A Deficiency Screen of the Major Autosomes Identifies a Gene (*matrimony*) That Is Haplo-insufficient for Achiasmate Segregation in Drosophila Oocytes

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ABSTRACT

In Drosophila oocytes, euchromatic homolog-homolog associations are released at the end of pachytene, while heterochromatic pairings persist until metaphase I. A screen of 123 autosomal deficiencies for dominant effects on achiasmate chromosome segregation has identified a single gene that is haplo-insufficient for homologous achiasmate segregation and whose product may be required for the maintenance of such heterochromatic pairings. Of the deficiencies tested, only one exhibited a strong dominant effect on achiasmate segregation, inducing both *X* and fourth chromosome nondisjunction in *FM7/X* females. Five overlapping deficiencies showed a similar dominant effect on achiasmate chromosome disjunction and mapped the haplo-insufficient meiotic gene to a small interval within 66C7-12. A *P*-element insertion mutation in this interval exhibits a similar dominant effect on achiasmate segregation, inducing both high levels of *X* and fourth chromosome nondisjunction in *FM7/X* females and high levels of fourth chromosome nondisjunction in *X/X* females. The insertion site for this *P* element lies immediately upstream of *CG18543*, and germline expression of a UAS-*CG18543* cDNA construct driven by *nanos*-GAL4 fully rescues the dominant meiotic defect. We conclude that *CG18543* is the haplo-insufficient gene and have renamed this gene *matrimony* (*mtrm*). Cytological studies of prometaphase and metaphase I in *mtrm* hemizygotes demonstrate that achiasmate chromosomes are not properly positioned with respect to their homolog on the meiotic spindle. One possible, albeit speculative, interpretation of these data is that the presence of only a single copy of *mtrm* disrupts the function of whatever "glue" holds heterochromatically paired homologs together from the end of pachytene until metaphase I.

THE accurate disjunction of homologs during meiosis is accomplished by a series of highly coordinated processes beginning in meiotic prophase. In the chiasmate system of segregation, centromeres are oriented toward opposite poles by chiasmata, which restrict the movement of the chromosomes and serve to orient the centromeres toward the poles (Nicklas 1974; Hawley 1988). However, *Drosophila melanogaster* females also employ a highly efficient backup mechanism, known as homologous achiasmate segregation, to ensure the separation of homologous chromosomes that, for one reason or another, fail to undergo exchange (Hawley and Theurkauf 1993). In this system, the physical association of homologs at the end of pachytene is maintained not by chiasmata, but rather by the perdurance of heterochromatic pairing, which is both necessary and sufficient to ensure centromere co-orientation (Hawley *et al.* 1992; Karpen *et al.* 1996). These heterochromatic pairings are established in early prophase and maintained until metaphase I, despite the dissolution of euchromatic synapsis at the end of pachytene (Carpenter 1979; Dernburg *et al.* 1996).

The first evidence that heterochromatic homology is crucial to the segregation of achiasmate homologs came from studies of the effects of homologous duplications on the segregation of two normal fourth chromosomes (Hawley *et al.* 1992). These duplications, designated as *Dp(1;4)*'s, carry some or all of the heterochromatic base of the fourth chromosome and range in size from the size of a normal fourth chromosome to 3.5 times its length. The more fourth chromosome heterochromatin carried by a *Dp(1;4)*, the higher the level of induced fourth chromosome nondisjunction, regardless of the size of the duplication. Removing a block of the fourth chromosome heterochromatin from the largest of these duplications ablates its ability to induce *Dp(1;4)* ↔ *44* disjunctions.On the basis of these data, Hawley *et al.* (1992) suggested that fourth chromosome segregation is facilitated by the pairing of fourth chromosome heterochromatin. Similar results were also obtained using duplications for the pericentric heterochromatin of the *X* chromosome, validating this conclusion for larger achiasmate chromosomes as well.

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Karpen *et al.* (1996) presented stronger evidence that homologous achiasmate segregation is dependent on heterochromatic homology. They showed that the frequency with which two achiasmate deletion derivatives of *Dp(1;f)1187* segregate from each other in female meiosis is proportional to the amount of centric heterochromatic homology. Normal segregation requires 800 kb of overlap in the centric heterochromatin, whereas nearly random disjunction is observed with only 300 kb of overlap. A linear correlation between the amount of heterochromatic homology and segregation efficiency was observed in the range of 300–800 kb. They concluded that sequences found throughout the centric heterochromatin of *Dp(1;f)1187* act additively to ensure achiasmate meiotic segregation. Finally, Dernburg *et al.* (1996) used three-dimensional fluorescent hybridization to demonstrate that although euchromatic pairings dissolve following pachytene, heterochromatic pairings are preserved within the karyosome until prometaphase. Thus, in Drosophila female meiosis, heterochromatic pairings persist beyond the dissolution of the synaptonemal complex at pachytene until centromere co-orientation at prometaphase.

The fact that heterochromatic pairings are maintained after the dissolution of euchromatic synapsis suggests the existence of specific functions required to maintain heterochromatic associations. Obviously, the identification of such functions might provide important insights into the mechanisms by which heterochromatic alignments facilitate achiasmate segregations. Unfortunately, little is known about the mechanisms that underlie the maintenance of such homologous heterochromatic associations in the oocyte nucleus. In an attempt to identify the structural proteins that maintain these heterochromatic pairings, we set out to identify genes that showed a clear dosage effect on the efficacy of achiasmate segregation.

Our screen was partly based on the assumption that a reduction in the quantity of some essential "glue" or "binder" protein by 50% might have dramatic and direct effects on the ability of the oocyte to maintain heterochromatic associations and thus disrupt achiasmate segregation. Toward that end, we screened the Bloomington deficiency kit for deficiencies that exhibited a dominant effect on *X* and/or on fourth chromosome nondisjunction in females of the genotype *FM7/X; Df/+; spa^{pol}*. We focused our attention on deficiencies that impaired the proper segregation of both achiasmate *X* and fourth chromosomes, for which we could subsequently demonstrate a direct effect on fourth chromosome nondisjunction in females bearing chiasmate *X* chromosomes (*i.e.*, we were specifically interested in identifying genes whose ability to induce fourth chromosome nondisjunction as hemizygotes did not require high levels of *X* chromosome missegregation). This latter stipulation is meant to remove from consideration those mutants in which the effect on fourth chromosome segregation appears to be an indirect consequence of *X* chromosome nondisjunction (O'Tousa 1982; Zitron and Hawley 1989; Whyte *et al.* 1993).

Our initial screen of 123 deficiencies identified a single deletion, *Df(3L)66C-G28*, that exhibited a reproducible dominant defect in achiasmate segregation in females, inducing both *X* and fourth chromosome nondisjunction in *FM7/X* females. We were able to determine that this increase in meiotic nondisjunction is indeed the consequence of hemizygosity for a gene identified in the Drosophila genome sequence as *CG18543* (Celniker *et al.* 2002). We have renamed this gene *matrimony* (*mtrm*) because it appears to hold paired chromosomes together. In *mtrm* hemizygotes, achiasmate chromosomes are often improperly positioned on the spindle, relative to their homologs during both prometaphase and metaphase, suggesting that proper heterochromatic alignments or pairings either failed to occur or were not properly maintained. One possible interpretation of these data is that the presence of only a single copy of *mtrm* disrupts functioning of whatever "glue" holds heterochromatically paired homologs together from the end of pachytene until metaphase I.

MATERIALS AND METHODS

Drosophila stocks: Most of the deficiency lines screened in this study were obtained from the Bloomington *Drosophila* Stock Center. A list of the deficiencies tested is provided in Table 1. In addition to those deficiencies, we examined a number of other deficiencies that delete all or part of the 66C region. *Df(3L)66C-I65* (BL#209), *Df(3L)66C-G28* (BL #1541), and *Df(3L)66C-T2-10* were generated by loss of the transposon (*p{white-un2}30*) in the GR881 insertion line (J. E. Natzle, unpublished data). *Df(3L)66C-I65* and *Df(3L)66C-G28* were generated by imprecise transposon excision of the *p{white-un2}30* insertion by Δ*2-3*-mediated mobilization. *Df(3L) 66C-T2-10* was generated from the GR881 line by X-ray mutagenesis. *Df(3L)66C-B2-2* and *Df(3L)66C-E3-1* were recovered in a screen for diepoxybutane-induced mutations that were lethal *in trans* with the *Df(3L)66C-I65* deletion. Both deletions were induced on an isogenized third chromosome carrying the markers *mwh red ebony*. We also tested *Df(3L)BSC13*, which uncovers the region 66B12-C1;66D2-4. This deletion was kindly provided by Kevin Cook at the Bloomington *Drosophila* Stock Center, who produced this deficiency by recombining two *P* elements located at positions 66B10-11 and 66D6 on the map presented in Figure 2. *Df(3L)ZP1* (66A17-66C5) was received courtesy of P. Maroy (Szeged, Hungary). *l(3)F6* and *l(3)J1* were isolated by J. Natzle (unpublished data). *l(3)L3852, EP(3)3729*, and *EP(3)3616* were obtained from the Bloomington *Drosophila* Stock Center.

The basic cross scheme: To introduce marked *X* and fourth chromosomes, males from each of the second or third chromosome deficiency stocks were crossed to *y w; Sp^{1} Bl^{1} L^{rm} Bc^{1} Pu^{2}/ SM6a; spa^{pol}* or *y w; D/TM3; spa^{pol}*, respectively. To screen for haplo-insufficient meiotic loci, deficiency-bearing males resulting from these crosses were crossed to *FM7w; spa^{pol}* females to generate *y w/FM7w; Df/+; spa^{pol}* females. For each deficiency stock, we scored progeny from at least 20 such *y w/FM7w; Df/+; spa^{pol}* females, crossed *individually* to *attached-XY, y^{+} v f B; C(4),ci ey^{R}* males, and assessed the frequency of *X* and fourth chromosome nondisjunction. (The symbol *attached-XY* de-

notes the chromosome *C(1;Y), IN(1)EN*, whose structure is $Y^{S}X{\cdot}Y^{L}$.) The *FM7w* chromosome is marked with *y*, *w*, and *B*. Regular female progeny from this cross are yellow-plus Bar (*FM7w/attached-XY*) and yellow-plus Bar (*y w/attached-XY*). Regular male progeny are yellow-white Bar (*FM7,w/0*) and yellow-white (*yw/0*). All regular progeny carry three copies of chromosome *4*, the attached-*4* chromosome [*C(4)RM*], and a maternal fourth chromosome marked with spa^{pol}, and are wild type for spa^{pol}, *ci*, and ey^{R}.

