Identification of Novel Drosophila Meiotic Genes Recovered in a *P*-Element Screen

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ABSTRACT

The segregation of homologous chromosomes from one another is the essence of meiosis. In many organisms, accurate segregation is ensured by the formation of chiasmata resulting from crossing over. *Drosophila melanogaster* females use this type of recombination-based system, but they also have mechanisms for segregating achiasmate chromosomes with high fidelity. We describe a *P*-element mutagenesis and screen in a sensitized genetic background to detect mutations that impair meiotic chromosome pairing, recombination, or segregation. Our screen identified two new recombination-deficient mutations: *mei-P22*, which fully eliminates meiotic recombination, and *mei-P26*, which decreases meiotic exchange by 70% in a polar fashion. We also recovered an unusual allele of the *ncd* gene, whose wild-type product is required for proper structure and function of the meiotic spindle. However, the screen yielded primarily mutants specifically defective in the segregation of achiasmate chromosomes. Although most of these are alleles of previously undescribed genes, five were in the known genes α*Tubulin67C*, *CycE*, *push*, and *Trl*. The five mutations in known genes produce novel phenotypes for those genes.

BRIDGES' classic studies of nondisjunction in *Drosophila melanogaster* females provided the definitive proof that genetic material is carried on chromosomes and that the meiotic segregation of homologous chromosomes is the basis for Mendelian inheritance (Bridges 1916). Since that time, *D. melanogaster* females have continued to provide an excellent model system in which to study meiosis (reviewed in Hawley *et al.* 1993). The primary segregation system in Drosophila females follows a canonical recombination-based pathway in which exchange between homologous chromosomes results in chiasmata. Chiasmata are used to stabilize the association between homologues until anaphase, thus ensuring their proper disjunction from one another. In Drosophila females, achiasmate chromosomes, whether homologous or heterologous, are also segregated from one another accurately (Hawley and Theurkauf 1993). The segregation of homologous achiasmate chromosomes relies on pairing between regions of heterochromatic homology (Hawley *et al.* 1992; Dernburg *et al.* 1996; Karpen *et al.* 1996).

Only a year after the publication of Bridges' paper, Gowen and Gowen (1922) discovered the first meiotic mutant, *crossover suppressor on 3 of Gowen* [*c(3)G*], which spontaneously arose in a Drosophila stock. Meiotic crossing over is eliminated in *c(3)G* mutants, which results in high levels of nondisjunction at the first meiotic division (Gowen 1928; Hall 1972). In the next four decades, only a single additional mutant affecting meiotic chromosome behavior in Drosophila females was discovered: *claret-nondisjunctional* (*cand*, Sturtevant 1929; Lewis and Gencarella 1952). This prompted Sandler *et al.* (1968) to conduct the first systematic screen for such mutants (reviewed in Hawley 1993). They tested 118 viable and fertile *2;3* constellations isolated from the wild for effects on *X* chromosome exchange and *X* and *4* segregation in both females and

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males, and they recovered mutants defective in meiotic recombination (*e.g.*, *mei-S282*), sister chromatid cohesion (*mei-S332*), and achiasmate homologous segregation (the synthetic mutation *mei-S51*).

After the success of the screen conducted by Sandler *et al.*, Baker and Carpenter (1972) made use of newly developed methods for feeding the mutagen ethyl methanesulfonate (EMS) to Drosophila males to induce mutations. They screened females homozygous for each of 189 EMS-treated *X* chromosomes for *X* and *4* nondisjunction. In spite of the small number of mutagenized chromosomes screened, Baker and Carpenter recovered several mutants with defects in recombination (*e.g.*, *mei-9* and *mei-218*) and one with impaired ability to segregate achiasmate chromosomes (*nod*).

Genetic analyses of the meiotic genes discovered by these workers and others have yielded a wealth of information about the meiotic process in Drosophila females (reviewed in Baker and Hall 1976; Baker *et al.* 1976). More recently, molecular analyses of several of these genes have provided new insights into their functions in the meiotic process (Kerrebrock *et al.* 1992; Hari *et al.* 1995; Sekelsky *et al.* 1995; Bickel *et al.* 1996; McKim *et al.* 1996; McKim and Hayashi-Hagihara 1998). However, the number of known meiotic genes in Drosophila is probably only a small fraction of the total. We therefore conducted a new and larger screen for mutants exhibiting chromosome misbehaviors during female meiosis. Our aim was to design a screen that would not be biased for particular types of meiotic genes. We used *P* elements to facilitate localization and subsequent molecular cloning. We recovered mutations both in known genes and in previously unknown genes.

MATERIALS AND METHODS

Drosophila stocks and culture: Except where noted, genetic markers are described in Lindsley and Zimm (1992) and Flybase (1998). Flies were reared on standard cornmeal-molasses-dextrose medium at 25°.

Balancers: Many of the mutations described here exert their effects on achiasmate chromosomes. These effects are most easily detected in females in which exchange along the *X* is suppressed because of heterozygosity for a multiply inverted balancer chromosome. We used two different balancers, *Bwinscy* [*In(1)sc*^{8L}*sc*^{S1R}, *y*^{C4} *w B*] and *FM7w*. *Bwinscy* was made by selecting yellow, white, singed^{+}, Bar recombinants between *Binsn* [*In(1)sc*^{8L}*sc*^{S1R}, *sn*^{X2} *B*] and *winscy* [*In(1)sc*^{8L}*sc*^{S1R}, *y*^{C4} *w*]. *FM7w* is a derivative of *FM7a* that carries *w*^{1} rather than *w*^{a}. It was generated by selecting recombinants between *FM7a*, *y*^{31d} *sc*^{8} *w*^{a} *v*^{Of} *B* and *Bwinscy*.

Generation of single *P*-element inserts: We performed single *P*-element mutagenesis using *P*{*lacW*} (Bier *et al.* 1989). This construct carries the eye color marker gene *mini-white* (*w*$^{+mC}$). The degree of expression of this gene is sensitive to chromosomal position effects and to copy number, so two copies of *w*$^{+mC}$ usually result in a more wild-type eye color than does a single copy. This property allowed us to detect homozygous individuals without balancing individual inserts. A second feature of *P*{*lacW*} is the ability to quickly clone the DNA flanking the element by the plasmid rescue technique.

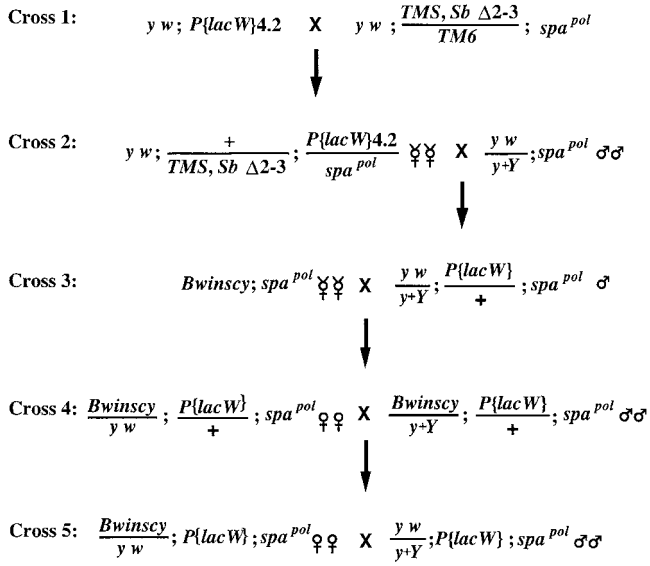

Figure 1.—Scheme 1 for generating new *P*{*lacW*} inserts and testing them as homozygotes for effects on *X* chromosome meiotic segregation in females. See materials and methods for details.

