Double or nothing: A Drosophila Mutation Affecting Meiotic Chromosome Segregation in Both Females and Males

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ABSTRACT

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

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

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

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

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

In *Drosophila* males there is no detectable recombination, and synaptonemal complex is not formed (BAKER and HALL 1976; MEYER 1960; RASMUSSEN 1973). Mutations affecting distributive segregation in the female have no effect on meiosis I in the male, thus a distinct pathway must exist for homolog segregation in males. This mechanism has been most fully investigated for the sex chromosomes in which specific pairing sites are responsible for pairing and proper segregation

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(COOPER 1964). The *cis*-acting pairing site for the X and Y chromosomes has been localized to part of the rDNA repeat (McKEE and KARPEN 1990; McKEE *et al.* 1992). It appears that pairing sites also mediate autosomal segregation (McKEE *et al.* 1993; YAMAMOTO 1979).

The specificity of meiotic mutations isolated in *Drosophila* provides strong evidence for multiple pathways of chromosome segregation in meiosis I. For example, with two exceptions, all of the mutations affect meiosis only in the female or only in the male. The majority of mutations affecting chromosome segregation in the female reduce recombination (BAKER and HALL 1976). Other mutations, also female specific, almost exclusively cause nondisjunction of nonexchange chromosomes. Mutations in the *nod*, *Axs*, *ald*, and *mei-S51* genes belong to this class (CARPENTER 1973; O'TOUSA 1982; ROBBINS 1971; ZHANG and HAWLEY 1990; ZITRON and HAWLEY 1989). The *ncd* gene is unusual in that mutations in this gene result in aberrant segregation of both exchange and nonexchange chromosomes (DAVIS 1969). *Trans*-acting mutations affecting homolog segregation specifically in the male are not well defined.

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

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

MATERIALS AND METHODS

Stocks: All *Drosophila* stocks and crosses were grown at 25° (unless otherwise noted) on a standard mix of cornmeal, brewer's yeast, molasses and agar. All balancer chromosomes and all mutations other than *Dub* are described in LINDSLEY and ZIMM (1992). *C(1)RM*, $y^2 su(w^a) w^a$ will be referred to in this paper as compound-X or $\bar{X}\bar{X}$. $Y^S X \cdot Y^L y^+$, *In(1)EN*, $y v f B$ was used as the compound-XY chromosome and is referred to as $\bar{X}\bar{Y}$ in this report. *C(4)EN*, *ci ey^R* is referred to as $\bar{4}\bar{4}$. These compound chromosomes, the *cu v f car* and the compound autosome stocks are described in KERREBROCK *et al.* (1992). The *FM7c* balancer has the markers $y^{31d} sc^8 w^a sn^{X2} v^{of} g^4 B$. The *c wt px* stock used in mapping was obtained from the Bloomington stock center. The deficiency *Df(2R)PC4* was obtained from R. LEHMANN. The *TM3, Sb/T(2;3)CyO*, *st Kg^V red Tb* stock was obtained from W. SAXTON.

Isolation of the *Dub* mutation: *Double or nothing* (*Dub*) is a mutation that was induced on a second chromosome, marked with *J Sco*, using the mutagen, ethyl methanesulfonate (EMS). It was isolated in a screen of 2034 chromosomes for

new alleles of *abo* (*abnormal oocyte*) (SANDLER 1970; TOMKIEL *et al.* 1991), and its isolation number was 1102. A female-specific meiotic defect as well as a maternal effect lethality are associated with *abo¹* (CARPENTER and SANDLER 1974; SANDLER 1970). While the *Dub* mutation complemented the maternal effect, the frequency of nondisjunction in *abo¹/Dub* females was double that of *Dub/+* females. However, no increase in nondisjunction was observed in *abo²/Dub* females, suggesting that either the *abo¹* interaction is allele specific or due to a locus elsewhere on the chromosome.

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

In the assay of female meiotic nondisjunction for Table 2, compound-XY, *v f B* males were crossed to *cu v f car/y* females. Normal ova yielded Bar females ($\bar{X}\bar{Y}/X$) and males wild-type for Bar (X/O). Exceptional-X ova yielded Bar males ($\bar{X}\bar{Y}/O$) and females wild-type for Bar (X/X). The number of exceptional progeny was doubled for the adjusted total and for calculation of the nondisjunction frequencies. The centromere-linked mutation, *carnation*, allowed diplo-X ova resulting from meiosis II nondisjunction (carrying two sisters) and those resulting from meiosis I nondisjunction (carrying two homologs) to be distinguished. To calculate map distances, exchange events on the X chromosomes were counted. This was done by recording the phenotypes of the XO males resulting from normal-X ova, and by crossing the F_1 females resulting from diplo-X ova to compound-XY males and recording the phenotypes of F_2 X/O males to determine the markers on the parental chromosomes in the F_1 females. Mapping distances for the diplo-X ova were calculated as if the chromosomes had been isolated from independent ova carrying a single X chromosome. Exchange rank distributions were calculated by the method of WEINSTEIN (1936) for regular-X progeny and by the method of DAVIS (1969) and MERRIAM and FROST (1964) for diplo-X progeny.

