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-S332* 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^2 mutants chromosome segregation is aberrant in the mitotic divisions that produce the spermatocytes. The strongest *ord* alleles, ord^2 and ord^5, 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 chiasmate 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 RIESS 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 mutant flies (DAVIS 1971; GOLDSTEIN 1980; MASON 1976; SANDLER *et al.* 1968). The majority of Drosophila meiotic mutations have effects limited to one sex, and meiosis I appears to be under different genetic control in males and females (BAKER and HALL 1976). Drosophila females undergo recombination and form synaptonemal complex. In contrast, recombination does not occur in males and no synaptonemal complex is formed. Rather, homolog segregation is insured through specific pairing sites termed collochores (COOPER 1964; MCKEE and KARPEN 1990). *ord* and *mei-S332* are exceptional in that mutations in these genes affect meiosis in both males and females; therefore these genes probably define functions in meiotic pathways common to both sexes.

Although mutations in both *ord* and *mei-S332* lead to premature sister-chromatid separation, the timing of this separation differs between the two. The *mei-S332* mutant phenotype is not observed until mid to late anaphase I, after the time of kinetochore doubling in males (GOLDSTEIN 1981; KERREBROCK *et al.* 1992). However, ord^1 appears to act earlier than *mei-S332* in two respects: meiosis I disjunction is aberrant in ord^1 flies but rarely so in *mei-S332* mutants; and reciprocal recombination is greatly reduced in ord^1 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^1 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^1 represented the *ord* loss-of-function or null phenotype. Additional alleles were needed to determine if the timing differences between ord^1 and $mei\text{-}S332^1$ 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^1 being involved in somatic (BAKER, CARPENTER and RIPOLL 1978) or germline (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 germline.

MATERIALS AND METHODS

Stocks: All Drosophila stocks and crosses were raised at 25° on standard cornmeal-brewer's yeast-molasses-agar food. The deficiency *Df(2R)3-70* was isolated in this laboratory by DANIEL MOORE by recovering chromosomes having undergone X-ray-induced loss of a *P* element carrying the *white* gene inserted into 59C (the P[(w,ry)A]3-1 transformant, obtained from R. LEVIS) (HAZELRIGG, LEVIS 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-*XY*, and compound autosome stocks were described in KERREBROCK *et al.* (1992). 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 $Df(2R)bw^{S46}$ (SIMPSON 1983) 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^1 males to *a px or* females, allowed recombination to occur in heterozygous F_1 daughters, and selected for F_2 sons which carried second chromosomes recombinant in the *px* (2-100.5) to *or* (2-107.0) interval. These males were individually crossed to $ord^1/SM1$ females to stock the recombinant chromosome and to generate sons carrying the px^- or^+ or

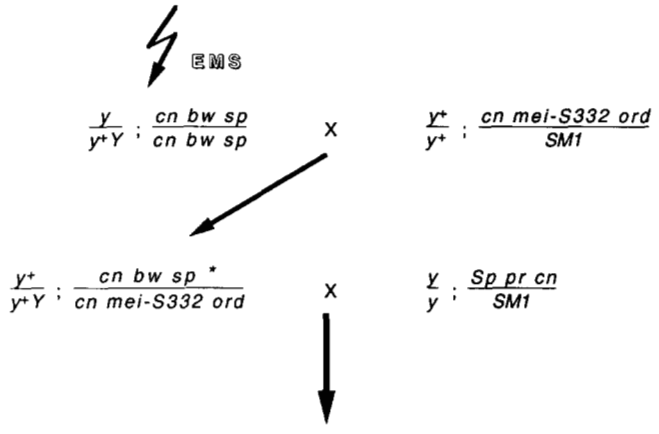

FIGURE 1.—Screen for new alleles of *ord* and *mei-S332*. Males with a y^+Y and a second chromosome marked with *cn bw sp* were mutagenized with EMS and crossed to a stock with a tester chromosome mutated for both *ord* and *mei-S332*. Single males were scored for mutations failing to complement either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