We used two schemes to generate new *P*{*lacW*} insertions. In screen 1 (Figure 1, described below), we generated transpositions of *P*{*lacW*} from a site on chromosome *4* to *X*, *2*, or *3*:

Cross 1: Flies homozygous for an insert of *P*{*lacW*} at the base of chromosome *4*, denoted *P*{*lacW*}4.2, which we recovered in a pilot screen, were crossed to a stock carrying the Δ2-3 source of transposase (Robertson *et al.* 1988) on a dominantly marked balancer chromosome, *TMS*.

Cross 2: Transposition occurs in the germlines of cross 1 progeny that carry *TMS* and *P*{*lacW*}4-2. We allowed transposition in female germlines to increase the chances of insertion into genes expressed in the female germline.

Cross 3: New inserts were recovered as poliert, white^{+}, stubble^{+} males from cross 2, with up to three males with different eye colors selected from each vial. These were mated singly to *Bwinscy* females to generate both male and female progeny bearing the new insertion. *X*-linked insertions were identified by their transmission to daughters only; these were made homozygous through two additional crosses (not diagrammed). The presence of multiple insertions on different chromosomes was detected by the generation of progeny with different eye colors. In this case, cross 3 was repeated with individual males believed to carry a single insert.

Cross 4: Males and females bearing a new insert were crossed to one another to generate progeny homozygous for *P*{*lacW*} (identified on the basis of eye color). The absence of progeny easily identified as being homozygous was taken as an indication that the insertion was likely to be lethal when homozygous.

Cross 5: For initial screening of new insert lines, females homozygous for a *P*{*lacW*} insert and heterozygous for *Bwinscy* (except for *X*-linked insertions) were placed into vials with several males from the stock. We usually made two or three vials, each containing two or three females, plus a stock vial with females heterozygous for the insert. Progeny were screened for the presence of yellow males (*XO* males derived from nullo-*X* ova or nullo-*XY* sperm) and yellow^{+} females (*XXY* females derived from diplo-*X* ova or *XY*-bearing sperm). Lines with more than two of these exceptional progeny (corresponding to an aberrant segregation rate of 1–2%) were retested once in the same manner. Those that transmitted the

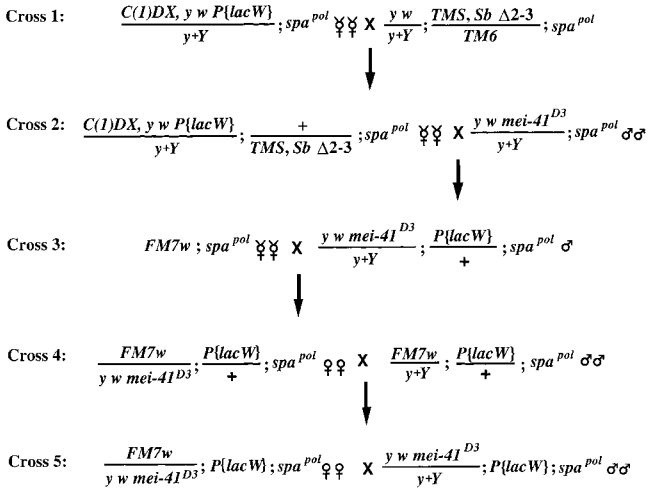

Figure 2.—Scheme 2 for generating new *P{lacW}* inserts and testing them as homozygotes for effects on *X* chromosome meiotic segregation in females. See materials and methods for details.

nondisjunction phenotype were analyzed further to determine whether the defect occurred in male and / or female germlines, and whether the phenotype segregated with the *P{lacW}* element.

Screen 2: As shown in Figure 2, this screen differs from screen 1 in two significant ways. First, the ammunition chromosome was *C(1)DX, y w f P{lacW}*, so we could not recover transpositions to the *X* chromosome from this scheme. Second, in cross 2, females were mated to *y w mei-41^{D3} / y^{+}Y*; *spa^{pol}* males so that insertions were picked up in males mutant for *mei-41^{D3}*, and segregation was tested in females heterozygous for *mei-41^{D3}*. In all meiotic mutants recovered from this scheme, heterozygosity for *mei-41* had no discernible effect on the mutant phenotype.

Additional screens: A secondary purpose of screen 2 was to identify mutations that were synthetically lethal with *mei-41*. Two such synthetic lethals, denoted *syn1* and *syn2*, were recovered. The *syn1* mutation is the result of a *P* insertion at 54C11-12. The *syn1* mutation is lethal to flies that are hemizygous or homozygous for *mei-41*, but *mei-41 / +* females carrying *syn1* are viable. Further characterization of *syn1* will be presented elsewhere. The *syn2* mutant was lost during characterization.

X-linked insertions that did not themselves cause nondisjunction were tested for their ability to dominantly enhance the meiotic phenotype of *nod^{DTW}* (Zimmerman 1998). *y w P{lacW} / FM7w* females were crossed to *nod^{DTW} / B^{S}Y* males, and the *y w P{lacW} / nod^{DTW}* progeny were crossed to *YSX · YL, v f B / 0; C(4)RM, ci ey^{R} / 0* males so that *X* chromosome segregation could be scored. Lines that increased nondisjunction at least threefold over the *nod^{DTW} / +* rate of 2% were retested and characterized. Eight lines showing enhancement of the *nod^{DTW}* phenotype were recovered from among 1500 screened. One of these, designated *P31*, is inserted into the *amnesiac* (*amn*) gene, which encodes the neuropeptide hormone PACAP38. Heterozygosity for *P31* or for a deficiency in the region increases *nod^{DTW}* nondisjunction approximately three to fourfold, but it has no effect on nondisjunction in the absence of *nod^{DTW}*.

Lines that displayed recessive female sterility were sent to Bill Theurkauf and Lynn Cooley. Among these lines was at least one allele of *grapes* (Sibon *et al.* 1997).

Scoring chromosome nondisjunction and loss: Nondisjunction values reported are summed from crosses of individual *P{lacW}*; *spa^{pol}* females to *YSX · YL, v f B / 0; C(4)RM, ci ey^{R} / 0* males. Progeny were scored and nondisjunction was calculated as described in Zitron and Hawley (1989). Diplo-*X* ova are recovered if fertilized by nullo-*XY* sperm (generating yellow females), but those fertilized by *YSX · YL* sperm are inviable. Conversely, nullo-*X* ova are recovered if fertilized by *YSX · Y* sperm (generating vermilion, forked, Bar males), but those fertilized by nullo-*XY* sperm are inviable. Hence, half of the *X* chromosome exceptions are inviable. Calculations of the percentages of diplo-*X* and nullo-*X* exceptions are adjusted for this inviability by doubling the number of viable exceptions counted.

Half of the chromosome *4* exceptions are also inviable. However, only half of the normal (haplo-*4*) ova are counted, namely those that are *spa^{pol} / C(4)RM, ci ey^{R}*. Haplo-*4* ova fertilized by nullo-*4* sperm are subviable; those that survive to adulthood are identified by their minute [because of *M(4)101*] and poliert phenotype, and they are excluded from progeny counts. Therefore, no adjustment for loss of half of the chromosome *4* exceptions is necessary (except when they are simultaneously *X* exceptions, in which case the normal adjustment for *X* exceptions applies).

Mapping of *P*-element insertions: Several methods were used to position mutations on the standard polytene chromosome map. For most inserts, *in situ* hybridization to polytene chromosomes was performed using biotinylated pCaSpeR as a probe; signals were detected with streptavidin-conjugated horseradish peroxidase (Life Technologies) and FAST DAB tablet solutions (Sigma, St. Louis). *mei-P24* and *mei-P44* were localized by using plasmid rescue sequences to probe a filter arrayed with Drosophila genomic P1 clones (Genome Systems). Where possible, cytological locations were confirmed or refined by deficiency mapping.