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

An unexpected class of progeny was noted in this cross, yellow Bar males with vermilion⁺ eyes. Although their external appearance was entirely male, these "males" were infertile and

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

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

For simultaneous measurement of the sex and fourth chromosome nondisjunction in males, y/y ; *C(4)EN*, *ci ey^R* females were mated with $y/y⁺Y$; *spa^{hot}* males. Normal sperm yielded yellow females (*X/X*; 44/4) and yellow⁺ males (*X/Y*; 44/4). As in the female test of *X* and 4 nondisjunction, any surviving haplo-4 Minute progeny were counted but were excluded from any calculations and are not reported in this paper. Sperm that were diplo or nullo for the sex chromosomes produced yellow⁺ females (*X/X/y⁺Y*) and yellow males (*X/O*). Exceptional-4 sperm produced sparkling-poliert progeny (4/4) or cubitus-interruptus eyeless-Russian progeny (44/O).

To determine the meiotic division affected in males, compound-*X*, $y^2 su(w^a) w^a$ females were mated with $y/y⁺Y$ males. Normal sperm yielded yellow⁺ females ($\bar{X}\bar{X}/y⁺Y$) and yellow males (*X/O*). Exceptional sperm yielded yellow or yellow² females (*X/X* and $\bar{X}\bar{X}/O$) and yellow⁺ males (*X/y⁺Y*). The females resulting from sperm carrying two sister chromosomes (*X/X*) were yellow and had a wild-type eye color, whereas exceptional females resulting from nullo-*XY* sperm ($\bar{X}\bar{X}/O$) were yellow² and had a darker eye color with no pseudo-pupil.

Mapping of *Dub*: The mutation was first mapped to the interval between *nw* and *Pin* in two small scale mappings (15 and 47 recombinants). Females heterozygous for *J Sco Dub* and *S Sp Tft nu^PPin* were mated with *abo¹* males, and the female progeny were mated with compound-*XY* males to test for skewed sex ratios or for nondisjunction events in the progeny. No sex ratio skewing was apparent, and nondisjunction events were used to map the mutation. *Dub* was later mapped to the smaller interval between *c* and *wt*. After mating *c wt px* males to *pr cn Dub/c wt px* or *pr cn Dub sp/c wt px* females, recombinant chromosomes from male progeny were

isogenized and tested for three phenotypes: inviability when *trans*-heterozygous with the original *pr cn Dub* chromosome, dominant meiotic nondisjunction in females, and dominant meiotic nondisjunction in males. In 33 recombinants between *c* and *wt* all three phenotypes mapped to 2–82.6 cM.

Lethal phase and phenotypes: The lethal phase of *Dub* homozygotes was assessed by mating parents heterozygous for *Dub* (*pr cn Dub/b pr*). As controls, heterozygous parents were outcrossed to *b pr* mates and, in addition, a mating of *b pr* males and females was set up. The females were allowed to lay their eggs overnight on apple juice-sucrose-agar Petri dishes with a wet yeast smear on the surface. The number of clear unfertilized eggs, the number of eggs that hatched, the number of pupal cases and the number of eclosed adults were all recorded. From these counts, a histogram of lethality was constructed.

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

Neuroblast squashes for mitotic chromosomes: Cytological preparations of larval brains were made by standard methods without colchicine (GONZALEZ *et al.* 1991; SUNKEL and GLOVER 1988). These were examined by phase-contrast microscopy using a Zeiss Axiophot equipped with Plan Neofluar 100 $\times$ and Plan Apochromat 63 $\times$ objectives.

RESULTS

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

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

TABLE 1

Dub is a dominant conditional mutation increasing female meiotic nondisjunction frequency

Ova type	Temperature and maternal genotype			
	25°		18°	
	+/+	<i>Dub</i> /+	+/+	<i>Dub</i> /+
Regular ova				
X; 4	1331	945	1063	822
X nondisjunctional ova				
X/X; 4	0	22	2	6
O; 4	2	45	0	1
4 nondisjunctional ova				
X; 4/4	1	192	3	39
X; O	2	247	24	41
X, 4 nondisjunctional ova				
X/X; 4/4	0	19	2	1
X/X; O	0	5	0	0
O; 4/4	1	6	0	0
O; O	0	39	0	2
Total progeny scored	1337	1520	1094	912
Adjusted total scored ^a	1340	1656	1098	922
% nondisjunction				
X	0.45	16.43	0.73	2.17
4	0.37	34.84	2.82	9.33

y/y; spa^{pol} females of the indicated genotype were crossed to *y/y⁺ Y; C(4)RM, ci ey^R* males.

