THE UTILIZATION DURING MITOTIC CELL DIVISION OF LOCI CONTROLLING MEIOTIC RECOMBINATION AND DISJUNCTION IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

To inquire whether the loci identified by recombination-defective and disjunction-defective meiotic mutants in Drosophila are also utilized during mitotic cell division, the effects of 18 meiotic mutants (representing 13 loci) on mitotic chromosome stability have been examined genetically. To do this, meiotic-mutant-bearing flies heterozygous for recessive somatic cell markers were examined for the frequencies and types of spontaneous clones expressing the cell markers. In such flies, marked clones can arise *via* mitotic recombination, mutation, chromosome breakage, nondisjunction or chromosome loss, and clones from these different origins can be distinguished. In addition, meiotic mutants at nine loci have been examined for their effects on sensitivity to killing by UV and X rays.—Mutants at six of the seven recombination-defective loci examined (*mei-9*, *mei-41*, *c(3)G*, *mei-W68*, *mei-S282*, *mei-352*, *mei-218*) cause mitotic chromosome instability in both sexes, whereas mutants at one locus (*mei-218*) do not affect mitotic chromosome stability. Thus many of the loci utilized during meiotic recombination also function in the chromosomal economy of mitotic cells.—The chromosome instability produced by *mei-41* alleles is the consequence of chromosome breakage, that of *mei-9* alleles is primarily due to chromosome breakage and, to a lesser extent, to an elevated frequency of mitotic recombination, whereas no predominant mechanism responsible for the instability caused by *c(3)G* alleles is discernible. Since these three loci are defective in their responses to mutagen damage, their effects on chromosome stability in nonmutagenized cells are interpreted as resulting from an inability to repair spontaneous lesions. Both *mei-W68* and *mei-S282* increase mitotic recombination (and in *mei-W68*, to a lesser extent, chromosome loss) in the abdomen but not the wing. In the abdomen, the primary effect on chromosome stability occurs during the larval period when the abdominal histoblasts are in a nondividing (G2) state.—Mitotic recombination is at or above control levels in the presence of each of the recombination-defective meiotic mutants examined, suggesting that meiotic and mitotic recombination are under separate genetic control in Drosophila.—Of the six mutants examined that are defective in processes required for regular meiotic chromosome segregation, four (*l(1)TW-6^{cs}*, *cand*, *mei-S332*, *ord*) affect mitotic chromosome

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behavior. At semi-restrictive temperatures, the cold sensitive lethal *l(1)TW-6^{cs}* causes very frequent somatic spots, a substantial proportion of which are attributable to nondisjunction or loss. Thus, this locus specifies a function essential for chromosome segregation at mitosis as well as at the first meiotic division in females. The patterns of mitotic effects caused by *cand*, *mei-S332*, and *ord* suggest that they may be leaky alleles at essential loci that specify functions common to meiosis and mitosis. Mutants at the two remaining loci (*nod*, *pal*) do not affect mitotic chromosome stability.

THE past decade has witnessed a concerted effort to dissect genetically the control of meiotic chromosome behavior in *Drosophila melanogaster*. Mutants have been isolated at approximately 40 loci that disrupt processes necessary for normal meiotic chromosome behavior. Previous studies of these meiotic mutants have focused on delineating the nature of the functions carried out by their wild-type alleles in insuring a normal meiosis (for reviews see BAKER *et al.* 1976a; BAKER and HALL 1976; LINDSLEY and SANDLER 1977; SANDLER and LINDSLEY 1974; SANDLER *et al.* 1972).

These studies have shown that a large majority of meiotic mutants are defective in processes essential for the first meiotic division and affect that division in only one sex, suggesting that the events of the first meiotic division are mediated by different sets of genes in males and females. Thus, divergence at the level of gene control parallels the long-recognized sexual differences in the events of the first meiotic division: recombination, distributive disjunction and synaptonemal complex formation are restricted to meiosis in females. A smaller number of mutants are defective in processes required for the second meiotic division, and all of these mutants affect both sexes, indicating that these events are common to both sexes. Mutants affecting the first meiotic division in females fall, with but one exception (MASON 1976), into two broad categories: (a) recombination-defective mutants —mutants in which the frequency and/or distribution of exchange is abnormal and (b) disjunction-defective mutants—mutants that have lesions in non-exchange processes required for normal meiotic chromosome segregation. Although all recombination-defective mutants also cause elevated frequencies of chromosomal nondisjunction and loss, this nondisjunction and loss is attributable to the normal behavior of nonexchange chromosomes, which are more frequent in these mutants (BAKER and CARPENTER 1972; HALL 1972; BAKER and HALL 1976).

In this study we have asked whether these meiotic loci are also required for normal chromosomal behavior during mitotic cell division. With respect to loci identified by recombination-defective meiotic mutants, there are two biological reasons why at least some of these loci might be expected to function in somatic cells. First, crossing over occurs not only during female meiosis, but also (although at a vastly lower rate) during mitotic cell division in both sexes. Each major chromosome arm, which has an average of one exchange per meiosis, has approximately 6×10^{-5} spontaneous exchanges per somatic cell division. The basic properties of mitotic exchange are similar to those of meiotic exchange in that mitotic exchange occurs at the four-strand stage, occurs only between

homologues and is reciprocal (STERN 1936). More recently, it has been shown that intragenic mitotic exchanges occur and that such exchanges can generate wild-type recombinants between heteroallelic mutants (STERN 1969; TOKUNAGA 1973; KELLY 1974). Furthermore, there is an interchromosomal effect of inversions on mitotic recombination (RONEN 1964) as there is meiotically (reviewed by LUCCHESI and SUZUKI 1968). By these criteria, mitotic and meiotic exchange are indistinguishable. Whether these similarities reflect a common genetic control is not known.

Mitotic and meiotic exchange differ not only in frequency, but also in the distributions of events along the chromosome: mitotic exchange is approximately proportional to physical distance (GARCIA-BELLIDO 1972; BECKER 1976), whereas meiotic exchange is relatively infrequent in proximal euchromatin and absent in heterochromatic regions. Since the mitotic map is very similar to the meiotic map obtained from the residual recombination that occurs in the presence of some recombination-defective meiotic mutants, it has been suggested that the difference between these two processes might result from the lack of involvement of these meiotic loci in mitotic exchange (CARPENTER and BAKER 1974).

Few experiments have been reported that compare the genetic control of mitotic and meiotic exchange. Minute mutants have been reported to increase mitotic crossing over in the arm on which they are present (STERN 1936; KAPLAN 1953), but do not alter meiotic crossing over. This effect of Minutes on mitotic recombination is, however, likely an artifact since clones arising from mitotic exchanges in these circumstances are larger than normal and thus more easily recognized (FERRUS 1975; MORATA and RIPOLL 1975). The one recombination-defective meiotic mutant whose effect on mitotic crossing over has been examined, *c(3)G*^{17}, was reported not to alter mitotic exchange (LECLERC 1946).

The second reason for inquiring whether loci identified by recombination-defective meiotic mutants function in somatic cells is that there is evidence in other organisms for common functions. Mutational analyses in prokaryotes have shown that the processes of recombination, DNA replication and repair utilize, in part, overlapping sets of gene functions (CLARK 1973; RADDING 1973; HANAWALT and SETLOW 1975). In eukaryotes, evidence that some genes are utilized in both meiotic and mitotic aspects of the chromosome cycle is available in maize (BEADLE 1932, 1937) and some fungal species (reviewed by BAKER *et al.* 1976a). In addition, there are limited data suggesting that some of the human genetic disorders causing increased mitotic chromosome instability may also confer sterility, implying a possible meiotic as well as mitotic function for these loci (for review see BAKER *et al.* 1976a).

An additional overlap in the genetic control of meiosis and mitosis would be expected in the functions mediating chromosome segregation. Disjunction-defective meiotic mutants recovered from conventional screens would be *a priori* unlikely candidates for defects in mitotic segregation functions since these screens require that a mutant be homozygous viable in order to be tested. However, one disjunction-defective meiotic mutant, *l(1)TW-6*^{cs} (initially recovered as a cold

temperature-sensitive lethal), is possibly defective in such a function (WRIGHT 1974). *l(1)TW-6^{cs}* disrupts chromosome segregation at the first meiotic division in females, but does not affect the first meiotic division in males or the second meiotic division in either sex. WRIGHT (1974) reports that *l(1)TW-6^{cs}* embryos developing under conditions where the temperature-sensitive lethality is expressed showed "abnormal mitotic figures, including multipolar spindles, in the majority of embryos inspected".

To inquire systematically whether the functions specified by meiotic loci in Drosophila are also utilized during mitotic cell division, the effects of 18 meiotic mutants, representing 13 loci, on mitotic chromosome stability have been examined genetically. To do this, we constructed meiotic-mutant-bearing flies that are heterozygous for recessive somatic cell markers (in this case, mutants affecting the color or morphology of the bristles and hairs of the cuticle). In such flies, clones of cells expressing these cell markers can arise through mitotic recombination, mutation, chromosome loss, nondisjunction or chromosome breakage (Figure 1). Thus, by examining the frequencies of such clones, effects of meiotic mutants on mitotic chromosome stability can be detected. Moreover, as discussed below, clones produced by each of the above mechanisms have distinctive properties, and thus, in those instances where instability is detected, the nature of the events producing instability can be inferred. In addition, some of these as well as other meiotic mutants have been studied with respect to their effects on sensitivity to killing by X-rays and ultraviolet (UV) light. A preliminary report of some of this work has been published (BAKER *et al.* 1976b).

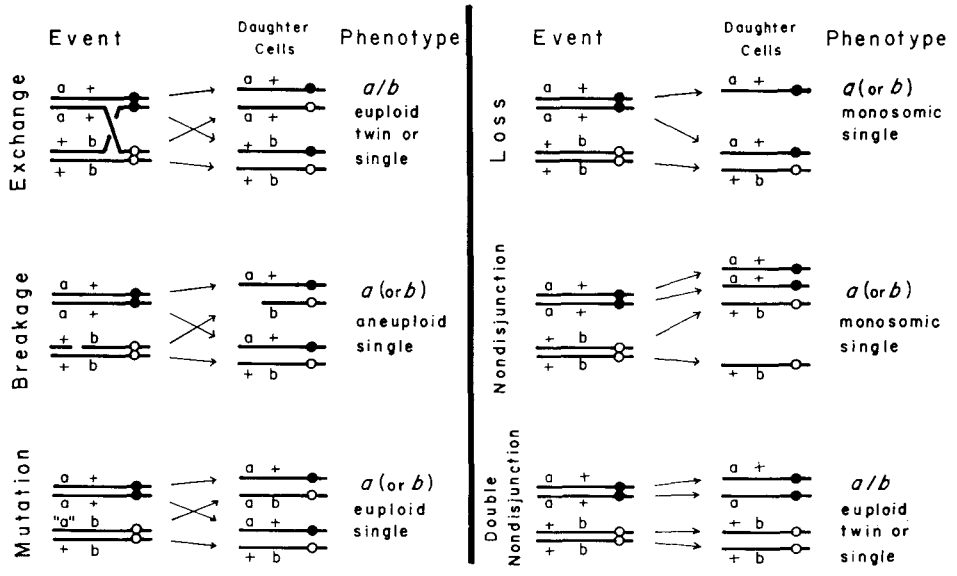

FIGURE 1.—Origins of marked clones in flies heterozygous for recessive cell markers (*a*, *b*).

MATERIALS AND METHODS

All flies were reared on standard Drosophila corn meal-molasses-brewers yeast-agar medium at 25° (unless noted otherwise). With the exceptions of the multiply inverted balancer chromosome *FM7* (MERRIAM 1968) and most meiotic mutants, descriptions of the mutants and chromosomes used in this study may be found in LINDSLEY and GRELL (1968). The meiotic mutants we have utilized and a summary of their characteristics are presented in Table 1. To confirm the presence of the appropriate meiotic mutant(s) in flies used to examine somatic chromosome stability and radiation sensitivity, sibs of these flies (or sibs of parents where appropriate) were crossed so as to ascertain the presence of the mutant by its effect on meiotic chromosome segregation.

Mitotic chromosome stability: The effects of meiotic mutants on the mitotic stability of both the *X* and third chromosome in the cells that produce the abdominal cuticle was examined. The stability of the third chromosome was also examined in the cells that produce the wing blade. *X*-chromosome stability was examined in flies that were heterozygous for the cell markers yellow (*y*) and forked (*f*) (Figure 2; *y* and *f* affect the color and morphology of bristles [chaetae], respectively). Third-chromosome stability was examined using the cell markers yellow (absence of *Dp(1;3)sc^{J4}*, *y+* in *y/y* or *y/Y* flies), multiple wing hairs (*mwh*) and javelin (*jv*) (Figure 2; *jv* affects chaeta shape; *mwh* produces two or more hairs [trichomes] per cell in the wing [wild type has one trichome per wing cell] but in the abdomen *mwh* deranges the normal pattern of trichomes without changing the number per cell). Some of the mutants have been examined for the presence of somatic nondisjunction using the centromere-spanning markers *Dp(1;3)sc^{J4}*, *y+ mwh* and *Ki* (Kinked, dominant bristle morphology marker). Flies homozygous for meiotic mutants were the progeny of heterozygous/hemizygous parents.

Flies to be scored for clones were preserved in three parts 70% ethanol and one part glycerol. Abdomens were prepared for scoring by dissection from the carcass, followed by cooking for 90 to 120 sec in 10% KOH at a temperature just below boiling. The KOH was replaced with water and the abdomens were flattened with forceps to expel soft tissues. They were then cooked an additional minute in 10% KOH then were placed in water, evisceration was completed, and the abdomens were cooked for five minutes in water. Water was replaced by passage through 95% ethanol, 100% ethanol and n-propanol, and the abdomens were mounted on glass slides in Euparal. Clones were scored in the second through sixth abdominal tergites at a magnification of 160–240× with bright-field optics and a blue filter. Since descendants of a single cell do not remain closely juxtaposed during the growth of abdominal histoblasts (GARCIA-BELLIDO and MERRIAM 1971b), all marked cells found in a hemitergite were recorded as products of the same event. The chaeta markers *y*, *f^{36a}*, *jv*, *Ki+*, and *Sb+* can be accurately scored in single cells of the abdomen; however, small *mwh* clones are not reliably identifiable and therefore only large *mwh* clones (greater than *ca.* 15 cells) were recorded.

For scoring *mwh* clones in the wing blade, wings were washed free of preservative, placed on a slide in n-propanol and mounted in Euparal. Wings were scored at a magnification of 400× with bright-field optics and a blue filter for the presence of *mwh* clones. Since *mwh* marks each cell in the wing blade and clonally related cells remain contiguous during development of the wing, the size of clones can be accurately determined here (GARCIA-BELLIDO and MERRIAM 1971a; BRYANT 1970).

Radiation-induced clones: To determine the size and pattern of marked clones arising at known times during development, we have repeated some of the experiments of GARCIA-BELLIDO and MERRIAM (1971a, b), BRYANT (1970), and GUERRA, POSTLETHWAIT and SCHNEIDERMAN (1973) and induced *mwh* clones in wings of *y/y*; *Dp(1;3)sc^{J4}*, *y+ mwh/jv* females and *y* and *f^{36a}* clones in abdomens of *y/f^{36a}* females by irradiating larvae and pupae at different stages of development. Cultures containing larvae of all ages or collections of pupae of known age were irradiated with 1000R from a ^{60}Co source. The age of larvae at the time of irradiation was determined following the procedure of GARCIA-BELLIDO and MERRIAM (1971b) by collecting pupae from the irradiated cultures at eight hr intervals.