X chromosome nondisjunction at meiosis I in the female results in yellow-white Bar females (*FM7,w/y w*, arising from diplo-*X* ova) or vermillion forked Bar males (attached-*XY/0*, arising from nullo-*X* ova). *X* chromosome nondisjunction at meiosis II results in *FM7/FM7* or *yw/yw* daughters. Meiosis II nondisjunction is extremely rare, and no such events were observed in the crosses reported here. The adjusted total is corrected for the inviability or reduced viability of certain progeny classes. Diplo-*X* ova are viable only when fertilized by a nullo-*XY*-bearing sperm, and nullo-*X* ova are viable only when fertilized by a sperm carrying the *attached-XY*. Therefore, only half of the nondisjunctional ova are recovered as exceptions. In the adjusted totals for crosses involving *y/y* and *FM7/y* females, we correct for this inequality by doubling the number of *X* chromosome exceptions. The frequency of nondisjunction was calculated as described in Hawley *et al.* (1992).

Fourth chromosome nondisjunction results in cubitus interruptus eyeless Russian progeny, arising from the fertilization of a nullo-*4* ovum by a sperm carrying the attached-*4* chromosome, or sparkling poliert offspring arising from the fertilization of a diplo-*4* ova by a nullo-*4* sperm. Nullo-*4* flies do not survive. Tetra-*4* flies are both rare and indistinguishable from regular progeny. Some crosses occasionally produce haplo-*4 Minute* progeny. The survival of these flies is poor and erratic. We recorded these flies, but do not list them in the tables or include them in our calculations. The cross involving the $P\{lacW\}exo^{mp1}$ insertion was done using females heterozygous for spa^{pol}; therefore the diplo-*4* exceptions could not be scored. In this cross, the calculated frequency of fourth chromosome nondisjunction was based on doubling the number of *ci* ey^{R} (nullo-*4*) exceptions and thus may represent an underestimate of the actual rate of fourth chromosome nondisjunction events. In several crosses, it became apparent that one or more of the tested females was triplo-*4*, as evidenced by a number of sparkling poliert exceptions that approximated 30% among progeny. Such vials also showed regular segregation of the sex chromosomes and produced few or no cubitus interruptus exceptions. The progeny of these females were excluded from consideration.

For crosses involving the 66C deletions we verified that the observed effects on achiasmate nondisjunction were not the effects of secondary nondisjunction events mediated by a cryptic *Y* chromosome by demonstrating that the expected regular *XO* sons were indeed sterile. In crosses involving the *P(SUPor-P) KG08051* insertion, the presence of both y^{+} and w^{+} genes on the *P* element precluded the identification of ~50% of the yellow-white matroclinous exceptions. To account for this, the number of yellow-white exceptions was doubled and the actual number of yellow-white exceptions was subtracted from the corresponding class of yellow-plus white-plus progeny. The numbers of yellow-white matroclinous exceptions observed were as follows: yellow-white females (18), yellow-white cubitus interruptus eyeless Russian females (17), and yellow-white sparkling poliert females (1). The corrected values were then used to create the list of progeny presented in Table 4.

Cytological studies: Oocytes were prepared and examined as previously described with minor modifications (Theurkauf and Hawley 1992; Matthies *et al.* 2000). Egg chambers from 3- to 7-day-old females were extracted by quick pulses of a blender using a modified Robb's medium. The mixture was passed sequentially through a loose and fine mesh to separate late-stage oocytes. The oocytes were fixed for 20 min on a rotator at room temperature in a hypertonic solution, thereby preventing hypotonic activation of the mature oocytes. After removal of the follicle cells, chorion, and vitelline membranes, the oocytes were permeabilized with 1% Triton X-100 in PBS. Oocytes were labeled with MAS078P (Harlan Sera-Lab at 1:500) and MAB1864 [Chemicon (Temecula, CA) at 1:500] rat antitubulin monoclonal antibodies. These antibodies were then labeled with Cy3 conjugated anti-rat secondary antibodies (1:250) purchased from Jackson Immunoresearch (West Grove, PA). Chromosomes were stained with 4′6-diamidino-2-phenylindole. Data were collected in 0.2-μm Z steps 3–5 μm above and below the subject of interest. The resulting data were deconvolved using the Softworx package (API).

RESULTS

Screening deficiency heterozygotes for their effects on achiasmate segregation: We initially screened 123 autosomal deficiencies obtained from the Bloomington *Drosophila* Stock Center, 66 second chromosome deficiencies, and 57 third chromosome deficiencies. An estimate of the autosomal genome coverage represented by this deficiency collection is ~64–73%. The effects of heterozygosity for each of these 123 deficiencies on *X* and fourth chromosome nondisjunction in *FM7/X; Df/+;* spa^{pol} females are listed in Table 1 and displayed graphically in Figure 1. Only four of the deficiencies, *Df(3L) 66C-G28*, *Df(2L)r10, Df(3L)Cat*, and *Df(3R)ea*, exhibited a strong dominant effect on achiasmate chromosome nondisjunction.

The highest frequencies of achiasmate chromosome nondisjunction were exhibited by females heterozygous for *Df(3L)66C-G28*. Similar high frequencies of *X* and fourth chromosome nondisjunction were also exhibited by five smaller deficiencies that overlap *Df(3L)66C-G28* (Table 2 and see below). None of the three smaller deficiencies that partially overlap the *Df(2L)r10* region [*Df(2L)osp29*, *Df(2L)TE35Bc-24*, or *Df(2l)H20*] exerted a dominant effect on chromosome segregation. This might suggest the presence of a dosage-sensitive region in the interval that is removed only by *Df(2L)r10* (35F-36A). The gene *c(2)M*, which encodes a synaptonemal complex component, lies within this interval (Manheim and McKim 2003). However, heterozygosity for a strong allele of this gene does not elevate the frequency of *X* and fourth chromosome nondisjunction in *FM7/X* females (data not shown). Similarly, the meiotic effects of *Df(3L)Cat* are not exhibited by the overlapping deficiency *Df(3L)W4*. Again, this might suggest the presence of a dosage-sensitive region in the interval that is removed only by *Df(3L)Cat* (75C-D). There are no obvious candidate genes in this interval. *Df(3R)ea* was not studied further.

The effects of *Df(3L)66C-G28* are also generated by overlapping deficiencies in region 66C: *Df(3L)66C-G28* removed all (or most) of region 66C. To map the haplo-

TABLE 1

Effects of the tested deficiencies on *X* and fourth chromosome nondisjunction

Deficiency name	Breakpoints	*N*	% *X* nondisjunction	% fourth chromosome nondisjunction
Df(2L)PMF	21A1; 21B7-8	929	0.4	0.0
Df(2L)a1	21B8-C1; 21C8-D1	153	2.6	0.0
Df(2L)S2	21C6-D1;22A6-B1	777	0.3	0.5
Df(2L)dp79b	22A2-3;22D5-E1	155	1.3	0.0
Df(2L) C144	23A1-4; 23C3-5	351	2.3	0.0
In(2LR)DTD16LDTD42R (Df(2L))	23C;23E3-6	812	7.4	3.0
Df(2L)JS32,dppd-ho	23C3-5;23D1-2	1124	0.4	0.0
Df(2L)S2590,P{ry[+t7.2]}FK1	23D2;23E3	1968	0.6	0.5
Df(2L)edSz-1	24A3-4;24D3-4	1224	0.5	0.0
Df(2L)GpdhA	25D7-E1;26A8-9	276	8.0	2.2
Df(2L)E110	3C1-2;20F;25F3-26A1; 26D3-11	767	2.6	0.5
Df(2L)J136-H52	27C2-9;28B3-4	633	1.3	0.6
Df(2L) spd	27D-E; 28C	899	2.0	0.2
Df(2L)wgCX3	27F2; 28A	480	4.6	3.3
Df(2L)Trf-C6R31	Within 28DE	821	1.7	1.9
Df(2L)N22-5	29C3-5; 30C4-D1	531	0.4	0.0
Df(2L)N22-14	29C1-2; 30C8-9; 30D1-2; 31A1-2	132	1.5	0.0
Df(2L)30C	29F7-30A1;30C2-5	473	2.1	2.5
Df(2L)S1402,P{w[+mc]=lacw}1402	30C1-2;30F,30B9-10	949	1.1	1.1
Df(2L)Pr1	32F1-3;33F1-2	391	1.5	1.5
Df(2L)esc10	33A8-B1;33B2-3	823	0.0	0.2
Df(2L)osp29	35B1-3;35E6	271	3.7	0.0
Df(2L)TE35Bc-24	35B4-6;35F1-7	376	6.4	0.8
Df(2L)TE35B-4	35C1-35D5	360	0.6	0.0
Df(2L)r10	35D1-2;36A6-7	401	14.5	8.5
Df(2L)H20(d12034)	36A8-9;36E1-2	141	2.8	0.0
Df(2L)TW137	36C2-4;37B9-C1	414	1.0	0.0
Df(2L)pr76	37D;38E	864	0.2	0.0
Df(2L)E55	37D2-E1;37F5-38A1	339	3.5	2.4
Df(2L)TW84	37F5-38A1;39D3-E1	492	3.3	0.4
Df(2L) C'	40h35; 40h38L	1113	0.7	0.0
Df(2R) nap1	41D2-E1; 42B1-3, 41A-B; 42BC	253	4.0	1.6
Df(2R)cn88b	42A;42E	1218	1.1	0.7
Df(2R)St1	42B3-5; 43E15-18	525	1.9	0.8
Df(2R)pk78s	42C1-7;43F5-8 (42B;42Cmax)	720	1.1	0.6
Df(2R)cn9	42E;44C	634	1.3	0.9
Df(2R)H3C1	43F; 44D3-8	748	1.9	0.8
Df(2R)44CE	44C4-5;44E2-4	344	8.1	3.5
Df(2R)H3E1	44D1-4; 44F12	470	0.9	0.0
Df(2R)Np5	44F10; 45D9-E1, 31B; 45D9-E1	969	0.6	0.2
Df(2R)w45-30n	45A6-7; 45E2-3	700	2.3	4.9
Df(2R) B5	46A;46C	610	0.7	0.0
Df(2R) X1	46C; 47A1	300	2.0	0.0
Df(2R)stan2	46F1-2;47D1-2	331	6.6	10.9
Df(2R) E3363	47A; 47F	590	0.3	0.7
Df(2R)en-A	47D3;48A5-6	1113	0.9	0.0
Df(2R)en30	48A3-4; 48C6-8	809	0.2	0.5
Df(2R)vg135	48D-E;49D-E	732	2.7	1.9
Df(2R) CB21	48E; 49A	799	1.3	0.5
Df(2R)trix	51A1-2;51B6	626	2.2	1.0
Df(2R)Jp1	51C3;52F5-9	329	1.2	0.0
Df(2R)Jp8	52F5-9;52F10-53A1	591	0.7	1.4
Df(2R)PcP7B	54E8-F1;55B9-C1	1260	1.4	1.4
Df(2R)Pc111B	54F6-55A1;55C1-3	242	0.8	0.8
Df(2R)PC4	55A;55F	622	4.8	5.8
Df(2R)AA21	Df 56F9-17;57D11-12, In 38E;56E	940	1.1	1.1
Df(2R)59AD	59A01-03;59D01-D04	520	1.5	0.0
Df(2R)or-BR6	59D05-10;60B03-08, 40;60E04[L]40F;59E[R]	275	1.5	0.0
Df(2R)Chi[g230]	60A3-7; 60B4	776	0.8	0.5

