

## MEIOSIS IN *DROSOPHILA MELANOGASTER*. III. THE EFFECT OF ORIENTATION DISRUPTOR (*ord*) ON GONIAL MITOTIC AND THE MEIOTIC DIVISIONS IN MALES

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### ABSTRACT

Orientation disruptor (*ord*), a meiotic mutant that is recombination defective in females and disjunction defective in males and females, has been analyzed using serial section electron and light microscopy. From analysis of primary spermatocytes we have confirmed that *ord* males are defective in some aspect of the mechanism(s) that holds sister chromatids together during meiosis. In addition, we have determined that *ord* causes high frequencies of nondisjunction during spermatogonial mitotic divisions, as well as during the meiotic divisions. Mitotic nondisjunction involves the large autosomes more frequently than the sex chromosomes or chromosome 4 and results in high frequencies of primary spermatocytes that are either monosomic or trisomic for chromosome 2 or 3. Abnormalities in spermatocyte cyst formation are also observed in males homozygous for *ord*. These abnormalities include loss of regulation of meiotic synchrony and the number of gonial cell divisions.

**O**RIENTATION disruptor (*ord*), an EMS-induced, semidominant meiotic mutation located on chromosome 2 in *Drosophila melanogaster*, was isolated and genetically characterized by MASON (1976). Females homozygous for *ord* are recombination defective and disjunction defective; the two defects are almost independent of each other. Males are disjunction defective. The effects of *ord* on meiotic chromosome disjunction occur at both meiotic divisions and result in high frequencies of both reductional and equational exceptional offspring (MASON 1976).

Most meiotic mutations in *Drosophila* are restricted in their effects to one or the other meiotic division and are sex specific (BAKER *et al.* 1976; BAKER and HALL 1976). Since *ord* deviates from this pattern, MASON (1976) proposed that the *ord*<sup>+</sup> allele must function at an early meiotic stage, prior to the separation of genetic control in the two sexes. GOLDSTEIN (1980) performed extensive cytological and genetic analyses on *ord* males and concluded that *ord* flies are deficient in sister chromatid cohesion throughout meiosis. BAKER, CARPENTER and RIPOLL (1978) demonstrated that *ord* also causes chromosome instability in at least abdominal histoblasts in females.

We have extended the cytological analysis of *ord* using light microscopy and serial section electron microscopy. We have confirmed the prediction of GOLDSTEIN (1980, 1981) that *ord* males are deficient in some aspect of the mechanism

that holds sister chromatids together during meiosis. Furthermore, we have determined that *ord* also causes high frequencies of nondisjunction during gonial mitotic divisions in addition to causing abnormalities in cyst formation, including loss of regulation of the number of cell divisions, loss of meiotic synchrony and missegregation of ring canals. High frequencies of cyst death were also observed in *ord* testes.

#### METHODS AND MATERIALS

**Stocks:** All stocks were maintained at room temperature on standard cornmeal molasses agar medium with propionic acid as a mold inhibitor. The majority of the observations was made on homozygous *ord* males from the stock  $y/y, y/y^+Y; SM1/pr\ ord; +/+; spa^{prot}/spa^{prot}$ . The stock was obtained from A. T. C. CARPENTER (who in turn had obtained it from L. GOLDSTEIN, then at the University of Washington). Some preliminary observations were made on a second *ord* stock ( $y/y, y/Y; SM1/ord; +/+; +/+$ ) also obtained from DR. CARPENTER. The stocks were maintained by selecting males and virgin females heterozygous for the meiotic mutant and crossing them. Observations were made on 0- to 2-day-old male flies homozygous for *ord* or heterozygous for *ord* selected from fresh bottles. The Oregon R (*Ore R*) wild-type stock was obtained from W. W. DOANE at Arizona State University.

**Electron microscopy:** Testes were prepared for electron microscopy and serially sectioned by the techniques previously described (CHURCH and LIN 1982). Micrographs (magnification approximately  $\times 18,500$ ) of each consecutive nuclear section were obtained for analysis. Micrograph tracings were used to reconstruct and karyotype the cells (LIN, AULT and CHURCH 1981). Ring canals were analyzed from low magnification electron micrographs of every fifth section through the cyst of spermatocytes.

**Light microscopy:** Complete testes, fixed and embedded by techniques previously described (CHURCH and LIN 1982), were serially thick sectioned (0.50  $\mu\text{m}$  sections) for observation in the light microscope. Sections were transferred (five at a time) to glass slides and stained with 1% methylene blue in 1% borax and 1% azure II. Photographs were taken of consecutive sections with a Zeiss phase-contrast microscope. The technique for observing chromosomes in primary spermatocytes from squash preparations was adapted from techniques used for monitoring *Drosophila* neuroblast cells (GATTI *et al.* 1979) and from LIFSCHYTZ and HAREVEN (1977). Testes from young males were incubated for 1 hr in 0.7% NaCl containing 10  $\mu\text{M}$  colchicine and then transferred to hypotonic solution (0.5% sodium citrate) for 15 min. They were stained for 2 min in a drop of 3% aceto-orcein and then were transferred to a small drop of 60% acetic acid on a subbed microscope slide. The tubules were cut at the midpoint with a tungsten needle, and the contents were allowed to empty. A cover slip containing a small drop of 3% lacto-aceto orcein was gently lowered onto the preparation. Preparations last approximately 1 mo if the cover slips are ringed with clear nail polish and the slide is refrigerated. Quantitative data were obtained from direct observations. Photographs were obtained of representative cells on Kodak technical pan film 2415 with a Zeiss phase-contrast microscope.

#### RESULTS

##### *Background and meiotic staging*

We define prometaphase I in electron micrographs of wild-type cells by the absence of complete bivalent congression, the presence of a rather large intact nucleolus and by the presence of a fourth chromosome bivalent in which the two homologues are physically touching. A cell is said to be in metaphase I if the bivalents are congressed, an intact nucleolus is absent and the dyads of the fourth chromosome bivalent are distance paired [*i.e.*, not physically touching (LIN, AULT and CHURCH 1981)]. Normal congression does not occur in *ord*

spermatocytes (GOLDSTEIN 1980; H.-P. LIN and K. CHURCH, personal observation); thus, for the purposes of this investigation we have not always attempted to distinguish between prometaphase I and metaphase I. Anaphase I has begun when one or more bivalents (in addition to the four) have disjoined, and telophase I has occurred when all chromosomes have reached a pole. During all stages the nucleus is surrounded by multiple layers of double membranes (TATES 1971; CHURCH and LIN 1982). During telophase a constriction occurs both in the cell membrane and the perinuclear membranes. The chromosomes then decondense and are gradually completely surrounded by double membranes (TATES 1971; H.-P. LIN and K. CHURCH, personal observation). Once decondensation and nuclear membrane reformation are complete the stage is defined as interkinesis following the first meiotic division. On completion of the division process, the two products (secondary spermatocytes) are connected by a ring canal that has a larger diameter than the ring canals produced during the spermatogonial divisions.

Cells in the first meiotic division are also characterized by the presence of fibrous material inside the nucleus, which most likely represents remnants of the RNP structures produced by the Y chromosome during prophase I (TATES 1971; H.-P. LIN and K. CHURCH, personal observation). These structures are not observed in the nucleus during interkinesis of the secondary spermatocyte nor during the second meiotic division, suggesting that they have been eliminated from the nucleus during the first meiotic nuclear division.