TABLE 1

Meiotic characteristics of the mutants studied

Meiotic process(es) defective in mutant	Mutant	Recombination frequency relative to control	Linkage group and map position	Major references
I. Female meiosis				
A. Recombination				
1. exchange precondition: altered distribution of exchanges	*mei-218*	0.08	*1*–57	BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974
	mei-218$^{6-7}$	0.08		unpublished—CARPENTER and BAKER
	mei-41	0.50	*1*–54	BAKER and CARPENTER 1972
	mei-41^{195}	0.65		BAKER and CARPENTER 1972
	mei-S282	0.35–0.68	*3*–5	PARRY 1973
	mei-W68	0	*2*–94	unpublished—BAKER
	mei-W68^{L1}	0.63		unpublished—LINDSLEY
	mei-352	0.98	*1*-unlocated	BAKER and CARPENTER 1972
	mei-251	0.82	*1*-unlocated	BAKER and CARPENTER 1972
	c(3)G^{68}	0	*3*–57.4	HALL 1972
	c(3)G^{17}	0		HALL 1972
	Df(3)sbd^{105}	0.66(as heterozygote)		HALL 1972
2. exchange: normal distribution of exchanges	*mei-9*	0.08	*1*–6	BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974
	mei-9^{b}	0.16		
B. Disjunction, meiosis I	*cand*	~1	*3*–100.7	DAVIS 1969
	l(1)TW-6^{cs}	1 (as heterozygote)	*1*–37	WRIGHT 1974
	nod	0.98	*1*–36	CARPENTER 1973
II. Male meiosis	*pal*	0.90	*2*–35.7	BAKER 1975
III. Male and female meiosis				
A. Recombination, disjunction: meiosis I and II	*ord*	0.06	*2*–103.5	MASON 1976
B. Disjunction, meiosis II	*mei-S332*	1.00	*2*–95	DAVIS 1971

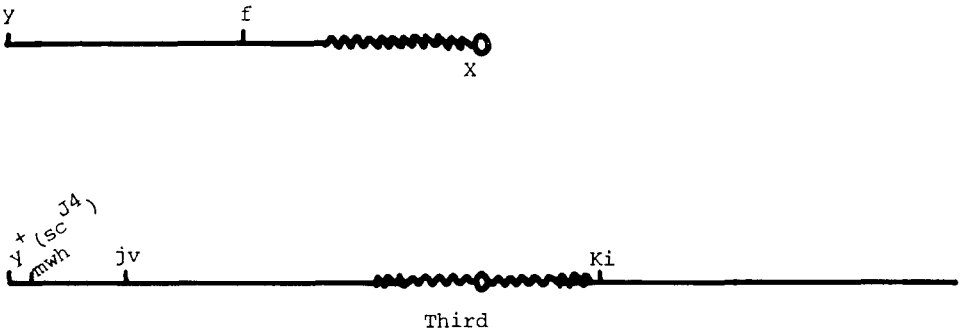

FIGURE 2.—Physical location on mitotic chromosomes of cell markers used to examine mitotic chromosome stability.

Radiation sensitivity: The effects of *X*-linked meiotic mutants on sensitivity to killing by UV- and X-irradiation were assessed by irradiating larvae produced by a cross of *y mei-*/y^+Y; spa^{pol}/spa^{pol} males to *C(1)DX, y f bb*$^-$/y^+Y; spa^{pol}/spa^{pol} females. The progeny from this cross are attached-*X* females that carry no meiotic mutant and males that are hemizygous for the meiotic mutant. Thus the nonmeiotic mutant females serve as an internal control against which the survival of males bearing the meiotic mutant is measured. To obtain larvae for irradiation, several thousand parents were placed in a plexiglass population cage, and eggs laid during 12-hr intervals were collected on 14 cm petri plates containing standard Drosophila food spread with a paste of live yeast. The first few egg collections were discarded to minimize the problem of females retaining fertilized eggs. Larvae were grown in the yeast paste on these plates until harvested for irradiation.

Survival curves following UV irradiation were determined for both first-instar larvae (33 ± 6 hr after egg collection) and third-instar larvae (102 ± 6 hr after egg collection). Larvae to be irradiated were washed and evenly dispersed on 12 cm filter paper disks by suspending them in water and collecting them on filter paper in a Büchner funnel. Larvae were transferred on the filter paper to a UV chamber and irradiated with UV germicidal lamps (GE bulb G8T5). UV dose was measured with an International Light Germicidal/Erythemal Radiometer IL570. Dose rates were 10 to 40 erg/mm^2/sec. After irradiation, the filter paper and larvae were transferred to quarter-pint bottles containing food, and emerged adults were counted through day 17 of culture. From the time of irradiation until adults were scored, cultures were maintained in the dark or in yellow light to prevent photoreactivation.

For determining the effect of meiotic mutants on X-ray sensitivity, larvae (90 ± 6 hr after egg deposition) were harvested from petri plates, and aliquots were placed in cloth-covered Stender dishes containing a heavy yeast paste. These larvae were X rayed with either a GE Orthovoltage X-ray unit (280 KV, 15 mA, 1.5 mm Cu filtration, 149 r/min) or a GE Maximar 250 (250 KV, 15 mA, 0.8 mm Cu filtration, 100 r/min). Since results with these two X-ray sources did not differ significantly, they have been pooled. After irradiation, larvae were washed free of yeast and placed in quarter-pint bottles. Emerged adults were scored through day 17 after egg deposition. For both UV and X-ray survival curves, 1000 to 2000 progeny were counted in the unirradiated samples, and at least comparable numbers of larvae were irradiated at each dose. All survival curves have been determined at least twice.

RESULTS

Recombination-defective mutants: general observations

Twelve recombination-defective meiotic mutants, representing seven loci, have been examined for their effects on the frequency of spontaneous mitotic chromo-

some instability. These particular mutants were chosen for study because they include representatives of all of the known types of defects in meiotic recombination and they produce the most severe disruptions of meiotic recombination available in these classes (Table 1). We will first present some general observations about the somatic roles of these loci and then consider in detail the effects of individual loci.

The frequency of clones of cells expressing recessive cell markers (conventionally termed spots) was examined in the abdominal tergites of *y/y*; *Dp(1;3)sc*^{J4}, *y*$^{+}$ *mwh/jv* females homozygous for recombination-defective mutants. These data show that in flies bearing mutants at six of the seven loci the frequency of spots was higher than in controls (Figure 3, Table 2). Only mutants at the *mei-218* locus had no effect on the frequency of spots. This suggests that the wild-type alleles of the six loci (*mei-41*, *mei-9*, *mei-352*, *mei-W68*, *mei-S282*,

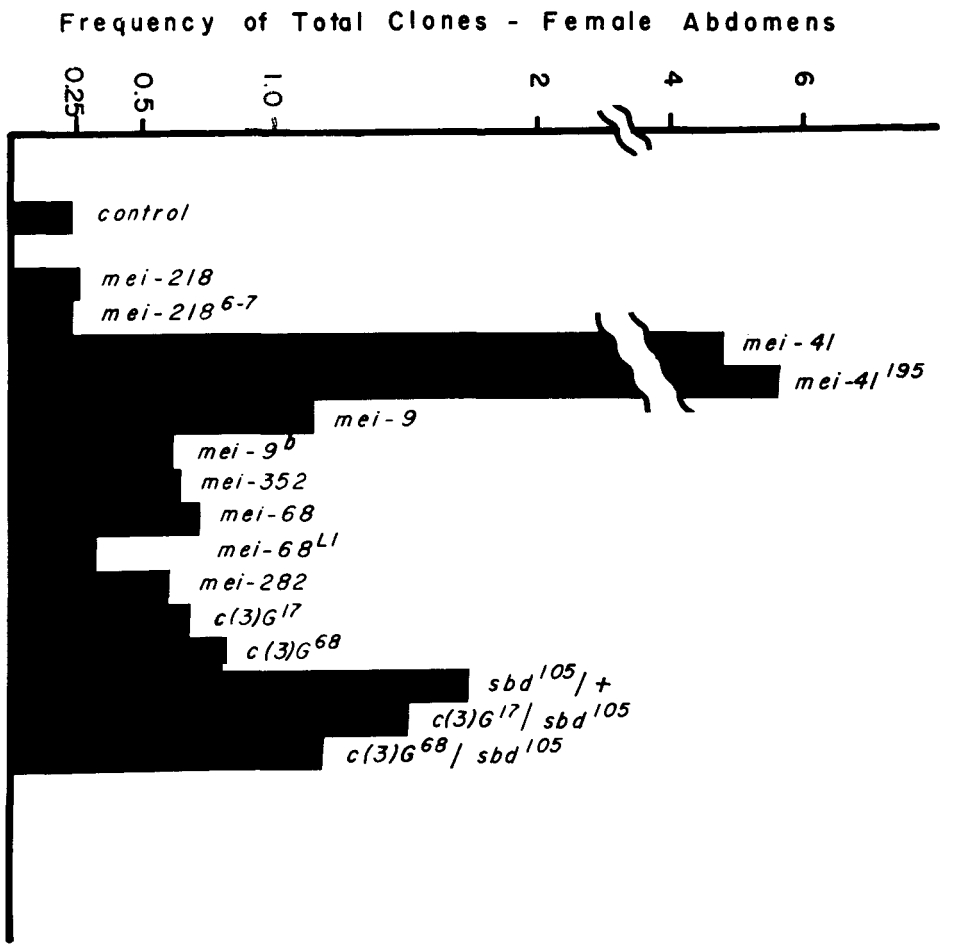

FIGURE 3.—Total frequencies of clones in abdomens of *y/y*; *Dp(1;3)sc*^{J4}, *y*+ *mwh/jv* females carrying recombination-defective meiotic mutants.

TABLE 2

Frequencies and types of clones in abdomens of y/y; Dp(1;3)sc^{J4}, y+ mwh/jv *females carrying the indicated meiotic mutant*

Bristle No./clone	*y* 1	*y* ≥2	*y jv* 1	*y jv* ≥2	*y//mwh* 1	*y//mwh* ≥2	*y jv//mwh* 1	*y jv//mwh* ≥2	*y+y jv*	*mwh*	y^{A*}†	*jv**	Male clones*	Total clones	Number abdomens	Frequency per abdomen of: Single	Twin	Total
Meiotic mutants																		
+/+	42(11)‡	4(3)	21(7)	4(1)	6	5	4	11	0	2	6	0	1	121	521	0.18	0.05	0.23
41/41	210(61)	73(24)	145(140)	48(29)	5	2	6	5	83	9	166	13	0	840	176	4.67	0.10	4.77
$41^{195}/41^{195}$	152(57)	51(11)	133(86)	58(28)	2(1)	12	5(2)	5(1)	123	7	94	3	0	734	127	5.56	0.22	5.78
9/9	72(33)	12(3)	55(39)	8(4)	5(1)	7	8(1)	11(1)	4	14	29	7	1	278	243	1.00	0.14	1.14
$9^{b}/9^{b}$	43(7)	4	19(37)	3(3)	1(1)	2	3(1)	9	0	1	29	0	0	134	218	0.54	0.08	0.62
218/218	9(2)	6	13(1)	3	1	6	1	6	0	3	0	0	1	51	193	0.19	0.07	0.26
$218^{6-7}/218^{6-7}$	26(8)	8	17(2)	4(1)	0	10	3	14	0	1	0	1	0	94	406	0.16	0.07	0.23
352/352	22(10)	10(2)	33(22)	4(5)	3(1)	6	3	5	2	3	15	2	1	131	204	0.55	0.09	0.64
W68/W68	32(7)	18(3)	31(7)	9(2)	3	3	7	13	2	17	14	4	17	154	211	0.61	0.12	0.73
$W68^{L1}/W68^{L1}$	19(5)	10(1)	14(1)	4	4	1	0	6	1	6	5	0	13	72	221	0.28	0.05	0.33
S282/S282	46(12)	23(6)	—	—	9	29	—	—	—	11	16	—	5	136	231	0.42	0.17	0.59
$c(3)G^{68}/c(3)G^{68}$	44(10)	31(4)	33(5)	3(2)	6	12	9(1)	19	1	11	8	2	0	191	238	0.60	0.20	0.80
$c(3)G^{17}/c(3)G^{17}$	35(11)	13(2)	11(10)	9(3)	2	5	4	7	1	20	13	0	12	133	198	0.58	0.09	0.67
$sbd^{105}/+$§	215(7)	69	—	—	6	21	—	—	—	5	9	—	1	323	190	1.56	0.14	1.70
$sbd^{105}/c(3)G^{68}$§	108(2)	33(1)	—	—	4	13	—	—	—	4	3	—	2	165	142	1.04	0.12	1.16
$sbd^{105}/c(3)G^{17}$§	180(7)	76(4)	—	—	8	11	—	—	—	5	8	—	3	291	195	1.39	0.10	1.49
nod/nod	9(1)	2	7	2	0	2	0	3	0	0	0	0	0	26	155	0.14	0.03	0.17
pal/pal	8(1)	1(1)	5(2)	1	1	1	0	4	0	1	8	0	0	27	142	0.15	0.04	0.19
ord/ord	20(5)	14(2)	5(6)	4	2	9	4	7	0	2	7	6	0	80	66	0.88	0.33	1.21
ca^{nd}/ca^{nd}	18(17)	3(2)	18(9)	7	1	5	5	9	0	15	3	3	2	109	255	0.35	0.08	0.43
l(1)TW-6^{cs}/ l(1)TW-6^{cs}	24(2)	6(1)	8(6)	5(1)	0	0	0(1)	6	0	3	12	0	1	63	100	0.56	0.07	0.63

* These clones are excluded from calculations of clone frequencies.

† y^{A} = severely abnormal *y* bristles.

‡ Numbers in parentheses are number of clones with short, thin (Minute-like) bristles; clones not in parentheses have normal bristles. For tergites with both normal *y* and short thin *y* bristles, clones are recorded as the majority type.

§ These flies not heterozygous for *jv*.

TABLE 3

Frequencies and types of clones in abdomens of y/Y; Dp(1;3)sc^{J4}, y mwh/jv *males carrying the indicated mitotic mutants*

Bristle No./clone	*y* 1	*y* ≥2	*y jv* 1	*y jv* ≥2	*y//mwh* 1	*y//mwh* ≥2	*y jv//mwh* 1	*y jv//mwh* ≥2	*y+y jv*	*mwh*	*y*^{A*}†	*jv**	Total clones	Number abdomens	Frequency per abdomen of: Single	Twin	Total
Meiotic mutants																	
+	16(3)‡	3	12(3)	2(1)	6	2	4	4	0	2	1	0	58	309	0.14	0.05	0.19
41	73(12)	17(1)	57(29)	15(5)	1	4	1	1	25	3	25	0	244	54	4.39	0.13	4.52
41^{195}	67(41)	23(4)	68(36)	17(8)	3	4	3	4	48	1	34	0	336	77	4.18	0.18	4.36
9	23(10)	0	37(6)	2(1)	3	0	2	3	0	3	10	1	90	121	0.68	0.07	0.74
9^{b}	32(6)	4	24(12)	0	0	3	3	6	0	3	6	0	93	124	0.65	0.10	0.75
218	5(2)	1	5	1	0	1	2	5	0	0	0	0	22	133	0.11	0.06	0.17
218$^{6-7}$	16	4	3(3)	1	2	0	2	3	0	2	2	0	36	151	0.19	0.05	0.24
352	21(11)	1	11(7)	4(1)	1	2	2	1	0	1	3	0	63	118	0.48	0.05	0.53
W68/W68	37(5)	10	13(5)	6(1)	5	3	4	5	0	2	5	1	96	180	0.44	0.09	0.53
W68^{L1}/*W68*^{L1}	4(1)	2	4(2)	7	2	1(1)	0	3	0	0	0	0	27	131	0.15	0.05	0.21
S282/S282§	32(7)	8	—	—	6	11	—	—	—	4	5	—	68	169	0.30	0.10	0.40
c(3)G^{68}/*c(3)G*^{68}	11(1)	10(1)	7(2)	2(1)	2	1	2	2	1	2	3	0	45	83	0.46	0.08	0.54
c(3)G^{17}/*c(3)G*^{17}	11(3)	4(1)	3(1)	1(1)	1	0	1	1	0	1	4	0	29	107	0.24	0.03	0.27
sbd^{105}/+§	130	57(1)	—	—	1	5	—	—	—	1	2	—	195	104	1.82	0.06	1.88
sbd^{105}/*c(3)G*^{68}§	129(6)	36(1)	—	—	3	2	—	—	—	5	2	—	182	108	1.64	0.05	1.69
sbd^{105}/*c(3)G*^{17}§	154(4)	65	—	—	10	14	—	—	—	0	4	—	247	131	1.70	0.18	1.88
nod	4(4)	1	1(1)	0	4	0	0	3	0	1	3	0	19	182	0.07	0.04	0.10
pal/pal	11(2)	1	4(2)	1	0	0	0	3	0	0	2	0	24	102	0.21	0.03	0.24
cand/*ca*nd	6(4)	2	5(1)	0	2	0	2	2	0	1	7	0	25	110	0.17	0.06	0.23
l(1)TW-6^{cs}	7(4)	2	5(1)	2	0	1	2	1	0	1	2	0	26	78	0.28	0.05	0.33

*, †, ‡, § = See Table 2.

c(3)G) that do increase the frequency of spots specify functions that are required for normal chromosomal stability in mitotic cells of females; the expression of the *mei-218* locus, on the other hand, may be restricted to female meiosis.