Plasmid rescue and DNA sequencing: Plasmid rescue was performed as described (Ashburner 1989). For most mutations, we determined the sequence adjacent to the *P* element on at least one side using the inverted repeat primer Pout (GGACCACCTTATGTTATTTC). Sequences were performed on an automated 377XL from Applied Biosystems (Foster City, CA).

TABLE 1

Summary of *P*-element screens

Cross	Number of vials		
	Screen 1	Screen 2	Total
Cross 2	26,358	12,533	38,891
Cross 3	21,627	7,676	29,303
Cross 4	12,830	6,288	19,118
Autosomal	10,357		16,645
X chromosome	2,473		2,473
Cross 5	13,382	5,176	18,558
Autosomal	11,071		16,247
X chromosomal	2,311		2,311

RESULTS

Overview and rationale of the *P*-element screen

We generated 21,627 new insertions of *P{lacW}* from a chromosome *4* site and 7676 insertions from a site on *C(1)DX* (Table 1). From these, we were able to screen

18,558 homozygous viable and fertile lines (16,247 autosomal and 2311 *X*-chromosomal).

Our initial screening for meiotic defects was based on a simple assay for *X* chromosome nondisjunction. We used a y^+-marked *Y* chromosome to detect *XXY* females (derived from diplo-*X* ova or *XY* sperm) and *XO* males (derived from nullo-*X* ova or nullo-*XY* sperm). This allowed us to screen for nondisjunction or loss without having to collect virgin females and set up test crosses and to recover dominant or recessive mutations altering *X* chromosome segregation in either the male or female germline, although only the latter were recovered.

For autosomal inserts, females screened for high levels of *X* chromosome nondisjunction were heterozygous for an *X* chromosome balancer, either *Bwinscy* or *FM7w* (both are referred to below as *X*/*Bal*, as opposed to females with isosequential *X* chromosomes, which are designated *X*/*X*). This step made it possible to identify mutants such as *nod* or *Axs* (Zitron and Hawley 1989; Zhang and Hawley 1990), which are specifically impaired in the segregation of achiasmate chromosomes.

In addition, we hoped that the use of such a balancer would facilitate the recovery of weak or hypomorphic recombination-defective mutants. By examining the effects of mutations that decrease exchange frequency to various degrees, Baker and Hall (1976) found that the frequency of *X* chromosome nondisjunction is roughly proportional to the cube of the frequency of nonexchange *X* tetrads. They suggested that this reflects the ability of the achiasmate segregation systems to properly segregate nonexchange major chromosomes when only one pair of homologues is nonexchange; high levels of nondisjunction result only when exchange is reduced to such an extent that there are frequently two pairs of nonexchange chromosomes (excluding the obligate nonexchange *4*). Our use of a balancer ensured that the *X* was essentially always nonexchange, and, thus, we required that only one of the major autosomes also be achiasmate to observe *X* nondisjunction.

We recovered 41 new mutants (~1 in 450 lines screened) affecting female meiotic chromosome segregation; 16 of these with more severe phenotypes (at least 5% *X* exceptions) were analyzed in greater detail (Table 2).

In the case of the *mei-P22* and *mei-P26* mutants, the observed high levels of nondisjunction are presumably a consequence of a defect in meiotic recombination. The remaining mutants appear to exert their effects by altering the segregation process itself. One of these, *mei-P39*, affects both exchange and nonexchange chromosomes, whereas the others appear to primarily affect achiasmate chromosome segregation.

The achiasmate segregation mutants can themselves be classed into three major groups. Members of the first group have phenotypes similar to that of the Axs^D mutant (see below; Zitron and Hawley 1989; Hawley and Theurkauf 1993; Whyte *et al.* 1993). Four other mutants define a previously unknown class in which there are high levels of achiasmate *X* chromosome nondisjunction but only low levels of *4* nondisjunction. Finally, several mutants cannot be lumped into an existing class of segregation-defective mutants, and they are referred to here as unique mutants.

Mutants that affect the recombination-based pathway

Three of the mutants we recovered have defects in the recombination-based pathway. These mutants display high levels of nondisjunction in isosequential *X* chromosome backgrounds (*X*/*X*) and in the presence of a multiply inverted balancer (*X*/*Bal*). A fourth mutant, *mei-P2*, may also belong to this group, but because it is an allele of the *CyclinE* gene (*CycE*), we describe it with the achiasmate segregation mutant *mei-P7*.

Recombination-defective mutants: Among the *X*-linked meiotic mutants recovered by Baker and Carpenter (1972), the largest class comprises those whose effects on chromosome segregation are secondary consequences of defects in recombination (Carpenter and Sandler 1974). We recovered only two such mutants, *mei-P22* and *mei-P26* (Tables 3 and 4). Brief descriptions follow, but further genetic analysis and molecular cloning of these genes has been (McKim *et al.* 1998) or will be described elsewhere.

mei-P22: Females homozygous for *mei-P22* display an absence of meiotic recombination. In an experiment measuring exchange along the *X* chromosome (using the markers *y*, *w*, *ct*, *m*, and *f*), there were only 25 recombinants among 1910 progeny derived from 75 *mei-P22* females. All were in the same interval (*w* to *ct*), and 23 of these occurred in clusters (18 from one female and 5 from another), suggesting that these events occurred premeiotically. In a similar experiment measuring exchange along *2L* (using the markers listed in Table 4), 17 recombinants were recovered from among 820 progeny. Again, all were in the same interval (*dp* to *b*), and all were produced by a single female. Thus, although germline mitotic recombination is increased in *mei-P22* females, meiotic exchange is completely ablated. Furthermore, meiotic gene conversion is absent in *mei-P22* mutants (McKim *et al.* 1998). The absence of interchromosomal recombination without an increase in sister chromatid exchange (McKim *et al.* 1998) indicates that *mei-P22* is required for the initiation of meiotic recombination.

As is the case for the two other recombination-null mutants in Drosophila, *c(3)G* and *mei-W68*, meiotic chromosome segregation in females homozygous for *mei-P22* is highly aberrant: both *X*/*X* and *X*/*Bal* females display frequencies of *X* chromosome nondisjunction that range from 29 to 35% and frequencies of *4* nondisjunction that exceed 20% (Table 3). However, two lines of evidence argue that the achiasmate segregation sys-

TABLE 2

Summary of *P*-element mutants

Line[a]	Gene	Ndj in X/X^b		Ndj in X/Bal		Cytological location	Sequence hits[c]
		X ndj	*4* ndj	*X* ndj	*4* ndj		
				Recombination-defective mutants			
P22	*mei-P22*	29.9	22.8	35.4	22.4	65E	None
P26	*mei-P26*	18.3	14.3	ND	ND	8C	None
				Segregation-defective mutants			
P39	*ncd*	20.9	65.2	52.5	53.6	99B	AQ026211, 153
				Axs-like mutants			
P8	*mei-P8*	2.5	1.0	20.7	0.5	96BC	ND
P19	*mei-P19*	0	1.2	16.7	6.7	88A	AQ034055, 127
P21	*push*	3.1	1.0	21.4	9.5	28E	AQ026494, 190
P38	*mei-P38*	ND	NS	9.3	5.6	91F	AC006091, 165,713[d]
P40	*αTub67C*	1.6	0.8	27.6	19.4	67C	M14646, 2165
				X-specific mutants			
P14	*mei-P14*	1.6	0.4	15.6	3.4	59B4-7	AC005650, 46240
P15	*mei-P15*	0.2	0	17.2	0	66F-67A	AA698279, (1)
P23	*mei-P23*	2.1	0.5	9.8	1.4	96A	Z27119, 110
P24	*Trl*	3.6	1.5	32.2	2.7	70F	AJ225042, 5685
				Mutants with unique phenotypes			
P2	*CycE*	6.6	0.5	6.4	0.5	35E	AC004362, 123
P7	*CycE*	1.6	1.3	8.1	5.6	35E	AC003700, (490)
P37	*mei-P37*	1.6	2.2	10.0	7.8	63F	AC004658, 87054; V01519, 33
P44	*mei-P44*	ND	ND	10.0	60	100F	None

ND, not done.