The spindle apparatus does not differ significantly between *ord* and wild-type spermatocytes at the ultrastructural level. The poles of first division *ord* cells are well defined by the presence of the double centrioles on opposite sides of the nucleus, and microtubular components and organization resemble wild type in all respects (LIN, AULT and CHURCH 1981; CHURCH and LIN 1982).

#### *Effect of ord on gonial mitosis and cyst formation*

A total of 23 primary spermatocytes in stages ranging from prometaphase I to anaphase I were completely examined from electron micrographs of serial sections of the testis of a homozygous *ord* fly (genotype  $y/y^+Y; pr\ ord/pr\ ord; +/+; spa^{pol}/spa^{pol}$ ). A high degree of aneuploidy was encountered. Of the 23 cells, seven were trisomic and nine were monosomic for one of the large autosomes, one was trisomic for both a large autosome and chromosome 4 and six cells were euploid.

Under normal conditions each cyst of meiocytes in *D. melanogaster* males contains 16 cells that are the result of four relatively synchronous spermatogonial divisions. Intercellular cytoplasmic bridges (ring canals), the products of incomplete spermatogonial cytokinesis, remain as a mitotic history of the cysts. The ring canals persist throughout the majority of spermatogenesis (LINDSLEY and TOKUYASU 1980). Since all of the cells we examined were obtained from one series of 1160 sections, our initial thought was that we had sampled cells from two different cysts, each of which had experienced a mitotic nondisjunctional event early in the formation of the cyst. Since it is usually possible to determine the lineage of each cell within a cyst by analyzing the ring canals

present (LINDSLEY and TOKUYASU 1980), we reconstructed the cyst from which our cells were obtained. Surprisingly, all of the cells were related to each other in terms of cell lineage (Figure 1). However, the cyst is very bizarre for several reasons. The cyst contains 32 cells rather than 16. One extra round of cell division could produce a 32-cell cyst. When conservative inheritance of pre-existing ring canals is assumed, such a cyst would be expected to contain two

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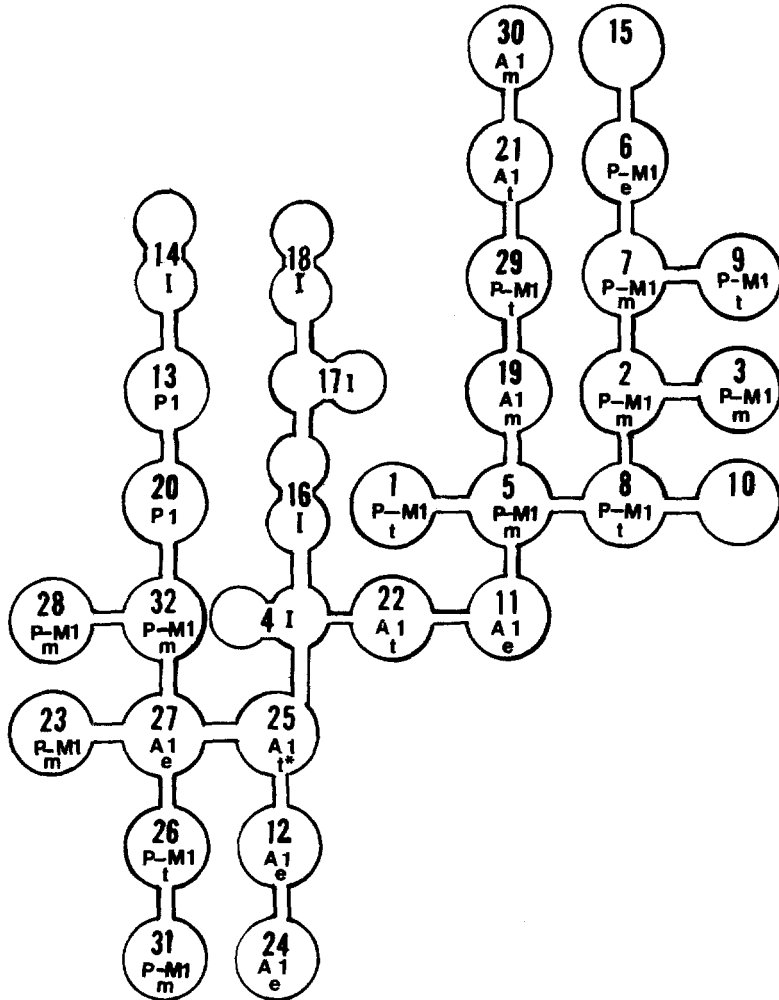


FIGURE 1.—Reconstruction from electron micrographs of 1160 serial sections through a cyst of *ord* spermatocytes. The cyst contains 32 cells (numbers) connected by ring canals. The stages encountered were prophase I (P1), prometaphase-metaphase I (P-M1), anaphase I (A1) and interkinesis (I) after the first meiotic nuclear division. Trisomy (t) and monosomy (m) involving the large autosomes occurred frequently. Cell number 25 was trisomic (t) for a large autosome and the 4th chromosome. Of 17 karyotyped cells, only five were euploid (e).

cells each with five ring canals, two with four, four with three, eight with two and 16 with one. Our cyst deviates from this pattern, suggesting that the pattern of ring canal inheritance is disrupted. Furthermore, the cells occupying the cyst are very asynchronous in development. The stages encountered range from meiotic prophase [mature spermatocyte according to TATES (1971) nomenclature] (cells 13 and 20) to interkinesis following the first meiotic division (cells 4, 14, 16, 17 and 18). This is in contrast to cysts we have examined in flies that are wild type for the *ord* locus. We have examined two complete cysts of division I cells from *Ore R* (light microscopic analysis of serial thick sections). One contained cells in prometaphase I only; the second, cells in anaphase I, telophase I and interkinesis. In addition we have examined two complete cysts from an *In(1)sc<sup>4L</sup>sc<sup>8R</sup>* stock in the electron microscope. Both contained prometaphase I and metaphase I cells only. Finally, we have examined (electron microscopy) approximately 25 partial cysts (eight or more complete cells). All follow the pattern of synchrony observed in the complete cysts. We have never encountered the degree of asynchrony that was observed in the *ord* cyst.

An additional line of evidence verifies that we have not sectioned an aberrant *ord* cyst in terms of cell numbers. We serially thick sectioned two additional *ord* testes and two *Ore R* wild-type testes for examination in the light microscope. Five *ord* cysts of primary spermatocytes in which the boundaries were well defined were analyzed from light micrographs of the serial thick sections. The number of cells observed was 14, 16, 16, 16 and 28. Another striking difference between the *ord* testes and the wild-type testes was the extent to which pycnotic cysts were encountered. Among the two wild-type testes, we observed a total of three degenerating cysts. Degenerating cysts among the *ord* testes were too numerous to count (Figure 2). Many examples of single densely staining cells were also encountered in the *ord* testes (Figure 2). Whether these represented partial cyst death or death of cells other than spermatogonia or spermatocytes could not be determined from the light micrographs. On gross examination *ord* testes are much smaller than wild-type testes, again suggesting that cyst (and perhaps cell) death commonly occurs.