These mutants do not affect male meiosis. To inquire whether they affected chromosome stability during mitotic cell division in males the frequency of spontaneous spots in the abdominal tergites of y/Y; *Dp(1;3)sc*^{J4}, y^+ *mwh/jv* males hemizygous or homozygous for these recombination-defective mutants was examined. The frequency of spots is increased in males carrying mutants

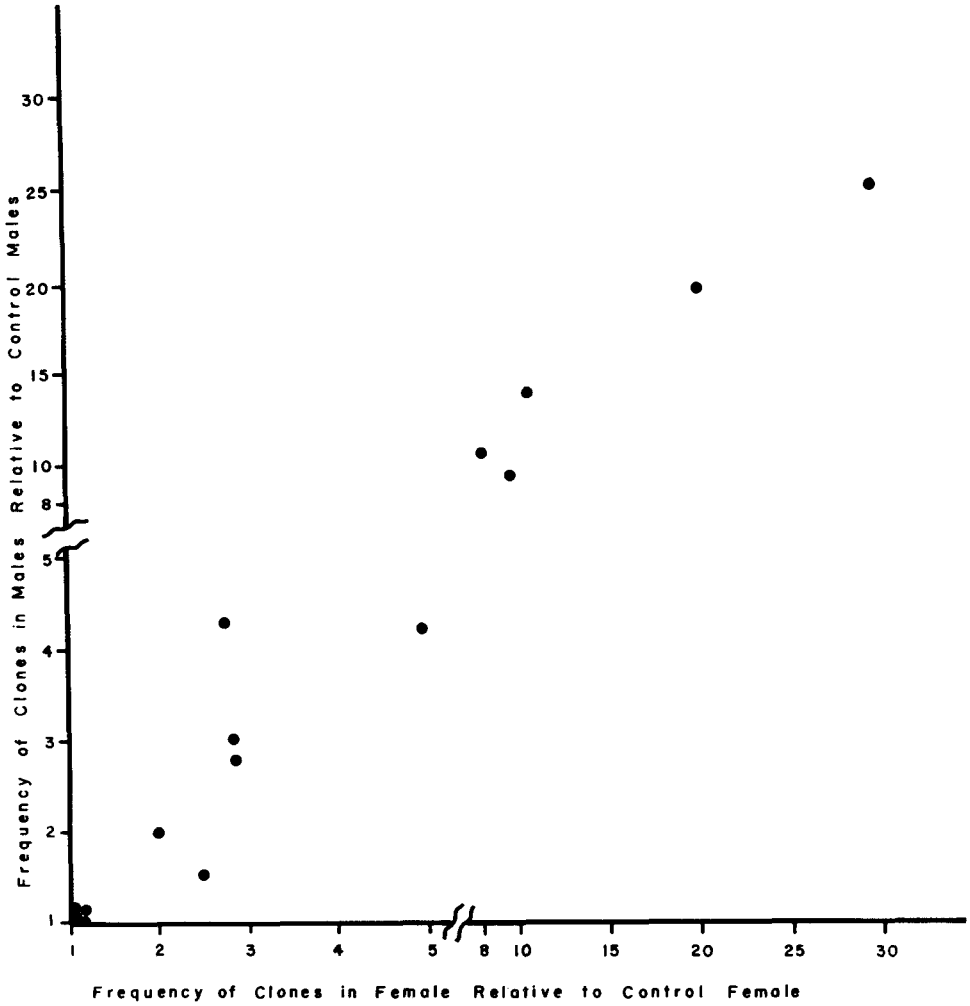

FIGURE 4.—Effects of recombination-defective meiotic mutants in males and females. Points are number of clones per abdomen in meiotic-mutant-bearing males carrying the markers y/Y; *Dp(1;3)sc*^{J4}, y^+ *mwh/jv* relative to control males plotted against the number of clones per abdomen of females carrying the same meiotic mutant and cell markers relative to control females.

at the same six loci that affected the frequency of spots in females (Table 3). A comparison of the total spot frequencies in males and females shows that the effect of each mutant is comparable in the two sexes (Figure 4). Thus, the *mei-41*, *mei-9*, *mei-352*, *mei-S282*, *mei-W68* and *c(3)G* loci specify functions essential for the maintenance of chromosomal integrity in the somatic cells of both sexes, as well as recombination during female meiosis.

For mutants at five of these loci (*mei-41*, *mei-9*, *mei-W68*, *mei-S282* and *c(3)G*), we have also examined their effects on the mitotic stability of *X* chromosomes in y/f^{36a} females. All of the mutants that increase third-chromosome instability also increase the frequency of spots expressing the *X*-linked cell markers *y* and f^{36a} (Table 4). From this we infer that the wild-type alleles of these loci specify functions that are essential for the integrity of the entire genome in mitotic cells, just as they are essential for recombination throughout the genome during female meiosis. Thus, these loci would appear not to belong to the category of genetic variants that control the structure or behavior of localized chromosomal regions, *e.g.*, the uncoiler locus in humans (YING and IVES 1968) and *cog* mutants in Neurospora (CATCHSIDE and ANGEL 1974), but rather to produce products that function in the chromosomal metabolism of the whole genome.

The progenitor cells of the abdominal tergites in which the marked clones scored in the above experiment arose have proliferation dynamics that are quite distinct from those of other imaginal tissues in Drosophila. In the embryo, groups of about eight cells, the abdominal histoblasts, are set aside as the progenitor cells for each hemitergite. The abdominal histoblasts do not divide during the approximately four-day larval period and are inferred to be resting in G2, since mitotic exchange can be induced throughout this period (GARCIA-BELLIDO and MERRIAM 1971b). At about the time of pupation, these cells begin a rapid series of divisions—nine during a 24-hour period (GARCIA-BELLIDO 1973)—to produce the cells that give rise to the abdominal tergites. In contrast, the cells of most prospective imaginal tissues divide more or less continuously throughout the larval and early pupal period, with cell cycles in the neighborhood of six to 15 hours (for review see NÖTHIGER 1972).

To inquire whether mitotic chromosome stability in a more typically growing population of imaginal cells is also dependent on the wild-type allele of recombination-defective meiotic mutants, the frequency of *mwh* clones in the wing blades of y/y; $Dp(1;3)sc^{J4}$, y^+ *mwh/jv* females homozygous for recombination-defective mutants was examined (Table 5). To enhance the validity of comparing effects in wing and abdomen imaginal cells, the abdomens of these females were also scored for spots and make up part of the data reported in Table 2. The two *mei-218* alleles, which do not affect the frequency of spots in abdomens, also have little, if any, effect on the amount of chromosome instability in the wing disc. All mutant alleles of the *mei-9*, *mei-41*, *mei-352*, and *c(3)G* loci examined caused an increased frequency of spots in the wing as well as in the abdomen. Since these four loci affect chromosome behavior in wing and abdomen imaginal cells, as well as meiotic recombination, we infer that these loci specify products

TABLE 4

Frequencies and types of clones in abdomens of y/f^{36a} *females carrying the indicated meiotic mutant*

Bristle No./clone	*y* 1	*y* ≥2	*f* 1	*f* ≥2	1/1	*y*//*f*† 1/≥2	*y*//*f*† ≥2/1	*y*//*f*† ≥2/≥2	Total	Male* clones	Number abdomens	Frequency per abdomen of: Single	Twin	Total
Meiotic mutant														
+/+	110(10)‡	42(5)	102	20	10	12	7	30	348	2	489	0.59	0.12	0.71
+/+ (1000R)	124(11)	60(3)	84	54	16	42	14	68	476	0	303	1.11	0.46	1.57
41/41	29(19)	44(17)	25	19	29	31	38	106	357	0	42	3.64	4.86	8.50
$41^{195}/41^{195}$	26(19)	35(17)	34	31	50	54	62	105	433	0	50	3.24	5.42	8.66
9/9	118(53)	60(6)	96	57	89	44	54	45	622	0	94	4.15	2.47	6.62
$9^b/9^b$	93(40)	29(4)	117	40	81	20	36	38	498	0	103	3.14	1.70	4.83
W68/W68	308(25)	223(17)	258	167	62	57	69	210	1396	35	707	1.41	0.56	1.97
$W68^{L1}/W68^{L1}$	147(13)	36(2)	140	51	22	26	26	42	505	1	479	0.81	0.24	1.05
$W68^{L1}/W68$	82(18)	36(4)	122	42	21	11	14	30	380	4	299	1.02	0.25	1.27
S282/S282	124(30)	72(19)	172	59	41	24	32	41	614	1	209	2.28	0.66	2.94
$c(3)G^{68}/c(3)G^{68}$	166(45)	77(6)	147	30	30	14	16	33	564	1	335	1.41	0.28	1.69
$c(3)G^{17}/c(3)G^{17}$	55(10)	18(7)	66	18	14	5	7	5	205	0	136	1.28	0.23	1.51
$c(3)G^{68}/c(3)G^{17}$	160(81)	69(25)	199	59	40	24	20	22	699	3	361	1.64	0.29	1.93
$sbd^{105}/+$	128(39)	31(9)	102	21	19	12	7	16	384	0	256	1.29	0.21	1.50
$sbd^{105}/c(3)G^{68}$	128(44)	29(6)	124	35	38	13	15	21	453	0	124	2.95	0.70	3.65
$sbd^{105}/c(3)G^{17}$	86(30)	20(6)	94	31	15	6	5	17	310	2	206	1.30	0.21	1.51
pal/pal	54(13)	24(2)	71	23	6	6	6	20	225	1	215	0.87	0.18	1.05
ord/ord	233(32)	81(2)	282	73	72	29	32	26	862	1	355	1.98	0.45	2.43
S332/S332	72(16)	31	69	25	20	20	17	78	348	2	200	1.06	0.68	1.74
ca^{nd}/ca^{nd}	203(52)	97(10)	263	129	47	35	26	50	912	0	342	2.21	0.46	2.67
l(1)TW-6^{cs}/ *l(1)TW-6*^{cs}	245(103)	50(15)	318	85	60	43	28	31	978	1	341	2.39	0.48	2.87

*, ‡ = See Table 2.

† Clones with *Minute*-like bristles not differentiated from those with normal bristles in summaries of twin spots.

TABLE 5

Frequency and size of mwh *clones in wings of* y/y; Dp(1;3)sc^{J4}, y$^+$ mwh/+ *females carrying the indicated meiotic mutant*

	Clone size*										
	1	2	3–4	5–8	9–16	17–32	33–64	Total clones	Number of wings	Clones/ wing	Frequency of clones relative to control
Meiotic mutant											
+/+	47	13	3	0	0	1	1	65	88	0.74	1
41/41	3403	966	156	24	7	3	2	4561	81	56.31	76.1
41^{195}/41^{195}	1919	388	55	9	3	1	0	2375	36	65.97	89.1
9/9	1325	256	18	10	7	0	1	1617	84	19.25	26.0
9^b/9^b	1333	205	19	16	3	4	1	1581	112	14.12	19.1
218/218	67	10	1	1	3	0	1	83	54	1.54	2.1
218$^{6-7}$/218$^{6-7}$	39	7	0	3	0	0	0	49	72	0.68	0.9
352/352	336	65	19	8	2	0	0	430	64	6.72	9.1
W68/W68	36	6	0	0	1	1	0	44	54	0.82	1.1
W68^{L1}/W68^{L1}	18	4	1	1	0	0	0	24	36	0.66	0.9
S282/S282	37	10	2	2	0	0	1	52	54	0.96	1.3
c(3)G^{68}/c(3)G^{68}	123	38	7	8	3	1	3	183	66	2.77	3.7
c(3)G^{17}/c(3)G^{17}	111	23	8	1	0	1	(1)†	145	78	1.85	2.5
sbd^{105}/+	108	10	8	3	2	0	0	131	48	2.73	3.7
sbd^{105}/c(3)G^{68}	168	49	9	2	2	0	1	231	78	2.96	4.0
sbd^{105}/c(3)G^{17}	132	58	11	8	4	2	(2)†	217	80	2.71	3.7
nod/nod	22	5	0	0	0	0	0	27	36	0.75	1.0
pal/pal	9	1	1	0	0	0	0	11	36	0.31	0.4
cand/cand	107	17	3	1	0	0	0	128	60	2.13	2.9
l(1)TW-6^{cs}/ l(1)TW-6^{cs} (25°)	33	5	2	0	0	0	0	40	36	1.11	1.5
l(1)TW-6^{cs}/ l(1)TW-6^{cs} (22°)	147	15	3	4	1	1	0	171	51	3.35	4.5

* Corrected for 0.38 clones (of one cell)/wing that mimic *mwh* in *mwh*+/*mwh*+ control wings.
† Clones of greater than 64 cells.

that function in all cells. In contrast to the above mutants, neither *mei-W68* nor *mei-S282* caused a significant increase in wing spots, although spots were increased in tergites. Thus, *mei-W68*$^+$ and *mei-S282*$^+$ are required for chromosome stability in abdominal histoblasts, but not in wing imaginal cells.

Several lines of evidence support the hypothesis that the spot frequencies observed in the flies bearing meiotic mutants are due to these mutants, not to polygenic differences in background genotype such as those shown by WEAVER (1960) to be responsible for the different frequencies of spots in the stocks she studied. First, and most generally, in our crosses to introduce cell markers into meiotic mutant or control genotypes, the chromosomes bearing the cell markers (*y*, *f*^{36a}, *Dp(1;3)sc*^{J4}, *y*$^+$ *mwh*, or *jv*) were all derived from the same initial stocks so that the backgrounds of these stocks are partly coisogenic.

More directly, in the case of the *X*-linked mutants *mei-41* and *mei-9*, the mutant responsible for the elevated frequency of spots and the mutant that produces a defective female meiosis have been inseparable by recombination. Females of the genotype *y w m mei-41*$^+$ *f*/*y w*$^+$ *m*$^+$ *mei-41 f*$^+$; *Dp(1;3)sc*^{J4}, *y*$^+$ *mwh*/+ were crossed to *FM7/Y* males and 115 *Dp(1;3)sc*^{J4}, *y*$^+$ *mwh*-bearing sons were picked at random and scored with respect to *w*, *m*, and *f*. To determine which of these chromosomes carried the locus (loci) responsible for an increased spot frequency and/or defective female meiosis, these males were individually crossed to *y mei-41/FM7* females for three days after which their wings were scored for *mwh* spots. The "recombinant" *X*/*y mei-41* daughters of these males were crossed to determine if the "recombinant" *X* chromosome carried the locus responsible for defective female meiosis. No case of separation of the spot and meiotic phenotypes was found among the 115 chromosomes tested, and the *mei-41* locus mapped 2.5 map units distal to *f* at 54 on the *X* chromosome. These data are in agreement with those of SMITH (1976), who found that the methylmethanesulfonate sensitivity and female sterility phenes of the allele *mei-41*^{A1} mapped to 54.1. Similar experiments have shown that the locus responsible for an elevated frequency of spots on the *mei-9* chromosome maps to the same location as its meiotic effect (T. LAWLOR, personal communication).

Finally, for five of the seven recombination-defective loci studied (*mei-41*, *mei-9*, *mei-218*, *mei-W68* and *c(3)G*) two alleles were studied. In the cases of *mei-9*, *mei-41* and *c(3)G*, both alleles were associated with an increased frequency of spots, and the types of events (*e.g.*, breakage, mitotic crossing over and chromosome loss) that produced these spots are the same in both alleles (see below). For the *mei-218* locus, neither allele had a significant effect on the frequency of spots. For the *mei-W68* locus, one allele, *mei-W68*, is associated with a clear increase in the frequency of spots, whereas a second allele, *mei-W68*^{L1}, has at best a marginal effect on chromosome stability. However *mei-W68*^{L1} is also a very weak mutant with respect to its meiotic effect: in homozygous *mei-W68*^{L1} females, only 5% of ova are nondisjunctional for the *X* chromosome, whereas in homozygous *mei-W68* females 40% of ova are nondisjunctional for the *X* chromosome. Thus the apparent disparity in mitotic effects between these

two alleles may simply reflect a difference in the severity of their defects. For the two loci studied at which only one mutant allele is extant (*mei-S282, mei-352*), there is as yet no direct evidence that the meiotic and mitotic effects of these chromosomes are due to the same mutant.

Although slight heterozygous effects on meiotic recombination are caused by those recombination-defective mutants examined carefully in this regard (HINTON 1966; LINDSLEY *et al.* 1968; and HALL 1972 for *c(3)G*. CARPENTER and SANDLER 1974 for *mei-9* and *mei-218*), all of these mutants are recessive in the generally accepted sense. However, these mutants exhibit clear heterozygous effects on mitotic chromosome stability (Table 6) and thus appear semidominant. The difference in degree of dominance of these mutants in meiotic and mitotic cells is in part a reflection of the different sensitivity of the tests used to monitor their effects. In meiotic cells a heterozygous effect of a mutant is detected as a change in the frequency and/or distribution of exchanges along a chromosome arm that has approximately one exchange per meiosis in wild type. Relatively substantial effects or carefully controlled large-scale experiments are needed for an effect to be detected against this high background. In mitotic cells, on the other hand, the control frequency of spots is quite low (in the wing, which has about 30,000 cells, there are about 0.7 *mwh* spots per wing in wild type). Thus errors in chromosomal metabolism that produce marked clones at rates as low as 10^{-4} per cell division are readily detectable in mitotic cells, whereas defects in meiotic recombination need to be orders of magnitude more frequent to be detected.

