). The \(c wt px\) stock used in mapping was obtained from the Bloomington stock center. The deficiency Df(2R)PC4 was obtained from R. Lehmann (Whitehead Institute, Cambridge, MA). The TM3, Sb/ T(2;3)CyO, st Kg\(^V\) red Tb stock was obtained from W. Saxton.

Isolation of the Dub mutation: Double or nothing (Dub) is a mutation that was induced on a second chromosome, marked with J Sco, using the mutagen, ethyl methanesulfonate (EMS). It was isolated in a screen of 2034 chromosomes for new alleles of abo (abnormal oocyte) (Sandler 1970; Tomkiel, Pimpinelli, and Sandler 1991), and its isolation number was 1102. A female-specific meiotic defect as well as a maternal effect lethality are associated with abo\(^1\) (Carpenter and Sandler 1974; Sandler 1970). While the Dub mutation complemented the maternal effect, the frequency of nondisjunction in abo\(^1\)/Dub females was double that of Dub/+ females. However, no increase in nondisjunction was observed in abo\(^2\)/Dub females, suggesting that either
the *abo¹* interaction is allele specific or due to a locus elsewhere on the chromosome.

**Nondisjunction tests, calculation of recombination frequencies and exchange ranks**

For simultaneous measurement of *X* and *4* nondisjunction in females, *y/y+Y; C(4)EN, ci eyR* males were crossed to *y/y; spa⁷⁰/spa⁷⁰* females. Regular ova yielded yellow females (*XX; 4⁴/4*) and wild-type males (*XY; 4⁴/4*). Progeny trisomic for chromosome 4 were viable, but progeny haploid for chromosome 4 were essentially inviable. Any surviving haplo-4 Minute progeny were counted and recorded, but they were excluded from any calculations and are not reported in this paper. Exceptional-*X* ova produced yellow+ females (*XY/y+Y*) and yellow males (*X/O*). The number of these progeny was doubled for the adjusted total and for calculation of the nondisjunction frequency, because half of the exceptional-*X* ova were not recoverable (those producing *X/XX* and *O/Y* progeny). Exceptional-*4* ova produced sparkling-poliert progeny (*4⁴/4*) or cubitus-interruptus eyeless-Russian progeny (*4⁴⁴*). Although only half of the exceptional-*4* progeny were recovered, it was not necessary to double their number for calculations of nondisjunction frequency because only half of the normal-*4* ova were recoverable.

In the assay of female meiotic nondisjunction for Table 2, compound-*XY, v f B* males were crossed to *cv v f car/ly* females. Normal ova yielded Bar females (*XY/X*) and males wild-type for Bar (*X/O*). Exceptional-*X* ova yielded Bar males (*XY⁴/O*) and females wild-type for Bar (*X/X*). The number of exceptional progeny was doubled for the adjusted total and for calculation of the nondisjunction frequencies. The centromere-linked mutation, *carnation*, allowed diplo-*X* ova resulting from meiosis II nondisjunction (carrying two
sisters) and those resulting from meiosis I nondisjunction (carrying two homologs) to be distinguished. To calculate map distances, exchange events on the X chromosomes were counted. This was done by recording the phenotypes of the XO males resulting from normal-X ova, and by crossing the F₁ females resulting from diplo-X ova to compound-XY males and recording the phenotypes of F₂ X/O males to determine the markers on the parental chromosomes in the F₁ females. Mapping distances for the diplo-X ova were calculated as if the chromosomes had been isolated from independent ova carrying a single X chromosome. Exchange rank distributions were calculated by the method of Weinstein (1936) for regular-X progeny and by the method of Davis (1969) and Merriam and Frost (1964) for diplo-X progeny.

In the assay of female meiotic nondisjunction for Table 5, compound-XY, v fB males were crossed to y/FM7c, y B females. Regular ova yielded yellow+ females (X/XY and FM7c/XY) and yellow males (X/O or FM7c/O). Exceptional ova yielded yellow females (FM7c/X and X/X) and yellow+ males (XY/O). Because particular classes of progeny from regular ova had reduced viability (the FM7c/O and FM7c/XY progeny), these classes were not used in the adjusted total and calculations. Consequently, the number of exceptional progeny did not need to be doubled.

An unexpected class of progeny was noted in this cross, yellow Bar males with vermilion+ eyes. Although their external appearance was entirely male, these "males" were infertile and their testes had a glittering appearance. This phenotype resembled the crystals observed in X/O males that result from overexpression of the Stellate protein in the absence of the Y chromosome (Livak 1984). We believe the "males" were actually intersexes (FM7c/X; 2/2/2; 3/3/3; 4/4 or 4/4/4) resulting from nondisjunction of autosomes
as well as the X chromosomes. The ova that produced the intersexes would have produced triploid females if fertilized by XY sperm, but these triploid females had a phenotype not easily distinguishable from the products of normal ova (X/X\(^Y\)). To ask if the triploid females were present, we outcrossed approximately 20 of the supposed X/X\(^Y\) females (excluding any vermilion-eyed FM7c/X\(^Y\) females), and we observed male progeny with the phenotype expected of the balancer, FM7c. These male progeny revealed the presence of one or more X/FM7c/X\(^Y\) triploid mothers among the 20 supposed X/X\(^Y\) mothers. We estimated that as many triploid females existed as intersexes, and the estimated number of the triploid females was subtracted from the normal ova for the adjusted total and for calculation of nondisjunction frequency. The intersexes were also not included in calculation of the X chromosome nondisjunction frequency.

In the nondisjunction assay performed for Table 6, y males were mated with compound-X/y+Y females. Normal ova yielded yellow females (X\(^X/X\)Y) and yellow+ males (X/y+Y). Exceptional ova yielded yellow+ females (X\(^X/y+Y/Y\)) and yellow males (X/O). Only half of the normal ova were recoverable, so doubling of exceptional classes was not necessary. However, females carrying two Y chromosomes have reduced viability (Lindsley and Zimm, 1992), so the number of exceptional ova (X\(^XY/Y\) and X/O) was estimated as twice the number of yellow males (X/O) for the adjusted total and calculation of the nondisjunction frequencies.

For simultaneous measurement of the sex and fourth chromosome nondisjunction in males, y/y; C(4)EN, ci ey\(^R\) females were mated with y/y+Y; spa\(^pol\) males. Normal sperm yielded yellow females (X/X; 4\(^4/4\)) and yellow+ males (X/Y; 4\(^4/4\)). As in the female test of X and 4 nondisjunction, any surviving haplo-4 Minute progeny were counted but were excluded from any
calculations and are not reported in this paper. Sperm that were diplo or nullo for the sex chromosomes produced yellow+ females \((X/X/y^+Y)\) and yellow males \((X/O)\). Exceptional-4 sperm produced sparkling-poliert progeny \((4/4)\) or cubitus-interruptus eyeless-Russian progeny \((4^4//O)\).