It seems clear that the unique cyst of cells that we have observed has resulted from a combination of abnormal events including a disruption of the pattern of ring canal inheritance and a loss of regulation of meiotic synchrony and the number of mitotic cell divisions. However, the most interesting conclusion drawn from this analysis is that nondisjunction involving the large autosomes did occur during the formation of this cyst. Our conclusion based on this analysis is that *ord* is a mutation that not only causes high frequencies of meiotic nondisjunction (MASON 1976) but also causes nondisjunction during gonial mitotic divisions, in addition to a syndrome of other cyst abnormalities.

These observations were surprising in view of the fact that *ord* spermatocytes have been previously analyzed cytologically at the light microscope level (MASON 1976; GOLDSTEIN 1980) and aneuploidy has not been detected. One explanation for this apparent discrepancy is that we had managed to section an aberrant *ord* cyst in terms of nondisjunction events. Therefore, we undertook our own light microscopic analysis of *ord* spermatocytes to determine whether

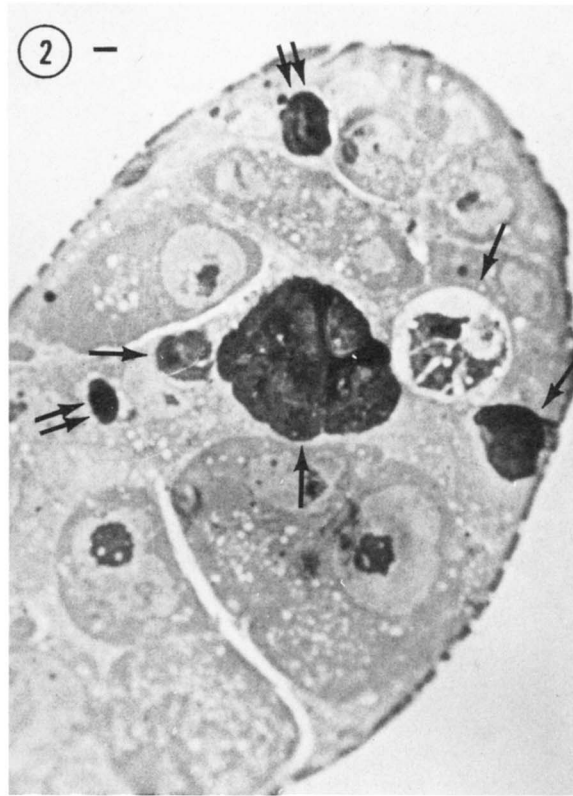


FIGURE 2.—Light micrograph of a section through an *ord* testis. Single arrows indicate degenerating cysts. Double arrows indicate degenerating cells. Bar = 1  $\mu$ m.

that was the case. To obtain unequivocal observations, it was necessary to observe cells in metaphase I and/or anaphase I. This proved to be difficult in *ord* since, at metaphase I, the synapsed chromosomes are small and very compact and we could not distinguish bivalents, trivalents and univalents with any degree of confidence. At anaphase I, the disjoined sister chromatids (monads) tend to lie on top of each other and often lag (MASON 1976; GOLDSTEIN 1980). To circumvent this problem we applied a dilute colchicine, hypotonic treatment (GATTI *et al.* 1979) to the spermatocytes. The technique allowed an unequivocal assessment of the ploidy of the large autosomal bivalents at metaphase I. The ploidy of the chromosome 4 bivalent and the XY bivalent could be determined with less confidence. Concerning chromosome 4, what we can say is that among 85 metaphase I cells examined, 71 clearly displayed a chromosome 4 bivalent with four chromatids visible (Figure 3a), 11 had the chromosome 4 bivalent either missing or obscured by the other chromosomes, and three may have been

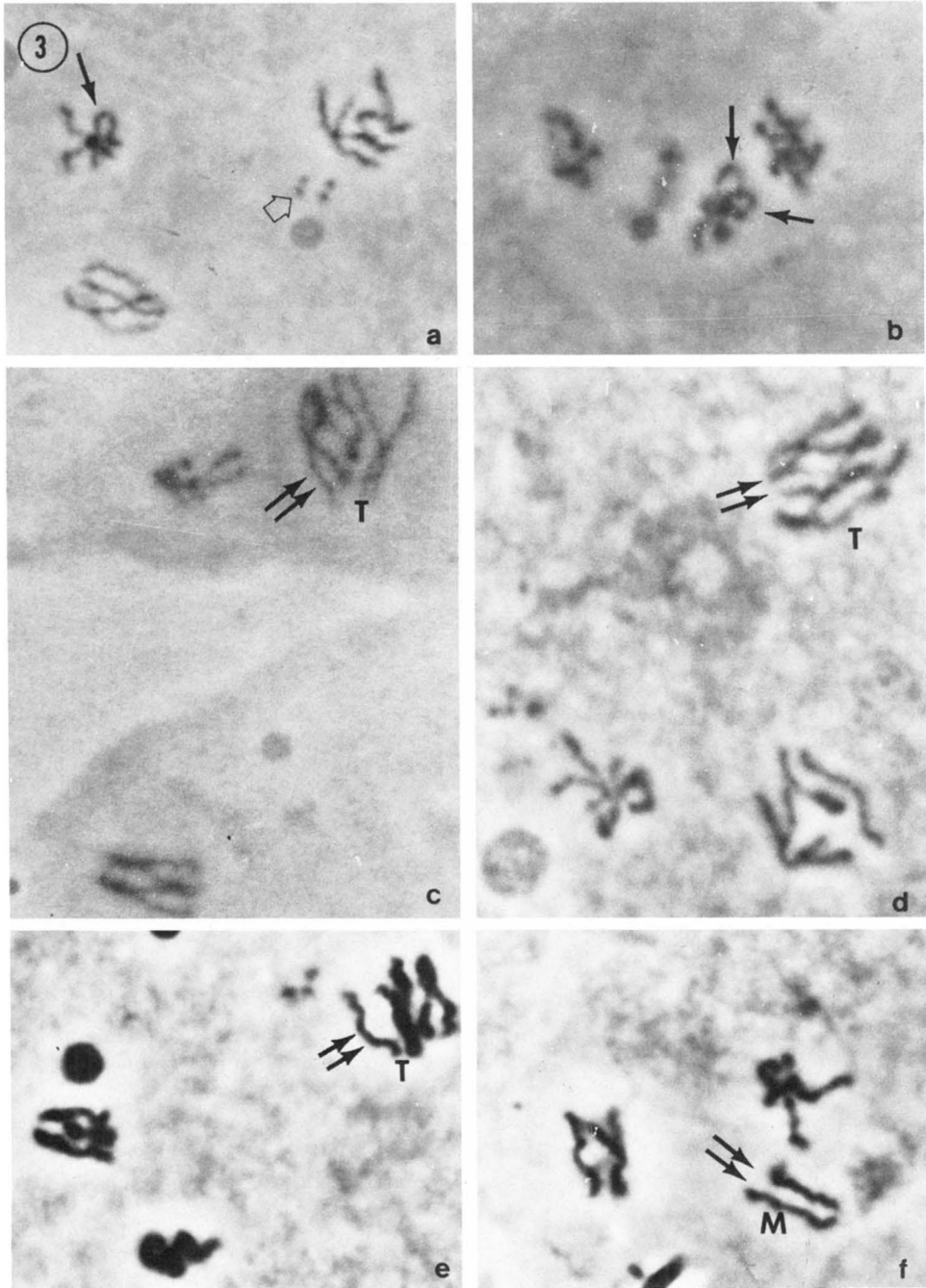


FIGURE 3.—Light micrographs of colchicine-treated *ord* primary spermatocytes in prometaphase-metaphase I. All four chromatids of the 4th bivalent (open arrow) can be resolved (a). The tip of the  $y^+Y$  chromosome participates in the pairing site forming a single loop (arrow, a) or a double loop (arrows, b). Euploid cells (a) are observed, as are trisomic (T) and monosomic (M) cells (c-f, respectively). Double arrows indicate trivalents and a univalent.