^a X nondisjunctional progeny were doubled for calculation of nondisjunction frequency (see MATERIALS AND METHODS).

much higher than the control frequency of 0.5%. Nullo-X ova outnumbered diplo-X ova.

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

To ascertain whether chromosome missegregation events were occurring in the first or second meiotic division, we mated *Dub* females to males carrying a compound-XY chromosome. The mutant females carried X chromosomes heterozygous for a centromere-linked marker, *carnation* (*car*), so that diplo-X excep-

TABLE 2

Dub female meiotic nondisjunction produces reductional exceptions

Ova type	Maternal genotype	
	+/+	<i>Dub</i> /+
Regular ova ^a		
X	5514	5268
X nondisjunctional ova		
O	3	246
X/X (<i>car</i> ⁺ / <i>car</i> ⁻)	1	335
X/X (<i>car</i> ⁺ / <i>car</i> ⁺) and (<i>car</i> ⁻ / <i>car</i> ⁻) ^b	0	1
Total progeny scored	5518	5850
Adjusted total scored ^c	5522	6432
% nullo-X	0.11	7.65
% diplo-X (<i>car</i> ⁺ / <i>car</i> ⁻)	0.04	10.42
% diplo-X (<i>car</i> ⁻ / <i>car</i> ⁻ and <i>car</i> ⁺ / <i>car</i> ⁺)	0.00	0.03
Total % nondisjunction	0.15	18.10

y/cv f v car females of the indicated genotype were mated to compound-XY, *v f B* males at 25°.

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

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

^c X nondisjunctional progeny were doubled for calculation of nondisjunction frequency (see MATERIALS AND METHODS).

tional progeny carrying two sister chromosomes could be distinguished from those carrying two homologous chromosomes. Nondisjunction occurred almost exclusively during the first meiotic division (Table 2), because essentially all of the exceptional ova carried two homologous chromosomes. The lower percentage of nullo-X relative to the number of diplo-X ova observed in Table 2 is likely due to cosegregation events of the X and 4, since the nullo-X nullo-4 ova are inviable in this assay. Cosegregation is discussed in further detail below.

In these matings of *Dub* heterozygous mothers there was a low but significant number of gynandromorphs. These result from chromosome instability in the early zygotic cleavages, either due to chromosome loss during the mitotic divisions or recovery by a mitotic spindle of a chromosome lost during a meiotic division. Other meiotic mutations, notably *nod* and *ncd*, show a similar phenotype (CARPENTER 1973; DAVIS 1969).

***Dub* has little effect on recombination:** Since the majority of mutations that affect the first meiotic division in *Drosophila* females cause a reduction in recombination, we examined the effect of *Dub* on recombination. The X chromosomes used in the cross for Table 2 were heterozygous for several recessive mutations, and map distances were calculated from the phenotypes of the regular XO male progeny. Surprisingly, although *Dub* causes reductional nondisjunction, it has relatively little effect on exchange. There were slight reductions in all of the intervals, but only one interval showed a significant dif-

TABLE 3
Dub has little effect on recombination in females

Genotype	Mapping interval				Total map distance (cM)	No. of progeny scored
	<i>y-cv</i> (cM)	<i>cv-v</i> (cM)	<i>v-f</i> (cM)	<i>f-car</i> (cM)		
Mono-X ova						
+/+	8.4	19.5	21.7	6.5	56.1	2445
<i>Dub</i> /+	7.6	19.2	18.5	5.5	50.8	2010
Diplo-X ova						
<i>Dub</i> /+	6.6	9.9	12.9	2.1	31.5	167 ^a

^a Both chromosomes in these progeny came from the mother, so a total of 334 chromosomes were scored for exchange.

ference (Table 3, Mono-X ova). The *Dub* and control values were significantly different for the *vermillion-forked* (*v-f*) interval (binomial distribution test, $P < 0.01$), but there was no significant difference for the other intervals (LINDREN *et al.* 1978).

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

Exchange in the proximal regions appeared to ensure proper disjunction better, because nondisjunction was more likely to be accompanied by exchange in the distal regions. This distribution of exchanges is reminiscent of that in the diplo-X and diplo-2 exceptions of *nod* and *nod*^{DTW} (CARPENTER 1973; RASOOLY *et al.* 1991).