To determine the meiotic division affected in males, compound-\(X, y^2su(w^a)\) \(w^a\) females were mated with \(y/y^+Y\) males. Normal sperm yielded yellow+ females \((XX/y^+Y)\) and yellow males \((X/O)\). Exceptional sperm yielded yellow or yellow\(^2\) females \((X/X\) and \(XX/O)\) and yellow+ males \((X/y^+Y)\). The females resulting from sperm carrying two sister chromosomes \((X/X)\) were yellow and had a wild-type eye color, whereas exceptional females resulting from nullo-XY sperm \((XX/O)\) were yellow\(^2\) and had a darker eye color with no pseudo-pupil.

**Mapping of Dub** The mutation was first mapped to the interval between \(nw\) and \(Pin\) in two small scale mappings (15 and 47 recombinants). Females heterozygous for \(J Sco Dub\) and \(S Sp Tft nw^D\) \(Pin\) were mated with \(abo^1\) males, and the female progeny were mated with compound-XY males to test for skewed sex ratios or for nondisjunction events in the progeny. No sex ratio skewing was apparent, and nondisjunction events were used to map the mutation. \(Dub\) was later mapped to the smaller interval between \(c\) and \(wt\).

After mating \(c\) \(wt\) \(px\) males to \(pr\) \(cn\) \(Dub/c\) \(wt\) \(px\) or \(pr\) \(cn\) \(Dub\) \(sp/c\) \(wt\) \(px\) females, recombinant chromosomes from male progeny were isogenized and tested for three phenotypes: inviability when transheterozygous with the original \(pr\) \(cn\) \(Dub\) chromosome, dominant meiotic nondisjunction in females, and dominant meiotic nondisjunction in males. In 33 recombinants between \(c\) and \(wt\) all three phenotypes mapped to 2-82.6 cM.
Lethal phase and phenotypes The lethal phase of *Dub* homozygotes was assessed by mating parents heterozygous for *Dub* (*pr cn Dub/ b pr*). As controls, heterozygous parents were outcrossed to *b pr* mates and, in addition, a mating of *b pr* males and females was set up. The females were allowed to lay their eggs overnight on apple juice-sucrose-agar petri dishes with a wet yeast smear on the surface. The number of clear unfertilized eggs, the number of eggs that hatched, the number of pupal cases and the number of eclosed adults were all recorded. From these counts, a histogram of lethality was constructed.

To examine the pupal lethal phenotype of *Dub*, heterozygous larvae and homozygous larvae were sorted by using the larval mutant phenotypes, Tubby and Kugel (Saxton *et al.* 1991). After *pr cn Dub/SM1* and *TM3, Sb/T(2;3) CyO, st KgV red Tb* flies were mated, the resulting *pr cn Dub/T(2;3) CyO, st KgV red Tb* progeny were crossed *inter se* to give *Dub* homozygotes. The non-Tubby, non-Kugel larvae were moved to new plates and the range of larval and pupal phenotypes was observed.

Neuroblast squashes for mitotic chromosomes Cytological preparations of larval brains were made by standard methods without colchicine (Gonzalez *et al.* 1991; Sunkel and Glover 1988). These were examined by phase-contrast microscopy using a Zeiss Axiophot equipped with Plan Neofluar 100X and Plan Apochromat 63X objectives.
RESULTS

{
Dub is a conditional dominant mutation that causes nondisjunction during meiosis I in females

The EMS-induced mutation, Dub, was discovered in a screen because it exhibited an increased frequency of X chromosome nondisjunction during female meiosis. We have examined meiosis in females carrying Dub, using genetic assays to ask whether all chromosomes are affected and which of the meiotic divisions is defective. Nondisjunction produces aneuploid ova, referred to as exceptional ova. By mating mutant females to males carrying compound chromosomes, exceptional gametes could be recovered and the frequency of nondisjunction quantified.

In a cross of heterozygous mutant females to males carrying marked sex chromosomes and a compound-4 (see Materials and Methods), the frequencies of meiotic nondisjunction of the X and fourth chromosomes were measured at two temperatures. Dub was found to increase nondisjunction of both chromosomes in a dominant and temperature-sensitive manner (Table 1). We were not able to test homozygous Dub females in this assay, because as described below, Dub has a recessive, temperature-sensitive lethality. The frequency of fourth chromosome nondisjunction was much higher in Dub females than in control females, yielding 34.8% exceptional ova relative to 0.3%. Nullo-4 ova outnumbered diplo-4 ova, suggesting that some chromosome loss occurred in addition to nondisjunction. Nondisjunction of the X chromosome occurred at a frequency of 16.4%, much higher than the control frequency of 0.5%. Nullo-X ova outnumbered diplo-X ova.

To assess whether nondisjunction of the large autosomes occurs in Dub females, males carrying compound autosomes were mated with mutant and
### TABLE 1.
**Dub** IS A DOMINANT CONDITIONAL MUTATION INCREASING FEMALE MEIOTIC NONDISJUNCTION FREQUENCY

<table>
<thead>
<tr>
<th>Ova type</th>
<th>Temperature and maternal genotype</th>
<th>25°C</th>
<th>18°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Regular ova</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X; 4</td>
<td></td>
<td>1331</td>
<td>945</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1063</td>
<td>822</td>
</tr>
<tr>
<td>X nondisjunctional ova</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XIX; 4</td>
<td></td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>O; 4</td>
<td></td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4 nondisjunctional ova</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X; 4/4</td>
<td></td>
<td>1</td>
<td>192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>39</td>
</tr>
<tr>
<td>X; O</td>
<td></td>
<td>2</td>
<td>247</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>41</td>
</tr>
<tr>
<td>X;4 nondisjunctional ova</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XIX; 4/4</td>
<td></td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>XIX; O</td>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O; 4/4</td>
<td></td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O; O</td>
<td></td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total progeny scored</td>
<td></td>
<td>1337</td>
<td>1520</td>
</tr>
<tr>
<td>Adjusted total scored</td>
<td></td>
<td>1340</td>
<td>1656</td>
</tr>
<tr>
<td>% nondisjunction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>0.45</td>
<td>16.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.73</td>
<td>2.17</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.37</td>
<td>34.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.82</td>
<td>9.33</td>
</tr>
</tbody>
</table>

*ly*, *spa*[,y] females of the indicated genotype were crossed to *ly*[,y]+; *C(4)RM*, *ci ey*[,] males.

*a* X nondisjunctional progeny were doubled for calculation of nondisjunction frequency (see Materials and Methods).
wild-type females in identical numbers, e.g. 10 males and 15 females per vial. This assay gave only a qualitative assessment of autosomal nondisjunction. Ova with the normal autosomal content will not yield viable progeny when fertilized by sperm from a male carrying a compound autosome. The sperm will carry the equivalent of either two or no copies of the autosome, and trisomy or monosomy for either the second or third chromosome is lethal in Drosophila zygotes. However, a female with frequent nondisjunction events will produce exceptional ova, and these may be fertilized by sperm with a compensatory number of autosomes such that viable zygotes are produced. Viable progeny resulted approximately ten-fold more frequently in vials containing mutant females. In crosses to C(2)EN the Dub females produced on average 27 progeny per vial, while the control females produced two. In crosses to C(3)EN, Dub females produced an average of 55 progeny per vial, but the control siblings produced only three. Therefore, Dub affects all four chromosomes.