(Figure 3a). Sometimes two loops are observed (Figure 3b). However, the two loops may represent the two long arm chromatids rather than two Y chromosomes. We suspect that the latter is the case since similar configurations were observed in *ord/+* heterozygous sibs. Our conclusion is that, if aneuploidy is occurring involving chromosome 4 or the sex chromosomes, the frequency is relatively low. The large autosomes present a different picture altogether. Table 1 presents the data obtained for *+/+*, *ord/+* and *ord/ord* primary spermatocytes in metaphase I. Approximately 30% of the homozygous *ord* cells are either trisomic (Figure 3c, d and e) or monosomic (Figure 3f) for one of the large autosomes. Among the 23 cells examined with the electron microscope 17 (74%) were aneuploid, a higher frequency than observed with the light microscope. The highest frequency of aneuploidy in an individual fly observed in the light microscope study was 43%. Thus, we happened to section a cyst with a higher-than-average frequency of aneuploidy. Nevertheless, our conclusions based on the electron microscope study appear to be valid. That is, chromosome misbehavior involving the large autosomes occurs frequently during gonial divisions in *ord/ord* males.

#### *Effect of ord on sister kinetochore cohesion*

GOLDSTEIN (1981) described the ultrastructure of the kinetochore in wild-type *Drosophila* primary spermatocytes. He concluded that half bivalent kinetochores appear as single bilaminar hemispheric structures throughout early meiotic prometaphase. As meiosis progresses the kinetochore is "differentiated" into a "double disk" structure. Our observations are in accord with those of GOLDSTEIN (1981) with one or two minor exceptions. (1) In the best fixed preparations, the kinetochore in normal cells appears to be a trilaminar structure (CHURCH and LIN 1982), and (2) we have on very rare occasions detected a hint of doubleness in early prometaphase I kinetochores. However, it should be emphasized that the early meiosis I wild-type kinetochores appear as single structures (Figure 4a-c); in late meiosis I, even though the kinetochores are visibly double, the sisters are invariably physically associated (Figure 4d-f).

The most obvious effect of *ord* in prometaphase I-metaphase I cells at the ultrastructural level is at the kinetochore. The majority of the dyads display sister kinetochores that are physically separated (Figure 4g-i). Even in those cases in which the sister kinetochores are physically touching the doubleness is always apparent (Figure 4j-l). Each chromatid kinetochore in *ord* primary spermatocytes is much smaller than the single dyad kinetochore observed in wild-type cells (compare Figure 4a-f with Figure 4g-i). To emphasize the magnitude of this effect some quantitative observations were made. Among the 24 prometaphase-metaphase I sex chromosome dyads observed, 20 displayed two physically separated sister kinetochores and four displayed visibly double kinetochores that were in contact. There were no cases among all dyads (autosomal as well as sex chromosomal) in which the doubleness of the kinetochore was not readily apparent. Thus, univalents usually display two, bivalents four and trivalents six distinct kinetochores (Figure 5) rather than the expectation of one, two and three, respectively. One cell (cell 9) was judged to



TABLE 1

Frequency of aneuploidy for chromosome 2 or 3 observed by light microscopy of primary spermatocytes

Genotype	Euploid	Trisomic	Monosomic	Frequency of aneuploidy	No. of flies observed
Ore R	50	0	0	0	5
<i>pr ord/SM1</i>	48	1	1	0.04	11
<i>pr ord/pr ord</i>	69	14	17	0.31	10

be in early prometaphase I by the presence of a large nucleolus and by the paucity of kinetochore microtubules (Figure 6a-c). The large autosomal bivalents and the XY bivalent all displayed a lack of sister kinetochore cohesion (Figure 6d). Thus, as far as we can determine, the defect in sister kinetochore cohesion occurs very early, although it is impossible to determine precisely how early since kinetochores cannot be unambiguously identified in stages prior to prometaphase I.

Synapsis of homologous chromosomes is not obviously altered in *ord* spermatocytes (GOLDSTEIN 1980). In the prometaphase-metaphase I cells we examined, euploid cells always contained bivalents. In trisomic cells, the three homologous chromosomes were always associated in a trivalent and never appeared as a bivalent plus a univalent. The bivalents and trivalents are abnormal in that sister chromatids can be resolved in proximal regions (see Figure 5). In wild-type primary spermatocytes, in contrast, it is virtually impossible to resolve sister chromatids in electron micrographs at this stage (H.-P. LIN and K. CHURCH, personal observation).

Although sister chromatid cohesion in *ord* is obviously abnormal during the first meiotic division, it may not be completely abolished. The sister chromatids of univalents found in monosomic cells are always near each other and may physically touch each other, even though the sister kinetochores may be physically separated by distances up to 1.6  $\mu\text{m}$  (Figure 7). This association lasts throughout prometaphase kinetochore orientation and is abolished when anaphase I ensues. At anaphase I, all chromosomes in *ord* are displayed as monads. Among the nine anaphase I cells examined with the electron microscope, none of the dyads had remained intact. Sister kinetochore and chromatid cohesion was completely absent. Thus, monosomic cells contain 14, euploid 16 and trisomic cells 18 monads.

#### *Effect of ord on chromosome segregation during the first meiotic division*

The genetic analysis of *ord* (MASON 1976; GOLDSTEIN 1980) revealed that the mutation causes high frequencies of nullo, reductional and equational exceptional sperm for the sex chromosomes. The analysis of GOLDSTEIN (1980) demonstrated that the reductional exceptions were accounted for by the equational division of the sex chromosomes during anaphase I rather than from nondisjunction of entire half bivalents.