Taken together the above observations suggest that a large majority of the functions utilized during meiotic recombination in Drosophila also function in somatic cells in processes requisite for the stability of mitotic chromosomes. Such a dual role for these loci is quite reminiscent of the findings in prokaryotes, where it has been shown that there is a common utilization of gene functions in recombination and the repair of induced chromosomal damage (for review see CLARK 1973). Moreover, some of the mutants studied in prokaryotes also exhibit alterations in DNA metabolism in untreated cells (*e.g.*, increased DNA turnover, altered mutation rates). By analogy with these findings, the increased mitotic chromosome instability in the recombination-defective mutants we have studied is understandable if the wild-type alleles of these loci specify functions that serve either to prevent discontinuities from occurring in the genome or to repair them. If such discontinuities, which could be either spontaneous lesions or normal intermediates in chromosomal metabolism, are not removed from the genome, they could serve to initiate a sequence of events that would, *via* chromosome breakage, mitotic crossing over, mutation, chromosome loss, or nondisjunction, produce marked clones of cells.

Origin of marked clones: general considerations

The elevated frequencies of marked clones produced by these recombination-defective mutants could be due to mitotic crossing over, mutation, chromosome loss, mitotic nondisjunction, or chromosome breakage (Figure 1). A consideration

TABLE 6

Frequencies and types of clones in abdomens of females heterozygous for meiotic mutants

Bristle No./clone	*y* 1	*y* ≥2	*y jv* 1	*y jv* ≥2	*y//mwh* 1	*y//mwh* ≥2	*y jv//mwh* 1	*y jv//mwh* ≥2	*y⁺y jv*	*mwh*	Male spots*	*y^A**†*	*jv**	Total clones	Number abdomens	Frequency per abdomen of: Single	Twin	Total
Meiotic mutant																		
+/+	42(11)‡	4(3)	21(7)	4(1)	6	5	4	11	0	2	1	6	0	121	521	0.18	0.05	0.23
41/+	24(34)	7	12(22)	2(1)	0	2	2(1)	3(1)	0	0	0	0	1	111	233	0.44	0.04	0.48
41^195/+	35(22)	6(1)	13(8)	1(1)	5(1)	6	5	4	0	3	0	0	2	111	267	0.34	0.08	0.42
9/+	23	2	9	1	1	2	0	4	0	1	0	0	0	43	167	0.22	0.04	0.26
9^b/+	12	2	7	1	0	3	3	3	0	3	0	0	0	34	123	0.20	0.07	0.28
218/+	3(2)	0	1(2)	1	1	1	1	0	0	0	0	0	0	12	64	0.14	0.05	0.19
218^6-7/+	8(5)	6	3(1)	1(1)	0	3	2	3	0	6	13	2	0	39	115	0.27	0.07	0.34
352/+	10(1)	1	7(5)	0	1	3	0	2	0	0	1	6	0	30	111	0.22	0.05	0.27
nod/+	5(1)	2	2(1)	2	2	2	1	4	0	1	0	1	0	23	101	0.14	0.09	0.23

	y 1	*y* ≥2	*f* 1	*f* ≥2	*y//f* 1/1	*y//f* 1/≥2	*y//f* ≥2/1	*y//f* ≥2/≥2	Male spots*	Total clones	Number abdomens	Frequency per abdomen of: Single	Twin	Total
+/+	116(4)	46(1)	102	20	10	12	7	30	2	348	489	0.59	0.12	0.71
W68/+	32(4)	20	34	7	4	4	11	20	1	136	107	0.91	0.36	1.27
W68^L1/+	24(3)	8(1)	22	8	4	0	3	5	0	78	78	0.85	0.15	1.00
S282/+	128(23)	40(11)	142	43	18	12	8	27	0	452	264	1.46	0.25	1.71
c(3)G^68/+	87(1)	33	74	16	14	4	9	12	0	250	194	1.09	0.20	1.29
c(3)G^17/+	52(18)	21(4)	82	26	18	11	9	22	0	263	192	1.06	0.31	1.37
ord/+	22(6)	12	40	12	6	6	3	12	0	119	133	0.69	0.20	0.89
ca^nd/+	42(12)	8(4)	51	15	2	5	4	9	1	152	117	1.13	0.17	1.30
!(1)TW-6^cs/+	23(2)	0(1)	14	4	3	1	3	1	0	52	77	0.57	0.10	0.67

*, †, ‡ = See Table 2 for explanation.
Females were either *y/y; Dp(1;3)sc^J4, y+ mwh/jv* (upper part of table) or *y/f^s6a* (lower part of table).

of the properties of clones produced by each of these mechanisms suggests that each should have distinctive characteristics.

Mitotic crossing over: In flies heterozygous for two cell markers in *trans* configuration, mitotic crossing over proximal to the markers can produce two descendent clones of cells, each of which is homozygous for one of the cell markers. Upon differentiation, these clones can produce a twin spot which is diagnositc of mitotic crossing over. However, mitotic crossing over can also give rise to single spots (spots without a twin) under two circumstances. First, if none of the descendent cells of one daughter can express the marker phenotype, then only one-half of the twin spot may be detected. This can occur with bristle markers (such as those we have utilized) in the abdominal cuticle, since only a minority of these cells produce bristles. Second, single spots are produced by exchanges between the two cell markers, since such exchanges can render only the distal marker homozygous.

The proliferation dynamics of the cells that will form the abdominal cuticle are such that members of a clone (or twin halves) are frequently not contiguous. This necessitates the adoption of a criterion other than contiguity for the scoring of abdominal clone size and presence of twin spots; following GARCIA-BELLIDO and MERRIAM (1971b), we consider all marked structures within a hemitergite to be the result of a single event. When the overall probability of an event is low, this procedure will accurately reflect the size of clones, presence and frequency of twin spots, and overall frequency of events; when the probability of an event is sufficiently high that the probability of two independent events in one hemitergite is not negligible, then this procedure will overestimate the size of clones and frequency of twin spots and underestimate the overall frequency of events. One can, however, determine whether the data for any given mutant are consistent with the hypothesis that only single spots are being produced, since the expected frequency of spots scored as twins should equal the product of the frequencies of the two types of single spots that comprise the twins. For such a comparison, data from y/f^{36a} are the most reliable since both y and f^{36a} are readily scored. Data from y^+ *mwh/jv* flies are less reliable both because of the low number of *mwh* single spots observed and the difficulty of detecting smaller *mwh* spots without a twin. Because coincident spots of the same type on a hemitergite are scored as one spot, the frequencies of single spots used in these calculations may be underestimates, and as a consequence the expected numbers of false twins are also underestimated.

Values for the numbers of false twins (coincident singles) expected from the numbers of total y and f^{36a} clones are presented in Table 7. For all mutants, expected coincident spots are fewer than the observed twin spots.

Breakage: Breakage followed by loss of the acentric fragment carrying the wild-type allele of a cell marker produces an aneuploid daughter cell that is hemizygous for that marker. Such cells will give rise only to single spots, and such spots should have several characteristic features.

TABLE 7

Comparisons between observed numbers of twin spots and the expected numbers of coincident spots calculated on the assumption of independent occurrence of y *and* f *clones*

Mutant	Observed	Expected	χ_1^2
+	59	8.4	332
mei-41	204	184.8	19.1
mei-41^{195}	271	247.3	26.2
mei-9	232	192.1	28.0
mei-9^{b}	175	109.9	85.1
mei-W68	398	113.0	943
mei-W68^{L1}	116	20.1	523
mei-S282	138	67.6	109
c(3)G^{68}	93	31.2	151
c(3)G^{17}	31	10.2	50.8
sbd^{105}/+	54	18.0	86.1

χ^2 values calculated from observed *vs.* expected numbers of all four classes of hemitergites (*y*, *f*, *y*//*f*, and unmarked). Data from Table 4.

The first feature of aneuploid spots involves bristle morphology. There are Minute loci scattered throughout the genome, in particular near the tip of each major arm; two doses of the wild-type allele of each Minute locus are required for normal bristle morphology (SCHULTZ 1929; LINDSLEY *et al.* 1972). Thus, most breakage events that delete the wild-type allele of a cell marker will also remove one or more Minute$^+$ loci, so that bristles produced by the cells descended from such an event may have the short, thin-bristle phenotype characteristic of Minutes. Not all such bristles need be Minute in phenotype, since if the breakage event occurs sufficiently close to the end of cell divisions there may be sufficient cytoplasmic Minute$^+$ product present in the cell to render it Minute$^+$ in phenotype, even though it is genotypically Minute (a phenomenon termed perdurance by GARCIA-BELLIDO and MERRIAM 1971c). STERN (1936) has suggested that in instances of extensive aneuploidy more severely abnormal bristles may be produced.

In all mutants and in the control, a proportion of the bristles marked with *y* or *y jv* in single spots have the short, thin, Minute-like phenotype we here consider an indication of heterozygous deficiencies (no attempt was made to score *f*^{36a} clones for these features since *f*^{36a} itself drastically affects bristle morphology). The high proportion of *y* and *y jv* bristles that are morphologically abnormal in some of the mutants could be due either to the *y* phenotype and the Minute-like phenotype being produced by the same event or else to the simultaneous occurrence in a cell lineage of two events— one producing the marked clone and the other conferring the abnormal bristle morphology. The positive correlation between the expression of *y* and abnormal bristle morphology (see analyses of *mei-41* and *mei-9* below) suggests that both phenotypes are produced by a single event.

It should be noted that in the nonmeiotic mutant controls about 20% of the clones marked with *y* had bristles with a Minute-like phenotype. We infer that these clones are also aneuploid since here too there is a striking concordance between the occurrence of marked clones and morphologically abnormal bristles. On 12 *y*/*y*; *Dp(1;3)sc*^{J4}, *y*$^{+}$ *mwh*/*jv* control abdomens, we scored all bristles as to their morphology, and, even with a very liberal criterion of Minute-likeness, only 56/7920 = 0.7% of bristles were morphologically abnormal. Thus, cells that have experienced an event leading to the expression of a recessive marker are at least 29 times more likely to produce morphologically abnormal bristles than are all other cells. These data, therefore, suggest that a substantial proportion of spontaneous single clones in control abdomens represent instances of chromosome breakage rather than mitotic crossing over. However, most of the twin spots in the control are real and therefore represent instances of mitotic exchanges: 26 twins were observed in *Dp(1;3)sc*^{J4}, *y*$^{+}$ *mwh*/*jv* control female abdomens, whereas only 0.7 twins would be expected if only single spots were occurring and they were distributed randomly.

The second major feature that should be characteristic of aneuploid clones is small size. Reduced clone size may come about both from the fact that Minutes lengthen the mitotic cell cycle in a cell-autonomous manner (MORATA and RIPOLL 1974) and also that aneuploid cells produced late in development (when few cell divisions remain) have a higher probably of surviving to produce cuticle than do cells possessing the same degree of aneuploidy generated earlier in development (P. RIPOLL, unpublished observations). Furthermore, the broken chromosome ends may participate in bridge-breakage-fusion cycles that can generate further aneuploidy as well as interfere mechanically with cell division.

Clone size distribution is best analyzed in the wing, since here members of a clone do remain contiguous. If the probability of an event generating a marked clone is the same for all cells throughout development, then clones of one cell should be twice as frequent as those of two cells, etc. Thus, a plot of clone size (as # of divisions) *vs.* the log of clone frequency should be a straight line with slope −0.30 ($10^{-0.3} = 0.5$) (Figure 5). It has been shown by GARCIA-BELLIDO and MERRIAM (1971a) that X-ray-induced *mwh* clones exhibit a clone size distribution consistent with these assumptions, and we have confirmed their finding (Figure 5). However, the clone size distribution of *mwh* clones resulting from aneuploidy should be skewed such that the slope is steeper (more than one-half of the clones of size one, etc.), since such *mwh* clones will proliferate more slowly than their euploid (*mwh*$^{+}$) neighbors under any (or all) of the above considerations. If the probability of the generating event is constant per cell division and all clones have the same average proliferation dynamics, then the clone size distribution will be linear. If there are two types of events (such as breakage and somatic crossing over) whose resulting clones have different proliferation dynamics (*e.g.*, aneuploid and euploid), then the clone size distribution will be bipartite.

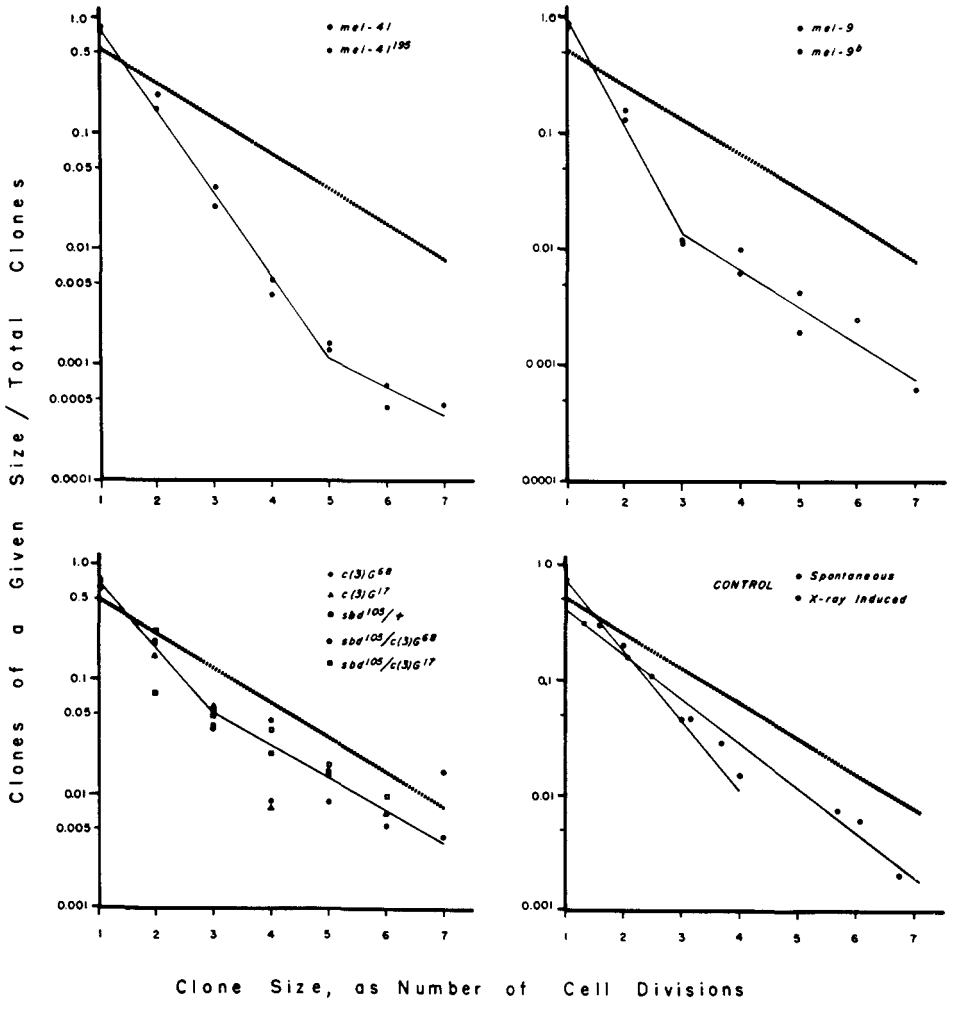

FIGURE 5.—Clone-size distributions of *mwh* clones in wings of y/y; $Dp(1;3)sc^{J4}$, y^+ *mwh*/*jv*, females carrying the indicated meiotic mutant. Heavy line = Theoretical Ideal Distribution. Control and mutant lines are least-square fits to the data.

Note that the clone size distribution from wild-type, unirradiated wings has a steeper slope than random, again suggesting that a substantial proportion of spontaneous clones result from breakage events.

Mutation: Mutation will produce only single spots. Such single spots should be euploid and exhibit normal bristle morphology and growth characteristics. In addition, when two cell markers are in the *cis* arrangement (as are y^+ and jv^+ in the experiments done to examine the stability of third chromosomes), then mutation will produce single spots expressing only one of the markers, *e.g.*, y or jv, but not y jv. Since all of the mutants examined produce approximately

equal numbers of *y* and *y jv* spots and very low frequencies of *jv* spots, mutation does not appear to be a major cause of chromosome instability in these mutants.

Loss: Loss of a chromosome during mitotic cell division will generate a daughter cell that is monosomic. For the major autosomes such monosomy is probably cell lethal since heterozygous deficiencies for more than about one-half of an arm of a major autosome are cell lethal (P. RIPOLL, unpublished results). In the case of the *X* chromosome, its loss during development of a female will generate a cell with one *X* and two sets of autosomes—the male genotype. Such cells would be expected to have normal viability and produce single spots expressing the markers on the remaining *X* chromosome. In addition, when such a clone encompasses a region of the cuticle that is sexually dimorphic, then that region should exhibit male sexual characteristics.