To ascertain whether chromosome missegregation events were occurring in the first or second meiotic division, we mated Dub females to males carrying a compound-XY chromosome. The mutant females carried X chromosomes heterozygous for a centromere-linked marker, carnation (car), so that diplo-X exceptional progeny carrying two sister chromosomes could be distinguished from those carrying two homologous chromosomes. Nondisjunction occurred almost exclusively during the first meiotic division (Table 2), because essentially all of the exceptional ova carried two homologous chromosomes. The lower percentage of nullo-X relative to the number of diplo-X ova observed in Table 2 is likely due to cosegregation events of the X and 4, since the nullo-X nullo-4 ova are inviable in this assay. Cosegregation is discussed in further detail below.
TABLE 2. *Dub* FEMALE MEIOTIC NONDISJUNCTION PRODUCES REDUCTINAL EXCEPTIONS

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>+</th>
<th>+</th>
<th><em>Dub</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ova type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular ova</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>5514</td>
<td>5268</td>
<td></td>
</tr>
<tr>
<td>X nondisjunctional ova</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>3</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>X/X  <em>car</em>/<em>car</em></td>
<td>1</td>
<td>335</td>
<td></td>
</tr>
<tr>
<td>X/X  <em>car</em>/<em>car</em> and <em>car</em>/<em>car</em></td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total progeny scored</td>
<td>5518</td>
<td>5850</td>
<td></td>
</tr>
<tr>
<td>Adjusted total scored</td>
<td>5522</td>
<td>6432</td>
<td></td>
</tr>
<tr>
<td>% nullo-X</td>
<td>0.11</td>
<td>7.65</td>
<td></td>
</tr>
<tr>
<td>% diplo-X (car+/car-)</td>
<td>0.04</td>
<td>10.42</td>
<td></td>
</tr>
<tr>
<td>% diplo-X (car-/car- and car+/car+)</td>
<td>0.00</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Total % nondisjunction</td>
<td>0.15</td>
<td>18.10</td>
<td></td>
</tr>
</tbody>
</table>

*yellow* *ey* *car* females of the indicated genotype were mated to compound-XY, *y f B* males at 25°C.

*a* The ratio of regular ova fertilized by nullo-XY sperm relative to *XY* sperm is 2445/3069 for the control females and 2010/3258 for the *Dub* females.

*b* *car*/*car* ova were distinguished from *car*/*car* ova by outcrossing a sample of 100 progeny that were non-carnation. No carnation+ homozygotes were observed. Consistent with this observation, the number of *car*/*car* ova should have been approximately equal to the number of *car*/*car* ova and only one carnation homozygote was observed.

*c* *X* nondisjunctional progeny were doubled for calculation of nondisjunction frequency (see Materials and Methods).
In these matings of *Dub* heterozygous mothers there was a low but significant number of gynandromorphs. These result from chromosome instability in the early zygotic cleavages, either due to chromosome loss during the mitotic divisions or recovery by a mitotic spindle of a chromosome lost during a meiotic division. Other meiotic mutations, notably *nod* and *ncd*, show a similar phenotype (Carpenter 1973; Davis 1969).

**Dub has little effect on recombination**

Since the majority of mutations that affect the first meiotic division in *Drosophila* females cause a reduction in recombination, we examined the effect of *Dub* on recombination. The *X* chromosomes used in the cross for Table 2 were heterozygous for several recessive mutations, and map distances were calculated from the phenotypes of the regular XO male progeny. Surprisingly, although *Dub* causes reductional nondisjunction, it has relatively little effect on recombination. There were slight reductions in all of the intervals, but only one interval showed a significant difference (Table 3, Mono-X ova). The *Dub* and control values were significantly different for the *vermillion-forked* (*v-f*) interval (binomial distribution test, p < 0.01), but there was no significant difference for the other intervals (Lindren, McElrath, and Berry 1978).

Recombination distances were also assessed in the diplo-*X* exceptional female progeny (Table 3, Diplo-*X* ova), and there was a significant reduction in exchange for all intervals except the most distal (binomial distribution test, p < 0.001, except the most distal interval p > 0.2). Since we were unable to score the recombination levels in nullo-*X* exceptional gametes, we could not detect whether nonexchange chromosomes were preferentially lost. If this were the case, the effect of *Dub* on recombination would be underestimated.
TABLE 3. *Dub* HAS LITTLE EFFECT ON RECOMBINATION IN FEMALES

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mono-X ova</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Number of progeny scored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mapping interval</td>
<td>y-cv (cM)</td>
<td>cv-v (cM)</td>
<td>v-f (cM)</td>
<td>f-car (cM)</td>
<td>Total map distance (cM)</td>
</tr>
<tr>
<td>+/+</td>
<td>8.4</td>
<td>19.5</td>
<td>21.7</td>
<td>6.5</td>
<td>56.1</td>
<td>2445</td>
</tr>
<tr>
<td>[D<em>ub</em>]</td>
<td>7.6</td>
<td>19.2</td>
<td>18.5</td>
<td>5.5</td>
<td>50.8</td>
<td>2010</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diplo-X ova</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Number of progeny scored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mapping interval</td>
<td>y-cv (cM)</td>
<td>cv-v (cM)</td>
<td>v-f (cM)</td>
<td>f-car (cM)</td>
<td>Total map distance (cM)</td>
</tr>
<tr>
<td>[D<em>ub</em>]</td>
<td>6.6</td>
<td>9.9</td>
<td>12.9</td>
<td>2.1</td>
<td>31.5</td>
<td>167&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Both chromosomes in these progeny came from the mother, so a total of 334 chromosomes were scored for exchange.
Exchange in the proximal regions appeared to ensure proper disjunction better, because nondisjunction was more likely to be accompanied by exchange in the distal regions. This distribution of exchanges is reminiscent of that in the diplo-X and diplo-2 exceptions of nod and nod$^{D T W}$ (Carpenter 1973; Rasooly et al. 1991).

**Dub primarily affects nonexchange but also exchange chromosomes**

Several observations suggested that Dub might not affect the exchange-mediated and achiasmate segregation systems equally. The greater nondisjunction of chromosome 4 relative to the X chromosome (Table 1) is consistent with disruption of the distributive system, since the fourth chromosomes are achiasmate in Drosophila. The exceptional progeny resulting from diplo-X ova showed a reduction in map distances while the normal progeny did not (Table 3), and the reduced amount of exchange was likely to be the result of a bias for nondisjunction of nonexchange chromosomes.

To address the question of whether Dub predominantly affects nonexchange chromosomes, we compared the percentage of nonexchange tetrads present in the ova having faithfully segregated chromosomes with the percentage in ova having improperly segregated chromosomes. When there is no exchange in a tetrad, chromosomes are segregated by the distributive system, so the number of nonexchange tetrads reflects the number of chromosomes that must be segregated in the distributive system. Therefore if nonexchange chromosomes were more likely to nondisjoin in Dub mutants, a greater proportion of the exceptional ova would be derived from nonexchange tetrads. The frequency of nonexchange, single exchange and multiple exchange tetrads (known as the tetrad or exchange rank) may be
estimated from the observed number of no crossover, single crossover and multiple crossover chromosomes. Appropriate equations have been developed for calculating the tetrad ranks from normal and diplo-X ova (Davis 1969; Weinstein 1936).

