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Cell autonomy of two DNA-repair mutations in *Drosophila melanogaster*

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SUMMARY

By means of genetic mosaics we have studied the cell autonomy of *mei-41* and *mei-9*, two loci involved in DNA metabolism. The frequency of spontaneous somatic spots resulting from unrepaired chromosome damage and the sensitivity of mutant cells to killing by X-irradiation – two traits indicative of deficient DNA repair – have been analysed at the cellular level. The results show that: (1) The effect of both mutations on chromosome stability is cell autonomous. (2) After 1000 r of X-irradiation practically all the cells homozygous for *mei-41* disappear while about one-third of the cells homozygous for *mei-9*^{a} survive the irradiation. The possibility of using these mutants as tools to approach developmental problems is discussed.

1. INTRODUCTION

Several viable mutations affecting DNA metabolism in *Drosophila* have been reported during the last few years (Smith, 1973, 1976; Baker *et al.* 1976; Baker, Carpenter & Ripoll, 1978; Boyd *et al.* 1976*a*). Two loci, *mei-41* and *mei-9* (Baker & Carpenter, 1972), have received special attention. As a consequence of a reduced ability to repair spontaneous or induced chromosome damage, mutants in these two loci show two phenotypic traits that will be the object of this work. One, which will be called the *mitotic effect*, consists of a high frequency of spontaneous chromosome breakage. It is inferred from an increased frequency of spontaneously arising somatic clones (Baker *et al.* 1976; Baker *et al.* 1978) and can be visualized in larval neuroblasts (Gatti, 1979). The other trait is a high mortality of mutant larvae exposed to ionizing radiation or chemical mutagens (Smith, 1973, 1976; Boyd *et al.* 1976*a*; Smith & Shear, 1974; Boyd, Golino & Setlow, 1976*b*; Baker *et al.* 1976; Baker *et al.* 1978; Nguyen, Green & Boyd, 1978). It will be referred to here as *X-ray sensitivity*.

In individuals heterozygous for a mutation it is possible by mitotic recombination to generate clones of cells homozygous for the mutant allele (Stern, 1936). If all the cells forming the clone, and only those cells, express the mutant phenotype, then the trait is said to be cell-autonomous. The aim of this work is to analyse

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the cell autonomy of two repair-defective mutations as well as the X-ray sensitivity of the mutant cells. Our results show that the mitotic effects of both *mei-9^a* and *mei-41* are cell autonomous and that while few of the homozygous *mei-41* cells survive after 1000 r of X-irradiation, about one-third of the homozygous *mei-9^a* cells are still able to divide and differentiate.

2. MATERIALS AND METHODS

Crosses. Flies were reared on standard cornmeal-agar yeast molasses medium at 25 °C. Except when noted, all mutations and aberrations are described in Lindsley & Grell (1968). Females of genotype *y; Dp(1;3)sc^{J4}, y$^+$ sc^{J4} mwh h* were mated to males of the following genotypes: (1) *y sn^{76} v mei-41/ Y*, (2) *y sn^{76} v/ Y*, (3) *mei-9^a f^{36a}/B^S Y* and (4) *f^{36a}/ Y*. All crosses yield male progeny of genotype: *y/ Y* (*B^S Y* in cross 3); *Dp(1;3)sc^{J4}, y$^+$ sc^{J4} mwh h/+*. The following female genotypes are recovered: (1) *y sn^{76} v mei-41/+*; *Dp(1;3)sc^{J4}, y$^+$ sc^{J4} mwh h/+* (sn mei-41), (2) *y sn^{76} v/+; Dp(1;3)sc^{J4}, y$^+$ sc^{J4} mwh h/+* (sn), (3) *mei-9 f^{36a}/+; Dp(1;3) sc^{J4} mwh h/+* (mei-9 f) and (4) *f^{36a}/+ ; Dp(1;3)sc^{J4}, y$^+$ sc^{J4} mwh h/+* (f). For the sake of simplicity in the tables we will refer to different genotypes as indicated in parentheses. In the text, genotypes 1 and 3 will be frequently referred to as 'mutant females' and genotypes 2 and 4 as 'nonmutant females'.

