Isolation and Cytogenetic Characterization of Male Meiotic Mutants of *Drosophila melanogaster*

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ABSTRACT

Proper segregation of homologous chromosomes in meiosis I is ensured by pairing of homologs and maintenance of sister chromatid cohesion. In male *Drosophila melanogaster*, meiosis is achiasmatic and homologs pair at limited chromosome regions called pairing sites. We screened for male meiotic mutants to identify genes required for normal pairing and disjunction of homologs. Nondisjunction of the sex and the fourth chromosomes in male meiosis was scored as a mutant phenotype. We screened 2306 mutagenized and 226 natural population-derived second and third chromosomes and obtained seven mutants representing different loci on the second chromosome and one on the third. Five mutants showed relatively mild effects (<10% nondisjunction). *mei(2)yh149* and *mei(2)yoh7134* affected both the sex and the fourth chromosomes, *mei(2)yh217* produced possible sex chromosome-specific nondisjunction, and *mei(2)yh15* and *mei(2)yh137* produced fourth chromosome-specific nondisjunction. *mei(2)yh137* was allelic to the *teflon* gene required for autosomal pairing. Three mutants exhibited severe defects, producing >10% nondisjunction of the sex and/or the fourth chromosomes. *mei(2)ys91* (a new allele of the *orientation disruptor* gene) and *mei(3)M20* induced precocious separation of sister chromatids as early as prometaphase I. *mei(2)yh92* predominantly induced nondisjunction at meiosis I that appeared to be the consequence of failure of the separation of paired homologous chromosomes.

MEIOSIS consists of two successive cell divisions following a single DNA replication, resulting in the production of haploid cells. Chromosome behavior in meiosis is complex and shows notable differences from that in mitosis. The orderly reduction of chromosome number is accomplished by segregation of homologous chromosomes at meiosis I. Sister chromatids segregate at meiosis II as in mitosis. To ensure proper orientation of chromosomes and the subsequent disjunction in meiotic divisions, two processes are essential: one is the pairing of homologous chromosomes at meiosis I and the other is the maintenance of sister chromatid cohesion at the centromere through metaphase II.

Male meiosis of *Drosophila melanogaster* is unusual in some respects. Genetic recombination is absent (Morgan 1912) and no chiasmata are formed in bivalents (Cooper 1964). Ultrastructural analyses have failed to demonstrate structural entities of meiotic pairing such as the synaptonemal complex between paired homologs (Meyer 1964; Rasmussen 1973; Ault *et al.* 1982; Ault and Rieder 1994). However, homologs pair with each other and segregate regularly to the opposite poles. The mechanism has been studied by determining chromosome regions important for chromosome pairing. Gershenson (1933) first pointed out that the centric heterochromatin of the *X* chromosome is important for sex chromosome meiotic pairing. Only part of the centric *X* heterochromatin pairs with the *Y* chromosome. The pairing regions are not evenly distributed throughout the *X* heterochromatin, but are restricted to particular regions (in blocks *hB*, *hC*, and *hD*; Cooper 1959, 1964). A mini-*X* chromosome consisting almost exclusively of *hA* does not pair with a copy of itself or with the *Y* chromosome, indicating that sex chromosome pairing requires special chromosome entities called "pairing sites" (Yamamoto and Miklos 1977). Appels and Hilliker (1982) and McKee and Lindsley (1987) proposed that the rDNA region functions as an *X-Y* pairing site. McKee and Karpen (1990) demonstrated the ability of a single copy rDNA to restore the pairing and disjunction of a heterochromatin-deleted *X* chromosome. McKee *et al.* (1992) delimited the sequence responsible for pairing to the 240-bp repeats in the nontranscribed region of the genes. Although the repeats function as a pairing site, other *X* heterochromatin regions in which rDNA is absent also promote *X-Y* pairing at a certain level. *X* chromosomes completely deleted for rDNA, such as $In(1)sc^{4L}sc^{8R}$, pair at a frequency between 55 and 80% depending on the genetic background with the normal *Y* chromosome (Cooper 1964; Peacock *et al.* 1975; Yamamoto and Miklos 1977; McKee 1996). Little is known about the nature of the *X* chromosome pairing sites other than the 240-bp repeats. Autosomal pairing in males also depends on the

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homology of limited sites of euchromatin (Yamamoto 1979, 1981; McKee *et al.* 1993).

Meiotic mutants that show high frequencies of nondisjunction would help to clarify the genetic mechanisms of homologous chromosome pairing and sister chromatid cohesion. Previous studies have demonstrated that in Drosophila females and males homologous chromosomes pair and segregate by different mechanisms (reviewed in Orr-Weaver 1995; Karpen and Endow 1998). All meiotic mutants that exclusively affect meiosis I exhibit a sex-specific effect. Because, in the male, chromosome-specific pairing sites play a crucial role in the association of the homologs and because nonhomologous pairing is totally absent (Yamamoto 1979; Hilliker *et al.* 1982), there must be a mechanism of homolog recognition and holding for each chromosome pair. However, the male-specific meiotic mutants recovered so far that disrupt chromosome pairing, such as *mei-O81*, *mei-1223*, and *teflon*, affect all or a subset of chromosomes rather than just one chromosome pair (Sandler *et al.* 1968; Yamamoto *et al.* 1993; Tomkiel *et al.* 2001; FlyBase 2003). These mutants emphasize the complexity of meiotic pairing in males and suggest that there must be a common aspect in the genetic control of bivalent formation among all chromosomes (Church and Lin 1988).

The other issue to be solved is the mechanism of cohesion of meiotic sister chromatids. Although cohesion along the chromosome arms is lost during meiosis I, centromeric cohesion is maintained until the transition from metaphase II to anaphase II. The meiotic mutants *mei-S332* (Sandler *et al.* 1968; Kerrebrock *et al.* 1992) and *orientation disruptor* (Mason 1976; Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1997) cause nondisjunction owing to premature separation of sister chromatids during meiosis I. These two mutants affect meiosis in both sexes.

The Drosophila genome has not yet been saturated for male meiotic genes, mainly because screenings for such mutants have been carried out only a few times (Sandler *et al.* 1968; Baker and Carpenter 1972; Gethmann 1974; Yamamoto *et al.* 1993). In this study, we have screened the second and the third chromosomes for male meiotic mutants. We examined flies carrying EMS-treated chromosomes or single *P*-element insertions and flies collected from natural populations. Nondisjunction of the sex and the fourth chromosomes was assayed. To assay the former, we also used a free minichromosome, *Dp(1;f)YP223*, which pairs with the compound-*XY* chromosome and disjoins faithfully during male meiosis (Park and Yamamoto 1993). Because this minichromosome carries no 240-bp spacer repeats of rDNA detected by fluorescence *in situ* hybridization (M.-T. Yamamoto, unpublished data) and yet retains the *X* chromosome pairing site within the part of *hB* (Figure 1), we designed experiments to isolate mutants that specifically affect the function of this pairing site. Altogether, eight male meiotic mutants were isolated. In this report we describe the genetic and cytological properties of these meiotic mutants.

MATERIALS AND METHODS

Chromosomes: Description of genetic markers, balancers, deletions, and compound chromosomes used in this work can be found in Lindsley and Zimm (1992) or in FlyBase (http://flybase.bio.indiana.edu/).

Ethyl methanesulfonate-treated chromosomes: EMS-mediated mutagenesis was carried out basically following the method of Lewis and Bacher (1968). We used two different concentrations of EMS (0.025 M and 0.013 M in 1% sucrose solution). Lines each carrying a separately mutagenized second and third chromosome were made using balancer chromosomes (*CyO* for the second chromosome and *TM3* for the third chromosome).

P-element-inserted chromosomes: P{lacW} lines were gifts from E. Nitasaka (Kyushu University) and R. Murakami (Yamaguchi University) and *P{GS}* lines were from T. Aigaki (Tokyo Metropolitan University). *P*-element-inserted lines were also newly established, using a *P{EP}* insertion at cytological location 32D, *EP(2)2478*, provided by H. Kose (Tokushima University). *EP(2)2478* was activated in the male germ line by using the *TMS* chromosome (obtained from the Bloomington Drosophila Stock Center) carrying the *Δ2-3* transposase source.

Chromosomes from natural populations: Iso-female lines established from flies caught in Ishigaki and Iriomote islands (Okinawa, Japan) in 1997 and 1998 and in Katsunuma (Yamanashi, Japan) in 1997 were kindly provided by M. Itoh and T. K. Watanabe (Kyoto Institute of Technology). From each line, balancer chromosomes were used to extract one second chromosome and/or one third chromosome.

Minichromosome: We used the predominantly heterochromatic free *X* duplication chromosome *Dp(1;f)YP223*, y^+ (hereafter referred to as *Dp223*; Figure 1). *Dp223* was generated by deleting a large portion of the $In(1)sc^{L8L}sc^{8R}$ chromosome using X-ray irradiation (Park and Yamamoto 1993). *Dp223* is about 0.4 times the size of a fourth chromosome (about 2 Mbp DNA) and retains the proximal half of *hA* and the distal half of *hB*. *Dp223* pairs with the compound-*XY* chromosome *C(1;Y)6* ($XY^L{\cdot}Y^S$, y^2 *sc cv v f*) and they disjoin from one another normally in male meiosis (see Table 2). Because *hA* does not have pairing ability (Yamamoto and Miklos 1977), *Dp223* must pair with the compound-*XY* only by the pairing site located in the half of *hB*. We consider this pairing site to be different from the 240-bp rDNA spacer repeats, because the repeats could not be detected by fluorescence *in situ* hybridization (M.-T. Yamamoto, unpublished data). To search for a mutation that specifically affects the minichromosome pairing, we compared segregation of the sex chromosomes in the males carrying *C(1;Y)6* and *Dp223* to those carrying the normal *X* and *Y* chromosomes.