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

To address the question of whether *Dub* predominantly affects nonexchange chromosomes, we compared the percentage of nonexchange tetrads present in the ova having faithfully segregated chromosomes with the percentage in ova having improperly segregated chromosomes. When there is no exchange in a tetrad, chromosomes are segregated by the distributive system, so the number of nonexchange tetrads reflects the num-

ber of chromosomes that must be segregated in the distributive system. Therefore if nonexchange chromosomes were more likely to nondisjoin in *Dub* mutants, a greater proportion of the exceptional ova would be derived from nonexchange tetrads. The frequency of nonexchange, single exchange and multiple exchange tetrads (known as the tetrad or exchange rank) may be estimated from the observed number of no crossover, single crossover and multiple crossover chromosomes. Appropriate equations have been developed for calculating the tetrad ranks from normal and diplo-X ova (DAVIS 1969; WEINSTEIN 1936).

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

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

We tested the effect of *Dub* on the achiasmate segregation system in one other way. An example of the distributive segregation system in *Drosophila* is the consistent and faithful segregation of a Y chromosome from a compound-X chromosome in females (GRELL 1976). These chromosomes are segregated by the achiasmate system even though exchange does oc-

TABLE 4

Exchange ranks of normal and exceptional ova

Exchange rank	Mono-X ova Maternal genotype		Diplo-X ova Maternal genotype
	+ / +	<i>Dub</i> / +	<i>Dub</i> / +
E ₀	0.13	0.10	0.51
E ₁	0.62	0.79	0.40
E ₂	0.25	0.10	0.09
E ₃	0.0	0.01	0.0
No. scored	(2445)	(2010)	(167)

TABLE 5

Dub females with a balancer X chromosome have very high meiotic nondisjunction frequencies

Ova type	Maternal genotype	
	+ / +	<i>Dub</i> / +
Regular ova ^a		
X	2156	1097
<i>FM7c</i>	737	371
X nondisjunctional ova		
O	9	545
<i>X/FM7c</i>	14	483
<i>X/X</i> and <i>FM7c/FM7c</i>	0	0
Autosome nondisjunctional ova ^b		
<i>X/FM7c</i> ; 2/2; 3/3; 4 or 4/4	0	160
Total progeny scored	2916	2656
Adjusted total scored ^c	2179	1965
% nullo-X	0.41	27.74
% diplo-X	0.64	24.58
Total % nondisjunction	1.05	52.32

y/FM7c females of the indicated genotype were mated with compound-XY, *v f B* males. The control females were *SM1/+*.

^a The ratio of regular X ova fertilized by nullo-XY sperm relative to $\bar{X}\bar{Y}$ sperm is 1225/931 for the control females and 457/480 for the *Dub* females (160 triploid female progeny have already been subtracted from the *X/X* progeny for the *Dub* ratio).

^b These progeny were observed as intersexes, and this number represented only half of the number of such ova (see MATERIALS AND METHODS).

^c Calculation of the X chromosome nondisjunction frequencies was done using adjustments described in MATERIALS AND METHODS. These adjustments compensate for the presence of autosomal nondisjunction and the reduced viability of the progeny resulting from regular ova carrying *FM7c*.

cur between the two X chromosome arms of a compound-X chromosome. Mutations such as *ncd*, *ald* and *Axs* have been shown to interfere with this segregation (DAVIS 1969; O'TOUSA 1982; ZITRON and HAWLEY 1989). In *Dub* females with a compound-X chromosome and a Y, the nondisjunction frequency was 40.9% compared to 0.6% in the control (Table 6).

These experiments demonstrate that *Dub* affects the segregation of nonexchange chromosomes, but the mutation causes nondisjunction of exchange chromosomes as well. *Dub* did not reduce recombination enough for all of the nondisjoined chromosomes to be nonexchange (Tables 2 and 3), and in the diplo-X exceptional gametes almost half of the tetrads have undergone at least one exchange (Table 4).

TABLE 6

Dub disturbs the segregation of the Y chromosome from the compound-X in females

Ova type	Maternal genotype	
	+ / +	<i>Dub</i> / +
Regular ova		
$\bar{X}\bar{X}$	1025	673
Y	1280	619
X Nondisjunctional ova		
$\bar{X}\bar{X}/Y$	1	61
O	7	447
Autosome nondisjunctional ova ^a		
$\bar{X}\bar{X}/Y$; 2/2; 3/3; 4 or 4/4	2	39
$\bar{X}\bar{X}$; 2/2; 3/3; 4 or 4/4	4	5
Total progeny	2319	1844
Corrected total progeny ^b	2319	2186
% nondisjunction ^b		
$\bar{X}\bar{X}/Y \leftrightarrow O$	0.60	40.90

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

^a These ova produced progeny that were either intersexes or triploid females.

^b See MATERIALS AND METHODS.