The percentage of nonexchange tetrads in the exceptional ova was much greater than the percentage in the normal ova (Table 4). The normal mono-X ova had a tetrad rank similar to the control, however there was a slight decrease in double exchange tetrads and a slight increase in single exchange tetrads. In contrast, the exceptional ova arising from Dub females had a decrease in all exchange tetrads and an increase in nonexchange tetrads. Therefore, nonexchange tetrads are more vulnerable to nondisjunction than are exchange tetrads in a heterozygous Dub background.

The hypothesis that the distributive system is disrupted in Dub females predicts that a chromosome pair that does not undergo exchange will experience higher rates of nondisjunction. To test this, we assayed nondisjunction of a balancer X chromosome heterozygous with a normal X chromosome. The rearrangements on the balancer FM7c have been estimated to suppress recombination completely (Hawley et al. 1993). In Dub females bearing FM7c and a normal X chromosome, the nondisjunction frequency dramatically increased to 52.3% compared to 16.4% for the normal X chromosome (Table 5). This suggests that the effect of Dub on distributive segregation was at least two- to three-fold greater than the effect on exchange-mediated segregation.

We tested the effect of Dub on the achiasmate segregation system in one other way. An example of the distributive segregation system in Drosophila is the consistent and faithful segregation of a Y chromosome from a compound-X chromosome in females (Grell 1976). These chromosomes are
<table>
<thead>
<tr>
<th>Exchange rank</th>
<th>Mono-(X) ova</th>
<th>Diplo-(X) ova</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal genotype</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Dub</td>
</tr>
<tr>
<td>(E_0)</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>(E_1)</td>
<td>0.62</td>
<td>0.79</td>
</tr>
<tr>
<td>(E_2)</td>
<td>0.25</td>
<td>0.10</td>
</tr>
<tr>
<td>(E_3)</td>
<td>0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Number scored</td>
<td>(2445)</td>
<td>(2010)</td>
</tr>
</tbody>
</table>
TABLE 5.
Dub females with a balancer X chromosome have very high meiotic nondisjunction frequencies

<table>
<thead>
<tr>
<th>Ova type</th>
<th>Maternal genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ (+)</td>
</tr>
<tr>
<td>Regular ova&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>2156</td>
</tr>
<tr>
<td>FM7c</td>
<td>737</td>
</tr>
<tr>
<td>X nondisjunctional ova</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>9</td>
</tr>
<tr>
<td>X/FM7c</td>
<td>14</td>
</tr>
<tr>
<td>XIX and FM7c/FM7c</td>
<td>0</td>
</tr>
<tr>
<td>Autosomal nondisjunctional ova&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>X/FM7c; 2/2; 3/3; 4 or 4/4</td>
<td>0</td>
</tr>
<tr>
<td>Total progeny scored</td>
<td>2916</td>
</tr>
<tr>
<td>Adjusted total scored&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2179</td>
</tr>
<tr>
<td>% nullo-X</td>
<td>0.41</td>
</tr>
<tr>
<td>% diplo-X</td>
<td>0.64</td>
</tr>
<tr>
<td>Total % nondisjunction</td>
<td>1.05</td>
</tr>
</tbody>
</table>

*F7/F7c females of the indicated genotype were mated with compound-XY, v f B males. The control females were SM1/+.

<sup>a</sup> The ratio of regular X ova fertilized by nullo-XY sperm relative to X<sup>+</sup> sperm is 1225/931 for the control females and 457/480 for the Dub females (160 triploid female progeny have already been subtracted from the XIX<sup>+</sup> progeny for the Dub ratio).

<sup>b</sup> These progeny were observed as intersexes, and this number represented only half of the number of such ova (see Materials and Methods).

<sup>c</sup> Calculation of the X chromosome nondisjunction frequencies was done using adjustments described in Materials and Methods. These adjustments compensate for the presence of autosomal nondisjunction and the reduced viability of the progeny resulting from regular ova carrying FM7c.
segregated by the achiasmate system even though exchange does occur between the two X chromosome arms of a compound-X chromosome. Mutations such as ncd, ald, and Axs have been shown to interfere with this segregation (Davis 1969; O'Tousa 1982; Zitron and Hawley 1989). In Dub females with a compound-X chromosome and a Y, the nondisjunction frequency was 40.9% compared to 0.6% in the control (Table 6). These experiments demonstrate that Dub affects the segregation of nonexchange chromosomes, but the mutation causes nondisjunction of exchange chromosomes as well. Dub did not reduce recombination enough for all of the nondisjoined chromosomes to be nonexchange (Tables 2 and 3), and in the diplo-X exceptional gametes almost half of the tetrads have undergone at least one exchange (Table 4).

Cosegregation of chromosomes in Dub mutant females

In Dub females when more than one chromosome was missegregated in the same ovum, these chromosomes were not segregated independently with respect to each other. By simultaneously following two chromosomes, the X and fourth (Table 1), we observed a strong tendency for the missegregating chromosomes to be incorporated into the same meiotic product. The double exceptions seen were not independently distributed among the possible classes: X/X; 4/4 and O;O double exceptions were more numerous than were X/X; O and O; 4/4 double exceptions. Such a non-random distribution among the double exceptions had been previously observed in the meiotic mutants nod and ncd for the X and fourth chromosomes (Carpenter 1973; Davis 1969; Wright 1974). This “cosegregation” behavior is in marked contrast to the non-random distribution of X; 4 double exceptions observed in Axs females,
<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ova type</td>
<td>+</td>
<td>Dub</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Regular ova</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\hat{X}\hat{X}$</td>
<td>1025</td>
<td>673</td>
</tr>
<tr>
<td>$Y$</td>
<td>1280</td>
<td>619</td>
</tr>
<tr>
<td>$X$ Nondisjunctional ova</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\hat{X}\hat{X}/Y$</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>$O$</td>
<td>7</td>
<td>447</td>
</tr>
<tr>
<td>Autosomal nondisjunctional ova$	extsuperscript{a}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\hat{X}\hat{X}/Y$; 2/2; 3/3; 4 or 4/4</td>
<td>2</td>
<td>39</td>
</tr>
<tr>
<td>$\hat{X}\hat{X}$; 2/2; 3/3; 4 or 4/4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Total progeny</td>
<td>2319</td>
<td>1844</td>
</tr>
<tr>
<td>Corrected total progeny$	extsuperscript{b}$</td>
<td>2319</td>
<td>2186</td>
</tr>
<tr>
<td>% nondisjunction$	extsuperscript{b}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\hat{X}\hat{X}/Y$ $\rightarrow$ $O$</td>
<td>0.60</td>
<td>40.90</td>
</tr>
</tbody>
</table>

Compound-$X$, $y^{2} su(w^{a}) w^{a}/y^{+}Y$ females of the indicated genotype were mated with $y$ males. The control females were $Sco^{+}$.

$	extsuperscript{a}$ These ova produced progeny that were either intersexes or diploid females.

$	extsuperscript{b}$ See Materials and Methods.
where the X bivalent is more likely to segregate away from the fourth bivalent, yielding X/X; O and O; 4/4 ova (Zitron and Hawley 1989).