We have also observed that equational kinetochore orientations at metaphase

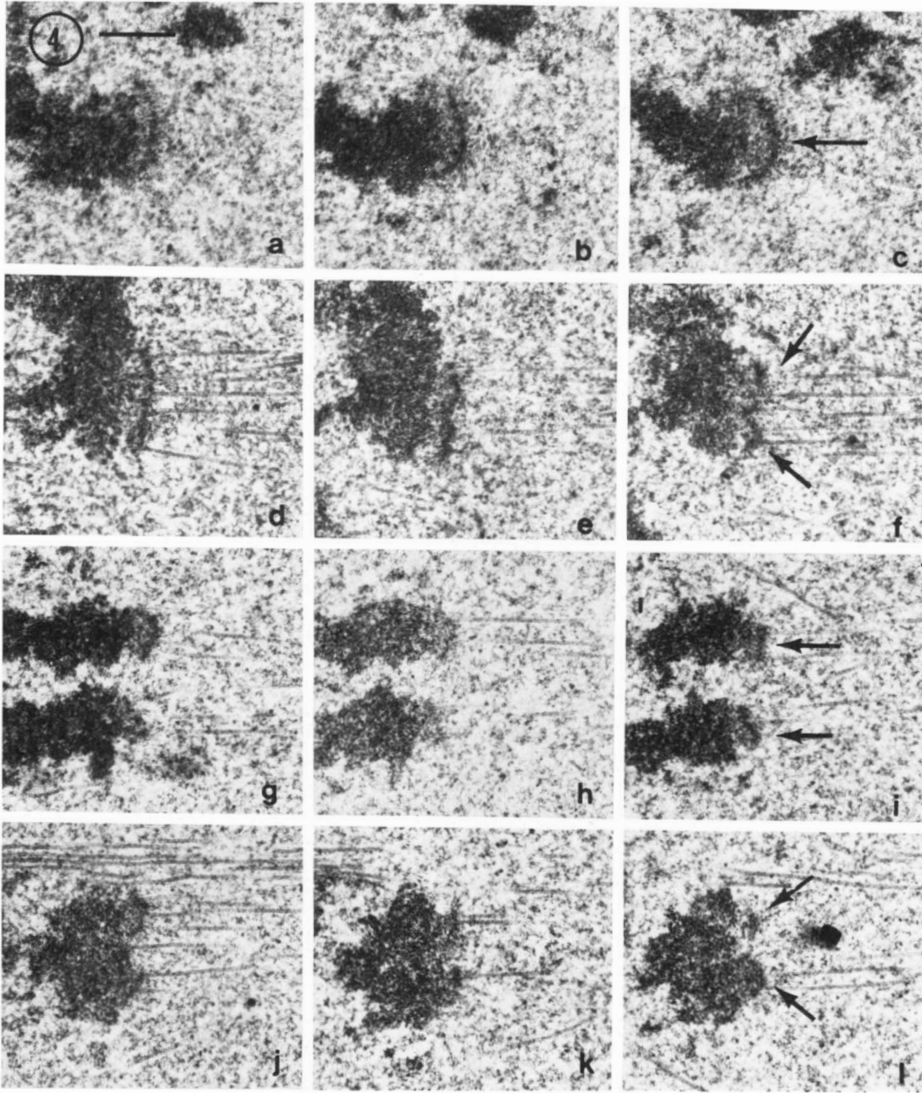


FIGURE 4.—Serial sections through a wild-type hemispheric dyad kinetochore at prometaphase I (a-c) and a wild-type, double disk kinetochore at anaphase I (d-f). The wild-type prometaphase I dyad kinetochore is visibly a single structure. By anaphase I the kinetochore is visibly double, although the two sister kinetochores are physically touching. The majority of *ord* sister kinetochores at prometaphase-metaphase I is physically separated (g-i). In those rare cases in which they are physically joined the doubleness is *always* apparent (j-l). Bar = 0.5  $\mu$ m.

I and segregations at anaphase I do occur frequently in *ord* males. The orientations of the kinetochores in metaphase I cells were determined in the electron micrographs, in addition to an analysis of segregation in anaphase I cells. The X kinetochore can be distinguished from the Y kinetochore in first division cells by reconstructing the bivalent from serial sections. The Y is a biarmed chromosome, and the arms (but not the chromatids) can sometimes be resolved in

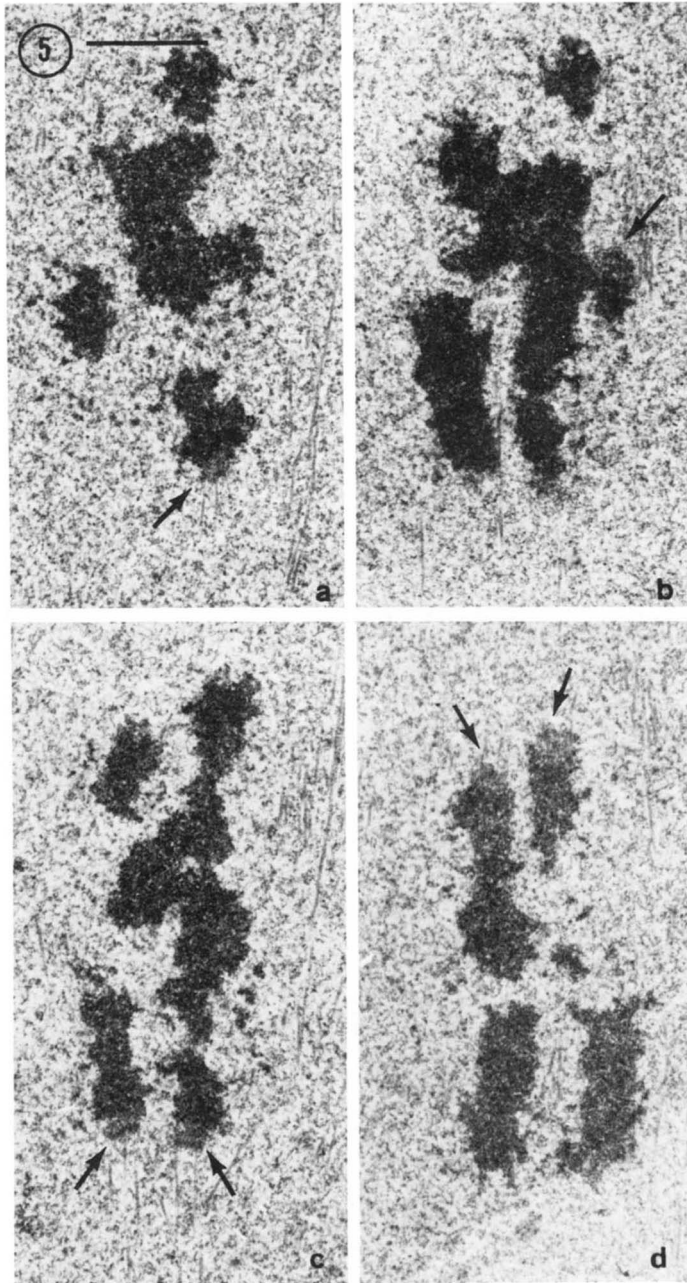


FIGURE 5.—Sections 21, 26, 30 and 35, respectively, through an autosomal trivalent from *ord/ord* at prometaphase-metaphase I (46 sections encompass the entire trivalent). Six distinct kinetochores are apparent (arrows). In this particular trivalent, three kinetochores are oriented toward one pole and three toward the opposite pole, suggesting equational separation of at least 1 dyad. Bar = 1  $\mu$ m.



FIGURE 6.—d, A partial reconstruction of cell 9 (see Figure 1). a-c, Serial sections through monad kinetochore  $A_3$  (see d). The cell was judged to be in early prometaphase I by the paucity of kinetochore microtubules (a-c) and the presence of a large nucleolus (Nu). The autosomal kinetochores ( $A_1$ - $A_4$ ) are noncohesive as are the X and Y kinetochores ( $X_1X_2Y_1Y_2$ ). Numbers are the section number in which the structure appeared. Bar = 0.5  $\mu$ m.