Cosegregation of chromosomes in *Dub* mutant females: In *Dub* females when more than one chromosome was missegregated in the same ovum, these chromosomes were not segregated independently with respect to each other. By simultaneously following two chromosomes, the X and fourth (Table 1), we observed a strong tendency for the missegregating chromosomes to be incorporated into the same meiotic product. The double exceptions seen were not independently distributed among the possible classes: *X/X*; 4/4 and *O/O* double exceptions were more numerous than were *X/X*; *O* and *O*; 4/4 double exceptions. Such a nonrandom distribution among the double exceptions had been previously observed in the meiotic mutants *nod* and *ncd* for the X and fourth chromosomes (CARPENTER 1973; DAVIS 1969; WRIGHT 1974). This "cosegregation" behavior is in marked contrast to the nonrandom distribution of *X*; 4 double exceptions observed in *Axs* females, where the X bivalent is more likely to segregate away from the fourth bivalent, yielding *X/X*; *O* and *O*; 4/4 ova (ZITRON and HAWLEY 1989).

Additional evidence indicated that cosegregation of all chromosomes occurred often. When a balancer X was introduced into *Dub* heterozygous females, intersexes and triploid females appeared among the progeny at a surprisingly high frequency (Table 5). The intersexes and triploid females resulted from ova carrying two copies of the major autosomes and one or two copies, respectively, of the X chromosome. Similarly, when a compound-X chromosome and a Y chromosome were present in a *Dub* heterozygous female, many intersexes and triploid females were found in the progeny (Table 6). Thus cosegregation of the sex chromosomes with the autosomes appeared to have occurred, although the number of *X/X* or $\bar{X}\bar{X}/Y$; 2/2; 3/3 ova could not be compared to the number of *O*; 2/2; 3/3

TABLE 7

Dub is a dominant conditional mutation increasing the male meiotic nondisjunction frequency

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

y/y⁺Y males of the indicated genotype were mated with *y*; *C(4)EN*, *ci ey*^R females.

ova, because the latter were not recoverable. It is interesting that in the $\widehat{XX}/Y$ cross, triploid females and intersexes were more likely to have received both the compound-X and the Y than to receive only the compound-X chromosome, as $\widehat{XX}/Y$; 2/2; 3/3 ova were more frequent than $\widehat{XX}$; 2/2; 3/3 ova.

***Dub* dominantly increases nondisjunction during meiosis I in males:** The first meiotic division in male *Drosophila* is distinct from the first division in females (BAKER and HALL 1976). There is no recombination, and assembled synaptonemal complex is not observed (MEYER 1960; RASMUSSEN 1973). Instead, segregation of the homologs employs specific pairing sites. All of the previously isolated *Drosophila* meiotic mutants are specific in affecting only females or only males, with the exceptions of *ord* and *mei-S332* (DAVIS 1971; KERREBROCK *et al.* 1992; MASON 1976; MIYAZAKI and ORR-WEAVER 1992). These two mutations cause premature sister-chromatid separation and have significant levels of meiosis II nondisjunction. *Dub* was striking because it caused meiotic chromosome nondisjunction in males and females, and in contrast to *ord* and *mei-S332*, meiosis I segregation was affected almost exclusively.

Meiotic nondisjunction in *Dub* males was characterized by genetic assays to test which chromosomes and which meiotic division were affected by *Dub*. In males, *Dub* acted to increase nondisjunction in a dominant and temperature-sensitive manner (Table 7). Both the sex chromosomes and the fourth chromosome were affected, and the frequency of fourth chromosome nondisjunction was lower than sex chromosome nondisjunction.

TABLE 8

Dub male meiotic nondisjunction yields primarily reductional exceptions

Sperm type	Paternal genotype	
	+/+	<i>Dub</i> /+
Regular sperm		
X or Y ^a	2816	5531
XY nondisjunctional sperm		
O	4	178
X/Y	3	105
X/X	0	15
Total progeny scored	2823	5829
% nullo-XY	0.14	3.05
% X/Y	0.11	1.80
% diplo-X	0.00	0.26
Total observed nondisjunction	0.25	5.11

y/y⁺Y males of the indicated genotype were mated with compound-X, *y*² *su(w^a) w^a* females. The control in this experiment was not done with siblings of the *Dub*/+ males.

^a In this assay, Y/Y exceptional sperm were indistinguishable from regular mono-Y sperm and are therefore included in these numbers.

tion. Sperm that were nullosomic for the sex chromosomes were more common than were X/X or X/Y sperm, indicating that chromosome loss also occurred. The overall frequency of nondisjunction was lower in males than in females, the difference in fourth chromosome segregation being particularly great.

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

By crossing test males to compound-X females we were able to assess the meiotic division in which missegregation was occurring (Table 8). The first meiotic division was primarily affected; however, missegregation did not appear to be as exclusive to the reductional division as it was in *Dub* heterozygous females. The number of equational exceptions was higher than observed in the control, although the frequency was still less than 1%. Because the progeny from Y/Y sperm were indistinguishable from normal progeny, only half of the equational exceptions were scored in this test. Consequently, the true frequency of equational missegregation was probably twice what we measured.