Meiotic mutants: The following previously known meiotic mutants, $mei\text{-}S332^1$ (2-99.5, 59D), $orientation\ disruptor^1$ (*ord*; 2-103.5, 58B), and *Df(2R)PC4* that uncovers the *subito* locus (2-82.6, 54D-54F), which were in the collection of the Drosophila Genetic Resource Center in Kyoto, were used in complementation tests. An allele of the *teflon* gene (2-80.0, 53F-54A), tef^{Z5549} (an EMS-induced nonsense mutation; J. E. Tomkiel, personal communication), and *Df(2R)P803-Δ15* deleted for the *tef* locus were kindly provided by J. E. Tomkiel (University of North Carolina, Greensboro, NC).

Genetic analyses: Flies were reared on a standard cornmeal-glucose-yeast-agar medium at 24 ± 1°. Three- to 5-day-old females and males were used for each cross. The parents were

transferred to new vials on day 3 (day 0 is the day of setup) and then were discarded on day 7. Progeny were scored on days 12 and 17 after the establishment of the cross for each vial.

Initial screenings were made by examining 5 single-pair matings for each line. Candidate lines (>2% nondisjunction, as defined below) were then rescreened by examining 10 single-pair matings. Those that behaved consistently were maintained and examined in this study.

Nondisjunction tests were carried out as described below. For simultaneous examination of sex and fourth chromosome segregation in *C(1;Y)6, y^2 sc cv v f/Dp223, y^+; spa^+/spa^+* males, these flies were crossed to *y/y; C(4)RM, spa^{pol}/O* females. Single-pair matings were performed.

The sex chromosomes [C(1;Y)6, y^2 sc cv v f/Dp223, y^+]: The tester females produce haplo-*X* (*y*) ova. The sperm produced by normal segregation bear either *C(1;Y)6* or *Dp223*, which results in y^2 females and y^+ males, respectively. The sperm produced by nondisjunction at meiosis I bear either both *C(1;Y)6* and *Dp223* or neither of them, resulting in y^+ female and y male progeny, respectively. In this cross, exceptional sperm bearing two compound-*XY* chromosomes could not be recovered and those bearing two *Dp223* chromosomes were phenotypically indistinguishable from regular ones. Nondisjunction frequency of the sex chromosomes was calculated as [(y^+ females + y males) × 100/total].

The fourth chromosome: The tester females produce compound-*4*, [*C(4)RM, spa^{pol}*]-bearing, and nullo-*4* ova. The sperm produced by normal segregation result in spa^+ progeny, trisomy-*4*, and monosomy-*4*, respectively. Nondisjunction of the fourth chromosome at meiosis I results in two classes of sperm, diplo-*4* and nullo-*4*. Progeny showing spa^{pol} phenotype are clearly the descendants of the nullo-*4* sperm, indicating meiotic nondisjunction of the fourth chromosome. Progeny that arose from diplo-*4* sperm are indistinguishable from regular ones. Flies lacking fourth chromosomes are inviable and those carrying a single fourth chromosome are weak and show strong Minute phenotype. Monosomy-*4* progeny are subviable. Although the haplo-*4* Minutes were counted (see Tables 2 and 3), they were excluded from any calculations because viability varied between females and males. On the assumption that all exceptional sperm result from meiosis I nondisjunction and that those bearing diplo-*4*, triplo-*4*, and tetra-*4* show equivalent viability, nondisjunction frequency was calculated as [spa^{pol} progeny × 2 × 100/spa^+ progeny]. In the case of *mei(2)yh137/tef^{Z5549}* and *mei(2)yh137/Df(2R)P803-Δ15*, *spa^{pol}/spa^+* males were crossed with *C(4)RM, ci ey^R/O* females. Nondisjunction frequencies were calculated on the basis of the number of ci ey^R progeny derived from nullo-*4* sperm.

To determine which meiotic division is disrupted in a given meiotic mutant, *y w/y^+ Y* males were crossed singly to females carrying a compound-*X* chromosome [*C(1)RM, y v f/O*]. In this cross all classes of sperm, two regular and seven exceptional, can be recovered upon fertilization with either *C(1)RM* (diplo-*X*) or nullo-*X* ova produced by the tester females. Normal segregation produces *X*-bearing and *Y*-bearing sperm. Nondisjunction at meiosis I produces *XY* and nullo-*XY* sperm and at meiosis II produces *XX, YY*, and nullo-*XY* sperm. Three classes of sperm, *XXY, XYY*, and *XXYY*, are diagnostic of nondisjunction in both meiosis I and meiosis II. If complete loss of sister chromatid cohesion occurs, all seven classes of exceptional sperm, as well as the regular two, will be produced. All classes were distinguished by the phenotypes of zygotes, except sperm bearing one *Y* chromosome and those bearing two. Provided that sufficient numbers of exceptional progeny are obtained in crosses using a mutant stock, we were able to distinguish whether the mutant affected predominantly, if not wholly, meiosis I or meiosis II. While metafemales [*C(1)RM/X; A/A*, where *A* represents a set of autosomes] usually die as larvae, escapers can be identified by their characteristic phenotype. Metafemales, triploid females [*C(1)RM/X(/Y); A/A/A*] and triploid intersexes [*C(1)RM(/Y); A/A/A*] were scored but were omitted from the table because of their highly variable recovery. The nondisjunction frequency was calculated as [exceptional progeny × 100/total].

In females, nondisjunction of the *X* and the fourth chromosomes was assayed separately. For the *X* chromosome, *y/y* females were crossed with *y pn/B^SY* males. Females were tested individually for *mei(2)yh15* and *mei(2)yh149*, while semisterile females of *mei(2)ys91* (*ord^{ys91}*) and *mei(3)M20* were tested in mass matings. Regular *X* (*y*) ova yielded y females and y B^S males. Two classes of exceptional ova were recoverable in this cross. Diplo-*X* ova that were fertilized by *Y* (*B^S*) sperm and nullo-*X* ova that were fertilized by *X* (*y pn*) sperm were recovered as y B^S females and y pn males, respectively. The nondisjunction frequency was calculated as [(y B^S females + y pn males) × 2 × 100/(total + y B^S females + y pn males)]. For fourth chromosome segregation *spa^+/spa^+* females were crossed with *C(4)RM, spa^{pol}/O* males and examined as described above for males.

Cytology: We made meiotic chromosome preparations without colchicine treatment using the air-dry procedure (Yamamoto 1992; Yamamoto *et al.* 1993). We used testes of 0- to 3-day-old adults. We stained the chromosomes with 4′,6-diamidino-2-phenylindole (DAPI) or Giemsa. We scored prometaphase I and metaphase I cells for the sex, second, and third chromosomes, but not for the fourth chromosome, since it is not always visible due to its small, dot-like morphology. *y w/y^+ Y* males were used as the control.

Embryo preparation: Females mated with *y w/y^+ Y* males were allowed to lay eggs on apple juice agar plates for 3 hr. Eggs were dechorionated in 50% bleach 2–3 hr later. Vitelline membrane permeabilization in heptane and fixation and devitellinization in a mixture of methanol/heptane were performed before staining with DAPI following the procedures described in Rothwell and Sullivan (2000). In separate tests, eggshell morphology was examined under the dissection microscope. All flies and embryos were kept at 24 ± 1°.

Inverse PCR: Genomic DNA preparation, restriction enzyme digestions, ligations, and inverse PCR were performed essentially following the protocol of Huang *et al.* (2000). Purified DNA was digested with *Msp*I, which makes cuts within the *P{GS}* vector sequence as well as in the 5′ flanking sequence. Following self-ligation, it was PCR amplified with primers 5′ CTGAATAGGGAATTGGGAATTCGACTAGTT and 5′ CTCC GTAGACGAAGCGCCTCTATTT. The product was then directly sequenced using ABI310 sequencer (Perkin-Elmer, Norwalk, CT) with a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and a sequencing primer, 5′ CACTGAATTTAAGTGTATACTTCGG.