Meiotic mutations. The cell autonomy of two recessive sex-linked meiotic mutations will be examined. As described above, *mei-41* (1–54) and *mei-9^a* (1–7) reduce meiotic recombination, and increase both chromosome instability in males and females and the sensitivity of mutant larvae to killing by ionizing radiations or chemical mutagens. *mei-41* is defective in postreplication repair of UV-induced DNA damage (Boyd & Setlow, 1976); *mei-9* is defective in excision repair of UV-induced pyrimidine dimers (Boyd *et al.* 1976*b*).

Cell marker mutations. To monitor the occurrence of mitotic recombination three cell marker mutations have been used. *Singed-76* (*sn^{76}*: 1–26, Kennison & Ripoll, 1981) and *forked-36a* (*f^{36a}* : 1–56·7), which alter the shape of trichomes covering the wing blade from straight to wavy, and *multiple wing hairs* (*mwh*: 3–0·0) which results in the production of several cuticular processes per wing cell instead of one. While clones formed by single *mwh* cells are easy to score, not all of the small clones marked with *sn^{76}* or *f^{36a}* are detected. From our experience while collecting the results presented here, we feel that all three cell marker mutations are equally reliable for clones larger than four cells.

Following mitotic recombination proximal to *mei-41* in females of genotype 1 (sn mei-41 mutant females), clones marked with *singed* are simultaneously homozygous for *mei-41*. In females of genotype 3 (mei-9 f mutant females) the homozygosity for *forked* is accompanied by homozygosity for *mei-9^a*. Clones marked with *multiple wing hairs* remain heterozygous for the meiotic mutations in both genotypes.

Irradiation. Females crossed to males of different genotypes were allowed to lay eggs for 24 h in split bottles. Larvae were exposed 48 h after removal of the parents to 500 r of X-irradiation (250 kV, 15 mA, 3 mm Al filter, 250 r/min) in the bottom halves of the bottles. Ninety-six hours after irradiation the larvae were separated

into two groups: one was kept to study the effect of the irradiation early in development (single irradiation experiments); the other was irradiated again with a dose of 1000 r under the same conditions as above (double irradiation experiments). At the time of the second irradiation the population of treated larvae is physiologically highly asynchronous, some larvae delaying pupariation at least three days. For this reason the larvae were also timed with respect to the time elapsed between the second irradiation and puparium formation. In both samples pupae were collected at daily intervals and dated with respect to time of puparium formation, discarding all individuals pupating after 3 days. In single irradiation experiments data from these intervals were pooled for analysis.

Scoring of clones. Adult wings of emerged flies were dissected, washed in alcohol and mounted in Euparal for examination under the compound microscope (400 ×). Only the distal compartment of the wing blade (García-Bellido, Ripoll & Morata, 1976) was scored for clones. From García-Bellido & Merriam (1971*a*) we estimate that we have scored approximately 20000 cells per wing. Since some of the interpretations of our results are based on the accuracy of this estimate, a direct count was made of the number of trichomes in the ventral surface on one wild-type wing from a scanning electromicrograph (500 ×) kindly provided to us by R. Hardy. Excluding the veins, the distal compartment was formed by 9182 cells. The growth parameters of the wing imaginal disc have been published by Bryant (1970) and García-Bellido & Merriam (1971*a*).

3. RESULTS

(*i*) *Single irradiation experiments*

Larvae of different genotypes were exposed to 500 r of X-irradiation and the adult wings scored for clones marked with *singed, forked* or *multiple wing hairs*. The aim of these experiments is threefold: to look at the cellular level for possible heterozygous effects of the meiotic mutations after irradiation, to analyse the cell autonomy of the mitotic effect, and to provide a control for double irradiation experiments. Results obtained from these experiments are presented in Table 1.

Heterozygous effects. For spontaneous events it has already been shown that females heterozygous for *mei-9*^{a} or *mei-41* do not show any increase in the frequency of either *mwh* clones in the wing (Kenninson & Ripoll, 1981) or chromosome breakage in larval neuroblasts (Gatti, 1979). Therefore both mutations are recessive as far as the mitotic effect is concerned. A possible slight semidominant effect in the abdomen has been reported (Baker *et al.* 1978). To our knowledge, no information is available on the heterozygous effects of these mutations with respect to their X-ray sensitivity. Several alleles of *mei-41* have been reported to exhibit semidominant sensitivity to killing by methyl methanesulphonate when larvae are fed high doses of this mutagen; nevertheless, they are recessive when treated with concentrations not affecting the survival of wild-type larvae (Boyd *et al.* 1976*a*).