(*continued*)

TABLE 1

(Continued)

Deficiency name	Breakpoints	*N*	% *X* nondisjunction	% fourth chromosome nondisjunction
Df(2R)Px2	60C5-6;60D9-10	1024	0.8	0.6
Df(2R)M60E	60E6-9; 60E11	1199	0.5	0.0
Df(2R)Kr10	60E10; 60F5	991	2.4	1.0
Df(3L) emc-E12	61A; 61D3	601	0.7	0.0
Df(3L) Aprt-1	62A10-B1; 62D2-5	1513	1.2	2.1
Df(3L)R	62B7;62B12	1161	4.5	0.5
Df(3L)R-G7	62B8-9;62F2-5	1850	1.9	1.2
Df(3L)HR232	63C1;63D3	954	1.0	0.2
Df(3L)HR119	63C6;63E	1580	1.9	0.3
Df(3L)GN24	63F4-7;64C13-15	245	13.1	7.3
Df(3L)XDI98	65A2;65E	1436	0.8	0.3
Df(3L)pbl-X1	65F3;66B10	1044	1.3	0.4
Df(3L)66C-G28	66B8-9; 66C9-10	1797	23.3	25.0
Df(3L)h-i22	66D10-11;66E1-2	587	0.0	0.0
Df(3L)Scf-R6	66E1-6; 66F1-6	1320	1.2	0.5
Df(3L)Rd1-2	66F5	840	0.2	0.2
Df(3L) Ixd6	67E1-2; 68C1-2	893	0.4	0.0
Df(3L)vin5	68A2; 69A1	784	3.8	0.3
Df(3L)vin7	68C8-11;69B4-5	167	7.2	3.6
Df(3L)iro-2	69B1-5; 69D1-6	819	8.5	3.4
Df(3L)Ly	70A2-3;70A5-6	784	4.3	2.0
Df(3L)fzGF3b	70B?;70D6	1163	1.0	0.7
Df(3L)fz[D21]	70D;71F	1058	1.1	0.0
Df(3L)BK10	71C;71F	471	0.4	0.0
Df(3L)st-f13	72C1-D1; 73A3-4	515	1.9	2.7
Df(3L)st[e4]	72D5-10; 73A5	558	1.1	0.7
Df(3L)W4	75B8-11; 75C5-7	1226	0.7	0.3
Df(3L) Cat	75B8; 75F1	482	14.5	2.1
Df(3L)VW3	76A3;76B2	677	0.9	0.0
Df(3L)rdgC	77A1;77D1	1130	2.3	3.4
Df(3L)ri79C	77B-C;77F-78A	893	0.9	1.1
Df(3L) Pc-2q	78C5-6; 78E3-79A1	662	0.6	0.6
Df(3L) Delta 1 AK	79F; 80A	893	1.1	0.4
DF(3R) ME15	81F3-6; 82F5-7	1367	0.6	0.0
Df(3R) w[11118]; Df(3R) 6-7	82D3-8; 82F3-6	1433	4.5	1.4
Df(3R) 3-4	82F3-4; 82F10-11	1647	4.1	3.2
Df(3R)Tp110, Dp(3;3)Dfd[rvX1]	83C1-2;84B1-2, 83D4-5;84A4-5;98F1-2	1095	1.1	0.5
Df(3R)Scr	84A1-2;84B1-2	1164	0.2	0.5
Df(3R)pXT103	84F14;85C-D	832	8.9	1.0
Df(3R)by10	85D8-12; 85E7-F1	276	0.7	0.0
Df(3R)M-Kx1	86C1;87B1-5	263	2.3	0.0
Df(3R)ry615	87B11-13;87E8-11	926	0.9	0.6
Df(3R) su[Hw]	88A9-B2	543	0.4	0.0
Df(3R)red[p52]	88A12-B1; 88B4-5	1167	3.4	1.7
Df(3R)red1	88B1;88D3-4	621	1.6	0.6
In(3R) Vbx [P18]/Df(3)R red P93 l	88A10-B1; C2-3	571	1.1	0.7
DF(3R) ea	88E7-13; 89A1	1573	13.2	3.3
Df(3R)P14	90C2-D1;91A1-2	806	1.5	0.5
DF(3R) Cha7	90F1-4; 91F5	544	1.1	1.8
Df(3R) H-B79	92B3; 92F13	503	2.4	2.4
Df3R) 23 D1	93D; 94F	406	3.0	3.0
Df(3R)hhE23	94A; 95	866	0.2	1.6
Df(3R) crb-F89-4	95D7-D11; 95F15	213	1.9	0.0
DF(3R) crb87-5	95F7; 96A17-18	400	3.0	1.5
Df(3R)96B	96A21;96C2	226	2.7	0.0
Df(3R)T1-P	97A;98A1-2	108	3.7	5.6
DF(3R) D605	97E3; 98A5	577	0.0	0.7
DF(3R) 3450	98E3; 99A6-8	188	0.0	0.0
DF(3R) Dr-rv1	99A1-2; 99B6-11	1150	0.0	0.0
Df(3R) faf-BP	100D; 100F5	779	1.0	0.5

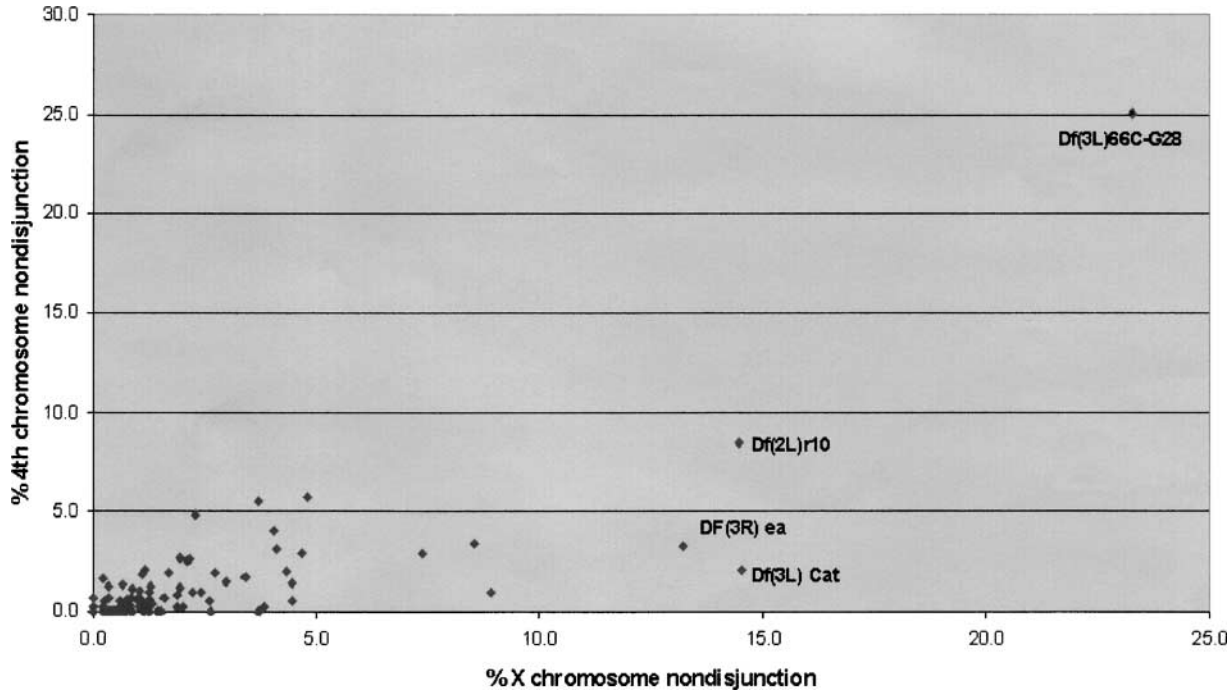

FIGURE 1.—*X* and fourth chromosome nondisjunction of regions screened for meiotic haplo-insufficiency. The effects of the deficiencies listed in Table 1 on the frequencies of *X* and fourth chromosome nondisjunction.

insufficient site, we tested a number of deficiencies that overlapped *Df(3L)66C-G28* for their effects on meiotic chromosome segregation (see Figure 2). As shown in Table 2a, *Df(3L)ZP1*, which uncovers the interval 66A17; 66C5, did not exhibit a dominant effect on meiotic nondisjunction, nor was any such effect observed for *Df(3L)pbl-X1* and *Df(3L)h-i22*. However, four overlapping deficiencies [*Df(3L)66C-I65*, *Df(3L)66C-T2-10*, *Df(3L)66C-B2-2*, and *Df(3L)66C-E3-1*] did show a dominant defect in achiasmate chromosome disjunction similar to that observed in *Df(3L)66C-G28* heterozygotes (Table 2a). These data restrict the putative haplo-insufficient meiotic gene to a small interval within 66C.