Nondisjunction: The final mechanism by which we can envisage spot production, nondisjunction, can generate marked clones in two ways. If the sister chromatids of one chromosome fail to disjoin while the chromatids of its homologue segregate regularly then a trisomic cell and monosomic cell will be produced. The monosomic cell will produce marked clones equivalent to those generated by chromosome loss. If, on the other hand, the sister chromatids of one chromosome go to one pole and those of its homologue both go to the other pole (double nondisjunction), then two euploid daughter cells will be produced. Each daughter cell will be homozygous for all markers on the chromosome and thus a twin spot will be produced. Such an event has the unique property that it leads to simultaneous homozygosis of markers on opposite sides of the centromere. In addition, with the marked third chromosomes used here, such nondisjunction should produce only *y jv//mwh* twins, whereas mitotic crossing over will produce *y//mwh* twins as well as *y jv//mwh* twins, depending on whether the exchange is proximal or distal to *jv*.

Origin of marked clones: individual recombination-defective loci

In this section we will examine the properties of the marked clones produced by the mutants at individual recombination-defective loci in order to infer the mechanism(s) by which the mutants evoke chromosomal instability.

mei-41: Of the recombination-defective mutants studied, the two alleles of *mei-41* produce the highest incidence of chromosomal instability (Tables 2 to 4). Marked clones expressing the *X*-linked cells markers in abdomens occur at 12 times control rates (Table 4), whereas clones expressing the third chromosome cell markers in abdomens are increased 20- to 25-fold (Tables 2, 3). The apparent insensitivity of the *X* chromosome to *mei-41*-produced instability is largely, if not entirely, an artifact due to the way abdominal spots are scored: all marked cells on a hemitergite are counted as coming from a single event. In *mei-41* and *mei-41*^{195} *y*/*f*^{36a} females, 85% of the hemitergites have marked bristles, which implies that many hemitergites have experienced more than one event. Assuming a Poisson distribution of events, 57% of hemitergites have had two or more such events, and thus our scoring procedure seriously underestimates the incidence of chromosomal instability. On the other hand, in *mei-41* and

mei-41^{195} females heterozygous for the third chromosomal markers, only 53% of hemitergites have marked clones, and thus the incidence of multiple events in single hemitergites is lower—18% of hemitergites, assuming a Poisson distribution. Strong support for that assumption, and direct evidence for the occurrence of more than one event producing marked clones in single hemitergites, is provided by the existence of hemitergites having both *y* and *y jv* bristles. These spots cannot be produced either by any single event or by a combination of exchanges within one cell, and thus must be the products of two or more events in separate cells. The numbers of hemitergites with both *y* and *y jv* bristles are in good agreement with the expectations from independence: for *mei-41* males, 25 *y* + *y jv* clones were observed *vs.* 31 expected; for *mei-41*^{195} males, 48 observed *vs.* 44 expected; for *mei-41* females, 83 observed *vs.* 106 expected; and for *mei-41*^{195} females, 122 observed *vs.* 127 expected.

To infer the nature of the event(s) that produce marked clones, it is important to know whether the event produces single or twin spots. Because the frequency of marked clones in abdomens of *mei-41* and *mei-41*^{195} individuals is so high, it is apparent that many of the spots scored as twin spots (Tables 2 to 4) are in fact due to the coincident occurrence of complementary single spots on one hemitergite. Although not all twin spots can be accounted for in this manner (Table 7), we believe that most spots produced by these mutants are single spots, for the reasons outlined below.

Of the three types of events that produce exclusively single spots (Figure 1), two (mutation, loss) will not give the arrays of types of spots observed (see above). Breakage, however, can give the arrays observed.

If most of the spots in *mei-41* and *mei-41*^{195} are the results of breakage and loss of the acentric fragment, then the spots should exhibit the two features of aneuploidy—morphologically abnormal bristles and small spot size.

In a high proportion of *y* and *y jv* single spots in *mei-41* and *mei-41*^{195}, the bristles are morphologically abnormal (Tables 2 to 4). That both phenotypes are produced by a single event is strongly suggested by the positive correlation between the expression of *y* and abnormal bristle morphology. On 12 abdomens of *y mei-41/y mei-41*; *Dp(1;3)sc*^{J4}, *y*$^{+}$ *mwh/jv* females, 326/7332 (= 5%) of all bristles were abnormal, whereas 36% of *y* bristles in such females were thin and an additional 19% were more severely abnormal. On these 12 abdomens, 30/362 (= 8.3%) of the abnormal bristles were *y*. This is in reasonable agreement with expectation if abnormal bristles are largely a consequence of aneuploidy in these flies and all major chromosome arms are undergoing breakage at comparable rates: since *y*$^{+}$ is present on only one of the ten arms, only 10% of breaks should produce a *y* spot. These data support the hypothesis that chromosome breakage is a major cause of the chromosome instability produced by *mei-41*, since 35 to 50% of *y* bristles have aneuploid characteristics. That approximately half of the marked bristles do not exhibit abnormal morphology could indicate that these clones arise *via* another mechanism(s). However, we favor the alternate interpretation that most of these spots are also aneuploid but are *Minute*$^{+}$ in phenotype due to perdurance.

That most of the large number of twin spots recorded in y/f^{36a} females homozygous for *mei-41* or *mei-41*^{195} are due to the coincident occurrence of single spots is supported by the observation that the y bristles in these twin spots are as frequency short and thin as are the bristles in y single spots: 42% of $y//f^{36a}$ twin spots in *mei-41* females had y bristles that were short and thin, while 34% of the y single spots in these females had Minute-like bristles. For *mei-41*^{195} y/f^{36a}, 43% of the y bristles in twin spots and 38% of the y bristles in single spots were Minute-like.

Further evidence that most clones produced by *mei-41* and *mei-41*^{195} are single clones comes from the third-chromosome data where total clones are substantially fewer than in the *X*-chromosome data, and hence the problem of coincident single clones is less. For the third chromosome, single clones are increased 30-fold, whereas twin spots are only three times more frequent than in the nonmutant controls.

The size distribution of *mwh* spots in *mei-41* and *mei-41*^{195} wings is skewed toward small spots (Figure 5), which strongly supports the hypothesis that most *mei-41*-induced clones are aneuploid. The apparent bipartiteness of the clone size distribution suggests that there may be more than one mechanism by which clones are generated. If there are two mechanisms of clone production, that which produces more than 95% of the clones generates only small clones, and we suggest that these are aneuploid clones produced by breakage. Although the data from which the second component to the clone size distribution is inferred are few, they are consistent with these clones having euploid growth characteristics and thus being the products of mitotic crossing over or mutation. Indeed, since a *mei-41* allele increases the spontaneous mutation rate meiotically (SMITH 1973), it would be surprising if mutation were not also elevated in mitotic cells. Mutation could in fact account for the few *jv* clones observed. It should be noted that to the degree that aneuploid cells fail to survive to produce clones, the proportion of the total clones that can be inferred to be euploid is an overestimate of the proportion of events that produce marked euploid cells. Data on the types of spontaneous chromosomal aberrations found in metaphases of *mei-41* larval neuroblasts suggest that most aneuploid cells divide poorly if at all (M. GATTI, personal communication) from which we infer that the proportion of events generating euploid clones is grossly overestimated by our data.

The above results all support the hypothesis that the chromosomal instability produced by *mei-41* alleles is primarily the consequence of chromosome breakage generating aneuploid clones. We envisage chromosome breakage occurring because *mei-41* mutants are defective in a process requisite for the repair of some array of spontaneous chromosomal lesions: if uncorrected these lesions result in chromosome breakage. A minimum estimate of how frequently *mei-41*+ acts to prevent breakage during mitotic cell division is provided by the frequency of marked clones in *mei-41*. There are about 60 *mwh* clones per wing in *mei-41* flies heterozygous for *mwh*. A wing has about 30,000 cells (GARCIA-BELLIDO and

MERRIAM 1971a). As *mwh* marks only one of the ten major chromosome arms, there are about $(60 \times 10)/30{,}000 = 2\%$ aneuploid cells in the mature wing.

Since *mei-41* alleles affect chromosome behavior in larval neuroblasts (M. GATTI, personal communication) and female meiotic cells (BAKER and CARPENTER 1972; SMITH 1976), as well as abdominal histoblasts and wing imaginal discs, it seems likely that the wild-type allele of *mei-41* functions in all cells.

mei-9: Both alleles studied at the *mei-9* locus cause substantial amounts of chromosome instability (Tables 2 to 4). For both alleles, instability of the *X* chromosome is increased more than that of the third chromosome. The difference between the responses of the *X* and third chromosomes is even greater than these figures suggest since, assuming clones are Poissonly distributed among hemitergites, 14% (*mei-9*^{b}) to 29% (*mei-9*) of hemitergites in the *X* chromosome experiments have more than one marked clone, whereas less than 1% of hemitergites have more than one clone in the third-chromosome experiments. We have no explanation for this differential sensitivity of the *X* and third chromosomes. It could be due either to a different frequency of spontaneous lesions on the two chromosomes that require *mei-9*$^+$ for their correction or to lesions on the *X* and third chromosome having different probabilities of giving rise to marked clones.

Both twin spots and single spots are increased by *mei-9* mutants. With the *X*-linked markers, there are more twin spots observed than would be expected if only single spots were produced and "twins" were due to the coincident occurrence on a hemitergite of two singles of the appropriate type (Table 7). Data for the third chromosome are: *mei-9*^{b} males, 12 twins observed and one expected; *mei-9* males, eight twins observed and 0.7 expected; *mei-9*^{b} females, 17 twins observed and one expected; *mei-9* females, 36 twins observed and four expected. Twin spots can be produced by either mitotic exchange or simultaneous nondisjunction of homologues (Figure 1). That the twin spots produced by *mei-9* alleles are due to mitotic exchange is strongly suggested by the fact that both *y//mwh* and *y jv//mwh* twins are increased in frequency, since *y//mwh* twins cannot be produced by nondisjunction.

Many of the single spots produced by *mei-9* alleles have the morphological characteristics indicative of aneuploidy: 20–40% of *y* bristles are short and thin and an additional 10 to 15% are more severely abnormal. In these mutants also there is a strong positive correlation between the presence of a marked clone and morphologically abnormal bristles. Of all bristles on the tergites of 12 abdomens of *y mei-9/y mei-9*; *Dp(1;3)sc*^{J4}, *y*$^+$ *mwh/jv* females, only $123/7632 = 1.6\%$ were abnormal. Yet 35% of *y* and *y jv* bristles on such females are short and thin. Thus, the chromosomal instability caused by *mei-9* alleles results from elevated frequencies of both chromosome breakage and mitotic crossing over.

Further evidence that *mei-9* alleles produce marked clones *via* two mechanisms, one of which is chromosome breakage, is provided by the bipartite clone-size distribution of *mwh* spots in wings (Figure 5). Between 5 and 10% of the clones have the clone-size distribution expected for euploid clones arising randomly throughout development. From the *mei-9* abdomen data, which show that *mei-9*

alleles increase mitotic crossing over, we suggest that the apparently euploid *mwh* wing clones also arise *via* this mechanism. However, the majority of *mwh* clones (90 to 95%) result from chromosome breakage. We should emphasize that the relative frequencies of detected spots arising *via* these two mechanisms may grossly overestimate the relative frequency of mitotic crossover events, since there is good evidence that many aneuploid cells fail to divide and hence do not produce clones (M. GATTI, personal communication).

These data, together with the demonstrations that *mei-9*+ is required in larval ganglia (M. GATTI, personal communication) and during female meiosis (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974), suggest that *mei-9*+ functions in all cells. The pattern of chromosomal instability caused by *mei-9*+ alleles is most easily understood if the wild-type gene product functions in the removal of a lesion(s) which, if not removed, can initiate events leading to chromosome breakage or mitotic exchange.

mei-218: Although both alleles of *mei-218* studied cause as great a reduction in meiotic recombination as *mei-9* (BAKER and CARPENTER 1972; CARPENTER and BAKER, unpublished) neither allele has any effect on chromosome stability in wing imaginal discs or abdominal histoblasts. This suggests that the wild-type gene product of the *mei-218* locus may function only during female meiosis.

mei-W68: Mitotic chromosome stability has been examined in the presence of two alleles at the *mei-W68* locus. These two alleles, *mei-W68* and *mei-W68^{L1}*, differ markedly in the degree to which they increase mitotic chromosome instability. In the presence of homozygous *mei-W68*, total spots on abdomens are increased approximately three-fold, whereas in homozygous *mei-W68^{L1}* flies spots are present at only 1.1 to 1.5 times control frequencies (Tables 2 to 4). This difference could be accounted for if the *mei-W68* chromosome carries a second-site mutation that affects mitotic chromosome stability or if *mei-W68^{L1}* is only a slightly defective allele at this locus. We favor the latter possibility for two reasons. First, *mei-W68^{L1}* produces a much less severe disruption of female meiosis than does *mei-W68*. Second, although the quantitative effect of *mei-W68^{L1}* on mitotic chromosome stability is marginal, the data do suggest that *mei-W68^{L1}* elicits the same pattern of effects as *mei-W68*. However, not readily understandable from this viewpoint are the heterozygous effects of these mutants. In *mei-W68^{L1}*/+ females, marked clones are increased as much as in homozygous *mei-W68^{L1}* females (1.4- and 1.5-fold, respectively) and in both *mei-W68*/+ and *mei-W68^{L1}f*/*mei-W68*, marked clones are present at 1.8 times the control rate (Tables 4 and 6). Regardless of the site of the locus on the second chromosome that affects mitotic chromosome stability, the effects are reproducible and striking.

Both single spots and twin spots are increased in frequency and twin spots are substantially more frequent than can be accounted for by the coincident occurrence of independently arising single spots (Table 7). Although the frequency of *y* bristles with the abnormal morphology indicative of aneuploidy is increased by *mei-W68*, such bristles make up a smaller proportion of all *y* bristles in

mei-W68 than they do in the controls (Tables 2 to 4). This suggests that the major increase in spots in *mei-W68* is *via* a mechanism that generates euploid clones. Since these clones are frequently twin spots, we infer that they are produced by mitotic recombination or double nondisjunction; that mitotic recombination is the mechanism by which spots are being produced is suggested by the observation that both *y//mwh* and *y jv//mwh* twins occur. The former type of twin cannot be produced by nondisjunction.

Nondisjunction was assayed directly by examining flies of the genotype *y/y* (or *y/Y*); *mei-W68/mei-W68*; *Dp(1;3)sc*^{J4}, y^+ *mwh/Ki*. Mitotic crossing over in *3L* will produce *y Ki//mwh Ki* twins, *y Ki* singles, and *mwh Ki* singles; mitotic crossing over in *3R* will produce Ki^+ singles (*Ki/Ki* and *Ki/Ki*$^+$ are indistinguishable). Nondisjunction will produce the unique classes *mwh* Ki^+*//y Ki* twins and *mwh* Ki^+ singles. We scored 174 abdomens (79 female, 95 male) for Ki^+ spots. There were ten Ki^+ spots, six of one bristle, three of two, and one of three. None were clearly of nondisjunctional origin. Two of the spots were on the sixth tergite and could not be scored for simultaneous presence of *mwh*; one shared a hemitergite with a (probably independent) *mwh* clone; one occurred with a *y Ki* bristle but no *mwh* hairs. The same abdomens had 15 *y Ki* and nine *y Ki//mwh Ki* clones of three or more bristles. The scarcity of *mwh* Ki^+ clones, and in particular the absence of large Ki^+ clones, indicates that nondisjunction is not a major contributor to the spots in *mei-W68*.

Because mitotic crossing over can produce single spots (*e.g.*, when one half of a twin is not able to express the cell marker for which it is homozygous) as well as twin spots, an increase in the frequency of mitotic crossing over could account for the production of both types of spots by *mei-W68*. To determine the relative proportions in which mitotic recombination produces single spots and twin spots, we have examined the types of spots induced by X rays in abdominal histoblasts of *y/f*^{36a} larvae. There is good evidence that X-ray-induced spots result from mitotic exchange (for review, see BECKER 1976). Our data (Table 4) show that the frequency of both twin spots and single spots is increased (3.85-fold and 1.88-fold, respectively; see also Table 7). Thus increased mitotic recombination in *mei-W68* could account for a substantial number of the single, as well as the twin, spots that are produced.

One of the most distinctive features of the spots produced by *mei-W68* is their large size (Table 8). This difference is not due to different densities of bristles on controls and *mei-W68* abdomens since counts of bristles revealed approximately the same number of bristles per abdomen in these two genotypes. Clones of two or more bristles also appear to be preferentially increased by *mei-W68*^{L1} (Tables 2 to 4).