It has been shown (Kennison & Ripoll, 1981) that most, if not all, of the small clones found after irradiation of larvae early in development derive from spontaneous events taking place late in development. Conversely, the large clones

Table 1. *Number of clones of different size classes in female and male wings from single irradiation experiments*

Genotype		Cell marker	Clone size in number of cells: 1	2	3 4	5 8	9 16	17 32	33 64	65 128	129 256	257 512	513 1024	1025 2048	No. of wings	*Mean frequency	*Mean size
sn																	
	♀	*sn*	—	—	—	1	0	6	0	1	7	14	6	4	289	0·13	450
	♀	*mwh*	95	25	6	3	1	2	1	1	3	5	1	0	289	0·04	258
	♂	*mwh*	49	13	2	2	3	1	2	2	10	5	4	0	304	0·08	296
sn mei-41																	
	♀	*sn*	—	—	—	5	3	4	2	3	8	9	8	0	204	0·18	333
	♀	*mwh*	187	48	4	7	1	1	1	1	6	3	4	1	204	0·08	417
	♂	*mwh*	57	18	7	1	0	1	1	1	1	1	3	0	228	0·04	374
f																	
	♀	*f*	—	—	—	0	0	0	0	3	8	13	3	1	188	0·15	354
	♀	*mwh*	219	35	4	0	1	0	0	0	6	1	1	0	188	0·04	252
	♂	*mwh*	79	6	1	1	0	1	0	0	1	4	1	0	155	0·05	342
mei-9 f																	
	♀	*f*	—	—	—	2	0	1	2	8	21	17	1	1	336	0·15	246
	♀	*mwh*	328	72	17	11	6	3	5	10	13	5	0	0	336	0·11	134
	♂	*mwh*	119	17	6	0	1	0	2	8	9	3	2	1	242	0·10	270

Genotypes are described in Materials and Methods. Males are sibs of the females above them.
* Mean frequencies and sizes refer to clones larger than 16 cells. —, not scored.

derive mainly from events induced by the irradiation. Frequencies and mean sizes of clones larger than 16 cells from single irradiation experiments are presented in Table 1. Several conclusions can be drawn from these data:

(1) In mutant females, the mean sizes of clones marked with *singed* or *forked* are similar to those of clones marked with *multiple wing hairs* appearing in the same sample of wings, differences being smaller than one cell division. This indicates that homozygous mutant cells (*mei-41* or *mei-9^a*) are as viable and have the same growth kinetics as heterozygous cells. Moreover, since clone size in mutant females is the same as that in nonmutant females or males, it can be concluded that cells homozygous, heterozygous or wild type for the meiotic mutants are equally viable.

(2) A comparison of frequencies of clones marked with the same cell marker in mutant and nonmutant females as well as in their male sibs does not reveal any differences above those found among males. Therefore *mei-41* and *mei-9^a* heterozygous cells are similar to wild-type cells with respect to their ability to generate marked clones in response to 500 r of X-rays.

(3) Frequencies and sizes of *sn^{76}* (or *f^{36a}*) clones are similar in mutant and nonmutant females, suggesting that *mei-41* and *mei-9^a* heterozygous cells are no more sensitive than wildtype cells to killing by X-irradiation. A similar conclusion is reached if *mwh* clones in mutant females are compared with *mwh* clones in their male sibs. At the cellular level, therefore, *mei-41* and *mei-9^a* are recessive with respect to their sensitivity to killing by 500 r of X-rays.

Mitotic effects. Homozygous *mei-41* cells are marked with *singed* and homozygous *mei-9^a* cells with *forked* following mitotic recombination in the first chromosomes of mutant females. These cells remain heterozygous for *multiple wing hairs*. If the mitotic effect of these mutations is cell autonomous, an elevated frequency of spontaneous *mwh* clones will be found within *sn^{76}* and *f^{36a}* clones. We will refer to those *mwh* clones that appear within other clones as 'internal' clones. The remaining clones will be referred to as 'external' clones. If the mitotic effect is not cell autonomous, the frequency of internal and external clones should be similar.