Because all five nondisjunction-inducing deficiencies were derived by mutagenesis of a *P*-element insertion in 66C (see MATERIALS AND METHODS), it is possible that observed defects were the result of some other dominant mutation carried by the original P-insertion chromosome and not due to haplo-insufficiency for one or more genes in region 66C. To eliminate this possibil-

TABLE 2

Evidence for a gene in region 66C that is haplo-insufficient for achiasmate chromosome segregation

Deficiency name	Cytological breakpoints	% nondisjunction		Adjusted total
		X chromosome	Fourth chromosome	
a. *y w/FM7, w; Df(3L)/+*				
Df(3L)66C-G28	66B8-9;66C9-10	23.3	25.0	1797
Df(3L)pbl-X1	65F3;66B10	1.3	0.0	1044
Df(3L)h-i22	66D10;66E1-2	0.0	0.0	587
Df(3L)ZP1	66A17;66C5	1.0	0.7	602
b. *y w/FM7, w; Df(3L)66C/+*				
Df(3L)66C-G28	66B8-9;66C9-10	23.3	25.0	1797
Df(3L)66C-I65	66C7-10	33.9	10.4	1250
Df(3L)66C-B2-2	66C7-10	31.3	6.7	1458
Df(3L)66C-E3-1	66C7-10	38.1	30.1	714
Df(3L)66C-T2-10	66C7-10	39.2	27.7	1307
Df(3L)66C-BSC13	66B12-C1;66D2-4	50.8	11.9	751

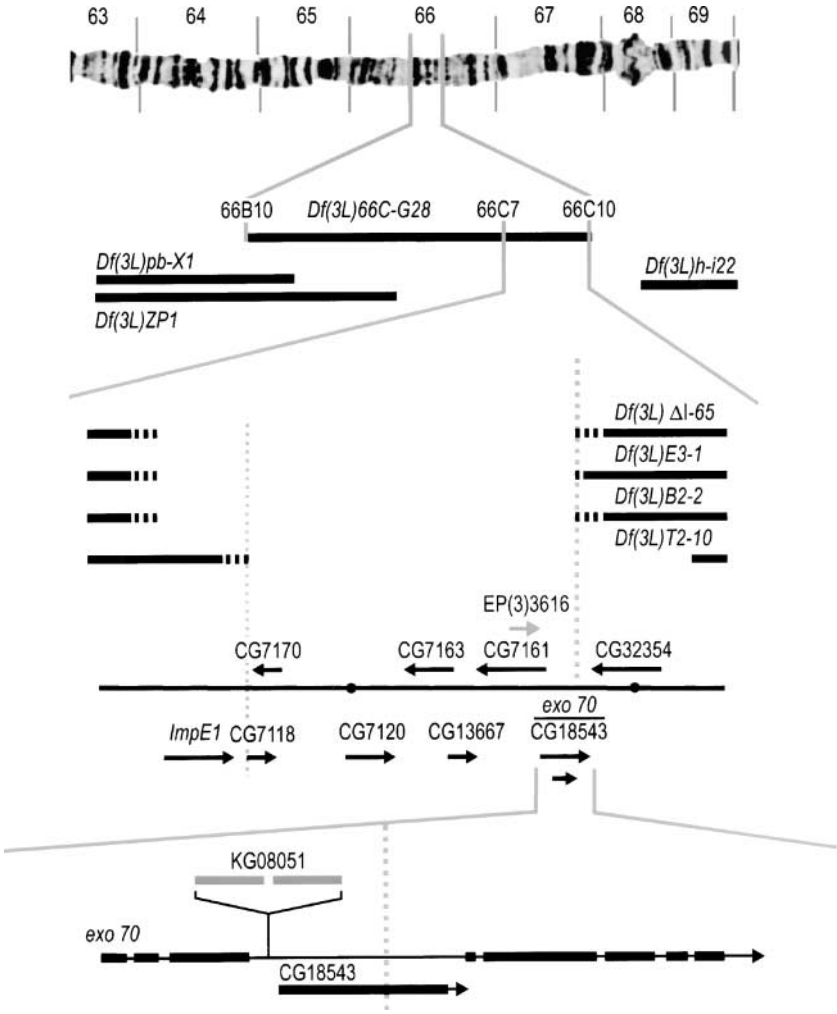

FIGURE 2.—A cytogenetic map of region 66C showing the breakpoints of the indicated deletions in reference to the polytene map (top); the known and predicted genes that lie within the limits defined by the deficiencies that are haplo-insufficient for achiasmate segregation (middle); and the site of insertion of the *P(SUPor-P)KG08051* element relative to *exo70* and *CG18543* (bottom).

ity, we tested an independently derived deficiency [*Df(3L)BSC13*] uncovering the region 66B12-C1;66D2-4 that was kindly provided by Kevin Cook. As shown in Table 2b, *FM7/X; Df(3L)BSC13/+* females displayed frequencies of *X* and fourth chromosome nondisjunction of 50.8 and 11.9%, respectively ($N = 751$). The fact that this independently derived deficiency shares no known background elements with the other tested deficiencies confirms that the effects of these deficiencies truly are a consequence of the deletion of some element in region 66C7-12.

As is obvious from Table 2b, the observed frequencies of *X* and fourth chromosome nondisjunction varied considerably among the six deficiencies tested (from 23.3 to 50.8% for the *X* chromosome and from 6.7 to 30.1% for the fourth chromosome). To some degree, this may well reflect the influence of genetic background. However, during the course of these experiments we also noted substantial quantitative variations in the observed levels of *X* chromosome and fourth chromosome nondisjunction exhibited by several of these deficiencies, but most notably for *Df(3L)66C-I65* and *Df(3L)B2-2*. For these deficiencies, the observed frequencies of *X* and fourth chromosome nondisjunction could vary as much as twofold from experiment to experiment. The highest and lowest values observed for several of these deficiencies over the course of numerous trials are displayed in Table 3 as experiment 1 or experiment 2. We can suggest only that our assays are extremely sensitive and that the experiment-to-experiment variation that we observe reflects the effects of subtle environmental variation and/or genetic background effects.

Characterizing the deficiency breakpoints: To determine the limits of the region that included the putative dosage-sensitive meiotic gene, we set out to position the ends of the deficiencies portrayed in Figure 1 on the physical map of the fly genome. This was accomplished by PCR analysis of homozygous deficiency-bearing embryos using pairs of primers selected to define specific intervals in the proximal region of 66C. As shown in Figure 3, the distal breakpoints of *Df(3L)66C-I65*, *Df(3L)66C-B2-2*, and *Df(3L)66CE3-1* lie distal to *ImpE1* (*CG7116* or *CG32356*). For *Df(3L)66C-T2-10*, the distal breakpoint lies immediately proximal to *ImpE1*. Thus the distal limit of the region containing the haplo-insuf-

TABLE 3

Variation in levels of *X* and fourth chromosome nondisjunction for four deficiencies in 66C

Deficiency name	Cytological breakpoints		% nondisjunction		Adjusted total
			X chromosome	Fourth chromosome	
Df(3L)66C-G28	66B8-9; 66C9-10	Experiment 1	23.3	25.0	1797
		Experiment 2	25.8	12.7	883
Df(3L)66C-I65	66C7-10	Experiment 1	33.9	10.4	1250
		Experiment 2	21.5	11.1	1461
		18°	24.9	23.7	587
		25°	17.4	21.2	665
		30°	13.7	22.8	702
Df(3L)66C-B2-2	66C7-10	Experiment 1	17.4	5.2	688
		Experiment 2	31.3	6.7	1458
Df(3L)66C-T2-10	66C7-10	18°	38.7	24.1	517
		25°	39.2	27.7	1307
		30°	31.6	24.4	664

ficient gene may be approximated to lie between 8,349,000 and 8,351,000 on the Release 3.1 sequence of the Drosophila chromosome 3L map (Celniker *et al.* 2002).

The proximal breakpoint of *Df(3L)66C-T2-10* lies immediately distal to or within *CG7015*, consistent with the failure of this deficiency to complement lethal alleles of the *Cbl* gene (*CG7037*). For *Df(3L)66C-B2-2*, the proximal breakpoint appears to lie within the *CG7127* (*exo70*) gene. Indeed, the most distal "positive" PCR results were obtained using primers corresponding to the *CG18543* gene that lies imbedded in the second intron of *exo70*. The breakpoints of *Df(3L)66C-I65* and *Df(3L)66C-E3-1* are more ambiguous and appear to lie between those of *Df(3L)66C-B2-2* and *Df(3L)66C-T2-10*. Nonetheless, the proximal limit of the region containing the haplo-insufficient gene may be approximated to lie between 8,371,000 and 8,373,000 on the Release 3.1 sequence of the Drosophila chromosome 3L map (Celniker *et al.* 2002).

Identifying the haplo-insufficient gene: As shown in Figure 3, the region defined by our deficiency analysis is composed of ~20–24 kb of genomic DNA. To identify the haplo-insufficient locus, we have tested a number of mutations that fall within the 66C10-11 interval for their ability to mimic the effects of the tested deficiencies with respect to achiasmate nondisjunction. The first mutations to be tested were *l(3)F6*, *l(3)J1*, *l(3)L3852*, *EP(3)3729*, and *EP(3)3616*. None of these mutants increased the level of *X* or fourth chromosome nondisjunction in *FM7/X* females above that observed in controls (data not shown).