[a] Lines 1–26 were from screen 1; others were from screen 2 (see materials and methods).

[b] Summed percentage progeny from nondisjunction (ndj) and chromosome loss.

[c] GenBank accession number and position of first base pair of 8-bp target sequence for our insertions, based on plasmid rescue sequences. The approximate position is in parentheses where the exact insertion position is unknown.

[d] When this article was submitted, this entry consisted of several unordered contigs. The sequence of the first 30 nucleotides after the *P*{*lacW*} in *mei-P38*, beginning with the 8-bp target sequence, is TGCGGCACGACTG CAAAACGCTTTTGAAAT.

tems are still functioning, albeit inefficiently. First, the frequencies of *X* and *4* nondisjunction, while high, are well below the value of 50% exhibited by mutants with random disjunction of achiasmate chromosomes (Zhang and Hawley 1990). Second, among chromosome *2* exceptions, which were recovered >500-fold more frequently from *mei-P22* females than from wild type, 57% were also nondisjunctional for the *X*, and 85% of these were the result of *XX* ↔ *22* segregation, a hallmark of the heterologous segregation system (data not shown).

mei-P26: Females homozygous for *mei-P26* exhibit meiotic recombination reduced by 70 to 90% (Table 4). Females bearing the *mei-P26* mutation display 18% *X* chromosome nondisjunction, a value commensurate with the residual level of exchange observed. Like most other recombination-defective mutants in Drosophila, the exchange defect observed in *mei-P26* females is polar; *i.e.*, distal regions are affected more strongly than proximal regions. The *mei-P26* locus may be semidominant, because heterozygosity for *mei-P26* or for a deficiency that removes the gene reduced exchange by one-third in this experiment (Table 4). The *mei-P26* mutation is probably hypomorphic, because *mei-P26*/*Df* females have more severe defects (K. S. McKim, B. Deneen, S. L. Page and R. S. Hawley, unpublished data).

Segregation-defective mutant: We recovered one mutant, *mei-P39*, that disrupts the segregation of chromosomes whether or not they have undergone exchange and without any direct effect on the level of exchange (Table 5 and data not shown). Our *in situ* hybridization placed *mei-P39* in 99B. Analysis of the *mei-P39* plasmid rescue showed that the *P* element is inserted ~20 bp upstream of the start of the longest reported cDNAs for *non-claret disjunctional* (*ncd*). The *mei-P39* mutant failed to complement ca^{nd} and *ncd*, two null alleles of this locus; accordingly, we denoted our allele ncd^{P39}.

The *ncd* gene encodes a kinesin-like motor protein required for formation and stabilization of the meiotic bipolar spindle (Endow *et al.* 1990; McDonald and Goldstein 1990; Matthies *et al.* 1996). Like other *ncd* mutations, ncd^{P39} causes high levels of *X* and *4* nondis-

TABLE 3

Segregation in recombination-defective mutants

Gamete types		Maternal genotype		
		mei-P26:	*mei-P22*	
Maternal	Paternal	*X/X*	*X/X*	*X/Bal*
Normal				
X; 4	*XY; 44*	159	219	274
X; 4	*0; 44*	145	198	282
X ndj				
0; 4	*XY; 44*	13	25	45
XX; 4	*0; 44*	9	45	74
4 ndj				
X; 0	*XY; 44*	6	15	27
X; 0	*0; 44*	6	13	12
X; 44	*XY; 0*	7	23	31
X; 44	*0; 0*	7	29	28
X,4 ndj				
0; 0	*XY; 44*	3	5	13
XX; 44	*0; 0*	3	5	6
0; 44	*XY; 0*	7	11	21
XX; 0	*0; 44*	2	15	20
Total progeny		367	603	833
Adjusted total		404	709	1012
% nullo-*X*		11.4	11.6	15.6
% diplo-*X*		6.9	18.3	19.8
% nullo-*4*		5.4	10.4	10.8
% diplo-*4*		8.4	11.2	11.6

TABLE 4

Map distances in *mei-P26*

Region[a]	Map distance (% of standard)		
	Standard	*mei-P26/+*[b]	*mei-P26*
	X chromosome		
pn-cv	12.9	2.5 (12.9)	0.1 (0.8)
cv-m	22.4	12.3 (54.9)	0.9 (4.0)
m-f	20.6	16.0 (79.6)	2.2 (10.9)
f-y$^+$	9.3	13.0 (140)	2.3 (24.7)
Total	64.7	43.8 (67.7)	5.5 (8.5)
No. scored		607	1044
	Second chromosome (left arm)		
net-ho	4.0	1.3 (32.5)	2.2 (55)
ho-dp	9.0	5.3 (58.9)	1.2 (13.3)
dp-b	35.5	26.2 (73.8)	6.8 (19.2)
b-pr	6.0	5.0 (83.3)	5.1 (85.0)
pr-cn	3.0	0.8 (26.6)	2.8 (93.3)
Total	57.5	38.6 (67.1)	18.1 (31.5)
No. scored		689	647

[a] Regions are listed from distal to proximal, with the final region on each chromosome spanning the centromere.

[b] For the second chromosome experiment, *Df(1)9a4-5/+* was used. This deficiency deletes *mei-P26* (data not shown).

junction in both +/+ and *FM7*/+ females (Table 5). However, the *ncd*^{P39} allele differs from other well-characterized *ncd* alleles in two major respects. First, in *ncd* and *ca*nd oocytes, most of the exceptions result from chromosome loss. In *ncd*^{P39} oocytes, however, we do not see any excess of *X* chromosome loss and see only a twofold excess of chromosome *4* loss. Hence, *ncd*^{P39} causes primarily nondisjunction of meiotic chromosomes, with only a low frequency of loss. Second, confocal microscopy of *ncd*^{P39} females reveals spindle abnormalities in only a small fraction (<10%) of the oocytes (H. J. Matthies, L. Messina and R. S. Hawley, unpublished results), in contrast to the frayed and monopolar spindles frequently observed in *ncd* or *ca*nd females (Matthies *et al.* 1996).

One interpretation of our results is that *ncd*^{P39} is a hypomorphic allele. The *ncd*^{D} mutation is a hypomorphic allele that produces lower frequencies of *X* and *4* missegregation than *ncd*^{P39} (Komma *et al.* 1991). However, unlike the case for *ncd*^{P39}, *ncd*^{D} homozygotes produce fivefold more nullo-*4* ova than diplo-*4* ova. Another difference is that *ncd*^{P39} females produce a high frequency of gynandromorphic offspring, which is similar to the frequencies observed for *ncd* or *ca*nd homozygotes (data not shown). Thus, it seems likely that *ncd*^{P39} represents the loss of a specific *ncd* function rather than being simply a weak allele.

Mutants that affect the achiasmate system

Most of the mutants we recovered specifically interfere with the segregation of achiasmate chromosomes. This is most clearly demonstrated by comparison of the levels of nondisjunction of isosequential *X* chromosomes (*X/X*), in which recombination is permitted, to the levels of nondisjunction in females with a normal sequence *X* and a multiply inverted balancer (*X/Bal*), in which exchange is suppressed. For achiasmate segregation mutants, heterozygosity for a balancer typically increases *X* nondisjunction 5- to 20-fold (Table 2).