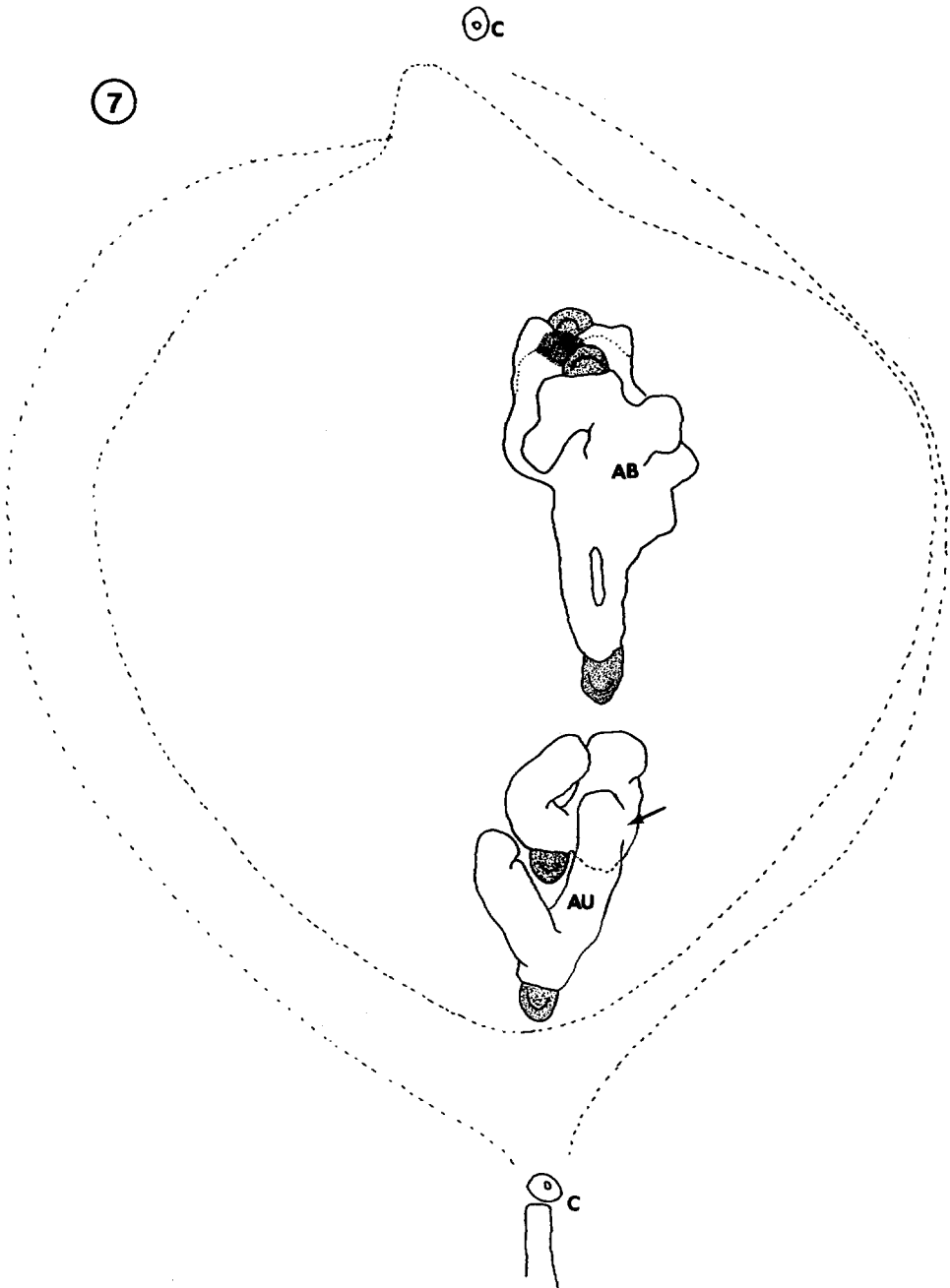


FIGURE 7.—Partial reconstruction of cell 3 (see Figure 1). The autosomal univalent dyad (AU) is located near one pole [marked by centrioles (C)] with both sister kinetochores oriented toward the same pole. The sister chromatids touch in one place (arrow) but the noncohesive sister kinetochores are separated by a distance of  $1.64 \mu\text{m}$ . The large autosomal bivalent (AB) displays a 3-1 kinetochores orientation.

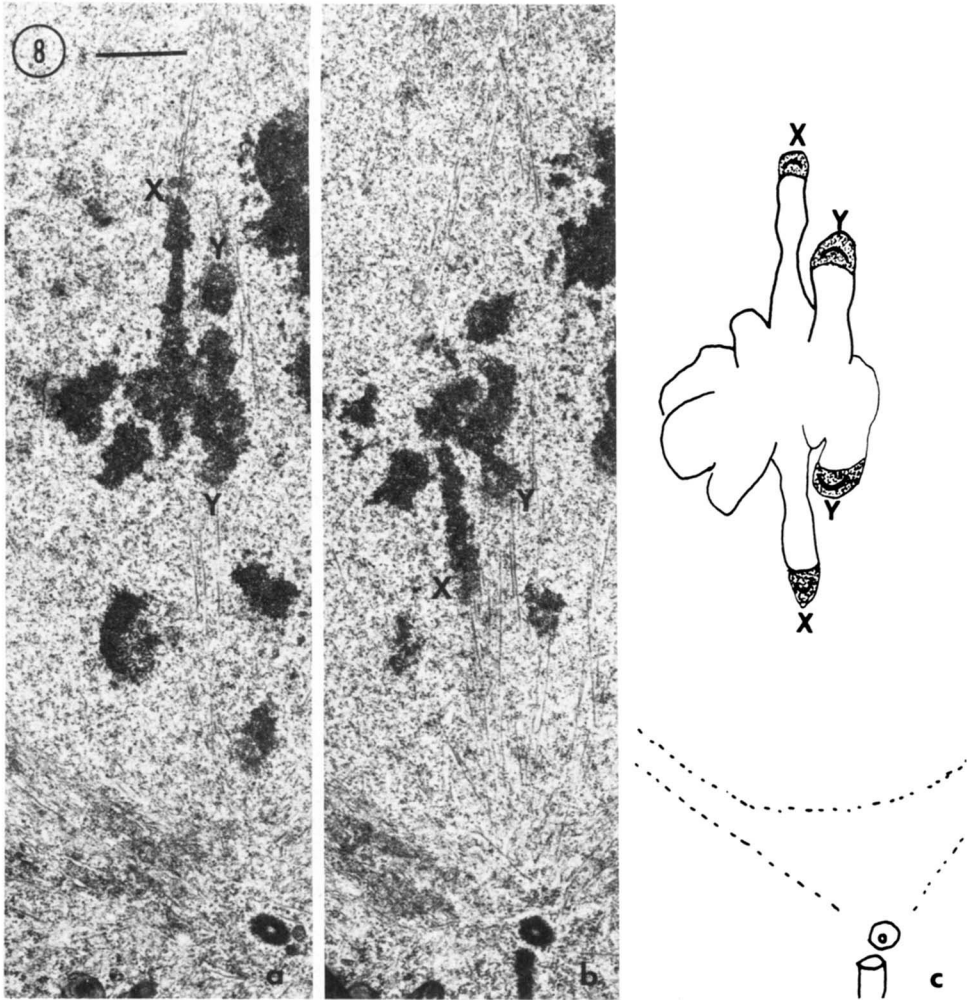


FIGURE 8.—Sections 15 and 17 through an XY bivalent and a reconstruction of the complete bivalent, respectively (30 sections encompassed the entire bivalent). Sister kinetochores of both the X dyad and the Y dyad are oriented toward opposite poles (equational separation). Bar = 1  $\mu$ m.