RESULTS

Screening: We first generated 1100 EMS-treated second chromosome lines and 913 such third chromosome lines. Of these, 493 second and 331 third chromosome lines that were homozygous viable and fertile in the male were screened for the presence of male meiotic mutants with increased nondisjunction frequencies. The males tested here carried a compound-*XY* chromosome [*C(1;Y)6 = $XY^L{\cdot}Y^S$*] and a free miniduplication chromosome (*Dp223*), because an aim was to recover mutations that might specifically affect the sex chromo-

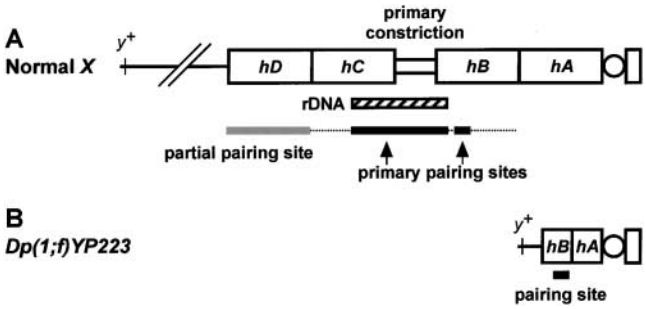

FIGURE 1.—Structure of the normal *X* chromosome and its derivative, *Dp(1;f)YP223*. (A) Normal *X* chromosome. The heterochromatin is divided into four blocks, *hA*, *hB*, *hC*, and *hD*, and the primary constriction (COOPER 1959), under which are indicated the rDNA region and pairing sites required for normal pairing of the sex chromosomes in male meiosis. The primary (strong) sites are in the rDNA region and *hB*, and the partial (weak) pairing site is in *hD*. There are no pairing sites in *hA* and euchromatin (COOPER 1964; YAMAMOTO and MIKLOS 1977). (B) *Dp(1;f)YP223*. This minichromosome consists of the centromere of the *X* chromosome and some of the proximal *X* heterochromatin plus a portion of the euchromatic tip marked with the *yellow*$^+$ gene (PARK and YAMAMOTO 1993). *Dp(1;f)YP223* retains a pairing site located in *hB*. Solid lines, euchromatin; white blocks, heterochromatin; open circles, centromeres.

some pairing site carried by the *Dp223* chromosome (see Figure 1). *C(1;Y)6, y*^{2}*/Dp223, y*$^+$*; spa*$^+$*/spa*$^+$ males were single-pair mated to *y/y; C(4)RM, spa*^{pol}*/O* females. This cross makes it possible to examine the segregation of the sex and the fourth chromosomes simultaneously. Three mutants were recovered. These were *mei(2)yh92*, *mei(2)yh149*, and *mei(2)yh217* (Table 1). Using the same mating scheme, we next screened 67 second and 33 third chromosome lines from the Ishigaki and Iriomote natural populations. Two mutants, *mei(3)M19* and *mei(3)M20*, were obtained (Table 1).

We also screened 1482 second and third chromosome *P*-element-insertion lines. Males that had unmarked *X* and *Y* chromosomes and were homozygous for a *P*-insertion chromosome were single-pair mated to *y/y; C(4)RM, spa*^{pol}*/O* females. Here only fourth chromosome nondisjunction could be detected. Two mutants, *mei(2)ys91* and *mei(2)yoh7134*, were recovered (Table 1). Similarly, 126 second chromosomes from the Katsunuma natural population were screened and two mutants, *mei(2)yh15* and *mei(2)yh137*, were recovered (Table 1).

For the sake of comparison, the mutants recovered solely on the basis of abnormal segregation of the fourth chromosome (Table 1) were reexamined in the mating scheme by which the other mutants (Table 1) were obtained. Table 2 shows the results for eight of the nine mutants recovered. The remaining mutant, *mei(2)ys91*, was not included in Table 2 since it was shown to be an allele of the well-characterized meiotic mutant *orientation disruptor* (*ord*), as described below. The two third chromosomal mutants, *mei(3)M19* and *mei(3)M20*, showed the same high frequencies of nondisjunction of the sex and the fourth chromosomes. Since they were recovered from the same Ishigaki natural population and since they showed the same high frequencies of nondisjunction in *mei(3)M19/mei(3)M20* males, they most probably are two independent isolates of the same mutation. We chose *mei(3)M20* for further characterization.

Table 3 shows the results of reexamination of all eight mutants recovered for segregation of the *X* and the *Y* chromosomes (instead of the compound-*XY* and *Dp223*) and of the fourth chromosomes by crossing *y/y*$^+$*Y; spa*$^+$*/spa*$^+$ males to *y/y; C(4)RM, spa*^{pol}*/O* females. Nondisjunction frequencies were calculated in a manner similar to that employed for *C(1;Y)6/Dp223; spa*$^+$*/spa*$^+$ males. As can be seen in Tables 2 and 3, each mutant examined showed similar nondisjunction frequencies in both *C(1;Y)6/Dp223* and *X/Y* males. Three mutants, *mei(2)ys91*, *mei(2)yh92*, and *mei(3)M20*, may be called severe meiotic mutants, producing >10% nondisjunction. They all affected the segregation of both the sex and the fourth chromosomes. The other five mutants recovered may be called mild meiotic mutants, producing <10% nondisjunction. Some of the mild mutants exhibited a possible chromosome-specific effect.

The *mei(2)yh92* gene was mapped to 2-40.9 (202 recombinants between *Sternopleural* and *Tufted* were scored), and *mei(3)M20* was mapped to 3-40 (37 recombinants between *Roughened* and *Dichaete* were scored).

TABLE 1

Summary of male meiotic mutant screenings

Nondisjunction test	Chromosomes screened (*n*)	Origin	Meiotic mutants recovered
C(1;Y)6 ↔ *Dp(1;f)YP223* and *4* ↔ *4*	Second (493)	EMS	*mei(2)yh92*, *mei(2)yh149*, *mei(2)yh217*
	Third (331)	EMS	—
	Second (67)	Natural populations	—
	Third (33)	Natural populations	*mei(3)M19*, *mei(3)M20*
4 ↔ *4*	Second and third (1482)	*P*-element insertions	*mei(2)ys91* (*ord*^{ys91}), *mei(2)yoh7134*
	Second (126)	Natural population	*mei(2)yh15*, *mei(2)yh137* (*tef*^{yh137})

TABLE 2

Segregation data of the sex and the fourth chromosomes in *C(1;Y)6/Dp223* males

Second or third chromosome genotype	N^a	Sperm classes: *X^Y* 4(4)	*X^Y* *O*	*Dp(Dp)* 4(4)	*Dp(Dp)* *O*	*X^Y Dp(Dp)* 4(4)	*X^Y Dp(Dp)* *O*	*O* 4(4)	*O* *O*	Total	Minute[b]	% ND: Sex chromosome	% ND: Fourth chromosome
+/+	55	937	1	1728	1	4	0	18	0	2689	1003	0.8	0.1
mei(2)yh92/mei(2)yh92	73	1187	108	1284	115	127	6	416	47	3290	1283	18.1	18.3
mei(2)yh92/CyO	42	1381	8	1449	6	7	0	16	1	2868	1406	0.8	1.1
mei(3)M19/mei(3)M19	25	177	46	278	68	99	33	291	47	1039	191	45.2	45.9
mei(3)M19/TM3	14	660	1	728	1	4	0	4	0	1398	433	0.6	0.3
mei(3)M20/mei(3)M20	33	379	110	477	103	176	56	447	83	1831	416	41.6	47.6
mei(3)M19/mei(3)M20	8	73	22	74	18	42	13	72	19	333	42	43.8	55.2
mei(2)yoh7134/mei(2)yoh7134	26	497	22	733	26	2	0	73	9	1362	922	6.2	8.7
mei(2)yoh7134/+	13	433	0	566	1	0	1	4	0	1005	656	0.5	0.4
mei(2)yh149/mei(2)yh149	47	618	11	993	9	1	0	37	0	2032	359	2.3	2.4
mei(2)yh149/+	15	604	0	852	0	0	0	5	0	1461	613	0.3	0.0
mei(2)yh217/mei(2)yh217	57	790	5	1811	6	12	0	106	2	2732	546	4.4	1.0
mei(2)yh217/CyO	21	988	1	999	3	4	0	16	0	2011	321	1.0	0.4
mei(2)yh15/mei(2)yh15	59	1439	14	2374	26	1	1	60	5	3920	1588	1.7	2.4
mei(2)yh15/CyO	26	830	2	1338	1	6	0	22	0	2199	722	1.3	0.3
mei(2)yh137/mei(2)yh137	78	1699	37	3249	51	2	0	38	0	5076	2397	0.8	3.5
mei(2)yh137/CyO	22	510	0	795	2	3	0	15	0	1325	550	1.4	0.3

C(1;Y)6, y^2 sc cv v f/Dp(1;f)YP223, y^+; spa^+/spa^+ males were single-pair mated to *y/y; C(4)RM, spa^{pol}/O* females. *X^Y* denotes the compound-*XY* chromosome *C(1;Y)6*. *CyO* and *TM3* are balancers. Data presented are based on the number of Minute$^+$ progeny (see MATERIALS AND METHODS). The possible presence of multiple doses of the indicated chromosomes that cannot be distinguished phenotypically is shown in parentheses in the sperm genotypes. ND, nondisjunction.

[a] Number of single-pair matings performed.

[b] All haplo-*4* Minutes were listed here without classification and were excluded from further analysis and from the calculation of nondisjunction frequencies.

A mild mutant *mei(2)yoh7134* was shown to be induced by a *P*-element insertion (see below) and mapped to the cytological interval 37A4-6 by inverse PCR. The remaining EMS-induced and natural population-derived mutants were difficult to map because they gave only mild nondisjunction. Complementation tests among the seven meiotic mutations on the second chromosome were carried out. In all cases, nondisjunction frequencies of the sex and the fourth chromosomes did not differ from the values observed in the respective heterozygous controls (data not shown). Thus the seven meiotic mutations represent different genes on the second chromosome.