***Axs*-like mutants:** The *Axs*-like meiotic mutants share several attributes: (1) only the segregation of achiasmate chromosomes is affected; (2) the rate of *X* nondisjunction is usually two- to threefold higher than that of *4* nondisjunction; (3) there is a strong positive correlation between *X* and *4* nondisjunction, such that most *4* nondisjunction occurs in oocytes in which *X* nondisjunction also occurs; (4) the vast majority of simultaneous *X,4* nondisjunction is manifested as nonhomologous *XX* ↔ *44* segregation (the two *X* chromosomes segregate to one pole and the two *4*'s to the other); and (5) exchange is normal.

We recovered five mutants with these characteristics: *mei-P8*, *mei-P19*, *mei-P21*, *mei-P38*, and *mei-P40* (Table 6). Two of these mutants represent insertions into previously described genes: *mei-P21* is an allele of *pushover* (*push*), and *mei-P40* is an allele of α*Tubulin67C* (α*Tub-67C*). A third mutant, *mei-P19*, is allelic to *l(3)03477*, a *P{PZ}* insertion in 88A. Compound heterozygotes between *mei-P19* and *l(3)03477* display the meiotic pheno-

TABLE 5

Segregation in *ncd* mutants

Gamete types		Maternal genotype[a]		
		ncd^{P39}		*ncd:*
Maternal	Paternal	*X/X*	*X/Bal*	*X/Bal*
Normal				
X; 4	*XY; 44*	137	147	118
X; 4	*0; 44*	216	127	136
X ndj				
0; 4	*XY; 44*	9	34	86
XX; 4	*0; 44*	33	60	42
4 ndj				
X; 0	*XY; 44*	93	77	249
X; 0	*0; 44*	102	41	63
X; 44	*XY; 0*	41	54	24
X; 44	*0; 0*	54	27	14
X,4 ndj				
0; 0	*XY; 44*	64	90	346
XX; 44	*0; 0*	44	43	19
0; 44	*XY; 0*	3	6	25
XX; 0	*0; 44*	7	28	48
Total progeny		803	734	1122
Adjusted total		963	995	1706
% nullo-*X*		15.8	26.1	53.6
% diplo-*X*		17.4	26.3	12.8
% nullo-*4*		35.0	35.6	64.5
% diplo-*4*		19.6	18.0	7.4

[a] *ncd* results taken from Knowles and Hawley (1991).

type of *mei-P19* (see Table 9). The *P*{*lacW*} in *mei-P19* is inserted 79 bp from the *P*{*PZ*} insertion in *l(3)03477*.

mei-P40, an allele of α*Tub67C: mei-P40* is one of only two dominant meiotic mutations recovered in our screen. Although females homozygous for *mei-P40* are sterile, we were able to recover this mutant after noticing high levels of nondisjunction in cross 4 (Figure 1). Our *in situ* hybridization placed *mei-P40* in 67C. Analysis of the *mei-P40* plasmid rescue showed that the *P* element is inserted into the coding region of α*Tub67C*, 36 bp from the 3′ end.

The α*Tub67C* gene encodes an ovary-specific α-tubulin required for female meiosis and early embryonic divisions (Matthews *et al.* 1993; Komma and Endow 1997; Mathe *et al.* 1998). Because *mei-P40* failed to complement existing α*Tub67C* alleles with respect to recessive sterility, we denote our mutation α*Tub67C*^{P40}.

Although numerous alleles of this gene have been characterized previously in terms of their effects on female fertility and embryonic development, none have been shown to directly affect achiasmate chromosome segregation. To ensure that the meiotic phenotype of α*Tub67C*^{P40} was indeed caused by the *P* insertion at 67C, we generated 20 transposase-induced, white-eyed derivatives. Of these, 3 revert both the female sterility and the meiotic effects, and all 3 were found to be precise excisions of the *P* element. The remaining 17, which all retain some part of the *P*{*lacW*}, continue to exert recessive female sterility and dominant effects on *X* and *4* nondisjunction in *X*/*Bal* females. Thus, both the female sterility and the meiotic defects are direct consequences of the *P* insertion into α*Tub67C*.

As shown in Table 6, the frequencies of *X* and *4* nondisjunction are greatly elevated in *mei-P40*/+ oocytes in *X*/*Bal* females. Cytological studies of this mutation reveal a defect in progression of the oocytes from metaphase I to anaphase I (L. Messina, H. J. Matthies and R. S. Hawley, unpublished results).

mei-P21, an allele of pushover: The *mei-P21* mutant exhibits both an *Axs*^{D}-like meiotic phenotype and male sterility. Our *in situ* hybridization placed the *P*{*lacW*} insertion in 28E. Therefore, we did complementation experiments between *mei-P21* and two male sterile *P* insertions in the region, *gelded*, which complemented, and *ms(2)03420*, which failed to complement. The *ms(2)03420* insertion has been shown to be an allele of *push*, a gene so named because mutant flies, when pushed onto their dorsal surface, require an average of 20-fold more time than wild type to right themselves (Richards *et al.* 1996). In addition to behavioral defects, *push* mutants have defects in synaptic transmission at neuromuscular junctions. Analysis of transposase-induced excisions of *push*^{P21} demonstrated that both the male sterility and the *Axs*-like phenotype were caused by the *P*{*lacW*} insertion in 28E.

***X* chromosome-specific mutants:** The second large class of achiasmate segregation mutants we recovered is a novel one: those with high levels of *X* nondisjunction but low levels of *4* nondisjunction (Table 7). Although in some cases chromosome *4* segregation is elevated, we refer to members of this class as *X* chromosome specific because the effect on this chromosome is so much more severe. This class includes *mei-P14*, *mei-P15*, *mei-P23*, and *mei-P24*.

mei-P24, an allele of Trithorax-like: The *mei-P24* insert was mapped to 70F by *in situ* hybridization and genomic P1 filter hybridization. Sequencing of plasmid rescue insertions revealed that the causative *P* element is inserted into the large (second) intron of *Trithorax-like* (*Trl*). *Trl* encodes the Drosophila GAGA-binding factor, a multipurpose transcriptional activator and chromatin remodeling protein that localized to heterochromatin and specific euchromatic sites (Farkas *et al.* 1994).

Null mutations in *Trl* are lethal, but homozygotes of *Trl*^{13C}, which is caused by a *P*-element insertion within 500 bp of the *Trl*^{P24} insertion position, have reduced viability, rough eyes, and a maternal-effect lethal phenotype (Farkas *et al.* 1994). In addition, *Trl*^{13C} is a dominant enhancer of homeotic mutants and position-effect variegation (Dorn *et al.* 1993). Our results with *Trl*^{P24} are the first demonstration of a meiotic chromosome segregation defect among trithorax group mutants.

mei-P14 and mei-P23: The *mei-P14* insertion was localized to 56F by *in situ* hybridization and is not uncovered