However, as shown in Table 4, a P insertion designated *P(SUPor-P)KG08051* fully mimicked the effects of deficiencies for 66C in terms of its dominant effect on achiasmate segregation. [Table 4 provides detailed seg-

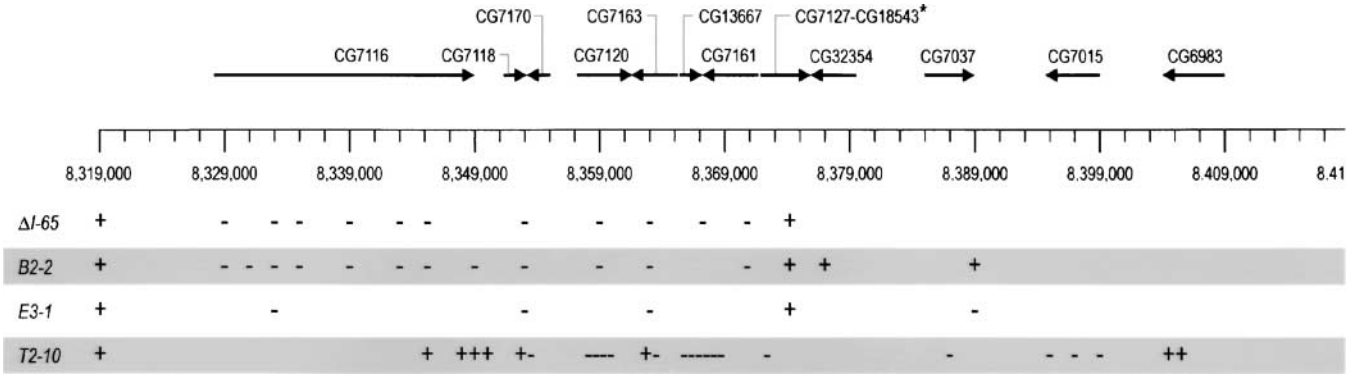

Figure 3.—PCR mapping of the endpoints of the deficiencies in 66C. DNA obtained from embryos homozygous for the indicated deletions was amplified using various primer combinations corresponding to the positions noted. Deficiency homozygotes were distinguished from siblings using a green fluorescent protein marker carried on the balancer chromosome. All primer pairs spanned an interval of no more than 1 kb. + indicates a clear positive signal and − indicates a failure to observe amplification. Numbering of the sequence is based on Release 3.1 of the Drosophila genome annotation (Celniker *et al.* 2002).

TABLE 4

Detailed segregational effects of heterozygosity for *KG08051* and its derivatives

Oocyte genotype	Paternal genotype	+[a]	*Df(3L)T2-10*	*KG08051*[b]	*Exc13*	*Exc21*	*Exc43*	*P{lacW} MP1*
Normal								
X; 4	*XY; 44*	8,115	293	137	101	274	360	456
X; 4	*O; 44*	6,063	290	140	89	182	254	370
X nondisjunction								
0; 4	*XY; 44*	10	109	41	47	0	3	1
XX; 4	*O; 44*	10	72	36	48	0	1	2
Fourth chromosome nondisjunction								
X; O	*XY; 44*	12	41	25	14	0	1	0
X; O	*O; 44*	2	12	7	6	0	1	0
X; 44	*XY; O*	2	86	11	52	0	1	0
X; 44	*O; O*	10	73	7	11	0	2	0
X:fourth chromosome nondisjunction								
O; O	*XY; 44*	1	6	3	4	0	2	0
XX; 44	*O; O*	0	5	2	5	0	0	0
O; 44	*XY; O*	0	30	46	18	0	0	0
XX; O	*O; 44*	0	34	34	9	0	0	0
Total progeny		14,225	1,051	488	404	456	625	829
Adjusted total		14,246	1,307	650	535	456	631	832
% nullo-*X*		0.1	22.2	27.7	25.8	0.0	1.6	0.2
% diplo-*X*		0.1	17.0	22.1	23.2	0.0	0.3	0.5
Total % *X* nondisjunction		0.2	39.2	49.8	49.0	0.0	1.9	0.7
% nullo-*4*		0.1	10.2	16.3	8.6	0.0	1.0	0.0
% diplo-*4*		0.1	17.5	17.5	20.3	0.0	0.4	0.0
Total % fourth chromosome nondisjunction		0.2	27.7	33.8	29.0	0.0	1.4	0.0
% nonhomologous segregations			8.1	23.0	6.7			

FM7/X; spa^{pol} females for each indicated *P*-element insertion were crossed to appropriate tester males (see MATERIALS AND METHODS) to allow the assessment of both *X* and fourth chromosome nondisjunction. Data for *Df(3L)T2-10* are provided for comparison.

[a] Control data are from ZITRON and HAWLEY (1989).

[b] See MATERIALS AND METHODS.

regation data for *Df(3L)T2-10* for comparison to *P(SUPor-P)KG08051*]. As shown in Figure 2, the *P(SUPor-P)KG08051* insertion falls into the third intron of the *exo70* gene, which encodes a component of a secretory structure known as the exocyst. However, a second predicted gene (*CG18543*) also falls within this intron and the insertion site for *P(SUPor-P)KG08051* is 90 bp upstream of the predicted start codon for *CG18543*. To demonstrate that the *P(SUPor-P)KG08051* insertion is indeed the basis of the segregational defect, we created both precise and imprecise excisions of this P insertion. *P(SUPor-P)KG08051-exc21* is a precise excision of the *P* element, as verified by DNA sequencing. As shown in Table 4, *P(SUPor-P)KG08051-exc21* no longer causes a defect in achiasmate segregation, confirming that the high levels of nondisjunction observed in females heterozygous for *P(SUPor-P)KG08051* are indeed a consequence of the P insertion.

The *P(SUPor-P)KG08051* insertion also exhibits recessive female sterility (associated with defects in oogenesis) and this sterility is also alleviated in the precise excision (*exc21*) described above. However, the *P(SUPor-P)KG08051-exc43* excision derivative, which retains the sterility defect but has lost the dominant meiotic defect, allows us to separate these two defects. Although *P(SUPor-P)KG08051-exc43* is associated with the loss of both y^+ and w^+ markers, it retains at least the two ends of the *P* element, and attempts to amplify the remaining *P*-element sequence by PCR using primers that span the insertion site were unsuccessful. Thus, this derivative may well be the result of an internal deletion that causes loss of the y^+ and w^+ markers while maintaining a sub-

stantial amount of the original sequence. Regardless of the exact nature of the lesion born by this derivative, the fact that it reverts only one of the two phenotypes exhibited by the original insertion is consistent with the view that the two types of defects may result from the disruption of two different genes (*exo70* and *CG18543*) by the *P(SUPor-P)KG08051* insertion.

Expression of *CG18543* in the germline from a transgenic insertion construct rescues the meiotic defects exerted by heterozygosity for *P(SUPor-P)KG08051*: The meiotic defects of *P(SUPor-P)KG08051* are largely ameliorated in females that also carry a transgenic construct bearing a CG18543 cDNA expressed under the control of a UAS promoter and driven by *nanos-GAL4* (Van Doren *et al.* 1998). The coding region of the *mtrm* cDNA was amplified by PCR using a 5′ primer tagged with a *Kpn*I restriction site and a 3′ primer tagged with an *Xba*I restriction site. The product was then digested and cloned into the pUASP vector using the above restriction enzymes. *FM7/X*; *P(SUPor-P)KG08051/+* females bearing both the *UAS-CG18543* construct and the *nanos-GAL4* driver display only 2.7% *X* nondisjunction and 7.1% fourth chromosome nondisjunction (adjusted total is 1266), demonstrating both that CG18543 is in fact the dosage-sensitive locus revealed in the original deficiency screen and that *CG18543* exerts its effects in the female germline. The *CG18543* transcription unit encodes a predicted protein of 217 amino acids. Although this gene is conserved within the Drosophila species group, at least as far as *D. pseudoobscura*, no significant homologs are observed in mosquitoes, and there are no obvious homologs in any other nonfly species (W. Gilliland, personal communication). We have named this gene *matrimony* (*mtrm*).

The identification of the *matrimony* gene as the meiotic haplo-insufficient locus is consistent with several other observations. First, mutants in other exocyst components (*sec5* and *exo84*) failed to exhibit dominant effects on meiotic chromosome segregation (data not shown) so it would have been surprising had such an effect been observed for *exo70*. Second, a *P*-element insertion (*P{lacW}exo70^{mp1}*) that lies in the 5′ untranslated region of *exo70*, 52 bp upstream of the predicted start codon, fails to exhibit a dominant effect on meiotic chromosome segregation (see Table 4). (The isolation and characterization of this mutant is described in materials and methods.) Third, the excision derivative, *P(SUPor-P)KG08051-exc13*, is associated with a large deletion of material upstream of the P insertion, including the first three exons of *exo70*. However, despite what might be considered to be a far more damaging mutational lesion in *exo70*, the effects of this derivative on meiotic chromosome segregation are similar, if not identical, to those observed for *P(SUPor-P)KG08051* (see Tables 4 and 5). The *P(SUPor-P)KG08051-exc13* derivative does display recessive semilethality. Less than 50% of the expected number of *P(SUPor-P)KG08051-exc13* adults hatch out of their pupal cases, and all of these die within 1–2 days. These phenotypes are similar to those observed for a loss-of-function mutant in another component of the exocyst (*sec5*), which also displays defects in oogenesis and recessive lethality (Murthy *et al.* 2003; M. Murthy and T. L. Schwarz, personal communication).

The observed segregational defects are the result of an impairment of the achiasmate segregation system: One could imagine that the reduction in the dosage of *mtrm* might occur by one of three general mechanisms. First, hemizygosity for *mtrm* might reduce the frequency of crossing over and thus increase the frequency of *X* and fourth chromosome nondisjunction in *FM7/X* females by overloading the distributive system. However, the recombination frequency along the length of the third chromosome did not show significant deviation from normal in females carrying a multiply marked third chromosome and either *Df(3L)66C-G28* or *Df(3L)B2-2*. [The map distances for the *ru–st* interval on 3L were 46.7, 45.0, and 52.2 cM, respectively, for females of the genotypes *+/ru h th st* (N = 169), *Df(3L)66C-G28/ru h th st* (N = 258), and *Df(3L)B2-2 /ru h th st* (N = 143)]. Second, although one could imagine a generalized defect in spindle function, none of these deficiencies increased the frequency of meiosis II nondisjunctional events or induced meiotic chromosome nondisjunction in the male, suggesting that the defect in segregation is restricted to meiosis I in females. Finally, it is possible that the observed defect represents a specific impairment of achiasmate segregation.