Table 2. *Frequencies of internal and external* mwh *clones of one and two cells in wings from single irradiation experiments*

	Internal clones			External clones		
Genotype	No. of *mwh* clones	No. of *sn^{76}* or *f^{36a}* cells scored*	*mwh* clones per *sn^{76}* or *f^{36a}* wing†	No. of *mwh* clones	No. of wings scored	*mwh* clones per wing
sn	2	17, 100	2·3	120	289	0·4
sn mei-41	59	11, 322	104·2	235	204	1·2
f	4	9, 912	8·1	254	188	1·4
mei-9 f	24	12, 546	38·3	400	336	1·2

* Calculated adding the size of all *sn^{76}* or *f^{36a}* clones larger than 16 cells.
† Calculations assume 20000 cells per wing.

Comparisons of the frequencies of internal and external clones are presented in Table 2. All of the internal clones consisted of one (79 cases) or two (10 cases) cells. Larger internal clones are expected to be rare since spontaneous *mwh* clones of one

or two cells represent 93–98% of the total number of *mwh* clones found in unirradiated females homozygous, heterozygous or wild type for the meiotic mutants (Baker *et al.* 1978; Kennison & Ripoll, 1981). All but two of the internal clones were found within sn^{76} or f^{36a} clones larger than 64 cells. Relative to external clones in the same wings, internal clones are 90 times more frequent in *mei-41* cells and 32 times more frequent in $mei\text{-}9^{a}$ cells. The homozygosity for either meiotic mutation is therefore accompanied by a dramatic increase in the frequency of spontaneously arising *mwh* clones, showing that the mitotic effects of both *mei-41* and $mei\text{-}9^{a}$ are cell autonomous.

(ii) *Double irradiation experiments*

Irradiation of mutant females early in development generates cells homozygous for the meiotic mutations marked with *singed* (*mei-41*) or *forked* ($mei\text{-}9^{a}$). Irradiation of the same larvae later in development, after proliferation of the homozygous cells, will therefore affect two genotypically distinct cell populations in each sample, one heterozygous and the other homozygous for a particular mutation and its accompanying cell marker mutation. Two types of clones will appear in individuals irradiated twice. Those produced by the first irradiation will be large, while those

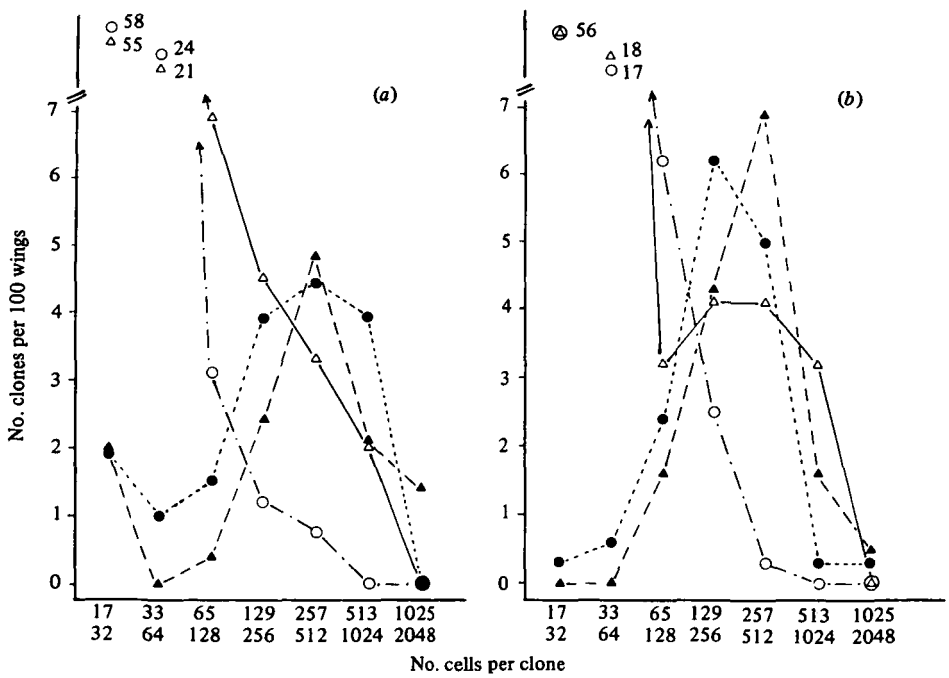

Fig. 1. Frequency distribution of clones larger than 16 cells marked with *singed* (*a*) or *forked* (*b*). Triangles, non-mutant females: $y\ sn^{76}\ v/+$ in (*a*) and $f^{36a}/+$ in (*b*). Circles, mutant females: $y\ sn^{76}\ v\ mei\text{-}41/+$ in (*a*) and $mei\text{-}9^{a}\ f^{36a}/+$ in (*b*). Full symbols, single irradiation experiments. Open symbols, double irradiation experiments. Numbers in upper left corners represent frequencies of clones of sizes 17–32 and 33–64 in double irradiation experiments.