Additional evidence indicated that cosegregation of all chromosomes occurred often. When a balancer X was introduced into Dub heterozygous females, intersexes and triploid females appeared among the progeny at a surprisingly high frequency (Table 5). The intersexes and triploid females resulted from ova carrying two copies of the major autosomes and one or two copies, respectively, of the X chromosome. Similarly, when a compound-X chromosome and a Y chromosome were present in a Dub heterozygous female, many intersexes and triploid females were found in the progeny (Table 6). Thus cosegregation of the sex chromosomes with the autosomes appeared to have occurred, although the number of X/X or XXY; 2/2; 3/3 ova could not be compared to the number of O; 2/2; 3/3 ova, because the latter were not recoverable. It is interesting that in the XXY cross, triploid females and intersexes were more likely to have received both the compound-X and the Y than to receive only the compound-X chromosome, as XXY; Y; 2/2; 3/3 ova were more frequent than XX; 2/2; 3/3 ova.

**Dub dominantly increases nondisjunction during meiosis I in males**

The first meiotic division in male Drosophila is distinct from the first division in females (Baker and Hall 1976). There is no recombination, and assembled synaptonemal complex is not observed (Meyer 1960; Rasmussen 1973). Instead, segregation of the homologs employs specific pairing sites. All of the previously isolated Drosophila meiotic mutants are specific in affecting only females or only males, with the exceptions of ord and mei-S332 (Davis 1971; Kerrebrock et al. 1992; Mason 1976; Miyazaki and Orr-Weaver 1992). These two mutations cause premature sister-chromatid separation
and have significant levels of meiosis II nondisjunction. Dub was striking because it caused meiotic chromosome nondisjunction in males and females, and in contrast to ord and mei-S332, meiosis I segregation was affected almost exclusively.

Meiotic nondisjunction in Dub males was characterized by genetic assays to test which chromosomes and which meiotic division were affected by Dub. In males, Dub acted to increase nondisjunction in a dominant and temperature-sensitive manner (Table 7). Both the sex chromosomes and the fourth chromosome were affected, and the frequency of fourth chromosome nondisjunction was lower than sex chromosome nondisjunction. Sperm that were nullisomic for the sex chromosomes were more common than were X/X or X/Y sperm, indicating that chromosome loss also occurred. The overall frequency of nondisjunction was lower in males than in females, the difference in fourth chromosome segregation being particularly great.

We have tested qualitatively whether the autosomes have an increased frequency of nondisjunction by crossing Dub males to compound autosome stocks by mating 10 males to 15 females in individual vials. The appearance of viable progeny was about ten-fold higher than what was observed when the same number of wild-type males were crossed to compound autosomal females. When Dub males were crossed to C(2)EN females an average of 26 progeny per vial were recovered, compared to four in wild-type controls. When Dub males were crossed to C(3)EN females an average of 30 progeny per vial were recovered, while less than one was produced by control males. Therefore, all chromosomes undergo nondisjunction in Dub heterozygous males.

By crossing test males to compound-X females we were able to assess the meiotic division in which missegregation was occurring (Table 8). The
TABLE 7.
Dub IS A DOMINANT CONDITIONAL MUTATION INCREASING THE MALE MEIOTIC NONDISJUNCTION FREQUENCY

<table>
<thead>
<tr>
<th>Sperm type</th>
<th>25°C</th>
<th></th>
<th>18°C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td><em>Dub</em></td>
<td>+</td>
<td><em>Dub</em></td>
</tr>
<tr>
<td>Regular sperm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X; 4</td>
<td>919</td>
<td>962</td>
<td>638</td>
<td>592</td>
</tr>
<tr>
<td>Y; 4</td>
<td>719</td>
<td>1064</td>
<td>421</td>
<td>413</td>
</tr>
<tr>
<td>XY nondisjunctional sperm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X/Y; 4</td>
<td>8</td>
<td>68</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>O; 4</td>
<td>6</td>
<td>91</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>4 nondisjunctional</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X; 4/4</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>X; O</td>
<td>5</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Y; 4/4</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Y; O</td>
<td>4</td>
<td>13</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>XY,4 nondisjunctional</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X/Y; 4/4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>X/Y; O</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O; 4/4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O; O</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total progeny scored</td>
<td>1668</td>
<td>2234</td>
<td>1074</td>
<td>1041</td>
</tr>
<tr>
<td>% nondisjunction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XY</td>
<td>0.96</td>
<td>7.48</td>
<td>0.93</td>
<td>2.59</td>
</tr>
<tr>
<td>4</td>
<td>0.96</td>
<td>2.19</td>
<td>0.56</td>
<td>1.06</td>
</tr>
</tbody>
</table>

\(y/y^+Y\) males of the indicated genotype were mated with \(y; C(4)EN, ci ey^R\) females.
TABLE 8. *Dub* MALE MEIOTIC NONDISJUNCTION YIELDS PRIMARILY REDUCTIONAL EXCEPTIONS

<table>
<thead>
<tr>
<th>Sperm type</th>
<th>+</th>
<th>Dub</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regular sperm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>X</em> or <em>Y</em></td>
<td>2816</td>
<td>5531</td>
</tr>
<tr>
<td><strong>XY nondisjunctional sperm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O</em></td>
<td>4</td>
<td>178</td>
</tr>
<tr>
<td><em>X/Y</em></td>
<td>3</td>
<td>105</td>
</tr>
<tr>
<td><em>X/X</em></td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total progeny scored</td>
<td>2823</td>
<td>5829</td>
</tr>
<tr>
<td>% nullo-XY</td>
<td>0.14</td>
<td>3.05</td>
</tr>
<tr>
<td>% <em>X/Y</em></td>
<td>0.11</td>
<td>1.80</td>
</tr>
<tr>
<td>% diplo-X</td>
<td>0.00</td>
<td>0.26</td>
</tr>
<tr>
<td>Total observed nondisjunction</td>
<td>0.25</td>
<td>5.11</td>
</tr>
</tbody>
</table>

*y/y+Y* males of the indicated genotype were mated with compound-*X, y2 su(wa)* *w* females. The control in this experiment was not done with siblings of the *Dub/+* males.

In this assay, *Y/Y* exceptional sperm were indistinguishable from regular mono-*Y* sperm and are therefore included in these numbers.
first meiotic division was primarily affected; however, missegregation did not appear to be as exclusive to the reductional division as it was in *Dub* heterozygous females. The number of equational exceptions was higher than observed in the control, although the frequency was still less than 1%. Because the progeny from *Y/Y* sperm were indistinguishable from normal progeny, only half of the equational exceptions were scored in this test. Consequently, the true frequency of equational missegregation was probably twice what we measured.

The cosegregation of heterologous chromosomes that nondisjoined was difficult to address in male *Dub* heterozygotes. Since the nondisjunction frequencies in *Dub* males were already low, the number of double exceptions was too low to conclude whether cosegregation of the sex and fourth chromosomes occurred. However, when *Dub* males were outcrossed, triploid females and intersexes appeared more frequently than in wild-type crosses (data not shown). Therefore, it appears that cosegregation of the autosomes occurred.