The most reasonable explanation for the large size of spots produced by *mei-W68* is that they are generated on the average at an earlier time in development than are spots in the control. Proliferation dynamics of the abdominal histoblasts are distinctive in that once they are set aside in the embryo no cell divisions occur until about the time of pupation, at which time a rapid series of divisions

TABLE 8

Clone size distribution in abdomens of y/f^{36a} females

Experiment	y bristles per clone 1	2	3	4	5	6	7	8	9	10	>10	Total no. clones	Average size
+/+ (spontaneous)	62.8	16.4	10.6	4.4	2.7	2.2	0.9					226	1.78
+/+* (spontaneous)	67.0	15.1	6.4	6.4	0.7	3.0	1.4					140	1.73
+/+ (1000R)	53.2	23.9	9.5	6.0	6.0	0	1.0	0.5				201	1.94
+/+* (1000R)	23.6	27.0	18.9	12.9	6.4	5.1	3.0	1.6	0.5	0.3		565	2.90
mei-W68	37.6	17.3	13.9	11.1	6.5	3.5	2.6	2.3	2.2	1.2	1.9	649	3.06
mei-S282	54.6	20.1	7.7	7.7	4.2	2.5	0.7	1.4	0.4	0	0.7	284	2.13
mei-S332	42.4	21.2	7.9	12.2	9.1	4.2	1.8	0	0	0.6	0.6	165	2.55
l(1)TW-6^{cs}	84.2	12.8	0.9	2.1								423	1.21
cand	66.4	18.2	4.5	4.8	2.0	1.7	0.8	0.6	0.8	0	0.3	357	1.78
ord	74.1	15.4	6.4	3.2	0.9							344	1.41

Experiment	f^{36a} bristles per clone 1	2	3	4	5	6	7	8	9	10	>10	Total no. clones	Average size
+/+ (spontaneous)	65.7	17.1	6.6	2.8	3.9	2.2	1.1	0	0.6			181	1.76
+/+ (1000R)	25.6	23.2	23.8	13.1	9.5	1.2	0.6	3.0				168	2.79
mei-W68	38.4	16.5	11.7	12.1	6.2	5.2	2.5	2.1	0.7	0.9	3.7	563	3.21
mei-S282	66.0	21.6	10.0	1.7	0	0.3	0	0	0	0	0.3	291	1.52
mei-S332	37.2	22.4	19.2	9.6	5.1	3.2	0	0.6	1.3	0	1.3	156	2.55
l(1)TW-6^{cs}	73.6	15.5	6.4	2.5	1.8	0	0	0.2				439	1.45
cand	59.5	22.0	10.2	4.5	1.9	0.7	0.7	0	0	0.2	0.2	422	1.76
ord	78.4	15.4	4.9	1.3								389	1.29

* Data from GARCIA-BELLIDO and MERRIAM 1971b.

Data for treatments and mutants have been corrected for spontaneous clones; values for each size class are expressed as percentage of total clones.

produces the cells that elaborate the abdominal cuticle (GARCIA-BELLIDO and MERRIAM 1971b). In order to define the time during development when *mei-W68* produces spots, the average sizes and the size distribution of *y* and f^{36a} clones occurring in the presence of *mei-W68* have been compared to those of clones induced by irradiation in y/f^{36a} nonmeiotic-mutant-bearing larvae. However, it should first be noted that the average size of radiation-induced *y* spots in our experiment was substantially smaller than that reported by GARCIA-BELLIDO and MERRIAM (1971b) for *y* clones induced in y/f^{36a} larvae (data reproduced in Table 8). As the average size and the size distribution of f^{36a} clones in our experiment agree with their data, we suspect there was selection against *y* clones in our experiment.

Clones produced by *mei-W68* are on the average as large or larger than those induced by irradiation of larvae (Table 8). The size distribution of *mei-W68*-produced clones shows a much higher proportion of large clones than is found among spontaneous clones and is quite similar to the size distribution of irradiation-induced clones (Table 8) except that a small proportion of *mei-W68*-produced clones are larger than any that were induced by the irradiation. We therefore conclude that some *mei-W68*-induced clones originate in the embryo during the divisions of the progenitor cells of the abdominal histoblasts. There may also be a slightly higher proportion of one-bristle clones in *mei-W68* than in the irradiated controls, suggesting that a few clones may arise during the pupal divisions. Altogether, these data suggest that the large majority of *mei-W68*-produced clones arise in the abdominal histoblasts during the larval period when they are in a nondividing state.

The abdomens of homozygous *mei-W68* flies are frequently morphologically abnormal, with large areas, up to several entire hemitergites per abdomen, missing; the frequency of these abnormalities is enhanced by low temperatures. Whether this is caused by *mei-W68* is not known.

Chromosome stability in cells of the wing imaginal disc—which divide throughout the larval and early pupal period—is not affected by *mei-W68* (Table 5), although the frequency of clones in the abdomen was increased in the same flies (Table 2). These results have several possible interpretations. The most straightforward (but to us least likely) explanation for these results is that the *mei-W68*+ function is utilized only in the abdomen, primarily during the nondividing period of the abdominal histoblasts. Alternatively, the apparent tissue specificity of *mei-W68* could be an artifact resulting from the different proliferation dynamics and/or physiologies of these two tissues. For example, if the *mei-W68*+ product were required for chromosome stability during G2, then the lack of this function should show a much more pronounced effect in the abdomen, where G2 is thought to be the resting stage of the histoblasts throughout the larval period (BECKER 1976), than in the continuously dividing wing imaginal disc. In addition, since wing cells do divide continuously, a number of enzymes involved in DNA synthesis and other aspects of chromosomal metabolism must be at least intermittently present throughout the larval period, whereas there is no apparent need for such functions in the resting abdominal

histoblasts. If one of these functions could supplant the *mei-W68*+ function, then it would mask an effect of *mei-W68* in the wing, but not in the abdomen.

In addition to the twin and single spots that we have ascribed to an increased frequency of mitotic recombination, *mei-W68* also produces a low frequency of clones indicative of nondisjunction or chromosome loss: 35 of the 707 y/f^{36a} homozygous *mei-W68* females scored had marked clones on the fifth or sixth tergites that were male by the criteria of pigmentation and hair pattern (Table 4). (These are the only tergites that are sexually dimorphic.) Male spots were also observed in homozygous *mei-W68* and *mei-W68*^{L1} females scored in other experiments (Table 2). Although y/f^{36a} homozygous *mei-W68*^{L1} females reared at 25° exhibit no increase in the frequency of male spots, 5 of 183 such females reared at 22° had a male spot and 3 of 203 such females reared at 18° had a male spot; no male spots were observed in 211 control females reared at 22° or in 154 control females reared at 18°. The frequency of male spots is also increased in *mei-W68* at low temperatures: 25 male spots in 189 females at 22° and 16 male spots in 102 females at 18°. These frequencies of male spots are minimum frequencies. 29 of the 35 male spots observed in y/f^{36a} homozygous *mei-W68* females at 25° encompassed bristles and, of these, six were y and 23 f^{36a} in phenotype, suggesting that our recognition of maleness in spots that are y may not be optimal. Of these 29 marked male spots, 28 occurred as single spots without a twin; one f^{36a} male spot had a y female twin.

One striking characteristic of these male spots is their large size. Of the 29 marked male spots, the average spot encompassed 5.9 bristles (range 1 to 22). This size is approximately the same size as the sum of both halves of twin spots induced in larval abdominal histoblasts. It can therefore be inferred that male clones arise either by a failure of both daughter cells of an abdominal histoblast to receive an X chromosome or by chromosome loss and/or nondisjunction in the embryo before the abdominal histoblasts enter their nondividing period.

Since only parts of the fifth and sixth tergites, and none of the second, third or fourth tergites have sexually dimorphic pigmentation, most instances of chromosome loss will generate marked clones that are not detectably male. To inquire if all large marked clones could arise *via* chromosome loss, we can estimate the expected number of large marked single spots from the number of male spots observed. There were 14 male spots with five or more f^{36a} bristles. Doubling this to account for y male spots gives 28 male spots. Male spots could be detected on roughly 25% of the abdomen, thus 112 single spots of five or more bristles would be expected due to chromosome loss; 95 such spots were observed. This suggests that all large single spots produced by *mei-W68* result from X-chromosome loss in the embryo. The apparent restriction of chromosome losses to the embryo could be either because *mei-W68*'s defect leads to chromosome loss only at this stage or because phenotypically male spots cannot be produced after this time. The latter would be true if, for example, the X-chromosome: autosome ratio determines sex at some early stage in development and altering this ratio after this point does not lead to a corresponding change in sex. Because male spots produced by mutants at other loci are similar in size to those

observed in *mei-W68*, we believe that the large size of male spots reflects a restriction on the time when phenotypically male spots can arise, rather than when these loci cause chromosome loss.

From these data, we suggest that the wild-type allele of *mei-W68* is required in abdominal histoblasts, as well as in meiosis, for normal chromosome behavior. Chromosome behavior in the wing imaginal disc is not affected by *mei-W68*, suggesting either that *mei-W68*$^+$ is not needed in the wing or else that a defect at this locus produces chromosome instability primarily during G2, the stage at which most marked abdominal clones arise. The major source of chromosome instability in *mei-W68* is mitotic recombination, with lesser contributions from chromosome loss and breakage.

mei-S282: The one allele of the *mei-S282* locus examined also increases mitotic chromosome instability. Marked abdominal clones expressing *X*-chromosome markers are increased somewhat more (4.1-fold) than are abdominal clones expressing the third-chromosome markers (2.2 to 2.5-fold).

Both single and twin spots are increased in frequency (Tables 2 to 4) and twin spots are more frequent than can be explained by the coincident occurrence of independent single spots (Table 7). Thus, mitotic crossing over is increased. The frequency of *y* bristles with the abnormal morphology that we believe to be indicative of aneuploidy is increased, suggesting that chromosome breakage is also occurring. Male spots, indicative of *X* chromosome loss, may also be increased in frequency; however, the number of such spots is quite small. The abdomens of homozygous *mei-S282* flies also exhibit abnormalities (missing areas) similar to those observed in *mei-W68*-bearing flies. The frequency of *mwh* wing clones is also increased slightly (1.4-fold) in *mei-S282* (Table 5). This suggests that *mei-S282* plays a less important role (if any) in chromosome stability in the wing than it does in the abdominal histoblasts. In these aspects of its phenotype, *mei-S282* is similar to *mei-W68*.

Data as to when *mei-S282* produces instability in the abdominal histoblasts are ambiguous. With third-chromosome markers, the increase in frequency of large clones ($\geq$ 2 bristles) is greater than the increase in frequency of one-bristle clones (Tables 2 and 3), suggesting that clones are generated earlier in development in *mei-S282* than they are in wild type. The *X*-chromosome data show that among single spots those of two or more bristles are increased more than those of size one, in agreement with the third-chromosome data. However, among twins the frequencies of single-bristle *y* or f^{36a} half-twins is increased substantially more than half-twins of larger size. For all spots expressing *X*-linked markers, both the average clone sizes and the clone size distributions of *mei-S282*-produced and spontaneous clones are similar (Table 8).

In summary, these data suggest that *mei-S282*$^+$ is required for chromosome stability in mitotic cells. Although the pattern of effects evoked by *mei-S282* is similar to that produced by *mei-W68*, the time at which these loci are utilized during development may differ.

c(3)G: Three genetic variants at the *c(3)G* locus have been examined: $c(3)G^{17}$, $c(3)G^{68}$ and a small deficiency that includes the *c(3)G* locus, *Df(3R)-*

sbd^{105}. In flies that are either homozygous for a mutant allele, have a mutant allele over *Df(3R)sbd*^{105}, or have one copy of each mutant allele, the frequency of clones expressing *X*-chromosome markers is increased 2.1 to 2.7 times (Table 4). In homozygous *c(3)G*^{17}, homozygous *c(3)G*^{68}, or *c(3)G*^{17}/*c(3)G*^{68} males and females, the frequencies of clones expressing third-chromosome markers are increased 1.5 to 3.4-fold (Tables 2 and 3). However, in flies that carry *Df(3R)-sbd*^{105} (with or without a mutant *c(3)G* allele) and the third-chromosome markers *Dp(1;3)sc*^{J4}, y^+ *mwh*, the frequency of spots is increased five- to ten-fold (Tables 2 and 3). This very large increase in spot frequency is due solely to a high frequency of *y* single spots; *y//mwh* twin spots and *mwh* single spots are no more frequent than in flies homozygous for a *c(3)G* allele. Thus, the high frequency of spots in these flies is attributable to an event that precludes the expression of the y^+ allele of *Dp(1;3)sc*^{J4} in some cells; the expression of *mwh*$^+$ is not affected although it is tightly linked (but *trans*) to *Dp(1;3)sc*^{J4}. Moreover, the frequency of events that preclude the expression of y^+ when it is in its normal position on the *X* chromosome is not disproportionately high. This leads us to suspect that the *Df(3R)sbd*^{105}-bearing chromosome alters the expression of the y^+ allele in *Dp(1;3)sc*^{J4} by a mechanism distinct from those we consider to be sources of chromosome instability, as is, for example, position-effect variegation.

Chromosome stability in the wing imaginal disc is also affected by *c(3)G* mutants. In all genotypes, the frequency of *mwh* clones on wings is increased 2.5- to 4.0-fold (Table 5).

The pattern of chromosome instability produced by *c(3)G* mutants is very similar to that generated by spontaneous events in non-meiotic mutant controls. The slopes of clone-size distributions in wings are parallel (Figure 5). Single spots and twin spots are increased comparable amounts, and twin spots are more frequent than can be accounted for by the occurrence of coincident single spots on a hemitergite (Table 7). For the third-chromosome data, the proportion of marked clones with bristles of abnormal morphology is about the same as in the controls. However, in the data from *X*-linked cell markers morphologically abnormal *y* bristles are a higher proportion of all *y* bristles than they are in the control.

These data suggest that *c(3)G*$^+$ is utilized in somatic cells (see also MARTENSEN and GREEN 1976). When the wild-type allele of this locus is absent in mitotic cells, chromosome instability is increased. Since no distinctive pattern of spot production can be identified, we infer that marked clones arise *via* a variety of mechanisms.

mei-352: In flies carrying the one existing mutant allele of *mei-352*, the frequency of spots is increased 2.8-fold (Tables 2 and 3). Twin spots are increased little, if any (0.9 to 1.5 times control rates), whereas single spots are increased more than three-fold. This suggests that spots are produced by either chromosome breakage or mutation. Single spots marked with both *y* and *jv* are increased as much as single spots marked with *y* alone, suggesting that marked clones are produced by breakage rather than mutation. Consistent with this inference is

the fact that in a third of all single spots marked with *y* the bristles are short and thin, suggesting that the cells producing these bristles are aneuploid.

Chromosome instability is also increased in the wing imaginal disc where *mwh* clones are nine times more frequent than in the control (Table 5); the distribution of clone sizes also suggests that the clones produced are aneuploid.

Together, these data suggest that *mei-352*$^+$ functions in somatic as well as meiotic cells. When the wild-type function of this locus is absent the frequency of chromosome breakage is increased.

Radiation sensitivity

Recombination and repair of induced chromosomal damage are under common genetic control in prokaryotes and fungi (for reviews see CLARK 1973; RADDING 1973; BAKER *et al.* 1976a). The demonstration that many of the loci necessary for meiotic recombination in Drosophila are also required to maintain chromosomal integrity in somatic cells suggests that they are good candidates for loci that function in the repair of induced chromosomal damage. To test this notion, we have examined the effects of 11 *X*-linked meiotic mutants, representing eight loci, on survival of larvae to adulthood following both UV and X irradiation. Survival curves were determined by irradiating larvae from a cross of *y mei-*/y^+Y; spa^{pol}/spa^{pol} males by attached-*X* [*C(1)DX*, *y f bb*$^-$] y^+Y; spa^{pol}/spa^{pol} females and counting the number of adults of each sex produced. In such a cross, all females carry the nonmeiotic mutant attached-*X* chromosome and all males the meiotic mutant. Thus, the females provide an internal control in each culture against which the survival of the meiotic-mutant-bearing males was measured.

The effects of X-ray sensitivity of recombination-defective mutants at the *mei-9*, *mei-41*, *mei-218*, *mei-352*, *mei-251* and *mei-152* loci, as well as disjunction-defective mutants at the *nod* and *l(1)TW-6*^{cs} loci were examined; only flies bearing *mei-9* or *mei-41* alleles showed a significant alteration in X-ray sensitivity (Figure 6; see also BAKER *et al.* 1976b; NGUYEN and BOYD 1976). Two alleles were studied for these two loci, and in each case males bearing the meiotic mutant are more sensitive to killing by X-rays than are their nonmeiotic mutant female sibs. That both alleles examined at the *mei-9* and *mei-41* loci are hypersensitive to killing by X rays strongly supports the conclusion that the radiation sensitivity is due to the mutant *mei-9* and *mei-41* loci. Thus, these two loci function in the repair of induced, as well as spontaneous, lesions. Of the recombination-defective mutants examined that are not X-ray sensitive, only *mei-352* is associated with an elevated frequency of mitotic chromosome instability. Although this may indicate that the wild-type allele of *mei-352* functions in the repair of spontaneous, but not X-ray-induced, lesions, it is the case that *mei-352* does not severely disrupt either meiotic or mitotic chromosome behavior and may thus be a leaky allele of a locus that is in fact utilized in the repair of X-ray-induced damage.