px^+ or^- recombinant chromosome in *trans* to the original ord^1 allele to test for the nondisjunction phenotype. These males carried a wild type copy of the *yellow* gene on the *Y* chromosome and thus nondisjunction of the sex chromosomes could be assayed by crossing these males to yellow females. We tested and stocked between 17 and 25 *px-or* recombinants for each allele and determined map positions of $ord^{1\text{-}6}$ from 102.0 to 103.8 cM. However, since the *ord* locus is located in the center of the *px-or* interval, the mapping results were also consistent with a new mutation being a second-site noncomplementer of *ord* that mapped far enough to be essentially unlinked. For this reason and also because of the low number of recombinants tested in the first experiment, we performed the mapping crosses described above again but instead scored for recombinants within the *px* to *bw* (2-104.5) interval, these flanking markers being positioned asymmetrically about *ord*. Recombinant chromosomes were tested for *ord* noncomplementation and stocked as before. The map positions obtained and the number of px^- bw^- and px^+ bw^+ recombinants are indicated for each allele: ord^2, 103.4 cM, 56 recombinants; ord^3, 103.1 cM, 56; ord^4, 103.6 cM, 55; ord^5, 103.4 cM, 53; ord^6, 103.3 cM, 47.

Construction of homozygous viable and fertile *ord* stocks: In order to test the new alleles of *ord* in the homozygous condition, we crossed off any extraneous lethal mutations which might have been induced during the EMS mutagenesis. *cn* ord^n *bw sp/a px or* females were backcrossed to *cn* ord^n *bw sp/SM1* males, and viable non-cn, bw sp sons were individually mated to *y; pr cn* ord^1*/SM1* females to test the nondisjunction phenotype. If positive for nondisjunction, flies of the genotype ord^n *bw sp/SM1* were stocked and the homozygous viability of the ord^n *bw sp* chromosome was assayed in the next generation. Several viable lines were generated in this manner for each new allele except ord^6.

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

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

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

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

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

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

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

For ord^6 closely linked lethal mutations did not allow viable ord^6 *bw sp/cn* ord^6 *bw sp* males to be selected in the scheme above. Therefore, *px* ord^6 *bw sp* and ord^6 *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^2 and ord^5, although we could obtain progeny from homozygous ord^3 and ord^4 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 (our 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 recombined off the marker *sp* on the ord^2 *bw sp* and ord^5 *bw sp*

chromosomes and found that the resulting recombinant chromosomes were now fertile when homozygous.

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*,*Y* stock; 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 $CaCl_2$ in Ringer's solution (EPHRUSSI 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 Axiophot equipped with Plan Neofluar 20, 40 and 100× objectives; all scoring was done using the 100× objective.

Definition of cytological terms: The term bivalent is used to denote a pair of replicated homologous chromosomes (*i.e.*, four chromatids) associated by collochores 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 "trio" of chromosomes. 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 precocious 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 × phosphate-buffered saline (PBS) and transferred to a solution of 1 × PBS containing 1 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI). Ovaries were stained for 5–10 min and washed twice in 1 × PBS for 5 min each. After transfer to a slide containing a drop of 70% glycerol/30% 1 × PBS, the individual ovarioles were teased apart with forceps and layered with a coverslip. Egg chambers were viewed under fluorescence microscopy on a Zeiss Axioskop or a Zeiss Axiophot equipped with a Plan Neofluar 20× 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^1 (2-103.5) and $mei\text{-}S332^1$ (2-99.5) (Figure 1; also previously described in KERREBROCK *et al.* 1992). Mutagenized *cn bw sp* males were crossed to females carrying the $mei\text{-}S332^1$ ord^1 double mutant chromosome, and progeny $mei\text{-}S332^1$ ord^1/*cn bw 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^1. 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 103.6 cM (see MATERIALS AND METHODS). ord^1 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^1 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.