**Dub** is a recessive, conditional lethal mutation

The dominant meiotic phenotype of *Dub* is linked closely to a conditional recessive lethality. At 25°C, homozygous *Dub* adults were rare. The rare escapers were very short-lived and had many defects: small rough eyes, etched tergites, crumpled or nicked wings, and bristles either missing or duplicated. At 18°C, homozygous *Dub* progeny were more common, although at most 20% of the expected number of homozygotes eclosed in bottles of the heterozygous stock. Homozygous adults raised at 18°C were more normal in appearance, except for patches of disorder in the eye facets. These flies were infertile.
Recessive lethality and the phenotype of the rare escapers are characteristics observed in mutations affecting mitotic chromosome segregation, such as *rough deal (rod)* (Karess and Glover 1989). The presence of gynandromorphs among the progeny of heterozygous *Dub* mothers also suggested that *Dub* product might play a role in mitosis. To test this, we determined the lethal phase and phenotype of *Dub* homozygotes, and we then cytologically examined neural cells of homozygous larvae for mitotic defects. Most known mitotic mutants have late-larval/pupal lethality, although a few embryonic lethal mitotic mutants are known (Edgar and O'Farrell 1989; Gatti and Baker 1989; Hime and Saint 1992).

To determine the lethal phase of *Dub* homozygotes, heterozygous parents were mated and the fate of their eggs was quantitated. One quarter of the progeny should have been homozygous, but about half of the progeny died (Figure 1). Therefore there appeared to be two causes of lethality, homozygous lethal animals and a dominant lethal effect of *Dub*. Control matings of a heterozygous parent and a wild-type parent showed 8-12% embryonic lethality. In contrast, when both parents were *Dub* heterozygotes, there was about 25% pupal lethality in addition to embryonic lethality (Figure 1). *Dub* homozygotes were most likely to account for the pupal lethality.

The embryonic lethality that occurred when either parent was a *Dub* heterozygote appears to have been the consequence of autosomal aneuploidy due to meiotic nondisjunction, rather than a semi-dominant lethal effect of

---

Figure 1. Lethal phase of *Dub* mutants at 25°C. The indicated crosses were done, eggs were collected, and lethality at the embryonic, larval, and pupal stages was scored. Flies designated here as wild type were *b pr*. The *Dub/+* flies were *pr cn Dub/b pr*. 885 fertilized eggs were examined for cross A, 1468 eggs for cross B, 430 eggs for cross C, and 1016 eggs for cross D.
Lethal Phase

- embryonic
- larval
- pupal

Percentage of Fertilized Eggs

Cross

A  B  C  D

Male Genotype:  +  Dub/+  Dub/+  +
Female Genotype: +  Dub/+  +  Dub/+
Dub, or a maternal-effect lethality. We found that Dub had no semi-dominant lethality by crossing pr cn Dub/ pr cn bw males to pr cn bw females and then counting the ratio of Dub+ and Dub progeny (data not shown). Maternal lethality seemed unlikely as there was a similar degree of embryonic lethality when either the mother or father was a Dub heterozygote (Figure 1).

Pupal lethality produced by heterozygous mothers (Figure 1, cross D) was five-fold greater than the pupal lethality seen in a cross performed in the opposite direction (Figure 1, cross C). This increased lethality was likely due to aneuploidy resulting from meiotic nondisjunction of chromosome 4. The frequency of nullo-4 gametes was much higher in females than in males (20.2% relative to 1.3%). The haplo-4 progeny that would result from such gametes are only rarely viable: many die during the pupal phase, and the rare survivors have a Minute phenotype.

To investigate the lethal phenotype of larval and pupal homozygotes, the dominant mutations Tubby and KugelV were used as larval markers for heterozygotes. The homozygous larvae were normal in size but were lethargic; they rarely wandered or pupated outside of the food. The larvae were missing some imaginal discs, and most discs were reduced in size. However, the brains appeared normal in size. The homozygous pupae showed a range of lethal phenotypes such as melanotic tumors, rough eyes, missing or duplicated bristles, and missing body parts (data not shown). We interpret these phenotypes as a result of random cell death.

To ask whether mitotic chromosome missegregation might be yielding aneuploid cells and consequent cell death, we examined larval neuroblast squashes from 10 Dub homozygotes. Surprisingly, these squashes did not
have any apparent chromosome segregation defects, and aneuploidy was not observed in any of the metaphase figures.

The nature of the *Dub* mutation

We identified a deficiency that uncovers *Dub* in order to determine if the dominant phenotype was due to a haplo-insufficient locus or if the mutation was hypermorphic. *Df(2R)PC4* was semi-viable when heterozygous to *Dub*. Moreover, the cytological location of the deficiency is consistent with the map position of *Dub*.

Many of the deficiency transheterozygotes died during the pupal phase and frequently could only eclose halfway. Adult transheterozygotes that did escape from the pupal case showed phenotypes similar to *Dub* homozygous pupae and to rare adult escapers raised at 25°C. Their eyes had a rough appearance with facets often fused and disorganized overall. The tergites were often etched, and the wings were frequently nicked along the edges or were blistered. Both males and females were sterile. The increased viability of *Dub* heterozygotes relative to *Dub* hemizygotes suggested that the mutation is not hypermorphic, at least with regard to the lethal phenotype.

We examined whether the locus is haplo-insufficient for the meiotic phenotype by mating females heterozygous for the *Df(2R)PC4* deficiency with males carrying the compound-XY. This test yielded no exceptional progeny, although approximately 850 progeny were scored (data not shown). Therefore it does not appear that the locus is haplo-insufficient for meiotic chromosome segregation. The mutation is most likely to be either antimorphic or neomorphic.
DISCUSSION

The Dub mutation

The dominant Dub mutation is the first mutation isolated in Drosophila melanogaster that affects the three known pathways of homolog segregation in meiosis I. Both nonexchange and exchange chromosomes in females undergo nondisjunction in Dub mutant females, and segregation of homologs is aberrant in mutant males. The segregation of all four chromosomes is disrupted in Dub mutant females and males.

Four results demonstrate that Dub causes nondisjunction of nonexchange chromosomes in females: 1) the achiasmate chromosome 4 undergoes nondisjunction at high frequencies in females; 2) diplo-X ova from Dub females show an increased percentage of nonexchange tetrads compared to normal, mono-X ova, indicating that nonexchange chromosomes are more likely to nondisjoin in the Dub mutant; 3) the segregation of compound-X chromosomes from a Y chromosome is affected by the Dub mutation, a segregation previously shown to be mediated by the distributive system (Grell 1976); and 4) nondisjunction frequencies for the X chromosome increase dramatically when it is made nonexchange by making it heterozygous with a balancer chromosome. The fact that both the segregation of chromosome 4 and the disjunction of a compound X from a Y chromosome are altered indicates that both the homologous and heterologous systems of achiasmate segregation are disrupted by the Dub mutation.

Although Dub predominantly affects nonexchange chromosomes, it also results in nondisjunction of exchange chromosomes. Dub reduces recombination frequencies only slightly, so the frequency of X chromosome nondisjunction (16-18%) in the female is too high to be the consequence of
failure of only nonexchange chromosomes to segregate. In addition, in diplo-
X exceptional ova, 49% of the tetrads had one or more exchange.