wild-type cells. In those cases in which the arms cannot be resolved in the proximal regions, the volume of chromatin is greater near the kinetochore for the Y than for the X. The two kinetochores can also be distinguished in *ord* division I cells using similar criteria. Equational segregations are readily apparent when observing the XY bivalents (Figure 8). They are also apparent when a 3-3 segregation occurs within a trivalent [three kinetochores directed to one pole and three to the opposite pole (see Figure 5)], a 1-1 segregation in a univalent or a 3-1 segregation in an autosomal bivalent (see Figure 7). Table 2 gives the frequencies observed. It is not always clear from autosomal bivalent topology whether a 2-2 orientation represents reductional separation of half bivalents or equational separation of sister chromatids. In addition, within the

TABLE 2

*Chromosome segregation patterns during meiosis I in primary spermatocytes of pr ord/pr ord flies as determined by kinetochore orientation<sup>a</sup>*

Chromosome	Segregation	Number	
XY Bivalent	XX-YY	9	
	XXY-Y	3	
	XYY-X	2	
	XY-XY	6	
	XXYY-O	1	
Chromosome 2 or 3	Bivalents	2-2	13
		3-1	4
		4-0	0
	Trivalents	3-3	3
		4-2	3
		5-1	0
		6-0	0
	Univalents	1-1	6
		2-0	2
	Chromosome 4	Bivalents	2-2
3-1			1
Trivalent		4-0	2
		4-2	1

<sup>a</sup> Kinetochore orientation defined as direction kinetochore faces in electron micrographs of serial sections.

colchicine-treated testes, numerous cells were observed that appeared to be in telophase I (Figure 9). Although colchicine treatment usually abolishes anaphase, we suspect that the short treatment of the meiotic cells did not drastically disrupt the architecture of cells that were in anaphase-telophase at the time the colchicine was administered. Unfortunately, in the colchicine-treated material it is not always possible to distinguish the X and Y chromosomes from the large autosomes due to the absence of a primary constriction and due to chromosome condensation. The chromosomes of the late anaphase-telophase cells are invariably displayed as monads (as they are in the electron micrographs of cells not treated with colchicine) due to the lack of sister kinetochore cohesion. In euploid cells there are 12 large elements, in trisomic cells 14 (Figure 9a) and in monosomic cells 10 (Figure 9b). Table 3 presents the segregations observed.

It is clear from both the electron and light microscopic observations that equational separation of dyad kinetochores occurs frequently during the first meiotic division in *ord* males.

#### DISCUSSION

Our observations have confirmed the proposal of GOLDSTEIN (1980) that *ord* is defective in some aspect of the mechanism that holds sister chromatids together. The wild-type meiotic dyad kinetochore that, at the ultrastructural

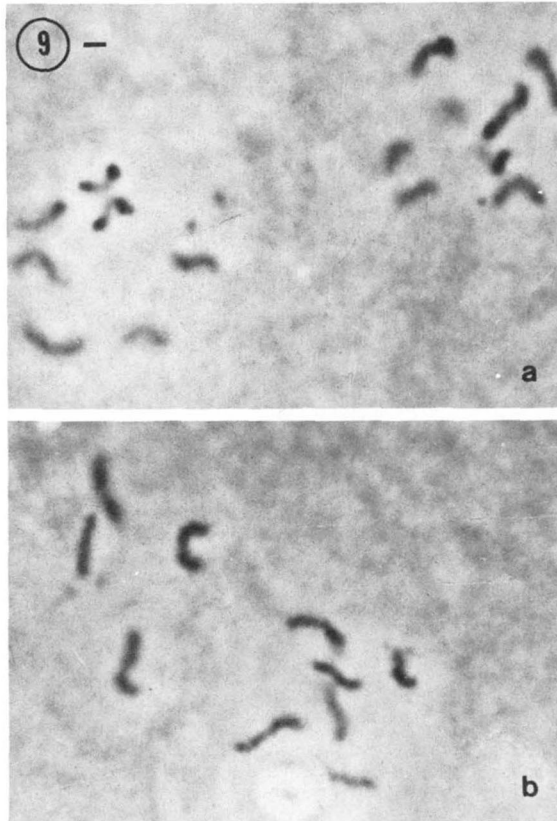


FIGURE 9.—Colchicine-treated telophase I cells from *ord* homozygotes. The trisomic cell (a) shows a 7-7 segregation for the large chromosomes (X, Y, 2 and 3), and the monosomic cell (b) shows a 6-4 segregation. Bar = 1  $\mu$ m.

level, is visibly a single hemispheric structure during prometaphase I is apparently composed of two kinetochores held together by some mechanism. As meiosis progresses the apparently single kinetochore is differentiated into a "double disk" structure (GOLDSTEIN 1980). It should be emphasized, however, that even at the double disk stage the two kinetochores are never observed to be physically uncoupled in wild-type primary spermatocytes (H.-P LIN and K. CHURCH, personal observation of more than 100 wild-type dyad kinetochores). Primary spermatocytes from *ord* males differ strikingly from their wild-type counterparts. The two sister kinetochores are displayed as small hemispheric structures each about half the size of the single wild-type dyad kinetochore. Each sister kinetochore can act more or less independently within the constraints of the bivalent structure resulting in interactions with the spindle that may result in disjunction of sisters in addition to disjunction of homologues at anaphase I. At metaphase and anaphase II the independent monad kinetochores are distributed to the poles at random (GOLDSTEIN 1980).

Our observations demonstrate that the defect in *ord* is most certainly not restricted to meiosis in males. MASON (1976) originally suggested that *ord* might



TABLE 3

Segregation pattern in telophase I cells from *pr ord/pr ord* flies by light microscope observation (excluding chromosome 4)

	Segregation pattern	No. of cells observed
Euploid	6-6	12
	7-5	12
	8-4	1
	9-3	0
	10-2	1
Trisomic	7-7	5
	8-6	1
Monosomic	5-5	2
	6-4	3

exert its effect as early as the gonial divisions since *ord* disrupts both recombination and disjunction in females and disjunction in males. BAKER, CARPENTER and RIPOLL (1978) demonstrated that *ord* causes increased chromosome instability in abdominal histoblasts of females. We have demonstrated that *ord* increases nondisjunction during gonial divisions in males.