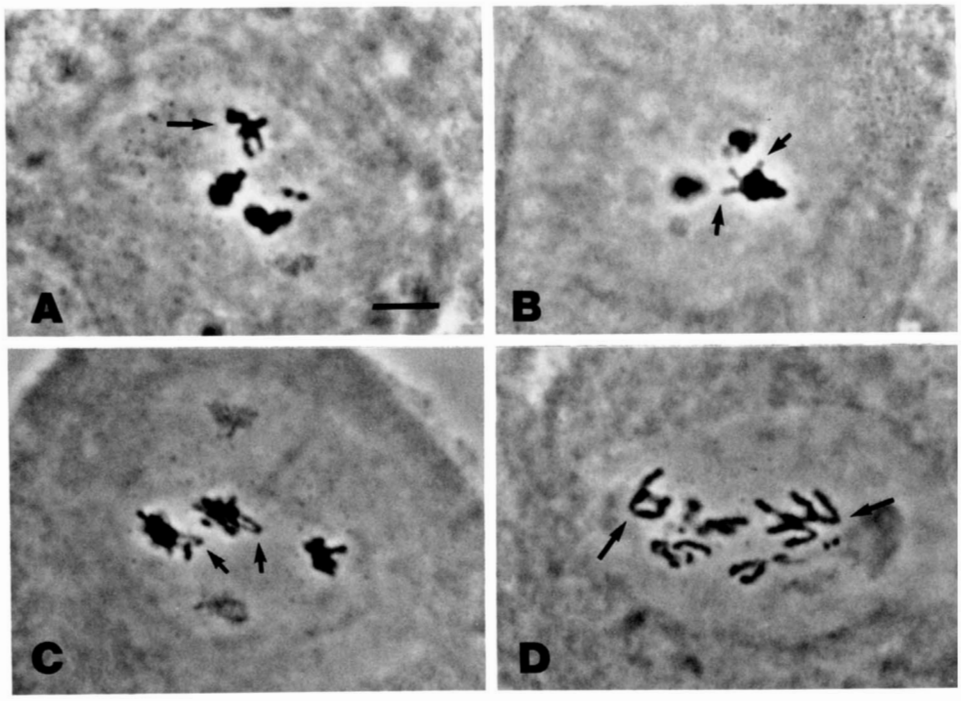

FIGURE 3.—Cytological analysis of meiosis in *ord* males. Meiotic figures in testis squashes were scored from wild type (A) and *ord²/Df(2R)3-70* males (B–D). (A) Prophase I chromosomes in wild-type cells pair and condense into compact bivalents. The sex bivalent is indicated by the arrow. (B) In *ord* cells chromosomes properly pair into bivalents but frequently protrusions extend from the bivalent mass (arrows). The protrusions have a distinct morphology that unambiguously distinguished them from the sex bivalent. (C) *ord²* bivalents also display a loosely packed appearance, and single kinetochores precociously separate from their sister kinetochores as early as prometaphase I (arrows). (D) By mid anaphase I precocious sister-chromatid separation is clearly evident in the *ord²* cell shown; arrows indicate dyads which have fallen apart to their constituent chromatids. The phenotypes shown in B and D were observed in the *ord⁴* and *ord⁶* alleles as well as *ord²*. Scale bar is 5 μm.

Thus, we chose to focus on the strong allele *ord²*, the moderate allele *ord⁶*, and the very weak allele *ord⁴*. 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 *ord²* 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 *ord¹* mutants (GOLDSTEIN 1980). Additionally, in the strong mutant *ord²* 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 *ord²* 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 *ord²* chromosomes were thick ropy structures.

In the *ord* alleles examined, bivalents congressed normally to the metaphase plate. However, it was frequently seen in *ord²* and *ord⁶* 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). *ord⁴* 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 *ord²* cells, only 28% appeared normal (Table 1). However, this same reduction in the frequency of normal-appearing cells was not seen in *ord⁶* 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 *ord⁶* 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 *ord²* 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 *ord⁶*, 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

TABLE 1

Cytological analysis of *ord* spermatocytes of the indicated genotype

Genotype	Prophase I		Metaphase I		Early→mid anaphase I		Mid→late anaphase I		Prophase II		Metaphase II		Anaphase II		Total number of cells scored (meiosis I and II)
	Normal	Abnormal	Normal	Abnormal	Normal	PSSC[a]	Normal	PSSC	Normal	PSSC	Normal	PSSC	Normal	Abnormal	
+/+	14	0	23	0	50	1	11	0	18	0	39	0	42	1	199
	(100)[b]	(0)	(100)	(0)	(98)	(2)	(100)	(0)	(100)	(0)	(100)	(0)	(98)	(2)	
ord²/Df[c]	11	17	3	10	16	42	4	22	0	11	0	0	14	104	254
	(39)	(61)	(23)	(77)	(28)	(72)	(15)	(85)	(0)	(100)	(0)	(0)	(12)	(88)	
ord⁴/ord⁴	74	16	32	3	31	1	11	1	71	4	32	0	62	2	340
	(82)	(18)	(91)	(9)	(97)	(3)	(92)	(8)	(95)	(5)	(100)	(0)	(97)	(3)	
ord⁶/Df[c]	41	27	16	25	21	23	8	25	10	9	11	19	6	30	271
	(60)	(40)	(39)	(61)	(48)	(52)	(24)	(76)	(53)	(47)	(37)	(63)	(17)	(83)	

[a] PSSC, precocious separation of sister chromatids.
[b] Values in parentheses are percent.
[c] The deficiency used in this study was *Df(2R)3-70*.

of chromosomes to the poles and chromosome laggards. The frequency of anaphase irregularities was high in both *ord²* and *ord⁶*, and very low in *ord⁴*.