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Table 3. *Number of* sn[76], f[36a], *and* mwh *clones of different size classes in wings from double irradiation experiments*

	Clone size in number of cells																						
	1		2		3–4		5–8		9–16		17–32		33–64		65–128		129–256		257–512		513–1024		1025–2048		
Age*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	*sn f*	*mwh*	No. of wings
Genotype: sn																									
0–24	—	493	223	591	232	377	223	209	46	28	13	4	0	1	0	0	8	1	5	0	3	2	0	0	93
24–48	—	64	10	42	35	57	122	69	120	61	56	33	6	3	1	1	1	1	1	0	1	0	0	1	59
48–72	—	53	2	22	2	10	15	16	58	33	65	39	45	33	16	7	1	0	2	2	1	0	0	1	91
Genotype: sn mei-41																									
0–24	—	767	442	741	611	604	617	422	149	117	7	4	2	2	0	2	2	2	1	1	0	1	0	0	130
24–28	—	106	27	49	44	58	109	72	124	98	59	43	11	9	0	0	0	0	0	1	0	1	0	0	53
48–72	—	79	9	29	9	18	25	30	65	33	81	53	47	18	8	4	1	3	1	1	0	0	0	0	71
Genotype: f																									
0–24	—	824	536	548	520	316	164	76	16	4	0	1	1	0	1	0	6	0	4	2	1	2	0	0	76
24–48	—	145	54	81	140	101	194	95	116	68	26	14	6	1	0	0	3	0	1	1	3	0	0	0	71
48–72	—	134	2	44	12	21	42	44	135	62	97	38	33	17	6	5	0	0	4	0	3	0	0	0	74
Genotype: mei-9 f																									
0–24	—	896	460	804	580	604	428	256	76	52	2	2	4	3	11	4	4	4	1	2	0	1	0	0	124
24–48	—	179	38	101	131	121	248	179	197	147	65	41	4	8	3	1	2	3	0	1	0	0	0	0	108
48–72	—	212	8	62	14	36	57	70	136	81	130	69	51	28	8	9	3	3	0	0	0	1	0	0	122

—, Not scored.

* Hours elapsed between the second irradiation and puparium formation.

produced by the second irradiation will be smaller and more frequent. Results from these experiments are presented in Table 3. As expected, there is some overlap in the size distributions of clones generated by the two different times of irradiation. The overlapping region corresponds to clones between 33 and 128 cells. In what follows we will consider that clones smaller than 33 cells derive primarily from the second irradiation, while clones larger than 128 cells are due to the first irradiation.

X-ray sensitivity. If the sensitivity to killing by X-rays is cell autonomous, the second irradiation should lead to the disappearance of cells homozygous for the radiation-sensitive mutations and, as a consequence, no large sn^{76} or f^{36a} clones resulting from the first irradiation should be found in mutant females. To study the effect of the irradiation on the viability of homozygous mutant cells, the sizes and frequencies of sn^{76} or f^{36a} clones in mutant and nonmutant females after one or two irradiations were compared (Fig. 1). In *mei-41* heterozygous females (Fig. 1*a*) there is a decrease in the frequency of sn^{76} clones larger than 64 cells after two irradiations. In *mei-9*^{a} heterozygous females there is a decrease in frequency after two irradiations for clones larger than 128 cells (Fig. 1*b*). It can be concluded from these data that cells homozygous either for *mei-41* or *mei-9*^{a} are more X-ray sensitive than cells nonmutant for these loci. Although this is qualitatively true, a few large clones are still found in both types of mutant females after two irradiations. To quantitate the extent to which X-irradiation affects the viability of homozygous mutant cells, it is important to know the origin of the 'surviving' clones.