TABLE 6

Segregation in *Axs*-like mutants

Gamete types		Maternal genotype								
		mei-P8		*mei-P19*		*mei-P38:*	*mei-P40/+*		*mei-P21*	
Maternal	Paternal	*X/X*	*X/Bal*	*X/X*	*X/Bal*	*X/Bal*	*X/X*	*X/Bal*	*X/X*	*X/Bal*
Normal										
X; 4	*XY; 44*	570	180	122	179	1132	479	586	205	173
X; 4	*0; 44*	648	205	197	193	558	719	517	170	134
X ndj										
0; 4	*XY; 44*	5	19	0	9	33	5	79	1	14
XX; 4	*0; 44*	8	26	0	18	34	4	78	3	14
4 ndj										
X; 0	*XY; 44*	1	2	3	2	23	1	13	0	3
X; 0	*0; 44*	2	1	0	5	10	3	13	0	0
X; 44	*XY; 0*	2	2	1	1	23	4	9	0	3
X; 44	*0; 0*	2	0	0	0	5	2	8	0	2
X;4 ndj										
0; 0	*XY; 44*	0	0	0	0	1	0	2	0	0
XX; 44	*0; 0*	0	0	0	0	0	0	9	0	0
0; 44	*XY; 0*	2	3	0	4	12	0	26	0	12
XX; 0	*0; 44*	1	3	0	7	10	0	24	2	0
Total progeny		1241	441	323	418	1841	1217	1364	381	355
Adjusted total		1257	492	323	456	1931	1226	1582	387	395
X nondisjunction		2.5	20.7	0	16.7	9.3	1.5	27.6	3.1	20.3
4 nondisjuction		1.0	3.5	1.2	6.6	5.5	0.8	10.4	1.0	8.1
X; 4 ndj expected			0.7		1.1	0.5		2.9		1.6
X; 4 ndj observed			2.4		4.8	2.4		7.7		6.1
% nonhomologous			100		100	96		82		100

by *Df(2R)AA21* (56F9-11; 57D12). We mapped *mei-P23* to 96A by *in situ* hybridization. The *P* element in this mutant is within a blastopia transposable element. Although we have not determined whether this insertion causes the mutant phenotype, a deficiency for the region [(*Df(3R)crbS87-5*, 95F7;96A17-18] does uncover *mei-P23* (data not shown), indicating that the meiotic defect is at least tightly linked to this insertion.

mei-P15: The *mei-P15* mutation is fully dominant and may be neomorphic, because heterozygosity for a deficiency from the region does not cause the same meiotic phenotype (data not shown). Each of the ten transposase-induced, white-eyed derivatives had completely lost the dominant meiotic chromosome segregation defect. Sequence from one side of the *mei-P15* insertion overlaps the 5′ sequence from expressed sequence tag (EST) HL04203.

Mutants with unique phenotypes: We also recovered mutations that confer achiasmate segregation phenotypes unlike those of previously known Drosophila meiotic mutations (Table 7). These include *mei-P2* and *mei-P7* (both alleles of *CycE*), *mei-P37*, and *mei-P44*.

mei-P2 and mei-P7, alleles of CycE: Sequences adjacent to the insertions *mei-P2* and *mei-P7* both match sequences from P1 clones in the *cact* contig in 35D (Kimmerly *et al.* 1996; Berkeley Drosophila Genome Project, personal communication). Further analysis showed that they are inserted into *CycE* in 35D.

Cyclin E regulates entry into and progression through S phase in both mitotic and endoreduplication cycles (reviewed in Follette and O'Farrell 1997). Null mutations in cyclin E are lethal, but hypomorphic alleles that confer female sterility or developmental defects are also known (Lilly and Spradling 1996; Secombe *et al.* 1998). Our results provide the first evidence for defects in meiotic chromosome segregation in *CycE* mutants.

$CycE^{P7}$ is unique in that it causes high levels of chromosome loss with very little nondisjunction for *X* and *4* (Table 8; note the large excess of progeny derived from nullo-*X* or nullo-*4* ova over diplo-ova). $CycE^{P2}$ has slightly lower levels of *X* chromosome exceptions, but unlike $CycE^{P7}$, a substantial fraction of these are the result of nondisjunction. $CycE^{P2}$ displays a similar phenotype in *X/X* and *X/Bal*, suggesting that its effects are not specific to the achiasmate segregation system.

Both $CycE^{P2}$ and $CycE^{P7}$ contain a *P*{*lacW*} inserted into sequences that correspond to the first intron for the maternal (type II) cyclin E, downstream of the second of the two known alternative first exons. Other *P*-element insertions in this region confer either lethality or female sterility. The lethal allele $CycE^{05206}$ is caused by a *P*{*PZ*} insertion within 20 bp of the target site of $CycE^{P2}$, with

TABLE 7

Segregation in *X*-specific mutants

Gamete types		Maternal genotype							
		mei-P14		*mei-P23*		*mei-P24*		*mei-P15*	
Maternal	Paternal	*X/X*	*X/Bal*	*X/X*	*X/Bal*	*X/X*	*X/Bal*	*X/X*	*X/Bal*
Normal									
X; 4	*XY; 44*	748	444	97	259	89	249	1222	753
X; 4	*0; 44*	738	453	85	254	99	272	1255	783
X ndj									
0; 4	*XY; 44*	3	27	1	15	2	51	1	68
XX; 4	*0; 44*	3	13	1	9	1	73	1	92
4 ndj									
X; 0	*XY; 44*	3	0	0	0	0	3	0	0
X; 0	*0; 44*	0	0	0	0	2	7	0	0
X; 44	*XY; 0*	1	1	1	0	1	0	0	0
X; 44	*0; 0*	2	1	0	0	0	5	0	0
X,4 ndj									
0; 0	*XY; 44*	0	0	0	0	0	0	0	0
XX; 44	*0; 0*	0	0	0	0	0	0	0	0
0; 44	*XY; 0*	0	0	0	2	0	3	0	0
XX; 0	*0; 44*	0	1	0	2	0	0	0	0
Total progeny		1498	940	185	541	194	663	2479	1696
Adjusted total		1504	981	187	569	197	790	2481	1856
X nondisjunction		0.8	8.4	2.1	9.8	3.0	32.2	0.2	17.2
4 nondisjunction		0.4	0.4	0.5	1.4	1.5	2.7	0.0	0.0

both being oriented such that the 5′ end of *P* is nearer the 3′ end of *CycE*. The different phenotypes probably stem at least in part from the different types or sizes of the constructs (*P*{*PZ*} is 14,545 bp; *P*{*lacW*} is 10,691 bp, but the element in $CycE^{p2}$ is apparently rearranged).

mei-P37: In *mei-P37* females, *X* and *4* exceptions are caused by nondisjunctions that are apparently independent of one another. Exchange is normal in *mei-P37* mutants (data not shown). Our *in situ* hybridization results placed *mei-P37* in 63F, and the sequence from the plasmid rescue matches the genomic P1 clone DS00079. At present, no genes or ESTs have been reported within the region from 10 kb 5′ to this site to the end of the sequenced region, 7700 bp 3′ to this site (relative to the *P*-element ends on *P*{*lacW*}). The only substantial open reading frames in this region are two that are adjacent to one another ~4800–7200 bp 5′ of the *P* element, reading away from the element. Conceptual translation of this region does not detect significant similarities to anything in the sequence databases. Curiously, the first 44 bp to the right of *mei-P37* on the plasmid rescue match the 3′ end of the *hobo* transposable element, but this element is not present in the DS00079 sequence. We do not know whether this *hobo* element preexisted at this location in our stocks or had inserted there simultaneously with the *P*{*lacW*}, or whether the *hobo* insertion contributes to the mutant phenotype.

mei-P44: The mutation *mei-P44* causes high levels of *X* missegregation and extremely high levels of *4* missegregation. Curiously, there is a large excess of diplo-*4* ova over nullo-*4* ova (Table 8), unlike any meiotic mutant described previously. The original *mei-P44* stock carried two *P*{*lacW*} elements, one inserted into an intron of the Drosophila UDP-glucuronosyltransferase gene at 86C and another at 100F, ~100 kb from the tip of *3R*. We have not yet determined with certainty which, if either, of these elements causes the *mei-P44* phenotype.

Genomic accommodation to meiotic mutation

A striking characteristic of many of the achiasmate segregation mutants is that the severity of the mutant phenotype diminishes very rapidly when the mutations are kept in stock (Table 9). For example, nondisjunction in *mei-P19* resulted in 16.7% *X* nondisjunction ($n = 456$) shortly after its isolation, but when retested after ~15 mo in a balanced stock, the level had dropped to 5.6% ($n = 313$). In most or all cases, some level of phenotypic severity can be recovered after outcrossing. For example, the *mei-P26* nondisjunction phenotype had decreased from 18 to 9% when the mutant chromosome was maintained as a *C(1)DX* stock. When a recombinant that replaced the proximal half of the *X* chromosome was used in a retest, however, the level was again 18%. We believe that this phenotypic erosion is a unique characteristic of mutations that affect heterochromatin-based processes (see discussion).