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 *ord²* and *ord⁶* 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 (*ord²*, *ord³*, *ord⁵*) exhibited levels of nondisjunction of 49–51% which were stronger than the 42% observed in the original allele *ord¹*. Two alleles (*ord⁴*, *ord⁶*) were weaker than *ord¹*, giving levels of 1% and 28%, respectively. The weak nondisjunction seen in *ord⁴* 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 (*ord²* > *ord⁶* ≫ *ord⁴*). The strong alleles *ord²* and *ord⁵* were statistically alike by a 6 × 2 χ^2 contingency analysis (d.f.= 5, $0.1 > P > 0.05$) (LINDREN, MCELRATH and BERRY 1978). Moreover, the frequencies of aneuploid gametes recovered from *ord²* and *ord⁵* 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 *ord⁵* exhibited a ratio of 4.6:1, while the weaker *ord⁶* allele was only 1.8:1. This lower level of meiosis I nondisjunction for *ord⁶* as compared to the stronger *ord* alleles is consistent with the conclusion reached in the cytological analysis: sister chromatids in *ord⁶* cells precociously disjoined on average later than those in *ord²* 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 *ord³*, *ord⁴*, and *ord⁶* 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 χ^2 analysis (*ord³*, $0.5 > P > 0.1$; *ord⁴*, $0.9 > P > 0.5$; *ord⁶*, $0.5 > P > 0.1$). 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 2

Sex chromosome nondisjunction in males

	+	ord^1	ord^2	ord^3	ord^4	ord^5	ord^6	$\frac{ord^5}{Df(2R)bw^{S46}}$	$\frac{ord^4}{Df(2R)bw^{S46}}$	$\frac{ord^6}{Df(2R)bw^{S46}}$	Theoretical Values[a]
Regular sperm											
X	1312	808	288	434	1255	293	919	156	1206	1232	
Y(Y)	1504	927	314	478	1113	394	896	160	1102	1205	
Exceptional sperm											
O	4	787	358	708	21	427	442	230	26	666	
XY(Y)	3	327	157	186	12	233	160	76	12	263	
XX	0	150	43	53	1	51	89	19	4	156	
XXY(Y)	0	16	8	4	0	5	6	4	0	9	
Total progeny	2823	3015	1168	1863	2402	1403	2512	645	2350	3531	
% Nullo-*XY*	0.1	26.1	30.7	38.0	0.9	30.4	17.6	35.7	1.1	18.9	32.9
% *XY(Y)*	0.1	10.8	13.4	10.0	0.5	16.6	6.4	11.8	0.5	7.4	17.1
% *XX*	0.0	5.0	3.7	2.8	0.04	3.6	3.5	2.9	0.2	4.4	3.6
% *XXY(Y)*	0.0	0.5	0.7	0.2	0.0	0.4	0.2	0.6	0.0	0.3	2.4
Total observed non-disjunction	**0.2**	**42.4**	**48.5**	**51.0**	**1.4**	**51.0**	**27.7**	**51.0**	**1.8**	**31.0**	**56.0**

[a] See MATERIALS AND METHODS for calculations of theoretical values.

TABLE 3

Sex chromosome nondisjunction in females

	+	ord^1	ord^3	ord^4	ord^6	$\frac{ord^5}{Df(2R)bw^{S46}}$	$\frac{ord^6}{Df(2R)3\text{-}70}$	Theoretical Values[a]
Regular ova								
X	3538	1373	554	2324	1990	424	741	
Exceptional ova								
O	0	480	238	45	403	134	203	
XX	1	368	181	53	319	117	225	
Total progeny	3539	2221	973	2422	2712	675	1169	
Adjusted total[b]	3540	3069	1392	2520	3434	926	1597	
% nullo-*X*	0.00	31.3	34.2	3.6	23.5	28.9	25.4	33.3
% diplo-*X*	0.03	24.0	26.0	4.2	18.6	25.3	28.2	22.2
Total *X* nondisjunction	**0.03**	**55.3**	**60.2**	**7.8**	**42.1**	**54.2**	**53.6**	**55.5**

[a] See MATERIALS AND METHODS for calculations of theoretical values.
[b] The progeny total is adjusted to correct for recovery of only half of the exceptional progeny.