All of our data taken together indicate that the large autosomes are more likely to misbehave during gonial mitosis in *ord* males than the small 4th chromosomes or the sex chromosomes. This is in spite of the fact that during the first meiotic division the noncohesiveness of the sister kinetochores is apparent in all dyads and equational chromosome segregation at meiosis I involves the sex chromosomes as well as the autosomes. It may be that, due to the sex-determining mechanism in *Drosophila*, any aneuploid condition involving two X chromosomes is lethal in spermatogonial cells. More enigmatic is the absence of XO cells and cells involving Y chromosome aneuploidy. We are certain that none of the 23 cells examined with the electron microscope were aneuploid for the sex chromosomes. We are less certain about our light microscope analysis. We have observed that the sex chromosome bivalent responds differently than the large autosomal bivalents to the colchicine-hypotonic treatment. The monads in large autosomal univalents, trivalents and bivalents are often completely separated, whereas the sister chromatids can never be resolved in the pairing region of the XY bivalent (see Figure 3). We suspect that the sister chromatids may be more cohesive near the kinetochore of the Y chromosome for reasons other than the sister chromatid cohesion specified by the *ord*<sup>+</sup> allele [perhaps due to the unique structure of the pairing sites (COOPER 1965)] and that this cohesiveness is enough to allow proper auto-orientation of the sister kinetochores during mitosis but not co-orientation during meiosis. The mitotic behavior of the kinetochores during the gonial divisions in *ord* homozygotes will be the topic of a future ultrastructural investigation.

GOLDSTEIN (1980) failed to detect aneuploidy for the large autosomes in primary spermatocytes of homozygous *ord* males. It is possible that our obser-

vations and his are not strictly comparable. GOLDSTEIN (1980) used the same original *ord* stock that we used; however, his stock was outcrossed to Canton-S, the mutants were allowed to pass through one generation of free recombination with Canton-S chromosomes, and the mutant-bearing chromosomes were reisolated. We did not perform this manipulation. We believe, however, that a more reasonable explanation for the apparent discrepancy in the two sets of observations is that the technique used in GOLDSTEIN'S light microscopic analysis could not detect this aneuploidy. We were able to demonstrate aneuploidy unequivocally with the light microscope only when we applied the colchicine-hypotonic treatment. Furthermore, we have made preliminary observations on a second *ord* stock, *y/Y; ord/SM1*, and aneuploidy is readily apparent in homozygotes derived from this stock also. Among 22 prometaphase-metaphase I cells from three flies, we observed three cells monosomic for a large autosome, three trisomic cells and one cell that was monosomic for both large autosomes. None of these cells displayed aneuploidy for the sex chromosomes or chromosome 4.

GOLDSTEIN (1980) analyzed the chromosome constitution of cells in anaphase II in *ord* flies and, assuming that the first division cells were euploid, inferred the consequences of the first division. The data obtained by GOLDSTEIN (Table 8 of GOLDSTEIN 1980) can be compared with our data if we separate the chromosomes at the two poles in our telophase I cells (Table 3). The data sets are strikingly similar (Table 4) and appear to have been drawn from the same distribution. We conclude, therefore, that the stock used by GOLDSTEIN had not changed significantly when later used by us. It should be emphasized that our observations do not invalidate any of the conclusions drawn from the GOLDSTEIN investigation; in fact, we have verified the majority of his conclusions. GOLDSTEIN (1980) was primarily concerned with the genetic and cytological behavior of the sex chromosomes, and our observations on the gonial disjunctional behavior of the large autosomes do not bear on the situation concerning the sex chromosomes.

Several studies have been undertaken to determine whether loci known to be involved in meiotic recombination and/or disjunction are utilized during the mitotic cell cycle (BAKER *et al.* 1976; BAKER, CARPENTER and RIPOLL 1978). The genetic approach involves an analysis of somatic clones produced in flies heterozygous for somatic cell markers and homozygous for the meiotic mutant of interest. Such clones are the result of somatic mutation, recombination, nondisjunction, chromosome breakage or loss. BAKER, CARPENTER and RIPOLL (1978) analyzed *ord* and observed an elevated frequency of clones attributable to nondisjunction and/or mitotic recombination in abdominal histoblasts. The distribution of clone size suggested that *ord* affects chromosome stability primarily in the terminal burst of division of the histoblasts. The observation that *ord* affects somatic mitosis was surprising since it was considered that disjunction-defective mutants that affect mitosis would be lethal (BAKER *et al.* 1976; BAKER, CARPENTER and RIPOLL 1978).

Our observation of high frequencies of trisomy and monosomy for large autosomes is also surprising. Such aneuploidy might be considered to be

TABLE 4

Comparison of chromosome number distributions resulting from anaphase I segregation of sex chromosomes and large autosomes in *pr ord/pr ord* males

Number chromosomes present	2	3	4	5	6	7	8	9	10
Number of telophase I poles (From Table 3 this study)	1	0	4	16	28	20	2	0	1
Number of anaphase II cells (From GOLDSTEIN 1980, Table 8)	1	0	7	23	29	13	4	0	1

$G_H = 1.7258^a$   
 $\chi^2 0.05[4] = 9.488^a$

<sup>a</sup> Interaction of heterogeneity G test (SOKAL and ROHLF 1981)

incompatible with cell viability. However, RIPOLL (1980) analyzed the effects of terminal duplications and deficiencies on the proliferation dynamics of epidermal cells by means of mitotic recombination and demonstrated that hyperploidy involving up to at least 45% of the euchromatin on the right arm of chromosome 3 is cell viable, although the effects of hypoploidy were more drastic. In these studies cell viability was defined as being synonymous with clone recovery. Whether the hypoploidy is actually cell lethal or simply blocks cell division or lengthens the cell cycle time leading to smaller clone size and, thus, to a reduced recovery of clones could not be determined.

Genetic tests for monitoring mitotic chromosome events may underestimate the absolute frequencies of such events. A more direct approach involves cytological analysis of dividing cells. Recently, several investigations have demonstrated that many meiotic mutants that increase mitotic chromosome instability in epidermal or wing derivatives as monitored by genetic tests may cause high frequencies of chromosome aberrations in dividing neuroblast cells (GATTI 1979; BAKER *et al.* 1980). For example, up to 14% of the neuroblast cells in flies homozygous for an allele of *mei 41* (a recombination-defective mutant) show chromosome aberrations (GATTI 1979). Interestingly, only chromatid and isochromatid breaks were observed, and in most cells the accompanying fragments were present, indicating that the vast majority of the aberrations scored in mutant cells occurred in the same division cycle in which they were scored. This suggests that such aberrations are either cell lethals or that cells possessing such aberrations do not progress to the next metaphase. If the effects observed occur in all mitotic cells, then up to 14% of the cells can be lost during each mitotic cell cycle and a viable fly can still be produced.

Since *ord* has been demonstrated to affect mitotic events in both female abdominal histoblasts and male gonial cells it is likely that *ord* affects all mitoses. We can say with confidence (based on this investigation) that in one population of mitotic cells (spermatogonial cells) trisomy and monosomy for the large autosomes occur frequently and are not necessarily cell lethals. The evidence also suggests that such aneuploid cells can divide (see Figure 1).

However, the aneuploidy probably does affect the length of the cell cycle as evidenced by the loss of synchrony and regulation of cell division during the production of spermatocyte cysts.

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