We next asked if these second chromosomal mutants were allelic to the four male meiotic genes previously reported, *mei-S332* (SANDLER *et al.* 1968), *ord* (MASON 1976), *subito* (GIUNTA *et al.* 2002), and *teflon* (*tef*; TOMKIEL *et al.* 2001). Two mutants recovered in this study, *mei(2)ys91* and *mei(2)yh137*, were found to be new alleles of *ord* and *tef*, respectively (see below). The remaining five mutants were shown not to be allelic to any of the genes already known. Further, there is no known meiotic mutant around the *mei(3)M20* gene on the third chromosome. Thus the male meiotic mutants isolated in this study, five on the second and one on the third, represent novel genes. Below we present the results of genetic analyses of each of these mutants and cytological analyses for the three severe mutants and a *tef* allele, *mei(2)yh137*.

Severe mutants: *mei(2)ys91:* The mutation, recovered from a *P*-element-insertion line, was found to be allelic to *ord*, which is known to be required for normal sister chromatid cohesion in meiosis (MASON 1976; MIYAZAKI and ORR-WEAVER 1992; BICKEL *et al.* 1997). A heteroallelic combination, *mei(2)ys91/ord^1*, produced 26.2% nondisjunction between the *X* and *Y* chromosomes (total 237). The severity of nondisjunction seen in the homozygotes and the hemizygotes (over a deficiency) was equivalent (Table 3). We cytologically examined and confirmed the *ord* phenotype in primary spermatocytes of *mei(2)ys91*. In the control prometaphase I cells, sister chromatids were attached to each other at the centromeres (Figure 2A). In the mutant cells, precocious separation of sister chromatids was observed for all chromosomes. Figure 2B shows an example of *mei(2)ys91* mutant cells in which the early separation in autosomes is evident. We also observed severely reduced fertility associated with this mutant [Table 3, compare *N* (number of pair matings) and Total (total number of M$^+$ progeny)], most probably reflecting the production of aneuploid

TABLE 3

Segregation data of the sex and the fourth chromosomes in *X/Y* males

Second or third chromosome genotype	N^a	Sperm classes: *X* 4(4)	*X* O	*Y(Y)* 4(4)	*Y(Y)* O	*XY(Y)* 4(4)	*XY(Y)* O	*O* 4(4)	*O* O	Total	Minute[b]	% ND: Sex chromosome	% ND: Fourth chromosome
+/+	87	1924	2	1905	4	1	0	2	0	3838	1910	0.1	0.3
mei(2)ys91/mei(2)ys91	51	25	15	37	5	15	5	20	8	130	51	36.9	68.0
mei(2)ys91/Df(2R)bw-S46	140	86	25	96	29	23	9	80	29	377	81	37.4	64.6
mei(2)yh92/mei(2)yh92	91	1736	69	1663	66	280	10	427	34	4285	746	17.5	8.7
mei(2)yh92/SM1	40	1365	3	1473	5	9	0	13	0	2868	457	0.3	0.6
mei(3)M20/mei(3)M20	57	510	117	563	147	271	75	436	83	2202	555	39.2	47.4
mei(3)M20/DcxF	21	513	2	446	0	0	0	3	0	964	327	0.3	0.4
mei(2)yoh7134/mei(2)yoh7134	17	483	15	468	19	7	0	21	0	1013	620	2.8	6.9
mei(2)yoh7134/CyO	13	508	0	493	1	0	0	0	0	1002	482	0.0	0.2
mei(2)yh149/mei(2)yh149	74	1558	11	1441	4	0	0	39	0	3053	606	1.2	1.0
mei(2)yh149/SM1	16	533	0	482	0	0	0	2	0	1017	118	0.2	0.0
mei(2)yh217/mei(2)yh217	22	1813	3	1174	1	7	0	77	0	3075	1108	2.5	0.3
mei(2)yh217/SM1	27	1302	1	1189	3	1	0	4	0	2500	1043	0.2	0.3
mei(2)yh15/mei(2)yh15	54	2139	20	1822	14	0	0	15	1	3996	2255	0.4	1.8
mei(2)yh15/SM1	22	1292	0	1283	1	0	0	7	0	2583	1021	0.3	0.1
mei(2)yh137/mei(2)yh137	83	2108	46	2183	54	4	0	11	0	4406	1291	0.3	4.6
mei(2)yh137/SM1	40	988	5	1035	2	1	0	4	0	2035	486	0.2	0.7
mei(2)yh137/tef^{Z5549} [c]	29	1122	38	1120	37	2	0	1	0	2320	635	0.1	6.7
tef^{Z5549}*/tef*^{Z5549}	24	794	179	789	190	1	0	2	1	1956	570	0.2	46.7

y/y$^+$*Y; spa*$^+$*/spa*$^+$ males were single-pair mated to *y/y; C(4)RM, spa*^{pol}*/O* females. *SM1*, *CyO*, and *DcxF* are balancers. Data presented are based on the number of Minute$^+$ progeny (see MATERIALS AND METHODS). The possible presence of multiple doses of the indicated chromosomes that cannot be distinguished phenotypically is shown in parentheses in the sperm genotypes. ND, nondisjunction.

[a] Number of single-pair matings performed.

[b] All haplo-*4* Minutes were listed here without classification and were excluded from further analysis and from the calculation of nondisjunction frequencies.

[c] *y/y*$^+$*Y; spa*^{pol}*/spa*$^+$ males were single-pair mated to *y/y; C(4)RM, ci ey*^{R}*/O* females. *mei(2)ys91* turned out to be an allele of *ord*, and *mei(2)yh137* turned out to be an allele of *tef* (see text).

sperm for the second and third chromosomes at high frequencies, which should generate lethal aneuploid zygotes. We will designate *mei(2)ys91* as *ord*^{ys91}. *ord* mutants have been shown to cause higher levels of nondisjunction in meiosis I than in meiosis II (MIYAZAKI and ORR-WEAVER 1992).

mei(2)yh92: The EMS-induced mutant *mei(2)yh92* showed relatively high nondisjunction (~20%). To examine which meiotic division this mutant affects, *y w/y*$^+$*Y; mei(2)yh92/mei(2)yh92* males were mated to compound-*X* females, in which *XX* sperm that result from nondisjunction in meiosis II as well as *XY* sperm that result from nondisjunction in meiosis I are detectable by using the genetic markers we employed. Essentially all of the exceptional sperm were the *XY* and nullo-*XY* classes, although a small number of the *XX* and *XXY* classes were produced (Table 4). We conclude that *mei(2)yh92* predominantly disrupts meiosis I.

Cytological examinations of the mutant showed that homologous chromosomes faithfully paired in prometaphase-metaphase I cells (Figure 2C, number of cells examined is 405). We noticed characteristic abnormali-

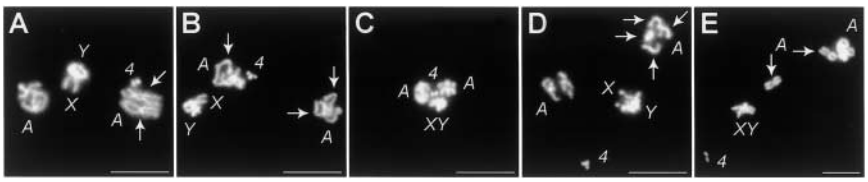

FIGURE 2.—Chromosome behavior in mutant meiosis I cells. (A) Wild type. (B) Homozygous *mei(2)ys91* (*ord*^{ys91}). (C) Homozygous *mei(2)yh92*. (D) Homozygous *mei(3)M20*. (E) Hemizygous *mei(2)yh137* (*tef*^{yh137}). (A, B, D, and E) Prometaphase I. (C) Metaphase I. Chromosomes were stained with DAPI. In the control, homologous chromosomes are paired and sister chromatids are attached to each other at the centromeres (arrows in A). Arrows in B and D point to prematurely separated chromatids. Bivalents are of normal appearance and congress properly in *mei(2)yh92* (C). Univalents of a major autosomal homologous pair are observed in the cell mutant for the *teflon* gene (arrows in E). *X*, *X* chromosome; *Y*, *Y* chromosome; *A*, second or third chromosome; *4*, fourth chromosome. Bars, 10 μm.

TABLE 4

Segregation data of the sex chromosomes at meiosis I and meiosis II in *X/Y* males

Second or third chromosome genotype	Sperm classes						Total	% ND
	X	*Y(Y)*	*XY(Y)*	*XX*	*XXY(Y)*	*O*		
+/+	3785	3552	3	0	0	5	7345	0.1
mei(2)yh92/mei(2)yh92	2696	2717	359	7	3	471	6253	13.4
mei(2)yh92/CyO	3653	3735	10	1	0	15	7414	0.4
mei(3)M20/mei(3)M20	493	458	353	63	10	334	1711	43.8
mei(3)M20/DcxF	766	757	0	0	0	2	1525	0.1
mei(2)yoh7134/mei(2)yoh7134	424	449	9	0	0	10	892	2.1
mei(2)yh149/mei(2)yh149	1094	1011	0	4[a]	0	12	2121	0.8
mei(2)yh217/mei(2)yh217	4629	3518	53	0	0	170	8370	2.7
mei(2)yh217/CyO	1838	1763	0	0	0	1	3602	0.0

y w/y$^+$ *Y* males were individually crossed to *C(1)RM, y v f/O* females. *CyO* and *DcxF* are balancers. The possible presence of multiple doses of the *Y* chromosome that cannot be distinguished phenotypically is shown in parentheses in the sperm genotypes. ND, nondisjunction.