Dub mutant males also exhibit nondisjunction. The frequencies of
nondisjunction in the male are considerably less than in the female. As
discussed below, the interpretation of this difference depends on whether the
Dub mutation is antimorphic or neomorphic. If the mutation is antimorphic,
the requirement of the gene product in male meiosis may be lower than in
female meiosis, or redundant functions may exist in the male. If the allele is
neomorphic, it may not interfere with meiosis in the male to as great an
extent as in the female.

Dub differs from mutations in the ord and mei-S332 genes, which also
cause nondisjunction in both sexes, in that Dub causes nondisjunction in
meiosis I almost exclusively. In ord mutants, nondisjunction occurs in both
meiosis I and II in a ratio suggesting that the four sister chromatids of the
bivalent separate prematurely and then segregate randomly through two
divisions (Mason 1976; Miyazaki and Orr-Weaver 1992). Indeed, precocious
sister-chromatid separation is observed as early as prometaphase I in ord
mutants (Miyazaki and Orr-Weaver 1992). In contrast, mei-S332 mutations
result primarily in meiosis II nondisjunction (Kerrebrock et al. 1992).
Although the sister-chromatids also prematurely disjoin in mei-S332
mutants, the sister-chromatids do not separate until late in anaphase I
(Kerrebrock et al. 1992). Thus the ord and mei-S332 genes control the
behavior of sister chromatids, whereas the Dub mutation causes aberrant
segregation of the homologs.

The Dub mutation is conditional lethal when homozygous. The
homozygous larvae and pupae exhibit phenotypes indicative of extensive cell
death such as small or missing imaginal discs, melanotic tumors, rough eyes,
etched tergites, and missing bristles. This suggests that when homozygous the *Dub* mutation affects mitotic chromosome segregation. We observed gynandromorphs in the progeny of *Dub* mutant females, consistent with abnormal mitotic chromosome segregation. However, abnormal mitotic figures were not found in neuroblast squashes from homozygous *Dub* larvae at a frequency that could account for the observed cell death. One possibility is that *Dub* affects mitosis in tissues other than the brain. This is consistent with our observation that while the imaginal discs are small or missing in homozygous *Dub* larvae, the brain appears normal in size. An alternative possibility is that the homozygous mutation affects other cell processes in such a manner that results in cell death.

**Comparison of Dub with other mutations affecting nonexchange chromosomes**

Since few Drosophila mutations have been identified that cause nondisjunction of nonexchange chromosomes in the female, the relationship between *Dub* and these genes is of particular interest. Five previously characterized mutations affect achiasmate chromosomes: *ald, Axs, mei-S51, nod,* and *ncd.* *Dub* is most similar to *nod* and *ncd* in its phenotypes.

The *ald, Axs,* and *mei-S51* mutants differ from *Dub* in that in a background of normal X chromosomes they have low frequencies of chromosome 4 missegregation. Furthermore, segregation of a compound-X chromosome from a Y chromosome is more faithful in *ald* and *Axs* than in *Dub* mutants. *ald, Axs,* and *mei-S51* show nonhomologous disjunction of the X chromosomes from the fourth chromosomes, in contrast to *Dub* (O'Tousa 1982; Robbins 1971; Zitron and Hawley 1989).

*Dub* is similar to *nod* and *ncd* in showing high chromosome 4 nondisjunction and cosegregation of nondisjoined X and fourth chromosomes.
to the same pole (Davis 1969; Zhang and Hawley 1990). However, there is considerably less loss of chromosome 4 in Dub mutants than in nod or ncd. In terms of its effect on exchange and nonexchange chromosomes, Dub can be viewed as being intermediate between nod and ncd. nod causes almost exclusively nonexchange chromosomes to nondisjoin, whereas exchange chromosomes will nondisjoin in Dub mutants. ncd does not affect nonexchange chromosomes to as great an extent as does Dub. Dub, nod, and ncd all produce gynandromorph progeny.

It is interesting that both the nod and ncd genes encode proteins with homology to the kinesin microtubule motor, and the Ncd protein has been shown to have motor activity in vitro (McDonald and Goldstein 1990; McDonald, Stewart, and Goldstein 1990; Walker, Salmon, and Endow 1990; Zhang et al. 1990). Aberrant meiotic spindles are present in nod and ncd mutant oocytes (Hatsumi and Endow 1992; Theurkauf and Hawley 1992). Achiasmate chromosomes are not confined to the spindle in nod mutants, while in ncd oocytes the spindle structure itself is abnormal. The ends of the spindle do not taper to the pole, suggesting that the Ncd protein may act to bundle microtubules into a functional spindle. The similarities among the phenotypes of Dub, nod, and ncd in females, particularly the cosegregation of nondisjoined chromosomes that occurs in these mutants, raise the possibility that the meiotic spindle is defective in Dub mutants as well.

**Possible functions of the Dub gene in chromosome segregation**

The phenotypes of the Dub mutation support a role for the gene in an aspect of meiotic chromosome segregation common to female and male meiosis. However, the mutation we have characterized is a dominant allele that may be antimorphic or neomorphic. If Dub were antimorphic, its phenotype would be similar to loss-of-function alleles and would reflect the
function of the wild-type gene. Antimorphic and neomorphic alleles can be distinguished by the properties of the mutation in the presence of a duplication of the wild-type gene, but unfortunately a duplication covering \textit{Dub} does not exist.

Three other dominant meiotic mutations have been identified in \textit{Drosophila}, and these provide a precedent in the sense that the alleles have either been shown to be antimorphic or to have meiotic phenotypes similar to loss-of-function alleles. The initial allele of \textit{Axs} was dominant, while \textit{l(1)TW6cs} was shown to be a dominant mutation in \textit{nod} (now called \textit{nodDTW}). Revertants of these mutations were isolated and demonstrated to be loss-of-function mutations in the genes (Rasooly \textit{et al.} 1991, Whyte \textit{et al.} 1993). Analysis of the phenotypes of both the dominant and revertant alleles showed that in each case the dominant allele was antimorphic, and its phenotype provided an accurate indication of the role of the gene in meiosis. A third dominant mutation is an allele of \textit{ncd} that initially was dominant but has lost its dominance in the time since its isolation (Komma, Horne, and Endow 1991). Nevertheless, homozygotes for this allele showed the same meiotic effects as loss-of-function alleles.

It is possible that the \textit{Dub} gene regulates a fundamental aspect of homolog separation or spindle function that is used in the segregation of all classes of homologs in female meiosis and also in male meiosis. Since the dominant \textit{Dub} mutation has essentially no effect on meiosis II, it may control properties that are unique to the first meiotic division. Alternatively, redundant functions may exist in meiosis II, or the amount of wild-type \textit{Dub} product required for meiosis II may be lower than that needed for meiosis I.

The other possibility is that the wild-type \textit{Dub} gene controls only one pathway of homolog segregation, and the dominant allele may interfere with
segregation systems normally not controlled by the gene. Analogously, as a homozygote or a hemizygote \( nod^{DTW} \) affects mitotic chromosome segregation, even though loss-of-function alleles of \( nod \) affect only the segregation of nonexchange chromosomes in females (Rasooly et al. 1991). In addition, the dominant allele in higher dosage or at nonpermissive temperature will affect exchange chromosomes.