TABLE 8

Segregation in mutants with unique phenotypes

Gamete types		Maternal genotype						
		mei-P2		*mei-P7*		*mei-P37*		*mei-P44:*
Maternal	Paternal	*X/X*	*+/Bal*	*X/X*	*X/Bal*	*X/X*	*X/Bal*	*X/Bal*
Normal								
X; 4	*XY; 44*	654	343	419	269	305	770	461
X; 4	*0; 44*	729	326	647	263	178	842	536
X ndj								
0; 4	*XY; 44*	28	16	5	11	0	39	7
XX; 4	*0; 44*	20	7	1	1	2	52	9
4 ndj								
X; 0	*XY; 44*	4	0	3	6	4	14	97
X; 0	*0; 44*	2	2	0	2	2	20	80
X; 44	*XY; 0*	0	0	5	0	0	43	241
X; 44	*0; 0*	0	0	0	1	1	57	298
X,4 ndj								
0; 0	*XY; 44*	1	0	2	12	1	0	4
XX; 44	*0; 0*	0	0	0	0	0	0	6
0; 44	*XY; 0*	0	0	0	0	0	1	3
XX; 0	*0; 44*	0	0	1	0	1	5	0
Total progeny		1438	694	1083	565	494	1843	1742
Adjusted total		1487	717	1092	589	498	1940	1771
% nullo-*X*		3.9	4.5	1.3	7.8	0.4	4.1	1.6
% diplo-*X*		2.7	2.0	0.4	0.3	1.2	5.9	1.7
% nullo-*4*		0.5	0.3	0.8	5.4	2.0	2.3	10.4
% diplo-*4*		0	0	0.5	0.2	0.2	5.3	31.5

DISCUSSION

Screen design: The general strategy for single *P*-element mutagenesis is to bring an ammunition chromosome carrying one or more genetically marked *P* elements into the same germline as a source of transposase, resulting in mobilization of the elements to other chromosomal sites (Cooley *et al.* 1988). The progeny of an appropriate cross are then screened for individuals that express the marker gene but received neither the ammunition chromosome (thus ensuring that inserts are new) nor the transposase source (thus ensuring stability of the new insert). The screens we used here combined several features that greatly increased their ease and perhaps their efficacy.

First, the use of a *P*-element construct carrying the w^{+mC} marker gene allowed us to select homozygotes for each of the new inserts on the basis of additive effects of w^{+mC} on eye color and without the use of balancers.

Second, the use of a scheme that prevented balancer chromosomes helped to prevent the recovery of mutations not associated with a *P* element. We wished to avoid "hit-and-run" events in which an element inserts into a gene of interest, excises in a subsequent cell division, and leaves a residual mutation. By removing the need to maintain our insertions over balancers, we allowed each insert to recombine freely with a normal sequence homologue for at least two generations before being tested. Thus, nonassociated mutations could recombine or segregate away from the *P* element. In addition, we routinely tested several individual females for meiotic defects. This strategy proved to be successful, because in each case tested (at least 10 of the 16 mutations listed in Table 2), the meiotic defect was found to be caused by the *P*-element insertion.

Finally, the use of a *P*{*lacW*} element on chromosome *4* as our ammunition allowed us to efficiently recover new inserts on *X*, *2*, or *3*. The absence of exchange on chromosome *4* allowed us to induce transposition in the female germline, yet exclude the ammunition chromosome (without the need for a balancer) by scoring only progeny that expressed a recessive marker present on the homologue (spa^{pol}). In screen 2, we used a *C(1)-DX* chromosome carrying *P*{*lacW*} as our ammunition. Again, we could select for transpositions that occurred in the female germline, this time eliminating the ammunition chromosome by scoring only male progeny.

Evaluating the success of the screen: We screened 18,558 homozygous viable and fertile *P*{*lacW*} insertion lines and recovered 16 strong meiotic mutations, a rate of 1 in 1160. Compared to *P*-element screens for some other complex, multigenic phenotypes, this is a low rate [*e.g.*, Castrillon *et al.* (1993) recovered insertions causing male sterility at a rate higher than 1 in 30]. However, we did identify at least 10 novel meiotic genes.

TABLE 9

Phenotypic amelioration in meiotic mutants

Line	Date	Test	*X* nondisjunction (*n*)
mei-P19	3/95	Original test	16.7 (456)
mei-P19	7/96	Retest	5.6 (513)
mei-P19	9/98	*mei-P19/l(3)03477*	8.6 (349)
mei-P21	5/95	Original test	21.4 (401)
mei-P21	1/96	Retest	12.4 (518)
mei-P24	8/95	Original test	32.2 (790)
mei-P24	7/98	Retest	4.3 (1161)
mei-P26	5/95	Original test	18.3 (404)
mei-P26	4/98	Retest from *C(1)DX* stock	9.2 (888)
mei-P26	11/98	*y w mei-P26/y w mei-P26 m f*	18.3 (689)

Continued characterization of these genes will provide a wealth of information on the meiotic process in Drosophila females, especially the segregation of achiasmate chromosomes. An understanding of this process is important, because failure to cross over is the primary cause of spontaneous nondisjunction in both flies and humans (Koehler *et al.* 1996; Lamb *et al.* 1996).

In addition, our screen uncovered novel meiotic phenotypes for six previously identified genes: *CycE*, *Trl*, *amn*, *push*, α*Tub67C*, and *ncd*. For four of these genes, no meiotic phenotype has been described previously, and for the remaining two, we discovered novel meiotic phenotypes.

At the outset, we recognized that the insertional specificity of *P* elements meant that we would not obtain insertions into some meiotic genes. We found, however, that the converse was also true: many of the mutants we recovered would not have been generated easily through chemical mutagenesis. For example, $CycE^{P2}$, $CycE^{P7}$, ncd^{P39}, and $\alpha Tub67C^{P40}$ are all special types of hypomorphic alleles obtained most easily by transposable element insertion.

Of the 15 loci identified in our screens, *P*-element insertions have been reported previously for only five [*push*, *CycE*, *Trl*, α*Tub67C*, and *l(3)03477*]. Hence, most of the loci we identified have not been identified in screens for *P*-element-induced sterility or lethality. In three of the five cases noted above [*CycE*, *Trl*, and *l(3)03477*], previously identified insertions have resulted in lethality or female sterility, and, thus, the meiotic roles of these genes would not have been detected easily.

In spite of the large size of our screen, we did not approach saturation of the genome. Indeed, we had only one case of more than one insertion into the same gene ($CycE^{P2}$ and $CycE^{P7}$), and in this case, the two insertions were at different sites and resulted in different phenotypes.

The paucity of recombination-defective mutants recovered in our screen: Although our aim was to design a screen that could recover many types of meiotic mutants, we were much more successful at recovering mutants with defects in the achiasmate segregation pathway than mutants with defects in recombination. Indeed, we recovered only two mutants that are clearly recombination defective. It is possible that we biased our screen against some recombination mutants by demanding a high level of nondisjunction, and some of the weaker mutants we recovered, which were not analyzed in depth, may suffer from mild defects in recombination. Another reason for the scarcity of recombination-defective mutations is that some recombination genes are required for fertility (Baker *et al.* 1978; Ghabrial *et al.* 1998; Sekelsky *et al.* 1998), which would preclude us from detecting them as meiotic mutants.