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 (KEREBROCK *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, ord^2 and ord^5, were highly infertile in females and sufficient progeny could not be obtained for analysis. Of the remaining alleles, both ord^1 and ord^3 showed high levels of total nondisjunction, between 55% and 60%, ord^6 showed a slightly lower level, 42%, and ord^4 showed weak nondisjunction, 8%. Similar to the male analysis, we found that the levels of nondisjunction of the strongest alleles, in this case ord^1 and ord^3, 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

TABLE 4

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

	ord^1	ord^3	ord^4	ord^6	$\frac{ord^5}{Df(2R)bw^{S46}}$	$\frac{ord^6}{Df(2R)3\text{-}70}$
Reductional (car^+/car)	251	147	46	180	90	158
Equational (car^+/car^+)	76	19	2	81	20	40
(car/car)	41	15	5	58	7	27
Total	368	181	53	319	117	225
% reductional	**68.2**	**81.2**	**86.8**	**56.4**	**76.9**	**70.2**
% equational	**31.8**	**18.8**	**13.2**	**43.6**	**23.1**	**29.8**

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 reductional nondisjunction. However, ord^6 in females, as in males, gave weaker reductional nondisjunction than did the stronger alleles ord^1 and ord^3 ($0.564 \times 18.6\% = 10.5\%$ of total gametes *vs.* 16.4% and 21.1%, respectively, from Tables 3 and 4).

ord^3 and ord^6 were tested in *trans* to *ord* deficiencies (Table 3). In the *X* chromosome nondisjunction assay, ord^3 showed a slight decrease in total levels when hemizygous (from 60.2% to 54.2%), whereas nondisjunction in hemizygous ord^6 females increased from the level observed in the homozygote (from 42.1% to 53.6%) and was similar to hemizygous ord^3. Moreover, the percentage of exceptional diplo-*X* progeny increased in hemizygous ord^6 (28.2%) as compared to homozygous ord^6 (18.6%, Table 3). Of the 18.6% diplo-*X* progeny scored in the homozygous ord^6 test, the fractions representing reductional and equational exceptions were 10.5% and 8.1% respectively; these values in the hemizygous ord^6 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 reductional nondisjunction, suggesting that the timing of the ord^6 defect was shifted earlier in meiosis I with the 50% reduction of gene product. The increased severity of the *ord* phenotype when ord^6 is in *trans* to a deficiency suggests that ord^6 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^4 (data not shown). Chromosome *4* nondisjunction was tested in small numbers by crossing *ord; spa*^{pol} flies to a tester stain carrying the compound-*4* chromosome *C(4)RM, ci ey*^{R} (KERREBROCK *et al.* 1992). All alleles of *ord* exhibited chromosome *4* nondisjunction, although the levels for ord^4 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-S332* 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; cv-v; v-f; f-car*).

All four fertile alleles decreased recombination to varying degrees (Table 5). ord^1, ord^3 and ord^6 exhibited recombination at 10–13% of the wild-type control

TABLE 5

Recombination in females homozygous or hemizygous for the indicated allele

	+	ord^1	ord^3	ord^4	ord^6	$\frac{ord^3}{Df(2R)bw^{S46}}$	$\frac{ord^6}{Df(2R)3\text{-}70}$
Number scored	1536	609	205	829	886	180	264
Map distances (cM)							
1 *y-cv*	10.4	1.8	1.0	2.2	1.0	0.6	0.4
		(0.17)	(0.10)	(0.21)	(0.10)	(0.06)	(0.04)
2 *cv-v*	19.2	2.0	4.4	7.0	1.2	1.7	0.4
		(0.10)	(0.23)	(0.36)	(0.06)	(0.09)	(0.02)
3 *v-f*	19.5	1.1	1.5	14.8	3.2	0.0	1.5
		(0.06)	(0.08)	(0.76)	(0.16)	(0.00)	(0.08)
4 *f-car*	5.1	0.7	0.0	7.5	1.1	0.0	0.0
		(0.14)	(0.00)	(1.47)	(0.22)	(0.00)	(0.00)
Total map distance (cM)	**54.2**	**5.6**	**6.9**	**31.8**	**6.5**	**2.3**	**2.3**
	(1.00)	(0.10)	(0.13)	(0.59)	(0.12)	(0.04)	(0.04)

Numbers in parentheses indicate values for the map distances normalized to the control map distance.

level along the *X* chromosome. Using the binomial distribution test (LINDREN, MCELRATH 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^6 gave significantly better segregation than either ord^1 or ord^3 (Table 3), 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^1 was semidominant for the effect on recombination. However, none of the five new alleles was semidominant (data not shown).