[a] These four females may be rare surviving metafemales or triploids [*C(1)RM/X*], instead of meiosis II nondisjunctional females (*X/X/O*). These two classes of flies were not distinguishable phenotypically, because *y w*$^+$*/y*$^+$ *Y* males, instead of *y w/y*$^+$ *Y* males, were used in this particular cross.

ties in chromosome behavior in late stages of meiosis I. In the control, anaphase I cells showed synchronous chromosome movement to each pole (Figure 3A). In *mei(2)yh92*, however, some pairs of chromosomes showed a delay in migration to the poles or remained in the vicinity of the equator in anaphase I cells, while others had already moved a considerable distance to the poles (Figure 3B). Such disrupted chromosome segregation was observed in 43% of the anaphase I cells ($n = 83$), whereas it was never observed in the control cells ($n = 54$). The mutant phenotype can be characterized by the presence of daughter nuclei connected by a thin chromatin bridge (Figure 3C) or of nuclei associated with chromatin trailing behind at telophase I (Figure 3D). The chromatin lagging is likely a cytological basis of the nondisjunction induced by *mei(2)yh92*. The mutant shows a unique defect in separation of paired homologous chromosomes at the onset of anaphase I.

mei(3)M20: The natural population-derived mutant *mei(3)M20* induced ~40% nondisjunction of the sex and the fourth chromosomes (Tables 2 and 3). Genetic analysis similar to that described above for *mei(2)yh92* indicated that *mei(3)M20* primarily affected meiosis I, but the proportion of meiosis II nondisjunctional sperm was significantly higher in this mutant than in the other mutants (Table 4). Not included in Table 4 is the appearance of triploid intersex progeny [nine *C(1)RM/y*$^+$ *Y; A/A/A* and two *C(1)RM/O; A/A/A*] from the cross. Their occurrence indicates that the *mei(3)M20* mutation also affects the disjunction of the second and the third chromosomes at high frequencies.

Cytological analysis revealed that, although homologous chromosomes were apparently paired, sister centromeres were prematurely separated as early as prometaphase I in *mei(3)M20* cells (Figure 2D). Although *mei(3)M20* bears close resemblance to *ord* with respect to these phenotypes, the two genes are located on different chromosomes. Some *ord* alleles have been shown to disrupt mitotic segregation in the gonial cells, resulting in aneuploidy of primary spermatocytes (Lin and Church 1982; Miyazaki and Orr-Weaver 1992). However, this was not the case for at least this allele of the *mei(3)M20* gene. In >100 mutant prometaphase-metaphase I cells examined, all chromosomes paired as bivalents, and neither univalents nor trivalents were observed.

Mild mutants: The five mild mutants were subdivided into three categories defined by the chromosomes showing nondisjunction. Two mutants—*mei(2)yoh7134*, which was recovered from *P*-element-insertion lines (Toba *et al.* 1999), and *mei(2)yh149*, which was induced with EMS—affected both the sex and the fourth chromosomes (Tables 2 and 3). *P*-element-excision experiments were performed for *mei(2)yoh7134*. Males carrying a *P*-element-excised chromosome, when examined in the mating scheme employed in Table 3, did not produce nondisjunction at an appreciable frequency (total 512). Thus the mutation was clearly caused by the *P*-element insertion. This mutant exclusively affected meiosis I because *mei(3)yoh7134* males produced *XY* sperm but not *XX* sperm (Table 4). In contrast, *mei(2)yh149* induced either nondisjunction at meiosis II or chromosome loss at meiosis I and/or II, because no *XY* sperm were recovered (Tables 3 and 4).

The EMS-induced mutant *mei(2)yh217* elicited a stronger effect on the sex chromosomes than on the fourth chromosome in *C(1;Y)6/Dp223* males (Table 2), suggesting the possibility that this mutant specifically impairs the function of the pairing site in *hB* (Figure 1). If this is true, *X-Y* segregation might be compensated by other pairing sites on the *X* chromosome. However,

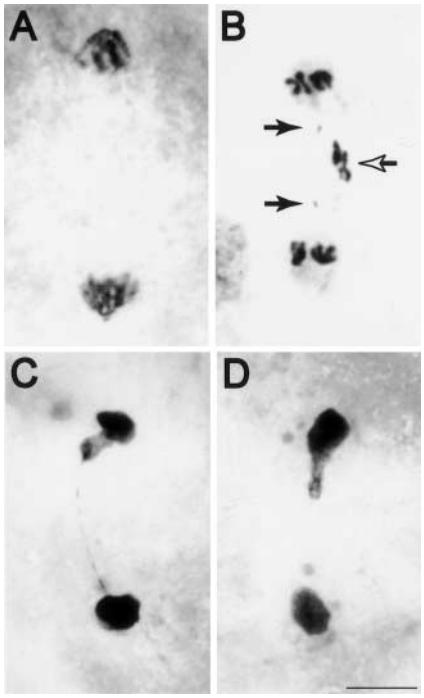

FIGURE 3.—Aberrant chromosome segregation in *mei(2)yh92* meiosis I. (A) Control. (B, C, and D) *mei(2)yh92*. (A and B) Anaphase I. (C and D) Telophase I. Chromosomes were stained with Giemsa. (A) Chromosomes migrate to each pole synchronously. (B) A cell showing asynchronous chromosome segregation. The large autosomes have moved to the poles. The sex chromosomes (solid arrows) and the fourth chromosomes (open arrows) show delays in migration. Note that the bivalent sex chromosome remaining in the vicinity of the equator appears to be oriented to the poles. (C) A thin chromatin bridge connecting daughter nuclei is visible. (D) The upper nucleus is associated with chromatin trailing behind. Bar, 10 μm.

nondisjunction took place between the *X* and the *Y* chromosomes (Table 3). Thus it is unlikely that the effect is specific to the pairing site in *hB*. This mutant was found to cause meiosis I-specific nondisjunction (Table 4). Another striking feature of *mei(2)yh217* was meiotic drive, that is, a discrepancy in the recovery of reciprocal products of meiotic segregation (Table 3). The recovery of *X*-bearing sperm (1816) exceeded that of *Y*-bearing sperm (1175), and recovery of nullo-*XY* sperm (77) exceeded that of *XY* sperm (7). The meiotic drive coefficients [$X/(X + Y)$ and $O/(XY + O)$] were 0.61 and 0.92, which were significantly different from those of the controls, +/+ (0.50 and 0.67) and *mei(2)yh217/SM1* (0.52 and 0.80), and from those of the other mutants that predominantly caused meiosis I nondisjunction, *mei(2)yh92* (0.51 and 0.61) and *mei(3)yoh7134* (0.51 and 0.75). Consistent results were obtained in separate tests (Table 4).

Two mutants derived from Katsunuma natural population, *mei(2)yh15* and *mei(2)yh137*, caused nondisjunction of the fourth chromosome but not of the sex chromosomes (Tables 2 and 3). *mei(2)yh15* represents a new locus as noted above, but was not examined further because of its only mild effect on nondisjunction. The *mei(2)yh137* mutation turned out to be allelic to a known male-specific meiotic gene, *tef*, which is required for the maintenance of homolog pairing (TOMKIEL *et al.* 2001). This gene has been characterized as having an autosome-specific effect. *mei(2)yh137/tef*^{Z5549} males showed an increased frequency of fourth chromosome nondisjunction (6.7%) compared to the value (4.6%) observed in homozygous *mei(2)yh137*, while sex chromosome segregation was unaffected (Table 3). Similar results (9.7% fourth chromosome nondisjunction, total 1100) were obtained in the male of *mei(2)yh137/Df(2R)P803-Δ15*. *Df(2R)P803-Δ15/+* males showed normal segregation (0.7% fourth chromosome nondisjunction, total 578), indicating no dominant effect of *tef*. The increased level of nondisjunction prompted us to carry out cytology. We observed univalents of autosomes at meiosis I in mutant cells (Figure 2E). Either one or both of the major autosomes were evidently unpaired in 12/102 cells hemizygous for *mei(2)yh137*. The sex chromosome pairing was intact. Such a defect in autosomes was never observed in 265 control cells examined (Figure 2A). We can infer from the genetic result that the fourth chromosomes also fail to form a stable bivalent at an increased frequency in mutant cells. Frequencies of fourth chromosome pairing were not scored because, when the punctiform chromosomes are visible, they frequently appear to be unconjoined even in control cells, although they are generally close to each other relative to other chromosomes (GUYÉNOT and NAVILLE 1929; GOLDSTEIN 1980). We will designate *mei(2)yh137* as *tef*^{yh137}.

Effects of male meiotic mutants on female gamete: We examined the effect of male meiotic mutants on female fertility: *mei(2)yh15* and *mei(2)yh149* were fully fertile; *ord*^{ys91} and *mei(3)M20* were semisterile; and *mei(2)yh137* (*tef*^{yh137}), *mei(2)yh92*, *mei(2)yh217*, and *mei(2)yoh7134* were completely sterile. We tested the two fertile mutants for their *X* and fourth chromosome segregations at meiosis (see MATERIALS AND METHODS). Control females of the genotype *y w/y w; spa*$^{+}$*/spa*$^{+}$ produced no nondisjunction in the *X* and the fourth chromosomes (total 2724 and 1760, respectively). In females homozygous for *mei(2)yh15* or *mei(2)yh149*, nondisjunction frequencies were comparable to those of the control: 0.1% *X* chromosome and no fourth chromosome nondisjunction in *mei(2)yh15* (total 1545 and 997, respectively) and no nondisjunction in either chromosome pair in *mei(2)yh149* (total 1799 and 1227, respectively). Thus, these two meiotic mutants are male specific.