If the effects of hemizygosity for *mtrm* indeed reflect an impairment of achiasmate segregation, one should observe a decrease in the effect on *X* nondisjunction in females bearing structurally normal (and thus usually chiasmate) *X* chromosomes. As shown in Table 5, *Df(3L)66C-T2-10*, *P(SUPor-P)KG08051*, and *P(SUPor-P)KG08051-exc13* were also tested in females bearing two normal sequence *X* chromosomes. In all three cases, the levels of *X* chromosome nondisjunction observed in *X/X* females were ~10-fold lower than the frequencies of *X* nondisjunction observed in *FM7/X* females. Moreover, the observed frequencies of *X* chromosome nondisjunction in females carrying two normal sequence *X* chromosomes (~4%) are ~40% of the predicted frequency of nonexchange *X* bivalents (~8–10%; Hawley *et al.* 1992), consistent with the view that even these low frequencies of nondisjunction arise as a consequence of the failed segregation of achiasmate *X* chromosome bivalents. These observations suggest that hemizygosity for *mtrm* primarily, if not exclusively, affects the segregation of nonexchange chromosomes.

A defect in achiasmate segregation that directly impairs partner maintenance would also be expected to exert a strong effect on fourth chromosome segregation that is independent of the effect on achiasmate *X* chromosomes. As shown in Table 5, the effects of hemizy-

TABLE 5

Comparison of the segregational effects of heterozygosity for *Df(3L)66C-T2-10*, *KG08051*, and *KG08051-exc13* in *FM7/X; spa^{pol}* and *X/X; spa^{pol}* females

Oocyte genotype	Paternal genotype	*FM7/X; Df(3L)T2-10*	*X/X; Df(3L)T2-10*	*FM7/X; KG08051*	*X/X; KG08051*	*FM7/X; Exc13*	*X/X; Exc13*
Normal							
X; 4	*XY; 44*	293	148	137	375	101	374
X; 4	*O; 44*	290	192	140	475	89	413
X nondisjunction							
0; 4	*XY; 44*	109	2	41	8	47	6
XX; 4	*O; 44*	72	2	36	4	48	7
Fourth chromosome nondisjunction							
X; O	*XY; 44*	41	33	25	43	14	72
X; O	*O; 44*	12	29	7	58	6	51
X; 44	*XY; O*	86	68	11	77	52	104
X; 44	*O; O*	73	107	7	101	11	128
X:4 nondisjunction							
O; O	*XY; 44*	6	1	3	4	4	0
XX; 44	*O; O*	5	3	2	4	5	3
O; 44	*XY; O*	30	2	46	4	18	2
XX; O	*O; 44*	34	2	34	2	9	0
Total progeny		1051	589	488	1155	404	1160
Adjusted total		1307	601	650	1181	535	1178
% nullo-*X*		22.2	1.7	27.7	2.7	25.8	1.4
% diplo-*X*		17.0	2.3	22.1	1.7	23.2	1.7
Total % *X* nondisjunction		39.2	4.0	49.8	4.4	49.0	3.1
% nullo-*4*		10.2	11.3	16.3	9.5	8.6	10.4
% diplo-*4*		17.5	30.8	17.5	16.4	20.3	20.5
Total % fourth chromosome nondisjunction		27.7	42.1	33.8	26.0	29.0	31.0
% nonhomologous segregations		8.1		23.0		6.7	

gosity for *mtrm* on chromosome *4* nondisjunction are similar in both *X/X* females and *FM7/X* females, demonstrating a direct effect of *mtrm* hemizygosity on fourth chromosome nondisjunction. Such a direct effect on fourth chromosome segregation has been previously demonstrated in only two other instances, namely mutants in the *nod* gene and overexpression of an *Axs^{D}* construct (driven by *nanos-GAL4*). In the case of mutants at the *nod* gene, nonexchange chromosomes precociously dissociate from the developing spindle during prometaphase (Carpenter 1973; Zhang and Hawley 1990; Theurkauf and Hawley 1992; Matthies *et al.* 1999), while in the case of the overexpression of *Axs^{D}*, chromosome organization in the karyosome is obviously disrupted and achiasmate chromosomes are often observed to be mispositioned relative to the center of the main mass (Kramer and Hawley 2003).

mtrm hemizygotes are similar to other meiotic mutants affecting achiasmate segregation in that simultaneous *X,4* nondisjunctional events usually occur more often than might be expected by chance and most cases of simultaneous nondisjunction result from *XX↔44* segregations (Sekelsky *et al.* 1999). As noted in the discussion, the excess of simultaneous exceptions and the preponderance of such exceptions that involve *XX↔44* segregations both likely reflect the function of a homology-independent third system of meiotic chromosome segregation. This process, referred to as the nonhomologous achiasmate segregation (Hawley and Theurkauf 1993), does not depend on the physical association of segregational partners (Dernburg *et al.* 1996) and may rather simply reflect the "crowding" of spindle poles by one pair of missegregating homologs forcing the remaining pair to choose the alternative pole (Sandler and Novitski 1956; Hawley and Theurkauf 1993).

Finally, we also observe an excess of diplo-*4* exceptions relative to nullo-*4* exceptions among the progeny of *mtrm* hemizygotes. This excess of diplo-*4* exceptions observed in the progeny of *mtrm* hemizygotes does not result from the inclusion of triplo-*4* mothers in the re-

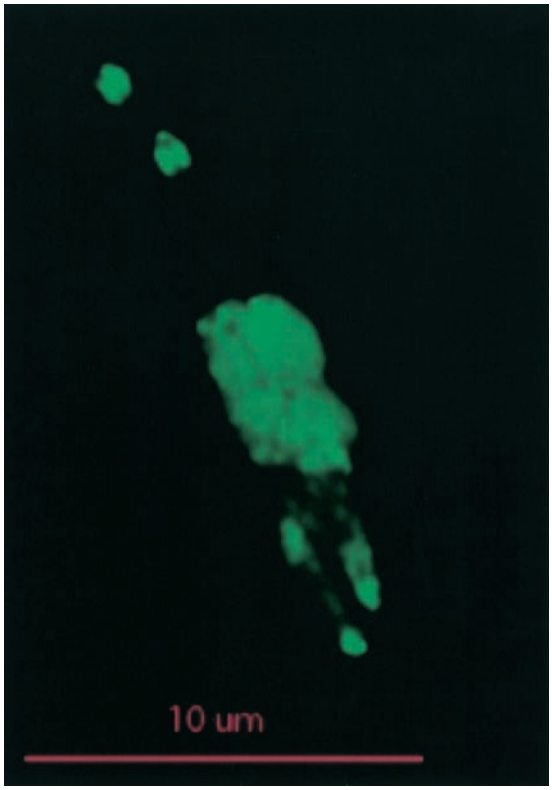

Figure 4.—Metaphase I image of an *FM7/X; Df(3L)T2-10* oocyte. The two chromosomes segregating to the upper pole are fourth chromosomes and the two segregating toward the lower pole are *X* chromosomes. The *X* chromosome on the left (with two obviously separated blocks of heterochromatin) is *FM7*. The *X* on the right is its normal sequence homolog.

ported crosses (see MATERIALS AND METHODS) and its basis is not understood. However, a similar excess of diplo-*4* exceptions was also observed in females heterozygous for Axs^{D} and is not understood in either case (ZITRON and HAWLEY 1989).

Cytological studies confirm a defect in achiasmate segregation: Cytological studies confirm a specific defect in the position of achiasmate chromosomes on the meiotic spindles of oocytes produced by *FM7/X; Df(3L)66C-T2-10* females (*i.e.*, females that are hemizygous for *mtrm*). The vast majority of prometaphase and metaphase figures observed in these oocytes are normal bipolar spindles, suggesting that these oocytes are competent to assemble proper bipolar spindles. However, as exemplified in Figure 4, achiasmate *X* and fourth chromosomes are often improperly placed on metaphase I spindles of these oocytes from females.

Among a sample of 19 metaphase figures in which the achiasmate *X* and fourth chromosomes were clearly discernible, the achiasmate *X* chromosomes are found on the same half-spindle 21% (4/19) of the time and the fourth chromosomes are found on the same half-spindle in 31% of the oocytes (6/19). In two of the cases of *X* chromosome nondisjunction, the two *X* chromosomes were on one-half of the spindle and the two fourth chromosomes were on the other, indicative of *XX* ↔ *44* segregation events. We also observed two cases involving the fourth chromosome and one involving the *X* chromosome in which, although the achiasmate homologs were found on opposite halves of the spindle, they were not observed to be on the same arc of the spindle. Thus it seems unlikely that these spindles reflect the segregation of the two homologs from their partners; rather they likely reflect the chance orientation of the two homologs to opposite poles. We did not observe *X* or fourth chromosomes dissociated from the spindle, as is observed in *nod* mutant oocytes (THEURKAUF and HAWLEY 1992), or defects in spindle assembly such as those observed in Axs^{D}-bearing oocytes or in oocytes heterozygous for mutants in the α-*tub67C* gene (MATTHIES *et al.* 1999; KRAMER and HAWLEY 2003). Indeed, the only obvious defect is the lack of proper positioning of achiasmate chromosomes on the meiotic spindle in a large fraction of the oocytes examined.