The cosegregation of heterologous chromosomes that nondisjoined was difficult to address in male *Dub* heterozygotes. Since the nondisjunction frequencies in

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

***Dub* is a recessive, conditional lethal mutation:** The dominant meiotic phenotype of *Dub* is linked closely to a conditional recessive lethality. At 25°, homozygous *Dub* adults were rare. The rare escapers were very short-lived and had many defects: small rough eyes, etched tergites, crumpled or nicked wings, and bristles either missing or duplicated. At 18°, homozygous *Dub* progeny were more common, although at most 20% of the expected number of homozygotes eclosed in bottles of the heterozygous stock. Homozygous adults raised at 18° were more normal in appearance, except for patches of disorder in the eye facets. These flies were infertile.

Recessive lethality and the phenotype of the rare escapers are characteristics observed in mutations affecting mitotic chromosome segregation, such as *rough deal* (*rod*) (KARESS and GLOVER 1989). The presence of gynandromorphs among the progeny of heterozygous *Dub* mothers also suggested that *Dub* product might play a role in mitosis. To test this, we determined the lethal phase and phenotype of *Dub* homozygotes, and we then cytologically examined neural cells of homozygous larvae for mitotic defects. Most known mitotic mutants have late-larval/pupal lethality, although a few embryonic lethal mitotic mutants are known (EDGAR and O'FARRELL 1989; GATTI and BAKER 1989; HIME and SAINT 1992).

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

The embryonic lethality that occurred when either parent was a *Dub* heterozygote appears to have been the consequence of autosomal aneuploidy due to meiotic nondisjunction, rather than a semi-dominant lethal effect of *Dub*, or a maternal-effect lethality. We found that *Dub* had no semi-dominant lethality by crossing *pr cn Dub/pr cn bw* males to *pr cn bw* females and then counting the ratio of *Dub*⁺ and *Dub*/+ progeny (data not shown). Maternal lethality seemed unlikely as there was a similar degree of embryonic lethality when either the mother or father was a *Dub* heterozygote (Figure 1).

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

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

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

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

The nature of the *Dub* mutation: We identified a deficiency that uncovers *Dub* in order to determine if the

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

Possible functions of the *Dub* gene in chromosome segregation: The phenotypes of the *Dub* mutation support a role for the gene in an aspect of meiotic chromosome segregation common to female and male meiosis. However, the mutation we have characterized is a dominant allele that may be antimorphic or neomorphic. If *Dub* were antimorphic, its phenotype would be

similar to loss-of-function alleles and would reflect the function of the wild-type gene. Antimorphic and neomorphic alleles can be distinguished by the properties of the mutation in the presence of a duplication of the wild-type gene, but unfortunately a duplication covering *Dub* does not exist.

Three other dominant meiotic mutations have been identified in *Drosophila*, and these provide a precedent in the sense that the alleles have either been shown to be antimorphic or to have meiotic phenotypes similar to loss-of-function alleles. The initial allele of *Axs* was dominant, while *l(1)TW6^{cs}* was shown to be a dominant mutation in *nod* (now called *nod^{DTW}*). Revertants of these mutations were isolated and demonstrated to be loss-of-function mutations in the genes (RASOOLY *et al.* 1991; WHYTE *et al.* 1993). Analysis of the phenotypes of both the dominant and revertant alleles showed that in each case the dominant allele was antimorphic, and its phenotype provided an accurate indication of the role of the gene in meiosis. A third dominant mutation is an allele of *ncd* that initially was dominant but has lost its dominance in the time since its isolation (KOMMA *et al.* 1991). Nevertheless, homozygotes for this allele showed the same meiotic effects as loss-of-function alleles.