When mated with wild-type males, females homozygous for *ord*^{ys91} and for *mei(3)M20* laid a large number of eggs. Gross morphology of the eggs was normal. Hatchability of the eggs laid by *ord*^{ys91} females (36/635) and *mei(3)M20* females (35/657) was ~5%. *X* chromosome nondisjunction of *ord*^{ys91} and *mei(3)M20* was estimated to be 40.0% (total 953) and 52.2% (total 408), respectively. No gynandromorph (*X/X-X/O*) was pro-

duced. We also examined embryonic development by staining with DAPI. The final preparation included embryos 2–6 hr old. Development beyond the syncytial blastoderm stage was observed in 97.1% embryos from the control females (number of embryos examined is 239), but only 57.7% embryos from *ord*^{ys91} females (total 286) and 51.0% embryos from *mei(3)M20* females (n = 204) reached the stage. Nondisjunction in *mei(3)M20* females and males appears to be caused by the same mutation, because the abnormalities were also manifested when *mei(3)M20* was placed over a chromosomal deficiency *Df(3L)vin6* (data not shown), although the possibility that two separate mutations are closely linked and located within the deletion (68C8-11; 69A4-5) remains.

Sterility of *mei(2)yh137* (*tef*^{yh137}) females is caused by another mutation on the same chromosome, because females *tef*^{yh137}*/Df(2R)P803-Δ15* restored fertility. The *tef* mutation has been shown to have no effect on female meiosis (Tomkiel *et al.* 2001). No further examination of *mei(2)yh137* females was thus performed. The remaining three mutants, *mei(2)yh92*, *mei(2)yh217*, and *mei(3)yoh7134*, laid a large number of eggs with normal eggshell morphology. In >100 embryos examined for each genotype, nuclear divisions became abnormal by the syncytial blastoderm stage. We do not have any evidence to demonstrate that the female sterility of *mei(2)yh92* and *mei(2)yh217* is caused by the same mutation responsible for male meiotic nondisjunction. The *P*-element-excision experiment for *mei(2)yoh7134* mentioned above showed that the female sterility was also attributable to the *P* insertion.

DISCUSSION

We screened 2532 second and third chromosome lines, derived from mutagenesis or from natural populations, that were homozygous viable and fertile in the male, and recovered nine male meiotic mutants. The efficiency was 3/824 for EMS-treated chromosomes, 4/226 for chromosomes from natural populations, and 2/1482 for *P*-element insertions. The rates were nearly equivalent to those reported previously: 2/160 for EMS-treated chromosomes (Gethmann 1974), 4/423 for chromosomes from natural populations (Sandler *et al.* 1968), and 41/18,558 for *P*-element insertions (Sekelsky *et al.* 1999, data on female meiotic mutants). Seven of the mutants recovered in this study were located on the second chromosome, each representing a different locus. Only one mutant [two lines, *mei(3)M19* and *mei(3)M20*] was on the third. Among the known autosomal male meiotic mutants, eight are on the second chromosome [*teflon* (*tef*, thought to be allelic to the extinct *mei-S8*), *mei-O81*, *mei-G17*, *mei-S332*, *orientation disruptor* (*ord*), *mei-G87*, *subito* (*sub*), and *sting*] and three are on the third chromosome [*mei-1223* (*mei-I1*), *mei-I3*, and *homeless* (*hls*); FlyBase 2003]. Because six of eight newly recovered mutants represent new loci, further screenings are needed to fulfill a set of genes required for male meiosis in *D. melanogaster*.

In this study we examined the segregation of the sex and the fourth chromosomes, but not of the second and the third chromosomes, in male meiosis. *mei(2)ys91* (named *ord*^{ys91}), *mei(2)yh92*, and *mei(3)yoh7134* disrupted both sex and fourth chromosome segregation. Preferential effects on specific chromosomes were observed in other mutants: *mei(2)yh217* showing a higher rate of nondisjunction in the sex chromosomes and *mei(2)yh15* and *mei(2)yh137* (named *tef*^{yh137}) showing a higher rate of nondisjunction in the fourth chromosome. Because, in male meiosis, pairing of homologs is mediated by chromosome-specific pairing sites, one can expect to recover pairing-defective mutants that exhibit a chromosome-specific effect. However, it has not been shown that Drosophila spermatocytes have such a chromosome-specific control of pairing by *trans*-acting proteins. Among meiotic genes analyzed to date, only two, *mei-1223* and *tef*, function specifically in the process of homologous chromosome pairing in meiosis of the male. All chromosome complements are affected in *mei-1223* mutant cells, albeit with different frequencies (Yamamoto *et al.* 1993). *tef* exclusively affects autosomes but not the sex chromosomes (Tomkiel *et al.* 2001; this study). It has been suggested that homologous pairing in male meiosis is related to the somatic pairing observed in spermatogonial cells (Cooper 1950; Vazquez *et al.* 2002). However, the *mei-1223*^{m144} and the *tef*^{Z5549} mutants, which severely disrupt meiotic pairing of all autosomes, have no detectable somatic pairing defects (K. Hirai and M.-T. Yamamoto, unpublished observations). Regulatory mechanisms of homologous chromosome pairing mediated by chromosome-specific pairing sites remain obscure.

Three mutants described here, *mei(2)yh217*, *mei(2)yh15*, and *tef*^{yh137}, showed potential chromosome specificity, although the effects were mild. Baker and Carpenter (1972) recovered 20 mutants on the *X* chromosome that induced sex chromosome-specific nondisjunction at rates of <10%. It should be mentioned that all male meiotic mutants causing significant anomalies in pairing and segregation have never been shown to affect any specific chromosomes. Thus we would postulate that the chromosome specificity in homologous chromosome pairing in the male of Drosophila may be generated by the results of an additive effect of multiple genes with mild influences.

To obtain mutants that exhibit a specific effect on the pairing site in *hB* carried by *Dp223*, but not on the other pairing sites of the *X* chromosome, we compared nondisjunction frequencies of the sex chromosomes between compound-*XY/Dp223* and *X/Y* males. Such mutants, if isolated, would have altered disjunction between the compound-*XY* and *Dp223* chromosomes (Table 2), but not between the normal *X* and *Y* chromosomes (Table 3). One mild mutant, *mei(2)yh217*, did produce possible sex chromosome-specific nondisjunction in

meiosis I, but it showed the same levels of nondisjunction in both compound-*XY/Dp223* and *X/Y*. *mei(2)yh217* may thus be a mutation affecting all pairing sites on the *X* chromosome or may be involved in a meiotic process other than pairing. The *Stellate* (*Ste*) elements (arrays of partially homologous and tandemly repeated sequences with an open reading frame encoding a 19,500-D protein) are expressed when the *Suppressor of Stellate* on the *Y* (also composed of tandemly repeated sequences) is deleted (Livak 1984, 1990). The expression of *Ste* causes, among other effects, chromosome nondisjunction in male meiosis. Nondisjunction is observed for the sex, second, and third chromosomes but not for the fourth chromosome (Hardy *et al.* 1984). How this type of chromosome-specific nondisjunction occurs is not known. Meiosis is disrupted after the formation of bivalents (Palumbo *et al.* 1994). Expression of the *Ste* elements is also regulated by other mutants such as *hls*. In the *hls* mutant males nondisjunction is produced in both meiotic divisions (Stapleton *et al.* 2001), whereas it is restricted to meiosis I in *mei(2)yh217* males. *Double or nothing* is an additional example of meiotic mutant in which nondisjunction frequency of the *X* and *Y* chromosomes is appreciably higher than that of the fourth chromosome (Moore *et al.* 1994). This mutation is an antimorphic allele of the *sub* gene encoding a kinesin motor protein required for normal spindle assembly (Giunta *et al.* 2002).

We also determined which meiotic division was defective in each recovered meiotic mutant by examining sex chromosome segregation. Three mutants, *mei(2)yh92*, *mei(2)yh217*, and *mei(3)yoh7134*, cause meiosis I nondisjunction almost exclusively. Normal function of these genes may be involved in homologous chromosome behavior. Two mutants, *ord*^{ys91} and *mei(3)M20*, disrupted both meiotic divisions, suggesting sister chromatid cohesion defects. Because almost all exceptional sperm produced by *mei(2)yh149* males were nullo-*XY* class, simple chromosome loss during meiotic divisions may be the major cause of the nondisjunction.