DISCUSSION

How might the Mtrm protein function? Both the haplo-insufficiency of the *mtrm* gene and its rather impressive phenotypic variability suggest to us that it encodes a structural protein whose concentration is critical to meiotic chromosome segregation. We propose that hemizygosity for the *mtrm* gene creates a "threshold defect," in which the level of Mtrm protein is just below some critical threshold for function, such that minor perturbations in *mtrm* expression can have dramatic effects on the final phenotype. To further characterize the phenotypic effects of altering the dosage of *mtrm*, it would be of interest to know the meiotic phenotype of *P(SUPor-P)KG08051* homozygotes. Unfortunately, homozygotes for *P(SUPor-P)KG08051* are female sterile and display multiple defects in oogenesis that preclude the cytological study of stage 13–14 oocytes.

In the absence of immunolocalization data, it is difficult to speculate on the function of the product of the *mtrm* gene. At present, we know only that *mtrm* is highly expressed in oocytes and early embryos (ARBEITMAN *et al.* 2002) and that overexpression of the Mtrm protein in *Schizosaccharomyces pombe* results in cell cycle arrest (EDGAR 1993). Our data suggest that in *mtrm* hemizygotes the *X* and fourth chromosomes are no longer competent to segregate from their partners and that the two homologs are often found on separate arcs of the spindle. One possible interpretation of these data is that the presence of only a single copy of *mtrm* disrupts functioning of whatever glue holds heterochromatically paired homologs together from the end of pachytene until metaphase I. (Our reason for suggesting that Mtrm is not required for euchromatic associations derives from the observation that meiotic recombination is normal in *mtrm* hemizygotes.) This hypothesis is testable

both by immunolocalization and by fluorescence *in situ* hybridization analysis of heterochromatic pairing in oocytes hemizygous for *mtrm*, and such studies are underway in the laboratory.

Are there other loci comparable to *mtrm* in the Drosophila genome? One could imagine two general means in which haplo-insufficient meiotic mutants might be identified in Drosophila. The first would be through the recovery of dominant loss-of-function meiotic mutants. Unfortunately, the vast majority of the known cases of dominant meiotic mutations (*e.g.*, sub^{Dub}, nod^{DTW}, and *Axs*) clearly reflect the effects of antimorphic mutations (Zitron and Hawley 1989; Komma *et al.* 1991; Rasooly *et al.* 1991; Matthies *et al.* 1999; Guinta *et al.* 2002; Kramer and Hawley 2003). However, Wasser and Chia (2003) have recently reported that loss-of-function mutations in the *X* chromosome *east* gene exert a dominant effect on achiasmate *X* chromosome segregation, but do not affect the segregation of the fourth chromosomes. As homozygotes these mutants also exhibit defects in chromosome condensation and organization during the cell cycle in both male meiosis and mitosis. Thus the *east* gene may well be an additional example of a gene that is haplo-insufficient for achiasmate segregation (at least for the *X* chromosome).

The second method of identifying haplo-insufficient meiotic loci is the type of deficiency screen reported here. Deficiency-based screens for haplo-insufficient genes have a long history in Drosophila genetics and are epitomized by the heroic screen performed by Lindsley *et al.* (1972) using segmental aneuploids. However, with the exception of the *Minute* loci, the number of haplo-insufficient loci that produce a discernible phenotype on their own is quite low. Most highly successful deficiency screens for haplo-insufficient genes are based on screening for enhancers or suppressors of mutants with "threshold" phenotypes (Halsell and Kiehart 1998; Lee *et al.* 2001). It is thus not surprising that we were aware of but two examples of loci that were haplo-insufficient for proper meiotic chromosome segregation at the time we initiated our screen: namely $Df(1)w^{rJ1}$, a deficiency spanning the *zeste-white* region (Robbins 1977, 1980, 1981), and $Df(3)sdb^{105}$ (Hinton 1966). Females heterozygous for $Df(1)w^{rJ1}$ show elevated levels of achiasmate nondisjunction and reduced recombination, while females heterozygous for $Df(3)sdb^{105}$ also display reduced levels of exchange. In neither case has the effect been narrowed to a single gene. These observations, as well as our own findings, strongly suggest that loci similar to *mtrm* are few in number.

Our working model for homologous achiasmate segregation in Drosophila: In most instances, meiosis is composed of three basic cellular processes: pairing, recombination, and segregation. Pairing identifies homologs, recombination acts to lock them together, and segregation moves them to opposite poles at the first meiotic division. In a more mechanistic sense, meiotic segregation can be viewed as the events that mediate the stable co-orientation of the homologous centromeres toward opposite poles of the meiotic spindle. Establishing stable co-orientation requires a balancing of plateward and poleward forces acting on the kinetochores. This balancing of forces is usually achieved by chiasmata, the physical consequence of recombination. Proper orientation results from the ability of chiasmata to constrain homologous kinetochores such that they are oriented in opposite directions (Nicklas 1974).

However, many organisms also possess systems that ensure the segregation of chromosomes that do not undergo exchange (Wolf 1994). In Drosophila two systems ensure the segregation of achiasmate chromosomes, homologous achiasmate segregation and nonhomologous achiasmate segregation. Although these systems are generally considered together and denoted by the terms "distributive pairing" or "distributive segregation" (Grell 1976), they differ in two fundamental ways. First, nonhomologous achiasmate segregation does not require or involve the physical association of segregational partners, while homologous achiasmate segregation is dependent on heterochromatic pairings (Hawley *et al.* 1992; Dernburg *et al.* 1996; Karpen *et al.* 1996). Second, homologous achiasmate segregations are disrupted mutants in genes such as *Axs, ald*, *mei-S51*, and α*tub67C*, while nonhomologous achiasmate segregation occurs normally in the presence of such mutants. The observations of very high levels of $XX \leftrightarrow 44$ segregations in *mtrm* homozygotes suggests that this defect is also restricted to homologous achiasmate segregations. Indeed, the only mutants known to affect both homologous and nonhomologous achiasmate segregations are *nod*, *ncd*, and *subito*. *nod* mutants cause nonexchange chromosomes to dissociate from the developing spindle during prometaphase, while *ncd* and *sub* mutants cause a failure to maintain a bipolar meiotic spindle (Wald 1936; Kimble and Church 1983; Theurkauf and Hawley 1992; Matthies *et al.* 1996; Guinta *et al.* 2002).

Our focus here is on the mechanisms that underlie homologous achiasmate segregations. We imagine that the pairing events underlying homologous achiasmate segregations are initiated by the same processes that allow the initial meiotic pairing of homologs in early meiosis. Thus in Drosophila females, synaptonemal complex (SC) formation does not require exchange, and nonexchange homologs pair and synapse normally (Carpenter 1973, 2003; McKim *et al.* 1998). However, at the end of pachytene, and concomitant with SC dissolution, the euchromatic arms of the chromosomes desynapse and nonexchange homologs nonetheless remain associated by heterochromatic pairings (Dernburg *et al.* 1996). Our current model is that the maintenance of these pairings requires the Mtrm protein, which serves to link homologous heterochromatic regions together.

One could imagine, for example, that the Mtrm protein might act by modifying some component of the SC that originally connected the heterochromatic regions of chromosomes during prophase. Although the primary function of the SC lies in mediating interhomolog exchanges (Page and Hawley 2003), in many meiotic systems the segregation of achiasmate chromosomes is mediated by the preservation of modified SC structures that persist after SC breakdown (Rasmussen 1977). Most recently, Page *et al.* (2003) have shown that proteins normally found in the lateral element of the SC create a "dense plate" that holds the achiasmate sex chromosome bivalent together in marsupial males. It thus does not seem unreasonable to suggest that Mtrm-dependent modifications of the heterochromatic SC might play an important role in achiasmate homologous segregations as well.

We also imagine that the maintenance of heterochromatic associations requires functions that serve to maintain the overall structure and compact form of the karyosome. We noted above that mutants in the nuclear protein East disrupt achiasmate segregation in meiosis and exhibit defects in chromosome condensation and organization (Wasser and Chia 2003). Perhaps East and other proteins assist in maintaining the integrity of the karyosome prior to nuclear envelope breakdown. The maintenance of karyosome integrity after nuclear envelope breakdown might involve the function of a membranous sheath that was recently shown to ensheath the developing meiotic spindle during prometaphase and metaphase (Kramer and Hawley 2003). The product of the *Axs* gene colocalizes with this sheath and dominant mutants in the *Axs* gene disrupt the segregation of achiasmate chromosomes during female meiosis.

Although chiasmate chromosomes achieve stability at the metaphase plate by balancing the tension of forces between two oppositely oriented kinetochores and the two poles of the spindle (Nicklas 1974), the segregation of achiasmate chromosomes poses a different problem. However, in principle, the manner in which achiasmate kinetochores become stably oriented should be functionally equivalent to that used by chiasmate chromosomes, *i.e.*, by balancing tension on the kinetochores, and thus should require the balancing of poleward force at the kinetochore by plateward force on the chromosome. Holding the achiasmate chromosomes on the developing spindle and preventing their precocious migration to the poles requires the Nod protein, which is localized along the arms of the meiotic chromosomes (Afshar *et al.* 1995a,b; Murphy and Karpen 1995).

The Nod protein is specifically required for the segregation of achiasmate chromosomes and functions to counterbalance the poleward forces acting at their kinetochores. When these poleward forces are unchecked, as a consequence of the absence of Nod or of chiasmata, chromosomes precociously move off the ends of the developing spindle. Mutations at the *nod* locus specifically impair achiasmate segregation, as assayed both genetically and cytologically (Carpenter 1973; Zhang and Hawley 1990; Theurkauf and Hawley 1992). Although Nod is a highly divergent member of the kinesin superfamily (Zhang *et al.* 1990; Rasooly *et al.* 1991, 1994), recent biochemical studies demonstrate that although Nod binds to microtubules and exhibits a microtubule-dependent ATPase activity, it is not capable of functioning as a motor (Matthies *et al.* 2001). The ability of *nod* mutants to suppress an effect in kinetochore poleward movement created by mutants in the α*tub67C* gene supports the conclusion that Nod serves to balance the poleward forces acting at the kinetochore (Matthies *et al.* 1999). We imagine that Nod serves this function by binding both microtubules and chromosomes as well by anchoring itself to a stable component of the spindle apparatus.