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

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

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

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LITERATURE CITED

- BAKER, B., and J. HALL, 1976 Meiotic mutants: genetic control of meiotic recombination and chromosome segregation, pp. 351-434 in *The Genetics and Biology of Drosophila*, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.
- BAKER, B., A. CARPENTER, M. ESPOSITO, R. ESPOSITO and L. SANDLER, 1976 The genetic control of meiosis. *Annu. Rev. Genet.* **10**: 53-134.
- CARPENTER, A., 1973 A meiotic mutant defective in distributive disjunction in *Drosophila melanogaster*. *Genetics* **73**: 393-428.
- CARPENTER, A. T. C., and L. SANDLER, 1974 On recombination-defective meiotic mutants in *Drosophila melanogaster*. *Genetics* **76**: 453-475.
- COOPER, K. W., 1964 Meiotic conjunctive elements not involving chiasmata. *Proc. Natl. Acad. Sci. USA* **52**: 1248-1255.
- DAVIS, B., 1971 Genetic analysis of a meiotic mutant resulting in precocious sister-centromere separation in *Drosophila melanogaster*. *Mol. Gen. Genetics* **113**: 251-272.
- DAVIS, D. G., 1969 Chromosome behavior under the influence of *claret nondisjunctional* in *Drosophila melanogaster*. *Genetics* **61**: 577-594.
- DAWSON, D. S., A. W. MURRAY and J. W. SZOSTAK, 1986 An alternate pathway for meiotic chromosome segregation in yeast. *Science* **234**: 713-717.
- EDGAR, B. A., and P. H. O'FARRELL, 1989 Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* **57**: 177-187.
- GATTI, M., and B. BAKER, 1989 Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. *Genes Dev.* **3**: 438-453.
- GONZALEZ, C., J. JIMENEZ, P. RIPOLL and C. E. SUNKEL, 1991 The spindle is required for the process of sister chromatid separation in *Drosophila* neuroblasts. *Exp. Cell Res.* **192**: 10-15.
- GRELL, R., 1976 Distributive pairing, pp. 436-486 In *The Genetics and Biology of Drosophila*, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.
- GUACCI, V., and D. B. KABACK, 1991 Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. *Genetics* **127**: 475-488.
- HATSUMI, M., and S. ENDOW, 1992 Mutants of the microtubule motor protein, nonclaret disjunctional, affect spindle structure and chromosome movement in meiosis and mitosis. *J. Cell Sci.* **101**: 547-559.
- HAWLEY, R. S., and W. E. THEURKAUF, 1993 Requiem for distributive segregation: achiasmate segregation in *Drosophila* females. *Trends Genet.* **9**: 310-317.
- HAWLEY, R. S., H. IRICK, A. E. ZITRON, D. A. HADDOX, A. LOHE, C. NEW, M. D. WHITLEY, T. ARBEL, J. JANG, K. MCKIM and G. CHILDS, 1993 There are two mechanisms of achiasmate segregation in *Drosophila*, one of which requires heterochromatic homology. *Dev. Genet.* **13**: 440-467.
- HIME, G., and R. SAINT, 1992 Zygotic expression of the *pebble* locus is required for cytokinesis during the postblastoderm mitoses of *Drosophila*. *Development* **114**: 165-171.
- JOHN, B., 1990 *Meiosis*. Cambridge University Press, Cambridge.
- KARESS, R. E., and D. M. GLOVER, 1989 *rough deal*: a gene required for proper mitotic segregation in *Drosophila*. *J. Cell Biol.* **109**: 2951-2961.
- KERREBROCK, A. W., W. Y. MIYAZAKI, D. BIRNBY and T. L. ORR-WEAVER, 1992 The *Drosophila mei-S332* gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. *Genetics* **130**: 827-841.
- KOMMA, D. J., A. S. HORNE and S. A. ENDOW, 1991 Separation of meiotic and mitotic effects of *claret nondisjunctional* on chromosome segregation in *Drosophila*. *EMBO J.* **10**: 419-424.
- LINDREN, B. W., G. W. McELRATH and D. A. BERRY, 1978 *Probability and Statistics*. Macmillan, New York.
- LINDSLEY, D., and G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, New York.
- LIVAK, K. J., 1984 Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics* **107**: 611-634.
- MASON, J. M., 1976 Orientation disruptor (*ord*): a recombination-defective and disjunction-defective meiotic mutant in *Drosophila melanogaster*. *Genetics* **84**: 545-572.
- MCDONALD, H. B., and L. S. B. GOLDSTEIN, 1990 Identification and characterization of a gene encoding a kinesin-like protein in *Drosophila*. *Cell* **61**: 991-1000.
- MCDONALD, H. B., R. J. STEWART and L. S. B. GOLDSTEIN, 1990 The kinesin-like *ncd* protein of *Drosophila* is a minus end-directed microtubule motor. *Cell* **63**: 1159-1165.
- McKEE, B., and G. KARPEN, 1990 *Drosophila* ribosomal RNA genes function as an X-Y pairing site during male meiosis. *Cell* **61**: 61-72.