Nonetheless, we screened 2311 *X* chromosome *P*-element insertions and recovered only a single mutant, *mei-P26*, whereas Baker and Carpenter (1972) screened 209 EMS-treated *X* chromosomes and found five recombination-defective mutations severe enough to have been recovered in our protocol (one allele of *mei-218* and two alleles each of *mei-9* and *mei-41*). At least some of the difference between the screens stems from the insertional specificities of *P* elements. On a genomewide scale, *P* elements insert in a nonrandom fashion such that insertions occur frequently in some genes and rarely, if ever, in others. Indeed, among our 2311 *X*-linked insertions, we recovered at least five alleles of *singed* and four alleles of *scalloped*. Yamamoto *et al.* (1990) screened more than 50,000 *X* chromosomes from a *P*-element mobilization cross (without selecting for insert-bearing chromosomes) for mutagen hypersensitivity and recovered three *mei-9* alleles and two *mei-41* alleles.

In addition to the bias between genes, *P* elements display a bias within a given gene, frequently inserting into 5′ regulatory regions and promoters in sequences corresponding to 5′ untranslated regions or in sequences corresponding to introns (Spradling *et al.* 1995). Insertion of a *P*{*lacW*} or a similar construct into these regions does not infrequently result in a hypomorphic mutation. Of the three *mei-9* alleles recovered by

Yamamoto *et al.* (1990), only *mei-9*^{RT1} has a *P* element within the gene. This element is inserted into sequences corresponding to the 5′ untranslated region, and although it causes severe mutagen hypersensitivity, there are no observable effects on meiotic crossing over or segregation (Sekelsky *et al.* 1995; J. J. Sekelsky and R. S. Hawley, unpublished data). We would not have recovered such an allele in our screen, though it should be noted that the large size of *P*{*lacW*} would likely increase its mutagenicity relative to internally deleted *P* elements such as the one in *mei-9*^{RT1}.

Conversely, insertion of a *P* element into a protein-coding region is likely to cause an amorphic mutation, but this can also be a disadvantage in our type of screen. The *P*-element insertions in the two *mei-41* alleles recovered by Yamamoto *et al.* (1990) are within the coding region, but both result in female sterility and, therefore, would not have been identified as meiotic mutants in our screen. In contrast, the EMS-induced *mei-41* alleles of Baker and Carpenter (1972) are fertile and, therefore, hypomorphic.

Recombination-defective mutants: The stronger of our two recombination-defective mutants, *mei-P22*, eliminates meiotic intergenic exchange as well as simple gene conversion (McKim *et al.* 1998) and is, therefore, a "recombination null." Two other genes in this class are known, *mei-W68* and *c(3)G*. Mutations in these genes cause similar phenotypes, including an absence of meiotic recombination and increased mitotic recombination. Recombination nulls are believed to identify genes whose products are required at or before the initiation of meiotic recombination. In support of this, *mei-W68* was recently shown to encode the Drosophila homologue of Spo11p (McKim and Hayashi-Hagihara 1998), a *Saccharomyces cerevisiae* enzyme responsible for making the double-strand break that initiates recombination (Keeney *et al.* 1997). Unlike the case in yeast, however, both *mei-W68* and *mei-P22* females have normal synaptonemal complexes (McKim *et al.* 1998). Molecular characterization of *mei-P22* should provide new clues to the initiation of recombination in Drosophila.

In our other recombination-defective mutant, *mei-P26*, exchange is reduced to only 10 to 30% of wild-type levels. The effect is polar, with medial regions of each arm being more severely affected than proximal regions. This characteristic would classify *mei-P26* as a "precondition mutation" according to Baker and Carpenter (1972). It is unclear at present, however, whether *mei-P26* truly belongs to this class because our *P*-element allele is apparently hypomorphic. Preliminary results indicate that the *P*{*lacW*} element in *mei-P26* is inserted into the first intron of a gene encoding a RING finger protein (S. L. Page, K. S. McKim, B. Deneen and R. S. Hawley, unpublished data). This raises the intriguing possibility that *mei-P26* encodes a transcriptional regulator of meiosis. If this is the case, MEI-P26 would be the first transcriptional regulator of Drosophila meiosis to be identified.

Achiasmate chromosome segregation mutations: We recovered 13 mutants representing 12 genes impaired in the segregation of achiasmate chromosomes. Our success in recovering this type of mutant certainly results from our screening for *X* chromosome nondisjunction in females heterozygous for an *X* chromosome balancer. Although one-eighth of the lines we screened harbored *X* chromosome insertions, we did not recover any achiasmate segregation mutants from these, presumably because we could not screen them in the presence of an *X* balancer.

It may seem surprising that so many genes are required for a segregation pathway that is secondary to the recombination-based pathway in most cases. Many of these genes may have additional functions outside of meiotic chromosome segregation. Indeed, two of the mutations we recovered are in genes known to affect heterochromatin structure. *Trl* is a dominant enhancer of position-effect variegation (Dorn *et al.* 1993), and *CycE* mutations cause defects in nurse cell polytenization and heterochromatin replication (Lilly and Spradling 1996). The achiasmate homologous segregation system relies on heterochromatic homology (Hawley *et al.* 1992; Dernburg *et al.* 1996; Karpen *et al.* 1996), so perhaps some of our mutants are defective in heterochromatin structure and function.

Two large classes of achiasmate-specific mutants are those that are similar to *Axs*^{D} and those that more drastically affect the *X* chromosome, a novel class in Drosophila. Although we refer to this latter class as *X* chromosome specific, we do not know whether the effect is truly chromosome specific or based on other factors, such as chromosome size. We have not tested these mutations for effects on large autosomes.

Genomic accommodation to meiotic mutations: As noted above, the phenotypic severity of many of the achiasmate segregation mutants diminished rapidly while they were kept in stock. This is not a characteristic of Drosophila meiotic mutants in general, because *mei-9* and *mei-218* have retained their high nondisjunction levels for >25 yr. Nor is it a characteristic of all *P*-element-induced meiotic mutations, because the severe phenotype associated with *mei-P22* has not diminished in >3 yr in stock, even though these stocks tend to become homozygous for *mei-P22*. Similar phenotypic erosion has been observed for the EMS-induced mutation *Axs*^{D} (T. Arbel and R. S. Hawley, unpublished data), suggesting that this characteristic is common among achiasmate chromosome segregation mutants. There are exceptions to this generalization, however: *mei-P26*, which exerts strong effects on recombination, has diminished in severity while in stock, but α*Tub-67C*^{P40}, which does affect achiasmate chromosome segregation, has not.

One possible explanation invokes the relationship

between achiasmate chromosome segregation and heterochromatic pairing. Perhaps the diminishing severity is a characteristic of mutations that affect heterochromatic pairing, and it is a reflection of the plasticity of heterochromatin and the factors that regulate it. Such an effect could be caused by *cis*-acting changes in heterochromatin (the sequence, arrangement, or higher-order structure), or by changes in *trans*-acting factors (the many genes that regulate heterochromatin structure and function). The effect cannot be limited to *cis*-acting changes because replacement of the *X* with chromosomes that have never been exposed to the mutation being tested does not entirely restore the original nondisjunction level (data not shown). Rather, we see a gradual change in phenotypic severity with additional generations of outcrossing (data not shown), indicating that numerous "suppressors" are located throughout the genome and that they act additively.

Conclusions: We screened >18,500 inserts and recovered 16 strong meiotic mutants. We did not saturate the genome for *P*-mutable meiotic genes, because there was only one gene in which we obtained more than one insertion (*CycE*, with two mutations with different phenotypes). Nonetheless, we recovered many different classes of meiotic mutants, several of which had not been described previously. Further genetic and molecular characterization of these mutations is sure to advance our understanding of meiosis in metazoan females.

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