Concluding remarks: Thus, we imagine that the basic process of achiasmate segregation can be described as chromosome pairing → maintenance of heterochromatic pairing → Nod-based segregation. We propose that the Mtrm protein plays a critical role in the second step of this process, that of holding homologs together long enough to ensure their co-orientation. We imagine that Mtrm does so by binding paired heterochromatic regions together long after the euchromatic intervals have desynapsed. If we are correct, we should be able to demonstrate that Mtrm protein binds to the heterochromatic regions of the chromosome early in meiotic prophase and stays bound until metaphase I. We are now conducting the experiments necessary to test this hypothesis.

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LITERATURE CITED

Afshar, K., J. Scholey and R. S. Hawley, 1995a Identification of the chromosome localization domain of the *Drosophila* Nod kinesin-like protein. J. Cell Biol. **131** (4): 833–843.

Afshar, K., N. Barton, R. S. Hawley and L. S. B. Goldstein, 1995b DNA binding and meiotic chromosomal localization of the *Drosophila* Nod kinesin-like protein. Cell **81** (1): 129–138.

Arbeitman, M. N., E. E. Furlong, F. Imam, E. Johnson, B. H. Null

et al., 2002 Gene expression during the life cycle of *Drosophila melanogaster*. Science **297** (5590): 2270–2275.

Carpenter, A. T. C., 1973 A meiotic mutant defective in distributive disjunction in *Drosophila melanogaster*. Genetics **73:** 393–428.

Carpenter, A. T. C., 1979 Synaptonemal complex and recombination nodules in wild-type *Drosophila melanogaster* females. Genetics **92:** 511–541.

Carpenter, A. T. C., 2003 Normal synaptonemal complex and abnormal recombination nodules in two alleles of the *Drosophila melanogaster* mutant *mei-W68*. Genetics **163:** 1337–1356.

Celniker, S. E., D. A. Wheeler, B. Kronmiller, J. W. Carlson, A. Halpern *et al.* 2002 Finishing a whole-genome shotgun: release 3 of the Drosophila melanogaster euchromatic genome sequence. Genome Biol. **3** (12): 0079.1–0079.14.

Dernburg, A. F., J. W. Sedat and R. S. Hawley, 1996 Direct evidence of a role for heterochromatin in meiotic chromosome segregation. Cell **86:** 135–146.

Edgar, B., 1993 *Drosophila melanogaster* Sevelin clone D52 cell cycle arrest protein gene, complete cds. GenBank/EMBL/DDBJ: U03288, FlyBase F Brf011401.

Grell, R. F., 1976 Distributive pairing segregation, pp. 436–486 in *The Genetics and Biology of Drosophila*, Vol. 1a, edited by M. Ashburner and E. Novitski. Academic Press, London.

Guinta, 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.

Halsell, S. R., and D. P. Kiehart, 1998 Second-site noncomplementation identifies genomic regions required for Drosophila nonmuscle myosin function during morphogenesis. Genetics **148:** 1845–1863.

Hawley, R. S., 1988 Exchange and chromosomal segregation in eukaryotes, pp. 497–527 in *Genetic Recombination*, edited by G. Smith and R. Kucherlapati. ASM Press, Washington, DC.

Hawley, R. S., and W. E. Theurkauf, 1993 Requiem for the distributive system: achiasmate segregation in *Drosophila* females. Trends Genet. **9:** 310–317.

Hawley, R. S., H. Irick, A. E. Zitron, D. A. Haddox, A. Lohe *et al.*, 1992 There are two mechanisms of achiasmate segregation in *Drosophila* females, one of which requires heterochromatic homology. Dev. Genet. **13:** 440–467.

Hinton, C. W., 1966 Enhancement of recombination associated with the c3G mutant of *Drosophila melanogaster*. Genetics **53:** 157–164.

Karpen, G. H., M. H. Le and H. Le, 1996 Centric heterochromatin and the efficiency of achiasmate disjunction in *Drosophila* female meiosis. Science **273:** 118–122.

Kimble, M., and K. Church, 1983 Meiosis and early cleavage in Drosophila melanogaster eggs: effects of the claret non-disjunctional mutation. J. Cell Sci. **62:** 301–318.

Komma, D. J., A. S. Horne and S. A. Endow, 1991 Separation of meiotic and mitotic effects of claret non-disjunctional on chromosome segregation in *Drosophila*. EMBO J. **10** (2): 419–424.

Kramer, J. J., and R. S. Hawley, 2003 Mutants in the spindle-associated transmembrane protein Axs impair meiotic spindle assembly in *Drosophila* oocytes. Nat. Cell Biol. **5:** 261–263.

Lee, L. A., L. K. Elfring, G. Bosco and T. L. Orr-Weaver, 2001 A genetic screen for suppressors and enhancers of the *Drosophila* PAN GU cell cycle kinase identifies cyclin B as a target. Genetics **158:** 1545–1556.

Lindsley, D. L., L. Sandler, B. S. Baker, A. T. Carpenter, R. E. Denell *et al.*, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71:** 157–184.

Manheim, E. A., and K. S. McKim, 2003 The synaptonemal complex component C(2)M regulates meiotic crossing over in *Drosophila*. Curr. Biol. **13** (4): 276–285.

Matthies, H. J., H. B. McDonald, L. S. Goldstein and W. E. Theurkauf, 1996 Anastral meiotic spindle morphogenesis: role of the non-claret disjunctional kinesin-like protein. J. Cell Biol. **134:** 455–464.

Matthies, H. J., L. G. Messina, R. Namba, K. J. Greer, M. Y. Walker *et al.*, 1999 Mutations in the *alpha-tubulin 67C* gene specifically impair achiasmate segregation in *Drosophila melanogaster*. J. Cell Biol. **147** (6): 1137–1144.

Matthies, H. J. G., M. Clarkson, R. B. Saint, R. Namba and R. S. Hawley, 2000 Analysis of meiosis in fixed and live oocytes by light microscopy, pp. 67–85 in *Drosophila: Protocols*, edited by W. S. Sullivan, M. Ashburner and R. S. Hawley. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Matthies, H. J., R. J. Baskin and R. S. Hawley, 2001 Orphan kinesin NOD lacks motile properties but does possess a microtubule-stimulated ATPase activity. Mol. Biol. Cell **12** (12): 4000–4012.

McKim, K. S., B. Green-Marroquin, J. J. Sekelsky, G. Chin, C. Steinberg *et al.*, 1998 Meiotic synapsis in the absence of recombination. Science **279:** 876–878.

Murphy, T. D., and G. H. Karpen, 1995 Localization of centromere function in a *Drosophila* minichromosome. Cell **82:** 599–609.

Murthy, M., D. Garza, R. H. Scheller and T. L. Schwarz, 2003 Mutations in the exocyst component Sec5 disrupt neuronal membrane traffic, but neurotransmitter release persists. Neuron **37** (3): 433–447.

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.

Page, S. L., and R. S. Hawley, 2003 Chromosome choreography: the meiotic ballet. Science **301** (5634): 785–789.

Page, J., S. Berrios, J. S. Rufas, M. T. Parra, J. A. Suja *et al.*, 2003 The pairing of X and Y chromosomes during meiotic prophase in the marsupial species *Thylamys elegans* is maintained by a dense plate developed from their axial elements. J. Cell Sci. **116:** 551–560.

Rasmussen, S. W., 1977 The transformation of the synaptonemal complex into the elimination chromatin of *Bombyx mori* oocytes. Chromosoma **60:** 205–221.

Rasooly, R. S., C. M. New, P. Zhang, R. S. Hawley and B. S. Baker, 1991 The *lethal(1)TW-6^{ts}* 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.

Rasooly, R. S., P. Zhang, A. K. Tibolla and R. S. Hawley, 1994 A structure-function analysis of NOD, a kinesin-like protein from *Drosophila melanogaster*. Mol. Gen. Genet. **242:** 145–151.

Robbins, L. G., 1977 The meiotic effect of a deficiency in *Drosophila melanogaster* with a model for the effects of enzyme deficiency on recombination. Genetics **87:** 655–684.

Robbins, L. G., 1980 Maternal-zygotic lethal interactions in *Drosophila melanogaster:* the effects of deficiencies in the *zeste-white* region of the *X* chromosome. Genetics **96:** 187–200.

Robbins, L. G., 1981 The meiotic behavior of some single-cistron mutants in the *zeste-white* region of the *Drosophila melanogaster X* chromosome. Mol. Gen. Genet. **183** (2): 214–219.

Sandler, L., and Novitski, E. 1956 Evidence for genetic homology between chromosomes *I* and *IV* in *Drosophila melanogaster*, with a proposed explanation for the crowding effect in triploids. Genetics **41:** 189–193.

Sekelsky, J. J., K. S. McKim, T. Arbel, G. Chin, R. L. French *et al.*, 1999 Identification of novel meiotic genes recovered in a *P* element screen. Genetics **152:** 529–542.

Theurkauf, W. E., 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** (5): 1167–1180.

Van Doren, M., A. L. Williamson and R. Lehmann, 1998 Regulation of zygotic gene expression in *Drosophila* primordial germ cells. Curr. Biol. **8** (4): 243–246.

Wald, H., 1936 Cytological studies on the abnormal development of the eggs of the claret mutant type of *Drosophila simulans*. Genetics **21:** 264–279.

Wasser, M., and W. Chia, 2003 The *Drosophila* EAST protein associates with a nuclear remnant during mitosis and constrains chromosome mobility. J. Cell Sci. **116** (Pt. 9): 1733–1743.

Whyte, W., H. Irick, T. Arbel, G. Yasuda, R. French *et al.*, 1993 The genetic analysis of distributive pairing in *Drosophila melanogaster*. III. The wild-type product of the *Axs* locus is required for achiasmate meiotic segregation. Genetics **134:** 825–835.

Wolf, K. W., 1994 How meiotic cells deal with non-exchange chromosomes. Bioessays **16** (2): 107–114.

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. 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. E., and R. S. Hawley, 1989 The genetic analysis of distributive segregation in *Drosophila melanogaster*. I. Isolation and characterization of aberrant X segregation, *Axs*, a mutant defective in chromosome partner choice. Genetics **122:** 801–821.

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