- McKEE, B. D., L. HABERA and J. A. VRANA, 1992 Evidence that intergenic spacer repeats of *Drosophila melanogaster* rRNA genes function as X-Y pairing sites in male meiosis, and a general model for achiasmate pairing. *Genetics* **132**: 529-544.
- McKEE, B. D., S. E. LUMSDEN and S. DAS, 1993 The distribution of male meiotic pairing sites on chromosome 2 of *Drosophila melanogaster*: meiotic pairing and segregation of 2-Y transpositions. *Chromosoma* **102**: 180-194.
- MERRIAM, J. R., and J. N. FROST, 1964 Exchange and nondisjunction of the X-chromosomes in female *Drosophila melanogaster*. *Genetics* **49**: 109-122.
- MEYER, G. F. 1960 The fine structure of spermatocyte nuclei of *Drosophila melanogaster*, pp. 951-954 In *Proceedings of the European Regional Conference on Electron Microscopy*, edited by A. L. HOUWINK and B. J. SPIT. Nederlandse Vereniging voor Electronen-Microscopie, Delft.
- MIYAZAKI, W., and T. L. ORR-WEAVER, 1992 Sister-chromatid misbehavior in *Drosophila ord* mutants. *Genetics* **132**: 1047-1061.
- NICKLAS, R. B., 1974 Chromosome segregation mechanisms. *Genetics* **78**: 205-213.
- O'TOUSA, J., 1982 Meiotic chromosome behavior influenced by mutation-altered disjunction in *Drosophila melanogaster* females. *Genetics* **102**: 503-524.
- RASMUSSEN, S. W., 1973 Ultrastructural studies of spermatogenesis in *Drosophila melanogaster* meigen. *Z. Zellforsch.* **140**: 125-144.
- RASOOLY, R. S., C. M. NEW, P. ZHANG, R. S. HAWLEY and B. S. BAKER, 1991 The *lethal(1)TW-6^{cs}* mutation of *Drosophila melanogaster* is a dominant antimorphic allele of *nod* and is associated with a single base change in the putative ATP-binding domain. *Genetics* **129**: 409-422.
- ROBBINS, L. G., 1971 Nonexchange alignment: a meiotic process revealed by a synthetic meiotic mutant of *Drosophila melanogaster*. *Mol. Gen. Genet.* **110**: 144-166.
- SANDLER, L., 1970 The regulation of sex chromosome heterochromatic activity by an autosomal gene in *Drosophila melanogaster*. *Genetics* **64**: 481-493.
- SAXTON, W., J. HICKS, L. S. B. GOLDSTEIN and E. C. RAFF, 1991 Kinesin heavy chain is essential for viability and neuromuscular functions in *Drosophila*, but mutants show no defects in mitosis. *Cell* **64**: 1093-1102.
- SEARS, D. D., J. H. HEGEMANN and P. HIETER, 1992 Meiotic recombination and segregation of human-derived artificial chromosomes in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **89**: 5296-5300.
- SUNKEL, C. E., and D. M. GLOVER, 1988 *polo*, a mitotic mutant of *Drosophila* displaying abnormal spindle poles. *J. Cell Sci.* **89**: 25-38.
- THEURKAUF, W., and R. S. HAWLEY, 1992 Meiotic spindle assembly in *Drosophila* females: Behavior of nonexchange chromosomes and the effects of mutations in the *nod* kinesin-like protein. *J. Cell Biol.* **116**: 1167-1180.
- TOMKIEL, J., S. PIMPINELLI and L. SANDLER, 1991 Rescue from the *abnormal oocyte* maternal-effect lethality by *ABO* heterochromatin in *Drosophila melanogaster*. *Genetics* **128**: 583-594.
- WALKER, R. A., E. D. SALMON and S. A. ENDOW, 1990 The *Drosophila claret* segregation protein is a minus-end directed motor molecule. *Nature* **347**: 780-782.
- WEINSTEIN, A., 1936 The theory of multiple-strand crossing over. *Genetics* **21**: 155-199.
- WHYTE, W. L., H. IRICK, T. ABEL, G. YASUDA, R. L. FRENCH, D. R. FALK and R. S. HAWLEY, 1993 The genetic analysis of achiasmate segregation in *Drosophila melanogaster*. III. The wild-type product of the

- Axs* gene is required for the meiotic segregation of achiasmate homologs. *Genetics* **134**: 825–835.
- WRIGHT, T., 1974 A cold-sensitive zygotic lethal causing high frequencies of nondisjunction during meiosis I in *Drosophila melanogaster* females. *Genetics* **76**: 511–536.
- YAMAMOTO, M., 1979 Cytological studies of heterochromatin function in the *Drosophila melanogaster* male: autosomal meiotic pairing. *Chromosoma* **72**: 293–328.
- ZHANG, P., and R. S. HAWLEY, 1990 The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the *nod* locus. *Genetics* **125**: 115–127.
- ZHANG, P., B. A. KNOWLES, L. S. B. GOLDSTEIN and R. S. HAWLEY, 1990 A kinesin-like protein required for distributive chromosome segregation in *Drosophila*. *Cell* **62**: 1053–1062.
- ZITRON, A., and R. S. HAWLEY, 1989 The genetic analysis of distributive segregation in *Drosophila melanogaster*. I. Isolation and characterization of *Aberrant X segregation* (*Axs*), a mutation defective in chromosome partner choice. *Genetics* **122**: 801–821.

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