Cytological examination as well as gene mapping were plausible for the severe effect mutants, *mei(2)yh92*, *mei(2)ys91*, and *mei(3)M20*. *mei(2)yh92* caused nondisjunction almost exclusively at meiosis I, although homologous chromosomes were paired as normal (Figure 2C). Double staining with a DNA dye and an anti-α-tubulin monoclonal antibody for chromosomes and microtubules, respectively, showed that meiotic spindles were morphologically normal and bivalents were normally aligned on the metaphase plate (data not shown). The *mei(2)yh92* mutation interferes with the fidelity of meiosis I disjunction. When homologous chromosomes begin to move to opposite poles in anaphase I, paired chromosomes are lagged behind (Figure 3B). The lagging chromosome pairs eventually reached both poles (normal disjunction) or a single pole (nondisjunction; Figure 3D). Thus, the ability of kinetochores to bind microtubules appears to be preserved in the mutant cells. Rather, the separation of paired homologs is defective in *mei(2)yh92*. The normal function of the *mei(2)yh92* gene product may thus be involved in proteolysis or dispersal of presumptive adhesive proteins at the pairing sites, for example "segregation bodies" (Wolf 1994), in a direct or indirect manner. Alternatively, it may be that transportation of homologous chromosomes upon dissolution of pairing is perturbed in *mei(2)yh92* anaphase I cells. Alignment of bioriented chromosome pairs at the metaphase plate is accomplished by the integration of antagonistic poleward forces and antipoleward forces, exerted by microtubule dynamics and microtubule-based motor proteins on the kinetochores and along the chromosome arms. Anaphase onset is permitted by downregulation of the antipoleward forces as well as disassociation of partner chromosomes (reviewed in McIntosh *et al.* 2002; Cleveland *et al.* 2003; Scholey *et al.* 2003). *mei(2)yh92* cells may thus be abnormal in continuous production of antipoleward forces during anaphase, blocking the movement of individualized homologous chromosomes to opposite poles. The predominant, but not exclusive, effect of this mutation on meiosis I could be explained if a larger amount of the gene product is needed to align a bivalent (tetrad) in meiosis I cells than to align a dyad in meiosis II cells, and/or if a redundant pathway exists enabling segregation of sister chromatids in the mutant meiosis II cells.

The remaining two mutants, *ord*^{ys91} and *mei(3)M20*, are both deficient in meiotic sister chromatid cohesion. The genes *ord* (Mason 1976; Goldstein 1980; Lin and Church 1982; Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1996, 1997, 2002; Balicky *et al.* 2002; this study) and *mei-S332* (Sandler *et al.* 1968; Goldstein 1980; Kerrebrock *et al.* 1992, 1995; Moore *et al.* 1998; Tang *et al.* 1998) are known to be defective in meiotic sister chromatid cohesion. Although a majority of the previously known Drosophila meiotic mutations affect only one sex, mutations in the *ord* and *mei-S332* genes result in nondisjunction in both sexes (reviewed in Orr-Weaver 1995). Similarly, *mei(3)M20* causes nondisjunction in both sexes. Meiotic sister chromatid cohesion, not homologous chromosome pairing, should depend on a common mechanism in the two sexes.

In wild-type spermatocytes, separation of sister chromatids along the chromosome arms occurs in mid-G_2, but centromeric cohesion is maintained throughout meiosis I (Balicky *et al.* 2002; Vazquez *et al.* 2002). The *ord* and *mei-S332* genes differ from each other in the stages at which precocious separation of sister centromeres occurs. Cohesion defects become detectable in late-G_2 in *ord* cells but in late anaphase I in *mei-S332* cells. This difference explains the result of genetic analyses that nondisjunction takes place in both meiosis I and meiosis II in *ord* but primarily in meiosis II in *mei-S332*. The ORD and MEI-S332 proteins are essential

to maintain centromeric cohesion until the onset of anaphase II. These proteins have no known homologs in other organisms (Kerrebrock *et al.* 1995; Bickel *et al.* 1996).

The phenotype of the newly recovered *mei(3)M20* mutant is similar to that of *ord* mutants. Prematurely individualized chromatids are observed in prometaphase I cells (Figure 2, B and D). Spermatogonia (data not shown) and meiosis I cells (Figure 2D) mutant for *mei(3)M20* carry the normal complement of chromosomes, indicating no effect of *mei(3)M20* on premeiotic mitosis. The *ord* alleles, ord^1 and ord^2, examined in homozygotes have been shown to cause nondisjunction not only in spermatocytes but also in spermatogonia (Lin and Church 1982; Miyazaki and Orr-Weaver 1992). However, such is not the case for the null allele ord^{10} (either over a chromosomal deficiency or over a strong allele of ord^5; Bickel *et al.* 1997). Complete loss of ORD activity is more similar to the *mei(3)M20* mutation.

A complex of proteins, the cohesin complex, establishes a structural link between sister chromatids during S phase in mitosis and meiosis (reviewed in Lee and Orr-Weaver 2001). The release of the cohesin complex from chromosomes in mitosis is permitted by the cleavage of one of the cohesin subunits, SCC1/MCD1/RAD21, at the transition from metaphase to anaphase. In meiosis this subunit is largely replaced with meiosis-specific REC8, which is not cleaved at the centromeres in meiosis I (Kitajima *et al.* 2003). This major effector of meiotic cohesion is known from diverse species, from fission yeast to humans (Parisi *et al.* 1999), but it has not been identified in Drosophila. How the cohesion of meiotic sister chromatids is established in Drosophila has not been understood. The *Drad21* gene appears to reside in heterochromatin of an unidentified chromosome (Vass *et al.* 2003). The *mei(3)M20* gene is mapped to the left arm of the third chromosome. Thus, *mei(3)M20* is not an allele of mitotic *Drad21* with strong meiotic effects in surviving mutant individuals. Indeed, we observed no effect of the *mei(3)M20* mutation on viability from the egg to the adult (data not shown). The phenotypes of *ord* (Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1997, 2002; this study) and of *mei(3)M20* suggest involvement of these gene products in the establishment as well as the maintenance of meiotic sister chromatid cohesion. However, ORD appears to play roles in chromatin condensation and maintenance of centromeric cohesion. This protein begins to accumulate on the chromatin in mid-G_2 of primary spermatocytes and then remains only at the centromeres of condensed chromosomes until the onset of anaphase II (Balicky *et al.* 2002). The mechanism of establishment of cohesion between meiotic sister chromatids in Drosophila would be revealed by further genetic and molecular characterization of the *mei(3)M20* gene.

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LITERATURE CITED

Appels, R., and A. J. Hilliker, 1982 The cytogenetic boundaries of the rDNA region within heterochromatin of the X chromosome of *Drosophila melanogaster* and their relation to male meiotic pairing sites. Genet. Res. **39:** 149–156.

Ault, J. G., and C. L. Rieder, 1994 Meiosis in *Drosophila* males. I. The question of separate conjunctive mechanisms for the *XY* and autosomal bivalents. Chromosoma **103:** 352–356.

Ault, J. G., H.-P. P. Lin and K. Church, 1982 Meiosis in *Drosophila melanogaster*. IV. The conjunctive mechanism of the XY bivalent. Chromosoma **86:** 309–317.

Baker, B., and A. T. C. Carpenter, 1972 Genetic analysis of sex chromosomal meiotic mutants in *Drosophila melanogaster*. Genetics **71:** 255–286.

Balicky, E. M., M. W. Endres, C. Lai and S. E. Bickel, 2002 Meiotic cohesion requires accumulation of ORD on chromosomes before condensation. Mol. Biol. Cell **13:** 3890–3900.

Bickel, S. E., D. W. Wyman, W. Y. Miyazaki, D. P. Moore and T. L. Orr-Weaver, 1996 Identification of ORD, a *Drosophila* protein essential for sister chromatid cohesion. EMBO J. **15:** 1451–1459.

Bickel, S. E., D. W. Wyman and T. L. Orr-Weaver, 1997 Mutational analysis of the Drosophila sister-chromatid cohesion protein ORD and its role in the maintenance of centromeric cohesion. Genetics **146:** 1319–1331.

Bickel, S. E., T. L. Orr-Weaver and E. M. Balicky, 2002 The sister-chromatid cohesion protein ORD is required for chiasma maintenance in *Drosophila* oocytes. Curr. Biol. **12:** 925–929.

Church, K., and H.-P. P. Lin, 1988 Drosophila: a model for the study of aneuploidy, pp. 227–255 in *Aneuploidy, Part B: Induction and Test Systems*, edited by B. K. Vig and A. A. Sandberg. Alan R. Liss, New York.

Cleveland, D. W., Y. Mao and K. F. Sullivan, 2003 Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell **112:** 407–421.

Cooper, K. W., 1950 Normal spermatogenesis in Drosophila, pp. 1–61 in *Biology of Drosophila*, edited by M. Demerec. John Wiley & Sons, New York.

Cooper, K. W., 1959 Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of "heterochromatin." Chromosoma **10:** 535–588.

Cooper, K. W., 1964 Meiotic conjunctive elements not involving chiasmata. Proc. Natl. Acad. Sci. USA **52:** 1248–1255.

FlyBase, 2003 The FlyBase database of the *Drosophila* genome projects and community literature. Nucleic Acids Res. **31:** 172–175.

Gershenson, S., 1933 Studies on the genetically inert region of the *X*-chromosome of *Drosophila*. I. Behaviour of an *X*-chromosome deficient for a part of its inert region. J. Genet. **28:** 297–313.

Gethmann, R. C., 1974 Meiosis in male *Drosophila melanogaster*. I. Isolation and characterization of mutants affecting second chromosome disjunction. Genetics **78:** 1127–1142.