mei-41 is located 28 map units proximal to *singed*. Mitotic exchange in this chromosome interval yields clones that are homozygous for the cell marker mutation but remain heterozygous for the meiotic mutation. From single irradiation experiments it is possible to estimate the fraction of sn^{76} clones due to mitotic recombination distal to *mei-41*. In females heterozygous for *mei-41*, 28 sn^{76} clones larger than 64 cells were found. Of these, 19 had at least one internal *mwh* clone. Assuming that only sn^{76} clones with internal *mwh* clones are homozygous for *mei-41*, 32 % of the clones marked with *singed* would have derived from exchanges distal to *mei-41*. This figure obviously is an overestimate since not all *mei-41* homozygous clones will contain internal clones. To estimate the expected number of sn^{76} clones in mutant females after two irradiations it is possible to use the relative frequencies of sn^{76} and *mwh* clones in their sibs irradiated only early in development. In mutant females irradiated once there are 1·8 (25/14) sn^{76} clones larger than 128 cells for every *mwh* clone of the same size class. In mutant females irradiated twice, 18 sn^{76} clones with more than 128 cells were expected from 10 *mwh* clones observed, and only five (28 %) were found. This figure is close to our previous estimate (32 %), suggesting that only clones heterozygous for *mei-41* survived the second irradiation. There is, however, one possible exception. Of these five clones, one has a somewhat abnormal phenotype: comprised of 131 cells, it included 13 internal *mwh* clones as well as a high number of cells either lacking trichomes or with very short ones. We consider this clone to be one of the rare homozygous *mei-41* clones that survived the second irradiation. In wings irradiated later in development only two other clones with abnormal hairs were found, comprised of 58 and 40 cells, respectively. Their origin will be discussed later.

mei-9 is distal to *forked* the cell marker used in these experiments. Therefore, most of the large f^{36a} clones found in mutant females from double irradiation experiments must be homozygous $mei\text{-}9^{a}$ clones that survived the irradiation. No clones with abnormal phenotypes as those described for *mei-41* have been observed in this case. It can be seen in Fig. 1*b* that clones between 65 and 128 cells in size are more frequent after two irradiations than in wings irradiated once. This increase in the frequency of clones of a given size at the expense of larger ones can be interpreted as a result of the disappearance of a fraction of the homozygous cells due to their X-ray sensitivity. Since the mean size of the large clones after the first irradiation is approximately 300 cells, the shift in clone size to an average of 90 cells suggests that about one-third of the homozygous $mei\text{-}9^{a}$ cells survived the second irradiation.

Double events. In all experiments involving two irradiations, clones with all of the cells marked simultaneously with *singed* (or *forked*) and *multiple wing hairs* are found. These probably arise from two independent mitotic recombinational events in the same cell, one in the first and another in the third chromosome. Depending on the segregation of centromeres, these events give rise to unmarked cells, cells homozygous for either of the cell marker mutations, doubly marked cells (coincident spots) and twin spots. Data on sizes and frequencies of coincident and twin spots in double irradiation experiments are presented in Table 4. Only clones that can unequivocally be termed coincident or twin are included and therefore the figures in the Table are doubtlessly underestimates. The most striking feature of the data in Table 4 is the high frequency of clones derived from double events in *mei-41* heterozygous cells. In single irradiation experiments double events were found only in *mei-41* mutant females. These events were one twin spot (400 sn^{76} cells, 600 *mwh* cells) and three coincident spots (1, 2 and 23 cells).

Table 4. *Sizes and frequencies of coincident and twin clones from simultaneous mitotic events in chromosomes 1 and 3 in double irradiation experiments*

	Clone size*						
Genotype	1–4	5–8	9–16	17–32	33–64	No. of wings	Frequency per 100 wings
sn	2	3	5	2	1	170	7·7
sn mei-41	34	17	10	7	3	144	49·3
f	1	4	5	2	0	164	7·3
mei-9 f	5	4	4	3	1	261	6·5

* Size refers to the number of *mwh* cells.

We do not know if *mei-41* itself is responsible for this dominant increase in the frequency of double events, since this new trait has neither been mapped nor other alleles of the locus have been studied. It is puzzling that the seven-fold increase in frequency of coincident and twin spots is not accompanied by an overall increase in clone frequency. We have not been able to find a reasonable explanation for this paradox, which deserves further investigation.