The nondisjunctionally weak allele ord^4 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^3 and ord^6 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^3 and ord^6 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^1, ord^3, ord^4 and ord^6 females, 2–4% exhibited homozygosis of *X*-linked markers (data not shown). These values are an underestimate of the actual number of E_1 (single crossover) tetrads undergoing meiosis I nondisjunction, since 50% of possible segregation products from such tetrads would have been two nonexchange 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 nonexchange tetrads. Thus similar to ord^1 (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 germline but has no effect on overall viability:** LIN and CHURCH (1982) had previously reported mitotic misbehavior in the germline gonial cells of ord^1 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^1 primary spermatocytes, suggesting that a nondisjunction event had occurred in some preced-

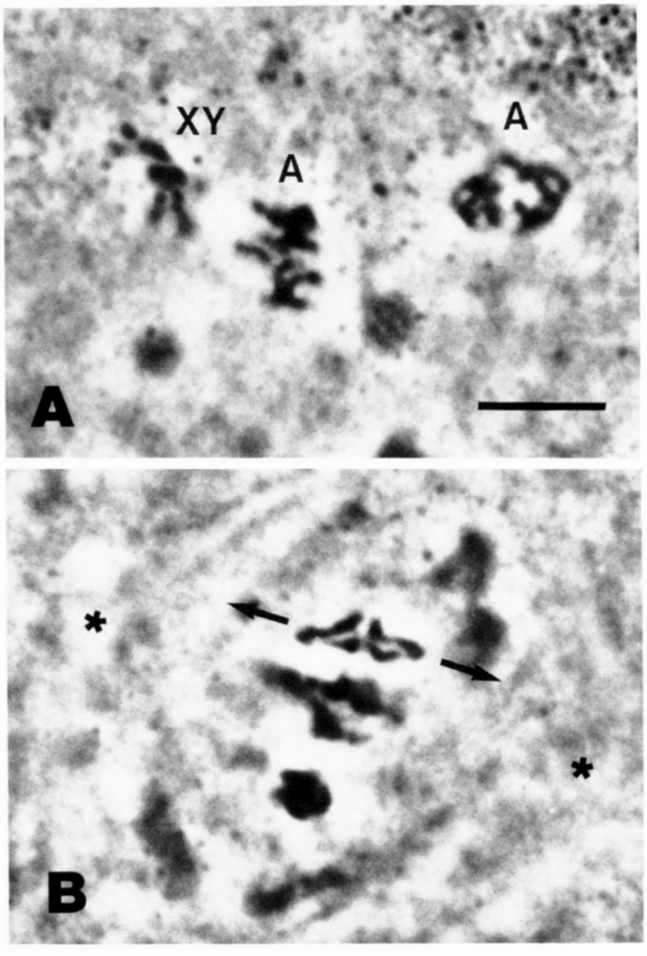

FIGURE 4.—Cytological analysis of hypotonically treated primary spermatocytes to determine ploidy. Chromosomes in primary spermatocytes subjected to a brief hypotonic treatment lose their tight associations and consequently allow determination of the number of chromosomes present (compare Figure 4A with Figure 3A). (A) A wild-type prophase I cell displays a normal diploid complement: the sex chromosome bivalent is labeled *XY* and the large autosome bivalents are labeled *A*. The fourth chromosomes are not visible in this preparation. (B) Early anaphase I segregation of an autosomal univalent is observed in an *ord²/Df(2R)3-70* cell. Asterisks denote the long axis of the spindle. The arrows indicate the direction of movement of each sister chromatid of the univalent. The other chromosomes are out of the plane of focus. Scale bar is 5 μm.

ing mitosis. We sought to determine if this phenotype was a general occurrence in *ord* mutant flies, or if *ord¹* was a special allele that affected the gonial mitotic divisions in addition to the meiotic divisions. We chose to analyze *ord²*, one of the strongest alleles in the male genetic nondisjunction tests.

When testis squashes were prepared according to the cytological techniques presented earlier (*e.g.*, Figure 3), prophase I to metaphase I bivalents in wild type and *ord* cells appeared as packed masses in which it was extremely difficult to determine the number of chromosomes present. However, treatment of intact testes with a hypotonic solution prior to fixation (see MATERIALS AND METHODS) resulted in a relaxation of the forces that held bivalents together, and thus we could score for aneuploid figures. After hypotonic treatment, sister chromatids of the major autosomes dissociated and resolved as single entities in *ord²* cells but not in wild type.