Loss-of-function mutations in the \( Dub \) gene, which can be obtained by reverting the dominant mutation, will reveal whether the wild-type gene is required in all pathways of meiotic chromosome segregation. These mutations will also permit possible functions of the gene in mitosis to be evaluated. Regardless of whether the dominant \( Dub \) mutation is antimorphic or neomorphic, understanding the manner in which it disrupts meiotic segregation will provide important insights into the mechanism of chromosome segregation in Drosophila meiosis.
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Appendix II.

The cloning of *ord*: A chromosome walk from *brown*

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In order to define molecularly the ord locus, we first mapped it cytogenetically by using various deficiencies in the area. We found that both Df(2R)bw$^{S46}$ and Df(2R)vir$^{130}$ failed to complement ord mutations; thus the breakpoints of these deficiencies would define the lower limits for the ord locus. By cytology of these deficiencies we knew that ord should lie in the salivary gland band 59D. The closely linked locus brown in 59E had been recently cloned (Dreesen et al., 1988), so we obtained a clone from the brown walk and began a walk proximally to try to cover the region of overlap between the deficiencies.

The cosmid library we used was made by John Tamkun and was unusual in two respects: it was made from DNA from a strain isogenic on all four chromosomes (and in fact, the 2nd chromosome was the one on which our new alleles of ord and mei-S332 were induced); and the vector contained NotI sites and P element ends flanking the insertion site, and also carried the screenable marker white$. These features made it quite easy to walk rapidly in this library, with the added bonus that isolated cosmids could be injected in germline transformation assays to ask for functional rescue of the mutant phenotype.

Five steps in this library were taken, and by in situ analysis we had crossed the Df(2R)vir$^{130}$ breakpoint in step 3 (Figure 1). However, a 'hole' was then encountered in this library, and also in two other independently constructed libraries. Luckily, Rolf Nöthiger was also interested in this

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**Figure 1.** Schematic drawing of the ord59D region. Cloned DNA is represented by heavy lines, whereas the shaded bars below indicate the DNA remaining in the deficiency stocks. The starting clone was obtained from a walk to isolate the brown locus; successive steps in the ord walk are numbered 1-5. The hole in the various cosmid libraries occurred after step 5, and could only be bridged with lambda clones from R. Nöthiger. All together, the region between the Df(2R)bw$^{S46}$ and Df(2R)vir$^{130}$ breakpoints is estimated to be about 150 kb.
Chromosome walk to *ord*

Cosmid Clones

Lambda Clones
(from R. Nothiger and R. Schneiter)

starting clone
(from T. Dreesen and S. Henikoff)

Deficiency Breakpoints

ord +  
Df(2R)HB132

ord -  
Df(2R)bwS46

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59D6-10

150 kb
region for the *virilizer* locus, and we obtained lambda and cosmid clones from him that eventually were shown to cover the region of overlap between *bw*<sup>546</sup> and *vir*<sup>30</sup>. Unfortunately, this region was estimated to be about 150 kb in length.

At this point Sharon Bickel, a post doc in the lab, began the task of isolating *ord* from this region. A deficiency generated by Dan Moore, a graduate student in the lab, allowed her to discount about 80% of that region from consideration. Rescue of the *ord* phenotype has been obtained in germline transformants with the remaining DNA, and transcript analysis is now underway.

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Appendix III.

Screen for P element alleles of *ord* and *mei-S332*

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My contribution to this work was in constructing the stocks, performing the pilot screen with UROP student Sarah Park, doing the screen crosses themselves, and doing the Southern blot testing for transposition.
Before the EMS alleles were isolated (Chapters 2 and 3), we had only a single allele each of ord and mei-S332. More alleles were needed to define further the functions of these genes in meiotic sister-chromatid cohesion. In addition, we wanted to be able to define these two loci molecularly. We decided to generate alleles using P element transposon tagging.

The crossing scheme for the P element screen is shown in Figure 1. We used the Birmingham 2 (Birm2) chromosome which contains 17 non-autonomous P elements that are unable to transpose by themselves, yet can be mobilized in trans by a transposase source. For the transposase source we selected Δ2-3, which can supply P element transposase yet which is itself unable to transpose (Robertson et al., 1988). Mutagenesis of the pr cn bw chromosome occurred when these two genetic factors were present in the same fly, and any mutations generated could be stabilized by the subsequent crossing away of the Δ2-3 chromosome. Because of the high level of transposition occurring when these two chromosomes were brought together (Engels et al., 1987), we had to perform the first cross at 16°C in order to prevent pupal lethality.

Our screen was designed to test for noncomplementation of both ord and mei-S332 by utilizing a double mutant chromosome. Noncomplementation was assayed by screening for nondisjunctional progeny of y/y+Y males mated to y/y females. Nullo-XY sperm from such males resulted in yellow males among wild-type brothers, and XY sperm resulted in yellow+ females among yellow sisters. 25,506 single male matings were set up to test for noncomplementation (Figure 1, cross 3). Of these crosses, approximately 18,000 were fertile enough to score for nondisjunctional progeny. Vials

Figure 1. Screen for P element alleles of ord and mei-S332
X1
MASS 16 C
\[ \frac{y}{y^+Y} : \text{Birm2} ; \text{ry}^{506} \]
\[ \text{SM1} \]
X
\[ \frac{\text{pr cn bw}}{\text{ry} \text{ Sb P[ry+\Delta2-3] TM6, Ubx}} \]

X2
MASS
\[ \frac{y}{y^+Y} : \text{Birm2} ; \text{ry Sb P[ry+\Delta2-3] ry}^{506} \]
\[ \text{SM1} \]
X
\[ y ; \frac{\text{cn mei-S332 ord ; spa}^{\text{pol}}}{\text{SM1}} \]

X3
SINGLE
\[ \frac{y}{y^+Y} : \frac{(\text{pr cn bw})^*}{\text{cn mei-S332 ord} \cdot \text{ry}^{506} \cdot \text{spa}^{\text{pol}}} \]
\[ + ; + \]
X
\[ y ; \frac{\text{Sp pr cn bw} ; \text{spa}^{\text{pol}}}{\text{CyO, bw}} \]

score for non-disjunction;
select males from vials giving high frequency of exceptional progeny

\[ \frac{y}{y^+Y} : \frac{(\text{pr cn bw})^*}{\text{Sp pr cn bw} ; \text{spa}^{\text{pol}}} \]
\[ \frac{\text{SM1}}{\text{cn mei-S332 ord ; spa}^{\text{pol}}} \]

re-test for non-disjunction phenotype

balanced stock
containing two or more such progeny were saved, and appropriate males were subsequently mated to generate a balanced stock and to re-test for the nondisjunction phenotype.

We did not recover any alleles of either ord or mei-S332 from this screen. P element transposition is not a random event; it might be the case that the ord and mei-S332 loci are refractory to P element transposition. To test molecularly for the efficiency of transposition, genomic DNA from 26 random lines (with the Birml2 and A2-3 chromosomes crossed away) were assayed by Southern blot with a P element probe. Fourteen of these lines contained any P element sequences at all, indicating that only about 50% of the 18,000 single male matings were mutagenized.

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