To ascertain whether the *mei-9*, *mei-41*, *mei-218*, *mei-352*, and *l(1)TW-6*^{cs} loci function in the repair of UV-induced lesions, the effects of mutations at

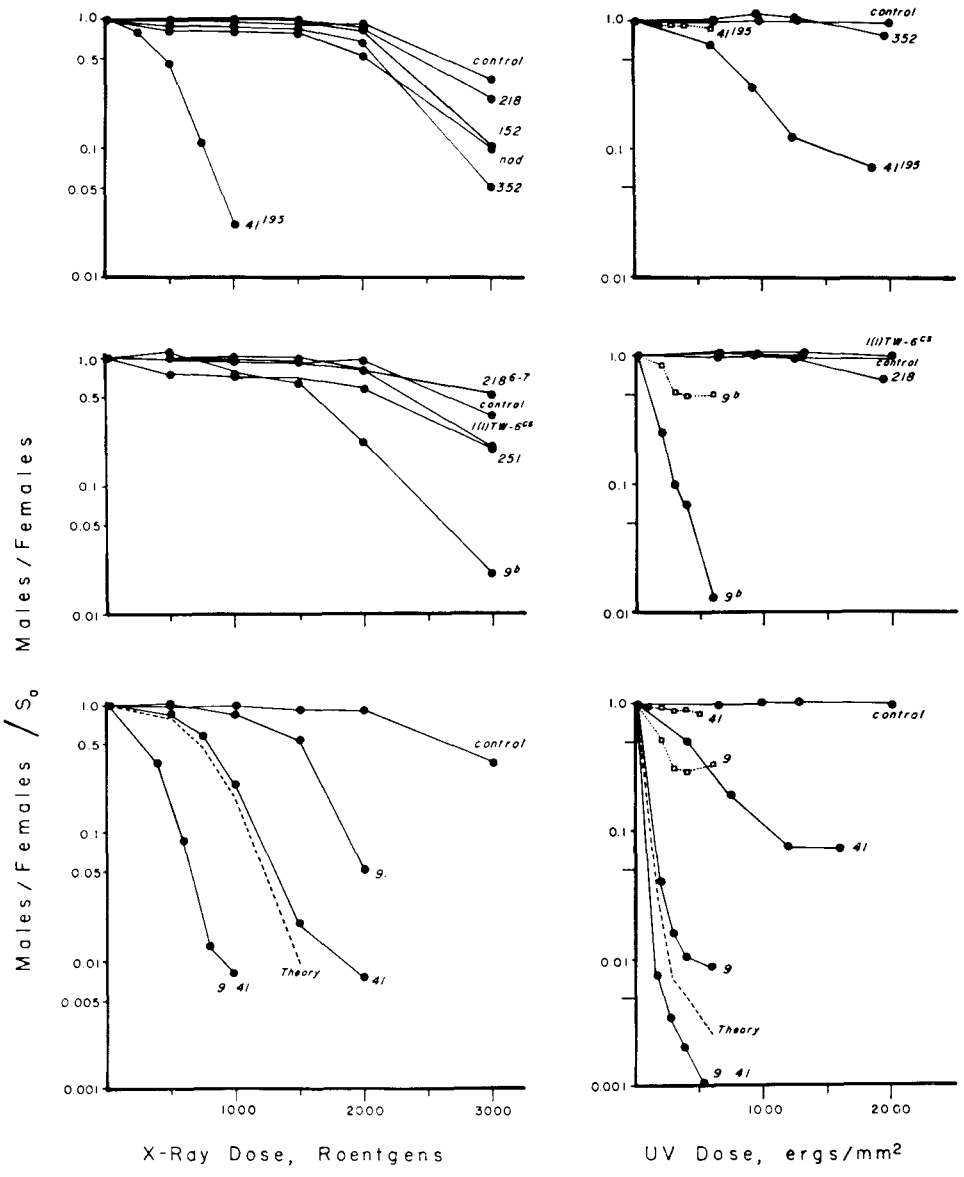

FIGURE 6.—UV and X-ray survival curves for selected meiotic mutants irradiated as third-instar larvae (solid lines) or as first-instar larvae (dashed lines). "Theory" curves in bottom panels represent the (logarithmic) sums of the *mei-9* and *mei-41* single-mutant curves.

these loci on survival following UV-irradiation were determined. Both first-instar and third-instar larvae produced by crossing *y mei-/y*$^{+}$*Y*; *spa*^{pol} males by *C(1)DX, y f bb*$^{-}$*/y*$^{+}$*Y*; *spa*^{pol} females were UV irradiated, and the survival to adulthood of meiotic-mutant-bearing males was measured relative to their non-meiotic mutant sisters.

The results from irradiating third-instar larvae show that *mei-9* and *mei-41* flies are hypersensitive to killing by UV (Figure 6; see also BAKER *et al.* 1976b; NGUYEN and BOYD 1976). Thus, these loci function in the repair of both X-ray and UV-induced lesions. Neither *mei-218*, *mei-218$^{6-7}$*, *mei-352*, *mei-251*, *nod*, nor *l(1)TW-6^{cs}*-bearing males are any more sensitive to UV irradiation than controls, suggesting that these loci do not function in the repair of UV-induced damage.

Although males bearing *mei-9* or *mei-41* alleles are markedly more sensitive than their nonmeiotic-mutant-bearing sisters to UV irradiation as third-instar larvae, when they are irradiated as first instar larvae the meiotic-mutant-bearing males are only slightly, if any, more sensitive to killing than their nonmutant sibs (Figure 6). Since the mothers of these larvae carry a nonmeiotic-mutant attached-*X* chromosome, one explanation for these results is that these females supply a sufficient amount of the *mei-41*$^+$ and *mei-9*$^+$ gene products to the egg to make *mei-9* or *mei-41* first-instar larvae nearly phenotypically wild type with respect to UV sensitivity. By the third instar, this maternal cytoplasmic contribution has been used up or sufficiently diluted so that the meiotic-mutant-bearing males are hypersensitive to killing by UV. Alternatively, first-instar larvae may have a longer time period to correct damage and replace killed cells, thus accounting for the relative insensitivity of *mei-41* and *mei-9* first-instar larvae. The latter possibility seems unlikely since at the highest UV dose with which first-instar larvae were irradiated approximately 90% of the nonmeiotic-mutant-bearing female larvae are killed (data not shown), and killed as effectively as their *mei-9* and *mei-41* sibs. Thus, in order to argue that the *mei-41* and *mei-9* first-instar larvae are phenotypically defective in their respective repair functions, but able to overcome this by the lengthened time during which repair can occur, it must also be argued that the possession of the *mei-9*$^+$ and *mei-41*$^+$ gene products by the nonmutant females contributes little to their ability to repair the quite evident UV damage they sustain as first-instar larvae.

Taken together the above results show that the *mei-9* and *mei-41* loci function in the repair of UV- and X-ray-induced damage as well as spontaneous chromosomal lesions. The mutant *mut^s*, which is an allele of *mei-41* (*mei-41^{A1}*, SMITH 1976), has also been shown to be sensitive to UV and X rays (SMITH and SHEAR 1974). The isolation of alleles of *mei-41* on the basis of their hypersensitivity to killing by methyl methanesulfonate (MMS) (SMITH 1973, 1976; BOYD *et al.* 1976) further demonstrates that these loci function in the repair of induced lesions. Some *mei-9* alleles have also been recovered from screens for MMS-sensitive mutants (M. GREEN and J. BOYD, personal communication). Mutants at the *mei-9* and *mei-41* loci have been shown to be hypersensitive to killing by nitrogen mustard, 2-acetylaminofluorene and gamma rays, as well as MMS (BOYD, GOLINO and SETLOW 1976). Thus, both of these loci function in the repair of a broad spectrum of chromosome lesions. One of the non-UV, non-X-ray sensitive mutants that we have studied, *mei-218*, has been treated with MMS, nitrogen mustard, and 2-acetylaminofluorene and shown not to differ from wild type in

its sensitivity to any of these compounds (BOYD, GOLINO and SETLOW 1976). Since mutants at the *mei-218* locus exhibit wild-type sensitivity to all mutagens examined as well as normal mitotic chromosome stability, the function of this locus appears to be restricted to meiotic recombination.

Since a number of different pathways for the repair of DNA damage exist in Drosophila (BOYD and PRESLEY 1974; BOYD and SETLOW 1976; BOYD, GOLINO and SETLOW 1976) as in other organisms, it is of interest to inquire whether the *mei-9* and *mei-41* loci function in the same or different pathway(s) of DNA repair. The logic for ascertaining whether mutagen-sensitive loci affect the same or different pathways of DNA repair has been developed from the comparison of yeast strains carrying single- and double-mutant combinations of mutagen-sensitive mutants (BRENDEL and HAYNES 1973). Briefly, if there exist amorphic mutagen-sensitive mutants at two loci and these loci control steps in the same pathway, then the double mutant should be as sensitive as the more sensitive of the two single mutants. However, if the two loci control steps in different pathways, then the sensitivity of the double mutant should exceed that of the more sensitive single mutant. In the latter case, if the repair of a particular type of lesion can normally proceed *via* either of the two pathways, then mutants at the two loci should interact synergistically. However, if the two pathways act on different types of lesions caused by a single agent, mutants would be expected to have a multiplicative interaction ("additive" on logarithmic plots of survival). This reasoning applies strictly only to the analysis of amorphic mutants. The "morphy" of the mutant *mei-9* and *mei-41* alleles is not known. Although the interaction of hypomorphic mutants is potentially more complex, the simplest interpretation of synergistic interactions is that the loci control steps in different pathways that compete in the repair of a particular type of lesion.

The sensitivity to killing by both X rays and UV of third-instar larvae carrying the *mei-9 mei-41* double mutant has been compared to that of larvae carrying these mutants singly (Figure 6; see also BAKER *et al.* 1976b). The double mutant is much more sensitive than either of the single mutants to both UV and X rays. The apparently synergistic interaction of these mutants suggests that these loci identify steps in two different pathways that provide Drosophila with alternative ways of repairing radiation damage in somatic cells. This conclusion is consistent with that reached from biochemical studies on the repair of UV irradiation damage in *mei-41* (BOYD and SETLOW 1976) and *mei-9* (BOYD, GOLINO and SETLOW 1976). The *mei-41* mutants exhibit defects in postreplication repair following UV treatment, whereas *mei-9* is defective in an early step in the excision repair of UV-induced lesions.

Disjunction-defective meiotic mutants: general considerations

Six other meiotic mutants have been examined for their effects on mitotic chromosome stability. These mutants include four disjunction-defective meiotic mutants: *nod* (CARPENTER 1973), ca^{nd} (DAVIS 1969), $l(1)TW\text{-}6^{cs}$ (WRIGHT 1974) and *mei-S332* (DAVIS 1971); a mutant that is both recombination and disjunction defective, *ord* (MASON 1976); and a male-specific meiotic mutant, *pal* (BAKER 1975).

From the similarities of chromosome segregation during meiosis and mitosis, a substantial overlap in the genetic control of chromosome segregation during these two types of cell division would be expected. However, because meiotic mutants must be viable and fertile to be detected in conventional mutant screens, we have argued that disjunction-defective meiotic mutants are likely to be defective in functions unique to meiosis (*e.g.*, BAKER *et al.* 1976a); mutants in loci utilized for both meiotic and mitotic chromosome segregation would be expected to be lethal, although leaky mutants at such loci might be recovered in conventional screens.

The meiotic effects of disjunction-defective mutants are consistent with these expectations. Two of the disjunction-defective mutants (*nod* and *mei-S332*) are defective in processes believed to be unique to meiosis: the segregation of distributively paired chromosomes in females in the case of *nod* (CARPENTER 1973) and keeping sister centromeres together between the first and second meiotic division in both sexes in the case of *mei-S332* (DAVIS 1971). Another disjunction-defective mutant (*l(1)TW-6^{cs}*) was recovered as a cold-sensitive lethal and subsequently shown to cause defective chromosome segregation at meiosis I in females (WRIGHT 1974); it is probably defective in a function common to mitosis and meiosis. The nature of the defects in the other two disjunction-defective mutants (*cand* and *ord*) is less clear. *cand* disrupts segregation during meiosis I in females (DAVIS 1969), and although its pattern of disruption (*i.e.*, frequencies and types of exceptional progeny) is similar to that caused by *l(1)TW-6^{cs}*, homozygous *cand* individuals have good viability. The recombination-defective and disjunction-defective mutant *ord* disrupts segregation at both meiotic divisions in males and females and greatly reduces recombination in females (MASON 1976). The mutant *pal* is defective in a function that is normally carried out during male meiosis and is necessary for the regular segregation of paternally derived chromosomes during the first few mitotic divisions of the embryo (BAKER 1975).

Because of these considerations, we had little expectation that any of these mutants, other than *l(1)TW-6^{cs}*, would disrupt mitotic chromosome behavior. However, the data suggest that several of these loci do function in mitotic cells.

Disjunction-defective mutants: individual loci

nod *and* pal: Neither of these mutants has a significant effect on mitotic chromosome stability (Tables 2, 3 and 5). Thus, it is likely that they specify functions that are unique to meiosis.

mei-S332: The other mutant thought likely to specify a function unique to meiosis, *mei-S332*, is, surprisingly, associated with a striking mitotic effect. In *y/f^{36a}* females homozygous for *mei-S332*, total spots are 2.5 times more frequent than in the control. Not all types of spots are increased equally: twin spots are present at 5.6 times the control rate, whereas single spots are increased only 1.8-fold. This suggests that the chromosome instability produced by *mei-S332* is the result of an elevated frequency of mitotic recombination (or nondisjunction). The size of the spots produced by *mei-S332* suggests that they arise primarily, if not exclusively, during the larval period when the abdominal histoblasts are in a nondividing state, since the average number of bristles per spot and the size

distribution of *mei-S332*-produced clones are very similar to those produced by *mei-W68* or irradiation of larvae (Table 8).

This increase in mitotic recombination in *mei-S332* is unexpected since the nature of its meiotic defect (failure to hold sister centromeres together between the first and second meiotic division) suggested that this locus specified a function unique to meiosis. As only one mutant allele of *mei-S332* is known and we have not attempted to map the site responsible for the increased frequency of mitotic recombination on the *mei-S332*-bearing chromosome, there is no direct evidence that the meiotic and mitotic effects are due to the same mutation. It is interesting to note, however, that *mei-W68* and *mei-S332* are the only mutants in which the major manifestation of mitotic chromosome instability is an increase in twin spots, and in both cases these spots arise during the period when the histoblasts are nondividing. *mei-S332* maps to 95 on the right arm of the second chromosome (DAVIS 1971) and *mei-W68* maps to 94 on the same arm (BAKER, unpublished results). The two mutants complement completely with respect to their meiotic phenotypes (BAKER, unpublished results). It seems unlikely that such a coincidence of phenotypes and map positions could be fortuitous.

To explain these results, we propose that the function of the *mei-S332*$^+$ locus is not to hold sister centromeres together between meiosis I and II, but rather to delay the separation of sister centromeres during all cell divisions. Because the extant *mei-S332* mutant is viable, we suppose either that it is a leaky mutant or else that the locus is but one of several of similar function. That a mutant in a locus that functions to hold sister centromeres together would have a more severe effect on meiosis than on mitosis could be explained by the fact that sister centromeres are held together through the first division and until anaphase of the second division during meiosis, but only until anaphase of mitosis. That abdominal histoblasts are primarily affected by *mei-S332* during the period they are nondividing is also consistent with this hypothesis since during this time these cells are thought to be resting in G2 (*i.e.*, with duplicated chromosomes and undivided centromeres). If this hypothesis is correct, then the marked clones produced by *mei-S332* should arise *via* mitotic nondisjunction; data to test this are not yet available.

l(1)TW-6^{cs} *and* cand: These mutants have similar mitotic effects. Chromosome instability is increased in both wing and abdomen imaginal cells (Tables 2 to 5), from which we infer that these loci function in all tissues.