TABLE 6

Primary spermatocyte aneuploidy in *ord* testes

	+	*ord²/Df(2R)3-70*
Sex chromosomes		
Bivalent	62	76
Univalent	0	0
Trivalent	0	2
Large autosome		
Bivalent	62	66
Univalent	0	11
Trivalent	0	1
Number of cells scored	62	78
Sex chromosome aneuploidy (%)	0.0	2.6
Large autosome aneuploidy (%)	0.0	15.4

For scoring criteria see MATERIALS AND METHODS and RESULTS.

We found that *ord²/Df(2R)3-70* cells exhibited univalent (Figure 4B) and trivalent figures in hypotonically treated primary spermatocytes (Table 6) in similar fashion to *ord¹* (LIN and CHURCH 1982). In contrast, only bivalent 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 *ord²*, like *ord¹*, 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, *ord²* and *ord⁵*.

TABLE 7

Numbers of progeny from the cross *ord*/+ ♂ × *Df(2R)bw*^{S46}/*CyO, bw* ♀

Genotype	*ord*^{1}	*ord*^{2}	*ord*^{3}	*ord*^{4}	*ord*^{5}	*ord*^{6}
Df(2R)bw^{S46} / +	422 (1.00)	136 (1.00)	209 (1.00)	60 (1.00)	140 (1.00)	144 (1.00)
Df(2R)bw^{S46} / *ord*	425 (1.01)	149 (1.10)	185 (0.89)	65 (1.08)	141 (1.01)	162 (1.13)
CyO, bw / +	518	164	213	111	175	176
CyO, bw / *ord*	564	184	202	63	155	158
Total	1929	633	809	299	611	640

Numbers in parentheses indicate values normalized to the recovery of *Df(2R)bw*^{S46}/+ siblings.

Although *ord*^{3}/*Df* flies were recovered at only 89% of the frequency of +/*Df* flies (Table 7), χ^2 analysis indicates that these values are statistically similar (d.f. = 1, $0.5 > P > 0.1$). Also, since *ord*^{1} 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*^{2}, *ord*^{3}, *ord*^{4} and *ord*^{6} 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*^{2} and *ord*^{5} 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*^{2} and *ord*^{5} 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*^{2} and *ord*^{5} mutations.

Because the female nondisjunction frequencies of strong yet fertile alleles such as *ord*^{1} and *ord*^{3} were already indicative of chromatids randomly segregating through two divisions (Table 3), we at first were puzzled how a more "severe" *ord*^{2} or *ord*^{5} defect could affect meiosis such that near sterility resulted. By analogy to the gonial mitotic defect seen in *ord*^{2} 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^2 egg chambers revealed striking phenotypes when analyzed in this manner. First, although *ord*^{2} heterozygotes only gave egg chambers containing 15 nurse cell nuclei, 30% of homozygous or hemizygous *ord*^{2} egg chambers had abnormal numbers of nurse cell nuclei (Table 8). The morphology of these polyploid nuclei was also altered in *ord*^{2} mutant females. Whereas heterozygous *ord*^{2} egg chambers gave uniformly spherical nuclei (Figure 5A), 80–90% of nuclei from homozygous or hemizygous *ord*^{2} 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*^{2} 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*^{2} 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

Genotype	Stage	Percent egg chambers with indicated numbers of nurse cell nuclei				Percent egg chambers with "sickled" nuclei	Percent egg chambers with nuclei of irregular size	Total egg chambers scored
		13	14	15	Other			
$\frac{ord^1}{ord^1}$	≤8	0	0	100	0	9	1	55
	9–10	0	3	97	0	29	1	68
$\frac{ord^2}{SM1}$	≤8	0	0	100	0	0	0	21
	9–10	0	0	100	0	0	0	21
$\frac{ord^2}{ord^2}$	≤8	18	5	73	5	77	27	22
	9–10	5	23	73	0	86	41	22
$\frac{ord^2}{Df^a}$	≤8	8	23	65	4	96	41	26
	9–10	11	19	70	0	89	63	27
$\frac{ord^5}{SM1}$	≤8	0	0	100	0	2	0	41
	9–10	0	0	100	0	9	0	22
$\frac{ord^5}{ord^5}$	≤8	0	0	99	1	11	0	71
	9–10	2	6	88	4	32	3	118
$\frac{ord^6}{Df^a}$	≤8	0	2	98	0	11	0	62
	9–10	0	1	96	3	16	0	74