4. DISCUSSION

(i) *Mitotic effects*

In a genetic mosaic, the phenotype of the cells homozygous for a mutant allele of a gene can, at times, be totally or partially suppressed by the influence of the surrounding wildtype tissue; if all the cells forming the clone express the mutant phenotype the mutant trait is said to be cell autonomous. Cell autonomy is easy to ascertain when the homozygosity for the mutation is associated with a clear morphological change. This is the case, for example, of cell marker mutations. To evaluate the cell autonomy of a phenotype that, like clone frequency, can only be measured in statistical terms poses some problems. It has been shown (Table 2) that the homozygosity for either *mei-41* or *mei-9^a* is accompanied by a drastic increase in the frequency of internal *mwh* clones, the estimated frequencies being 104 and 38 clones per wing, respectively. Both figures are of the same order as those previously reported for homozygous mutant flies (Baker *et al.* 1978). Since the increase in frequency of spontaneous *mwh* clones is restricted to cells made homozygous for the meiotic mutations we conclude that their mitotic effects are cell-autonomous. A partial nonautonomy cannot be ruled out, as we lack the means to measure it.

(ii) *X-ray sensitivity*

Larvae homozygous for either *mei-41* or *mei-9^a* are more sensitive to killing by X-irradiation than wild-type larvae. *mei-41* third instar larvae are about three times more sensitive than *mei-9^a* larvae of the same age to exposure to 1000 r (Baker *et al.* 1978). As we have shown above, the response of homozygous mutant cells to X-irradiation follows the same order, *mei-41* cells being more sensitive than *mei-9^a* cells.

Irradiation of *mei-9^a* or *mei-41* homozygous first instar larvae does not result in increased mortality relative to wild-type larvae, suggesting a maternal effect of the wild-type alleles of these mutations. Cell divisions seem to be required for the maternal wild-type products to be diluted and/or degraded before the irradiation is effective (Baker *et al.* 1978). A phenomenon similar to a maternal effect taking place late in development at the cellular level has been termed perdurance by García-Bellido & Merriam (1971*b*). Both phenomena are equivalent since when studying cell-autonomous lethal mutations there is a correlation between the strength of their maternal effect (indicated by their lethal effective phase) and their degree of perdurance, i.e. mutations with a stronger maternal effect need more cell divisions for the cell-lethal phenotype to show up in clones (Ripoll, 1977). In a similar way, cells made homozygous for DNA-repair mutations might require several cell divisions to deplete their levels of wild-type product enough to prevent the repair of the lesions caused by the X-rays at the time of the second irradiation. Nevertheless, aneuploid cells resulting from unrepaired or misrepaired DNA lesions might survive as long as their cytoplasm contains enough of the wild-type products of essential genes affected by the aneuploidy. It follows that death of homozygous mutant cells after irradiation may not occur under the following

circumstances: (*a*) if the second irradiation is performed shortly after generating homozygosity for the mutation; (*b*) if the homozygous cells are exposed to irradiation close enough to the end of development to allow differentiation before the effects of the generated aneuploidies are expressed.

Those sn^{76} clones that, due to their abnormal morphology, were considered to be formed by homozygous *mei-41* cells surviving the second irradiation could be examples of these possibilities. Two of them (comprising 131 and 40 *singed* cells, respectively) received the second irradiation at 0–24 h before puparium formation, close to the time when imaginal wing cells cease to divide. They might represent examples of possibility (*b*). If we use external *mwh* clones in the same wings as indicators of the time of development when the second irradiation was performed, in both cases the homozygous mutant cells had to go through a maximum of two cell divisions before reaching differentiation. The third clone, formed by 58 *singed* cells, was irradiated 24–28 h before puparium formation. In the same wing there were seven *mwh* external clones ranging from 18 cells to one cell in size (mean five cells). It had to divide an average of three or four times before the end of development. Had it undergone as many cell divisions as the largest external *mwh* clone, it would have been formed by only one or two cells at the time of the second irradiation. If this is so, it could represent a case of perdurance of $mei\text{-}41^{+}$ products as indicated by possibility (*a*). Since no other abnormal clones were found and the remaining large normal sn^{76} clones appearing in mutant females after two irradiations can be attributed to mitotic exchanges proximal to *singed* but distal to *mei-41*, we conclude that practically all *mei-41* homozygous cells are killed by 1000 r of X-irradiation.