Giunta, K. L., J. K. Jang, E. A. Manheim, G. Subramanian and K. S. McKim, 2002 *subito* encodes a kinesin-like protein required for meiotic spindle pole formation in *Drosophila melanogaster*. Genetics **160:** 1489–1501.

Goldstein, L. S. B., 1980 Mechanisms of chromosome orientation revealed by two meiotic mutants in *Drosophila melanogaster*. Chromosoma **78:** 79–111.

Guyénot, E., and A. Naville, 1929 Les chromosomes et la réduction chromatique chez *Drosophila melanogaster* (Cinèses somatiques, spermatogenèse, ovogenèse). Cellule **39:** 25–82.

Hardy, R. W., D. L. Lindsley, K. J. Livak, B. Lewis, A. L. Siversten *et al.*, 1984 Cytogenetic analysis of a segment of the *Y* chromosome of *Drosophila melanogaster*. Genetics **107:** 591–610.

Hilliker, A. J., D. G. Holm and R. Appels, 1982 The relationship

between heterochromatic homology and meiotic segregation of compound second autosomes during spermatogenesis in *Drosophila melanogaster*. Genet. Res. **39:** 157–168.

Huang, A. M., E. J. Rehm and G. M. Rubin, 2000 Recovery of DNA sequences flanking P-element insertions: inverse PCR and plasmid rescue, pp. 429–437 in *Drosophila Protocols*, edited by W. Sullivan, M. Ashburner and R. S. Hawley. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Karpen, G. H., and S. A. Endow, 1998 Meiosis: chromosome behavior and spindle dynamics, pp. 203–247 in *Dynamics of Cell Division*, edited by S. A. Endow and D. M. Glover. Oxford University Press, Oxford.

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.

Kerrebrock, A. W., D. P. Moore, J. S. Wu and T. L. Orr-Weaver, 1995 Mei-S332, a Drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions. Cell **83:** 247–256.

Kitajima, T. S., S. Yokobayashi, M. Yamamoto and Y. Watanabe, 2003 Distinct cohesin complexes organize meiotic chromosome domains. Science **300:** 1152–1155.

Lee, J. Y., and T. L. Orr-Weaver, 2001 The molecular basis of sister-chromatid cohesion. Annu. Rev. Cell Dev. Biol. **17:** 753–777.

Lewis, E. B., and F. Bacher, 1968 Method of feeding ethyl methane sulfonate (EMS) to Drosophila males. Dros. Inf. Serv. **48:** 193.

Lin, H.-P. P., and K. Church, 1982 Meiosis in *Drosophila melanogaster*. III. The effect of orientation disruptor (*ord*) on gonial mitotic and the meiotic divisions in males. Genetics **102:** 751–770.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

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.

Livak, K. J., 1990 Detailed structure of the *Drosophila melanogaster Stellate* genes and their transcripts. Genetics **124:** 303–316.

Mason, J. M., 1976 Orientation disruptor (*ord*): a recombination-defective and disjunction-defective meiotic mutant in *Drosophila melanogaster*. Genetics **84:** 545–572.

McIntosh, J. R., E. L. Grishchuk and R. R. West, 2002 Chromosome-microtubule interactions during mitosis. Annu. Rev. Cell Dev. Biol. **18:** 193–219.

McKee, B. D., 1996 The license to pair: identification of meiotic pairing sites in *Drosophila*. Chromosoma **105:** 135–141.

McKee, B. D., and G. H. Karpen, 1990 Drosophila ribosomal RNA genes function as an X-Y pairing site during male meiosis. Cell **61:** 61–72.

McKee, B. D., and D. L. Lindsley, 1987 Inseparability of *X*-heterochromatic functions responsible for *X:Y* pairing, meiotic drive and male fertility in *Drosophila melanogaster* males. Genetics **116:** 399–407.

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 achiasmatic 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.

Meyer, G. F., 1964 A possible correlation between the submicroscopic structure of meiotic chromosomes and crossing over, pp. 461–462 in *Proceedings of the 3rd European Regional Conference of Electron Microscopy*, edited by M. Titlbach. Czechoslovak Academy of Sciences, Prague.

Miyazaki, W. Y., and T. L. Orr-Weaver, 1992 Sister-chromatid misbehavior in Drosophila *ord* mutants. Genetics **132:** 1047–1061.

Moore, D. P., W. Y. Miyazaki, J. E. Tomkiel and T. L. Orr-Weaver, 1994 *Double or nothing*: a Drosophila mutation affecting meiotic chromosome segregation in both females and males. Genetics **136:** 953–964.

Moore, D. P., A. W. Page, T.-L. T. Tang, A. W. Kerrebrock and T. L. Orr-Weaver, 1998 The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. J. Cell Biol. **140:** 1003–1012.

Morgan, T. H., 1912 Complete linkage in the second chromosome of the male of Drosophila. Science **36:** 719–720.

Orr-Weaver, T. L., 1995 Meiosis in *Drosophila*: seeing is believing. Proc. Natl. Acad. Sci. USA **92:** 10443–10449.

Palumbo, G., S. Bonaccorsi, L. G. Robbins and S. Pimpinelli, 1994 Genetic analysis of *Stellate* elements of *Drosophila melanogaster*. Genetics **138:** 1181–1197.

Parisi, S., M. J. McKay, M. Molnar, M. A. Thompson, P. J. Van Der Spek *et al.*, 1999 Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. Mol. Cell. Biol. **19:** 3515–3528.

Park, H.-S., and M.-T. Yamamoto, 1993 Synthesis of free *X* duplications carrying a specific region of the centromeric heterochromatin in *Drosophila melanogaster*. Jpn. J. Genet. **68:** 83–95.

Peacock, W. J., G. L. G. Miklos and D. J. Goodchild, 1975 Sex chromosome meiotic drive systems in *Drosophila melanogaster*. I. Abnormal spermatid development in males with a heterochromatin-deficient *X* chromosome (sc^4sc^8). Genetics **79:** 613–634.

Rasmussen, S. W., 1973 Ultrastructural studies of spermatogenesis in *Drosophila melanogaster* Meigen. Z. Zellforsch. Mikrosk. Anat. **140:** 125–144.

Rothwell, W. F., and W. Sullivan, 2000 Fluorescent analysis of *Drosophila* embryos, pp. 141–157 in *Drosophila Protocols*, edited by W. Sullivan, M. Ashburner and R. S. Hawley. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sandler, L., D. L. Lindsley, B. Nicoletti and G. Trippa, 1968 Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics **60:** 525–558.

Scholey, J. M., I. Brust-Mascher and A. Mogilner, 2003 Cell division. Nature **422:** 746–752.

Sekelsky, J. J., K. S. McKim, L. Messina, R. L. French, W. D. Hurley *et al.*, 1999 Identification of novel Drosophila meiotic genes recovered in a *P*-element screen. Genetics **152:** 529–542.

Stapleton, W., S. Das and B. D. McKee, 2001 A role of the *Drosophila homeless* gene in repression of *Stellate* in male meiosis. Chromosoma **110:** 228–240.

Tang, T. T.-L., S. E. Bickel, L. M. Young and T. L. Orr-Weaver, 1998 Maintenance of sister-chromatid cohesion at the centromere by the Drosophila MEI-S332 protein. Genes Dev. **12:** 3843–3856.

Toba, G., T. Ohsako, N. Miyata, T. Ohtsuka, K. H. Seong *et al.*, 1999 The gene search system: a method for efficient detection and rapid molecular identification of genes in *Drosophila melanogaster*. Genetics **151:** 725–737.

Tomkiel, J. E., B. T. Wakimoto and A. Briscoe, Jr., 2001 The *teflon* gene is required for maintenance of autosomal homolog pairing at meiosis I in male *Drosophila melanogaster*. Genetics **157:** 273–281.

Vass, S., S. Cotterill, A. M. Valdeolmillos, J. L. Barbero, E. Lin *et al.*, 2003 Depletion of Drad21/Scc1 in *Drosophila* cells leads to instability of the cohesin complex and disruption of mitotic progression. Curr. Biol. **13:** 208–218.

Vazquez, J., A. S. Belmont and J. W. Sedat, 2002 The dynamics of homologous chromosome pairing during male *Drosophila* meiosis. Curr. Biol. **12:** 1473–1483.

Wolf, K. W., 1994 How meiotic cells deal with non-exchange chromosomes. BioEssays **16:** 107–114.

Yamamoto, A. H., K. Muramatsu, T. Otsuka and M.-T. Yamamoto, 1993 Meiotic mutants from natural populations of *Drosophila melanogaster*. Genetica **88:** 165–173.

Yamamoto, M., 1979 Cytological studies of heterochromatin function in the *Drosophila melanogaster* male: autosomal meiotic pairing. Chromosoma **72:** 293–328.

Yamamoto, M., 1981 The mechanism of meiotic chromosome pairing in the *Drosophila melanogaster* male. Jpn. J. Genet. **56:** 79–96.

Yamamoto, M., and G. L. G. Miklos, 1977 Genetic dissection of heterochromatin in *Drosophila*: the role of basal X heterochromatin in meiotic sex chromosome behaviour. Chromosoma **60:** 283–296.

Yamamoto, M.-T., 1992 Inviability of hybrids between *D. melanogaster* and *D. simulans* results from the absence of *simulans X* not the presence of *simulans Y* chromosome. Genetica **87:** 151–158.

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