[a] The deficiency used in these studies was $Df(2R)bw^{S46}$.

sterile allele, ord^5, did not show as strong an effect as did ord^2. 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 ord^5 were obtained for ord^1; and the weaker allele ord^6 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 cohesiveness 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 ord^1 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

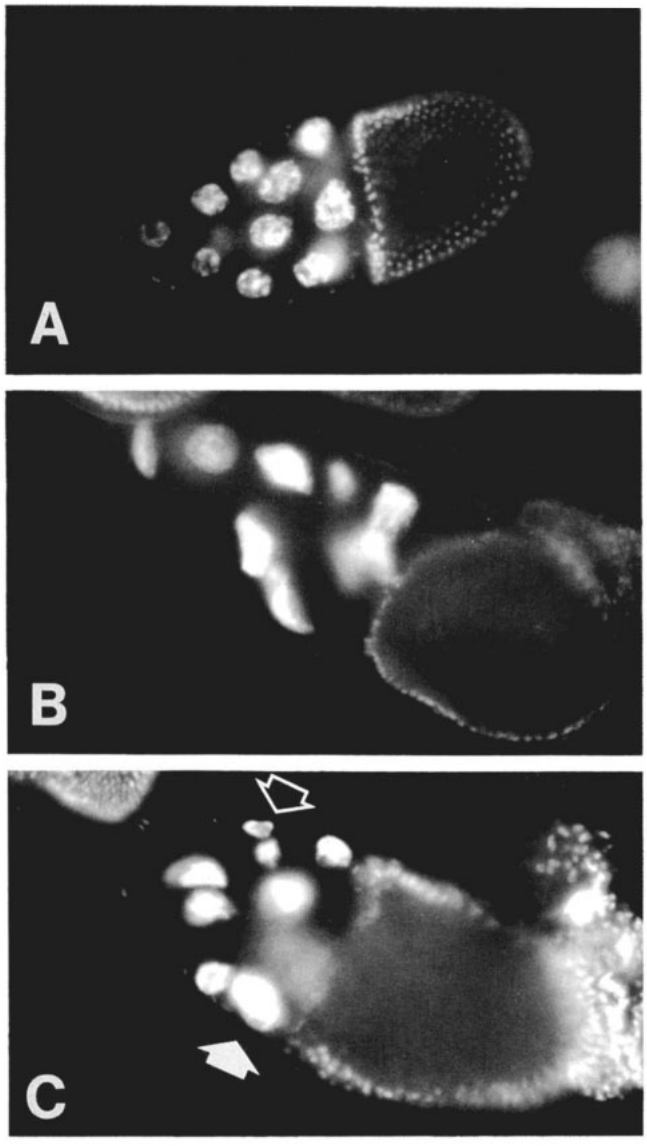

FIGURE 5.—Nurse cell nuclear phenotype in *ord* mutant females. Egg chambers were dissected from heterozygous control or homozygous ord^2 mutant females, stained with DAPI, and viewed under fluorescence microscopy. (A) Heterozygous ord^2 egg chambers reveal 15 large, roughly spherical, polyploid nurse cell nuclei. (B and C) Homozygous ord^2 egg chambers have nurse cell nuclei with an unusual sickled appearance. In (C) irregular-sized nuclei are present: the open arrow points to an abnormally small nucleus when compared to a larger nucleus in the same egg chamber (filled arrow) (compare to the less pronounced difference between the smallest and largest nuclei in wild-type egg chambers (A)).

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^1 (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^6 mutation demonstrates this separation of function, since recombination levels are reduced in ord^6 to the same extent as in ord^1 or ord^3 but nondisjunction is less frequent. Mutations in *ord* may have different consequences on recombination and segregation, and ord^6 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 *ord*^{2} 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 *ord*^{1} (LIN and CHURCH 1982). The two strongest *ord* alleles, *ord*^{2} and *ord*^{5}, 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 *ord*^{2} may cause a reduction in nurse cell number. The effect of these mutations on nurse cell phenotype is consistent with defects in the cystocyte mitotic divisions that give rise to the 15 nurse cell-oocyte 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 *ord*^{1} 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 *ord*^{1} 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 to 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 *ord*^{6} 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*^{6} 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 synaptonemal 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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