The temperature sensitivity of *l(1)TW-6*^{cs} has permitted a dramatic demonstration that this locus specifies a function that is crucial for regular mitotic chromosome behavior. In females homozygous for *l(1)TW-6*^{cs}, clones expressing *X*-linked cell markers are increased four-fold at 25°. When such females are reared at 20°, 23% of all abdominal bristles expressed the cell markers (654 *y* and 1223 *f*^{s6a} bristles among 8229 total bristles on 24 abdomens); in addition, 28% of the sexually dimorphic tergites contain marked male clones! Thus, many of the marked clones are the result of mitotic chromosome loss or nondisjunction. The mosaicism in these flies is very fine-grained, indicating that in a substantial

proportion of all cell divisions chromosome behavior is abnormal. The morphology of these abdomens at 20° is not normal; the number of bristles is about 60% of normal, the edges of tergites are frequently ragged, and there are frequently several small clear areas (holes?) in the cuticle of each abdomen. These morphological abnormalities could be the result of substantial cell death. The frequency of *mwh* clones in the wing is also increased in a temperature-sensitive manner. At 25° *mwh* clones are present at 1.4 times the control frequency, whereas at 22° they are 4.5 times more frequent than in the control. These data establish that the wild-type allele of *l(1)TW-6^{cs}* specifies a function of major importance for regular inheritance of chromosomes during mitosis.

Although chromosome loss (or nondisjunction) is an important source of marked clones in *l(1)TW-6^{cs}*-bearing flies at 20°, the frequency of marked bristles is so high under these conditions that other possible sources of marked clones could not be identified. In flies reared at 25° the much lower frequency of marked clones allows some inferences as to the events generating clones. In both *cand* and *l(1)TW-6^{cs}*, the frequency of *y*//*f^{s6a}* twin spots is increased, and twins are more frequent than can be accounted for by the number of coincident *y* and *f^{s6a}* single spots expected under the assumption that all events produce single spots (Table 7). This implies that mitotic recombination (or double nondisjunction) is increased in the presence of each mutant. Marked bristles with the abnormal morphology suggestive of aneuploidy are also increased in frequency. The average size and size distribution of clones expressing *X*-linked markers in *cand* are similar to those of spontaneous control clones (Table 8). The clones produced by *l(1)TW-6^{cs}* at 25° are smaller in size than spontaneous control clones (Table 8). The small average size of the *l(1)TW-6^{cs}*-induced spots suggests that they originate during the terminal burst of cell divisions of the abdominal histoblasts.

In *l(1)TW-6^{cs}* flies reared at 25°, the frequency of spots expressing *X*-linked cell markers is increased four-fold (Table 4), whereas spots expressing chromosome *3* markers are increased 2.7-fold in females, but only 1.2-fold in males (Tables 2 and 3). In homozygous *cand* flies the frequency of spots varies with chromosome and sex in a similar manner. At least *l(1)TW-6^{cs}* must act somatically in males since males bearing this mutant are killed at its restrictive temperature (17°). The greater apparent effect of these mutants on *X*-chromosome stability could be explained by *X*-chromosome aneuploidy being more compatible with cell viability than is autosomal aneuploidy.

For *cand* no direct evidence is available that links the increased mitotic chromosome instability to *cand*. However, the similar patterns of meiotic and mitotic chromosome misbehavior in *l(1)TW-6^{cs}* and *cand* suggest that *cand* does increase mitotic chromosome instability.

The finding that *l(1)TW-6^{cs}*, and by analogy perhaps *cand*, specify functions that are required for mitotic chromosome segregation and segregation during the first, but not the second, meiotic division in females suggests that they are representatives of a class of functions for which there is no cytological or genetic

precedent. Since the second meiotic division differs from both the first meiotic division and mitosis in having no DNA synthesis, one possibility would be that there is some special aspect of DNA synthesis that is required for segregation during meiosis I and mitosis. As neither mutant affects male meiosis, we imagine that the unusual nature of the first meiotic division in males precludes a need for these functions at that time.

ord: The recombination-defective and disjunction-defective meiotic mutant *ord* is also associated with increased mitotic chromosome instability (Tables 2 to 4). For both the *X* chromosome and chromosome 3, twin spots are increased somewhat more than are single spots (Tables 2 to 4), and twins are more frequent than expected from the coincident occurrence of single spots (Table 7). Thus, mitotic recombination (or nondisjunction) is increased. The frequency of spots attributable to breakage is also increased, but the proportion of this type of spot is about the same as that found in the nonmeiotic-mutant controls. Clones in abdomens of *ord* flies are on the average smaller in size than spontaneous clones in control flies (Table 8), suggesting that *ord* affects chromosome stability primarily in actively dividing cells (*e.g.*, the terminal burst of divisions of the histoblasts).

The effect of *ord* on chromosome stability in the wing imaginal disc could not be determined because in flies homozygous for the *ord*-bearing second chromosome and also homozygous for the wild-type allele of *mwh* there were a number of cells (*ca* 10–40) on each wing that had more than one hair and thus mimicked *mwh*.

CONCLUSIONS

These studies suggest that a substantial fraction of the loci that function during meiosis in Drosophila also function in somatic cells. Meiotic mutants at 10 of the 13 loci examined are associated with effects on mitotic chromosome stability.

Mutants at six of the seven recombination-defective loci examined affect mitotic chromosome stability. Although the meiotic expression of these loci is restricted to females, they function in the somatic cells of both sexes, suggesting that the control of mitotic chromosome stability is common to the two sexes.

For the *mei-41*, *mei-9* and *c(3)G* loci, the elevated frequency of spontaneous chromosome instability is accompanied by a hypersensitivity to damage by mutagens (BAKER *et al.* 1976b and this paper; BOYD and SETLOW 1976; BOYD, GOLINO and SETLOW 1976; SMITH and SHEAR 1974; SMITH 1976; WATSON 1969, 1972; HAENDLE, cited in BECKER 1976 and personal communication). Thus, these loci specify functions common to meiotic recombination and repair. Further evidence that meiotic recombination and the repair of mutagen-induced damage are under common genetic control in Drosophila is provided by the isolation of mutagen-sensitive mutants that increase meiotic nondisjunction in females and hence may be recombination-defective (SMITH 1973, 1976; BOYD *et al.* 1976). Mutants that are potentially defective in processes common to repair and meiotic exchange have been isolated in a number of fungal species by a variety of criteria (*e.g.*,

mutagen sensitivity, defective sporulation, nuclease deficiency, elevated meiotic nondisjunction, altered intragenic exchange; reviewed by BAKER *et al.* 1976a). Some of the mutants that are hypersensitive to mutagens also affect meiotic intragenic exchange or meiotic fertility and thus are likely candidates for defects in functions common to meiotic recombination and repair. However, we are aware of no case in which a mutant has been shown to affect both intergenic meiotic exchange and repair.

The synergistic interaction of *mei-9* and *mei-41* with respect to both UV and X-ray sensitivity suggests that they are defective in alternative pathways for the repair of the same lesions (BAKER *et al.* 1976b; this report). This is in agreement with the findings that *mei-41* is defective in postreplication repair (BOYD and SETLOW 1976) and *mei-9* is defective in excision repair of UV damage (BOYD, GOLINO and SETLOW 1976). The function of these loci in the repair of X-ray damage is not known; the rejoining of X-ray-induced single-strand breaks is normal in mutants at both loci (BOYD and SETLOW 1976; BOYD, GOLINO and SETLOW 1976). Thus, loci that function in several different repair pathways in somatic cells are utilized to carry out meiotic recombination. Moreover, the pathway(s) in which they function meiotically must overlap to some extent since in *mei-9* meiotic recombination is reduced to 8% of wild-type levels, in *mei-41* to 50% of wild-type levels, and in *c(3)G* there is no meiotic exchange.

The high incidence of spontaneous chromosome instability caused by these mutants shows that these loci specify functions that also participate in the normal chromosomal metabolism of Drosophila. Their effects are most straight-forwardly interpreted as resulting from an inability to repair spontaneous lesions: the presence in chromosomes of the discontinuities that are normally removed by the wild-type products of these loci can serve to initiate a sequence of events that results in chromosome instability. The mechanisms by which chromosome instability is generated by the different Drosophila recombination- and repair-defective mutants differ markedly. In *mei-41*, chromosome breakage is the immediate cause of instability, whereas in *mei-9* both breakage and, to a lesser degree, mitotic recombination are increased. Our inference that high frequencies of spontaneous breakage are caused by *mei-9* and *mei-41* alleles has been directly confirmed by M. GATTI (in preparation) in metaphases from ganglia of larvae bearing these mutants. In *c(3)G*, no predominant mechanism responsible for the elevated frequency of mitotic chromosome instability is discernible. For each of these loci, the same pattern of chromosome instability was produced by two or more mutant alleles, suggesting that the pattern of effects is a property of the locus. These results suggest that different abnormalities in DNA structure serve as substrates for the wild-type products of these three loci. Increased spontaneous mitotic chromosome instability is also caused by a number of the mutagen-sensitive mutants in fungi (*e.g.*, Neurospora, SCHROEDER 1970, 1974, 1975; Aspergillus, PARAG and PARAG 1975; PARAG and ROPER 1975; SHANFIELD and KÄFER 1969; JANSEN 1970; Ustilago, HOLLIDAY 1967; Saccharomyces, SNOW 1968; KOWALSKI and LASKOWSKI 1974; LEMONTT 1971; BORAM and ROMAN 1976;

HASTINGS, QUAH and VON BORSTEL 1976; reviewed by BAKER *et al.* (1976a) and humans (*e.g.*, Bloom's syndrome, GERMAN and CRIPPA 1966; GERMAN 1974; GERMAN, CRIPPA and BLOOM 1974; CHAGANTI, SCHONBERG and GERMAN 1974; Fanconi's anemia, GERMAN and CRIPPA 1966; SCHROEDER, ANSCHATZ and KNOPP 1964; DE GROUCHY *et al.* 1975; LATT *et al.* 1975).

For the three other loci at which recombination-defective mutants reduce mitotic chromosome stability (*mei-352*, *mei-W68* and *mei-S282*), the nature of the defective function is less clear. *mei-352* produces a pattern of instability similar to that elicited by *mei-41*, but it is no more sensitive than wild type to killing by either UV or X rays. Thus, it is not clear at present whether the *mei-352* locus also specifies a function common to recombination and repair.

The mitotic effects of *mei-W68* are quite distinct from those of the mutants that are known to be repair defective. *mei-W68* affects chromosome behavior in the imaginal cells of the abdomen but not the wing, and in the abdomen it increases mitotic recombination primarily during the larval period when the histoblasts are in a nondividing state. The data from *mei-S282* suggest that it may have a similar tissue specificity. We interpret this specificity to mean either that these loci are defective in functions that are needed during G2 (the resting stage of the abdominal histoblasts) or that these loci are expressed in all cells, but in cells other than the nondividing histoblasts there are other loci functioning that can compensate for the defects in the *mei-W68* and *mei-S282* mutants. Although we can rationalize the specificity of *mei-W68* and *mei-S282* in this way, we have no clear conception as to what type of functions are specified by the wild-type alleles of these loci; these mutants could be either slightly hypomorphic alleles of loci that carry out essential mitotic functions or, alternatively, amorphic mutants in loci that have only peripheral roles in mitotic cells.

One of our reasons for examining the effects of these recombination-defective meiotic mutants on mitotic chromosome behavior was to determine whether meiotic and mitotic recombination were under common genetic control. Mitotic recombination is clearly at, or above, control levels in the presence of mutants at all loci except *mei-41*; and even here we believe that mitotic recombination does occur. A small proportion of the clones produced by *mei-41* alleles in the wing appear to have the clone-size distribution expected for euploid clones and thus might be the consequence of mitotic exchanges. In addition, for some of the twin spots detected in *mei-41*, the arrangement and sizes of the two halves of the twin gave us the strong impression that they comprised real twin spots; not just two independent single clones.

The occurrence of spontaneous mitotic recombination in the presence of all of the mutants poses a problem, since it would be expected *a priori* that a locus involved in both meiotic recombination and somatic DNA metabolism would also be involved in mitotic recombination. However, if there is more than one pathway by which mitotic recombination can occur in Drosophila, then mutants such as these could be defective in one such pathway—and the resulting defect obscured by the functioning of alternative pathways on the lesions left unresolved

by the defect. Indeed, evidence for multiple pathways of induced mitotic recombination has been presented by HAENDLE (1971a,b, 1974), who has resolved two mechanisms by which X rays induced mitotic crossing over in wild type. The recombination defective mutant *c(3)G*^{17} lacks the ability to respond *via* one of these mechanisms, whereas the induction of mitotic recombination *via* the other mechanism is normal (HAENDLE, personal communication; cited in BECKER 1976).

Ex post facto, one of the most surprising results from our examination of the mitotic effects of meiotic mutants is that the disjunction-defective mutants *mei-S332*, *ca*nd, and *ord* also affect mitotic chromosome behavior. Such a finding is surprising because mutants defective in mitotic chromosome segregation would be expected to be lethal, as is *l(1)TW-6*^{cs}, which our data clearly indicate to be defective in a function essential for regular mitotic chromosome behavior. There are two basic explanations possible for the phenotypes exhibited by these mutants. One is that these loci specify functions that play a major role in meiotic chromosome segregation (since all three mutants have very strong meiotic effects), but only a minor role in the mitotic cell cycle (since these mutants exhibit good viability and, compared to *l(1)TW-6*^{cs} at semi-restrictive temperatures, relatively minor mitotic effects). Alternatively, these mutants could be leaky alleles of loci that specify functions essential for mitotic as well as meiotic chromosome segregation. In the latter case it is necessary to suppose that meiosis is much more sensitive than mitosis to the defects. This supposition is not unreasonable since in the case of *l(1)TW-6*^{cs} mitotic chromosome stability is affected very little under conditions (25°) where nearly every meiotic cell division in females is abnormal. For two of these disjunction-defective mutants, *mei-S332* and *ca*nd, our data on their mitotic effects provide some suggestion that they may, in fact, be leaky mutants in loci essential for mitotic as well as meiotic chromosome segregation. Thus, the distinctive patterns of meiotic and mitotic chromosome misbehavior produced by *ca*nd are strikingly similar to those produced at 25° by *l(1)TW-6*^{cs}, a mutant clearly defective in an essential mitotic function. In the case of *mei-S332*, our limited data on its mitotic effects have led us to speculate that it may be a leaky mutant in a function required to hold sister centromeres together during mitotic as well as meiotic cell divisions. That it affects the abdominal histoblasts during the larval period when they are not dividing, as well as meiosis, is consistent with this view since sister centromeres need to be held together longer at these times than they do in continuously dividing cells.

Our interest in these loci is stimulated by the possibility that they may control functions important for both mitotic and meiotic chromosome segregation. However, only in the case of *l(1)TW-6*^{cs} (and the recombination-defective mutant *mei-W68*) do our data directly demonstrate that increased mitotic nondisjunction or chromosome loss is a source of chromosome instability in any of these mutants. In these experiments we have relied on the male sexual characteristics of 1*X*2*A* cells as diagnostic of the occurrence of mitotic nondisjunction or chromosome loss in females. However, our analysis of all of the male abdominal clones that we

have observed strongly suggests that such clones can arise only in the embryo (possible reasons for this are explored in the following paragraph). This is of concern here because many embryonic functions are programmed by the maternal genome, and all of the flies we have scored for the presence of male clones have come from mothers that possessed a wild-type allele of the meiotic mutant being examined. The generation of mitotic chromosome loss or nondisjunction by these mutants might have been masked by such maternal effects.

That phenotypically male clones can arise only in the embryo is suggested to us by the sizes of such clones. In *mei-W68*, the average male clone on abdomens encompasses 5.9 bristles, which is substantially larger than the average size of marked (*e.g.*, y or f^{36a}) clones that are inducible in the abdominal histoblasts of larvae by X rays (*ca* 2.5 bristles). In y/f^{36a} females homozygous for $l(1)TW\text{-}6^{cs}$ at 20°, the immense number of marked bristles made it difficult to estimate accurately the number of marked bristles in each male spot. A conservative estimate would be about 4.5 bristles per male spot. Since the total number of bristles on these abdomens was only 60% of that found in wild type, the average male spot in $l(1)TW\text{-}6^{cs}$ is equivalent in size to a clone of about seven to eight bristles in wild type. In other meiotic mutants and controls heterozygous for y and f^{36a}, there were 17 marked male clones with an average size of 9.2 bristles (if one clone of 31 bristles is excluded, the average is 7.3 bristles). Thus, all of these data suggest that phenotypically male clones in the abdomen can be generated only prior to the cessation of division of the abdominal histoblasts in the embryo. We interpret these results to mean that an event occurs during the embryonic period that precludes the subsequent generation of $1X2A$ cells that express male sexual characteristics. These data do not distinguish between the possibilities (1) that $1X2A$ cells generated after this event are viable but female in phenotype (*i.e.*, the X:autosome ratio completes its role in sex determination in the embryo) or (2) that $1X2A$ cells arising subsequent to this event are not viable.

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