In contrast to *mei-41* homozygous cells, a large fraction of the cells homozygous for $mei\text{-}9^{a}$ survive irradiation. Comparing the size distributions of *forked* clones after one or two irradiations (Fig. 1*b*) we previously estimated that about two thirds of the cells homozygous for $mei\text{-}9^{a}$ disappear due to the second irradiation. After two irradiations it is still possible to find clones larger than 128 cells in $mei\text{-}9^{a}$ mutant females. The mean size of these clones provides additional evidence about the disappearance of $mei\text{-}9^{a}$ cells after the second irradiation. In mutant females irradiated once, the mean size of *forked* clones is 1·4 times that of *mwh* clones in the same wings. In mutant females irradiated twice, this relationship is reduced to 0·6, suggesting that an average of 58% (0·6/1·4) of the cells homozygous for $mei\text{-}9^{a}$ failed to reach differentiation after the second irradiation.

A similar estimate can be independently made using a different approach. After the second irradiation, internal clones are of two different origins: some will be spontaneous while others will be induced by the second irradiation. Twenty-four *forked* clones with internal *mwh* clones were found in nonmutant females after two irradiations. In $mei\text{-}9^{a}$ heterozygous females 42 *forked* clones with internal *mwh* clones were recorded. Since greater than 90% of all spontaneous *mwh* clones are of only one or two cells (Kennison & Ripoll, 1981), internal *mwh* clones larger than two cells can be assumed to result from the second irradiation. In nonmutant females, six of the 24 *forked* clones showing internal *mwh* clones have at least one that is larger than two cells (a maximum of eight cells). In heterozygous $mei\text{-}9^{a}$ females, eight of the 42 *forked* clones contained internal *mwh* clones of three or

more cells (a maximum of 22 cells). It is possible to estimate the minimal number of cells in a *forked* clone at the time of homozygosity for *mwh* simply by dividing the final number of *forked* cells by the number of cells in its largest internal *mwh* clone. In non-mutant females, these estimates vary from 5 to 110 cells (mean 59·6). In mutant females they range from 3 to 62 cells (mean 23·2). The ratio of these mean values suggests that 38 % of the irradiated homozygous *mei-9^a* cells survived exposure to 1000 r of X-irradiation. Although this estimate confirms those discussed previously, the argument is weakened by the variation in clone size shown in Table 1, which suggests that *mei-9^a* mutant females were slightly older than the nonmutant females used as controls at the time of the first irradiation. We have interpreted the reduction in cell number as due to death of cells homozygous for *mei-9^a* in response to the irradiation. An alternative explanation is that irradiated *mei-9^a* cells grow more slowly than wild-type cells, giving similar results even in the absence of cell death. If irradiated *mei-9^a* cells are growing more slowly than wild-type cells then the size of internal clones should be larger in nonmutant than in mutant females; this is, however, not the case. The mean size of internal *mwh* clones larger than two cells is 4·7 in non-mutant females and 7·7 in mutant females.

Irradiation of clones of cells made homozygous for the meiotic mutations leads to their elimination. The removal of these cells from the developing wing disc could affect its normal morphogenesis, but we have not found any morphological abnormalities in wings from double irradiation experiments. This is probably due to the small size the homozygous mutant clones had at the time the second irradiation was performed. A rough estimate of this size can be obtained comparing the most frequent size classes after one and two irradiations in *mei-41* mutant females, the case where cell death is more extensive (Tables 1 and 3). The homozygous mutant clones were irradiated between six (irradiation performed at 0–24 h) and four (irradiation at 48–72 h) cell divisions after homozygosity for the meiotic mutation. Therefore, as an average the irradiated clones comprised between eight and 32 mutant cells.

Most probably, the removal of large groups of cells would have an effect on the proliferating disc and it could be used as a technique to assess the regulative behaviour of developing tissues. This approach would be in essence identical to the induction of cell death using temperature-sensitive cell-lethal mutations (Russell, 1974; Simpson & Schneiderman, 1975; Postlethwait, 1978) but could overcome some of its limitations. Contrary to temperature-sensitive mutations, DNA-repair mutations are cell autonomous. Although X-irradiation affects all cells, the difference in sensitivity between homozygous and heterozygous cells is high enough to predict that all visible effects would be due to the removal of homozygous mutant cells; using double mutant combinations such as *mei-41 mei-9^a*, which show synergistic interactions (Baker *et al.* 1978), it should be possible to use doses of X-rays not affecting the viability of heterozygous cells. Only coherent groups of cells would be removed, and their approximate size and location within the disc could be known by means of twin spots. The average size of the mutant clone at the time of the second irradiation could be manipulated by varying the time elapsed between the first and the second irradiation. Experiments to test the usefulness of this technique are in progress.

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