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The synergistic effect of X-rays and deficiencies in DNA repair in P–M hybrid dysgenesis in *Drosophila melanogaster*

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Summary

X-rays and deficiencies in DNA repair had a synergistic effect on genetic damage associated with P-element mobility in *Drosophila melanogaster*. These interactions, using sterility and fecundity as endpoints, were tested in dysgenic males deficient in either excision or post-replication DNA repair. Three sublines of the Harwich P strain were used for the construction of hybrid males. These sublines differ in P-induction ability based on gonadal dysgenesis sterility (GD) and sn^{w} mutability tests, in P-element insertion site pattern, and in the types of defective P-elements, such as KP elements, they possess. A lower degree of gonadal dysgenesis was correlated with the presence of KP elements. GD sterility and sn^{w} mutability were not always correlated. Dysgenic hybrids originating from the standard reference subline, Harwich^{white}, were much more sensitive to the post-replication repair than the excision repair defect. In contrast, sterility of hybrids derived from the weak subline was least affected by, and that of hybrids of the strongest subline was most affected by either DNA repair deficiency. The exacerbation by X-rays of the effects of DNA repair deficiencies on genetic damage indicates that both repair mechanisms are required for processing DNA lesions induced by the combined effect of P activity and ionizing radiation.

1. Introduction

Transposons are ubiquitous in the genomes of both prokaryotes and eukaryotes. Thus the genetic consequences of their potential mutator activity merit investigation. In *Drosophila melanogaster*, the P-element family comprises about fifty transposable elements of heterogeneous size (reviewed in Bregliano & Kidwell, 1983; Engels, 1983, 1989) per genome. Hybrid dysgenesis, a syndrome of aberrant germ line traits (Kidwell, Kidwell & Sved, 1977) which results from the induced mobility of P-elements (Bingham, Kidwell & Rubin, 1982), includes temperature sensitive sterility, high mutability and chromosome breakage. The high activity of P-elements, which results from crosses of M cytotype (Engels, 1979*a*) females to P-cytotype males is an important source of recessive lethal mutations (Kidwell *et al.* 1977; *Simmons et al.* 1980; Margulies, Briscoe & Wallace, 1986, 1987) and chromosomal rearrangements (Simmons & Lim, 1980; Engels & Preston, 1984). It has been proposed (Engels, 1989) that dominant lethals, another trait associated with hybrid dysgenesis (Kidwell, 1984; Margulies *et al.* 1986, 1987) may be the major effect of chromosome breaks leading to gonadal dysgenic (GD) sterility. In addition to GD sterility, which results in the formation of rudimentary gonads (Engels & Preston, 1979; Kidwell & Novy, 1979), dysgenic hybrids can also manifest premature sterility, namely varying degrees of loss of fecundity (Kidwell *et al.* 1977; Margulies *et al.* 1989; Margulies, 1990).

P-element activity determined by genetic assays is apparently due to P-elements of heterogenous size (O'Hare & Rubin, 1983). Only the 2·9 kb (O'Hare & Rubin, 1983) intact elements, can however, supply a *trans*-acting function for the mobilization of both complete and defective P-elements (Spradling & Rubin, 1982; Rubin & Spradling, 1982; Engels, 1984). This function is the P-encoded transposase (Karess & Rubin, 1984; Rio, Laski & Rubin, 1986). The repression of P-element transposition in the P cytotype seems to be controlled by P-encoded repressor (Nitasaki *et al.* 1987; Engels, 1989; Misra & Rio, 1990) which may be a truncated transposase. KP elements, which are specific deletion derivatives of P-elements, have also been implicated in the repression of hybrid dysgenesis (Black *et al.* 1987; Jackson, Black & Dover, 1988). In P–M dysgenesis, P-element mobility is restricted to the germline, a characteristic which is controlled at the level of RNA splicing (Laski, Rio & Rubin, 1986).

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The molecular basis of GD sterility and the role of different DNA repair mechanisms in recovery from P-induced genetic damage is not understood. Previous studies have shown that only the deficiency in post-replication repair increased transmission distortion and zygotic lethality in dysgenic hybrids (Slatko, Mason & Woodruff, 1984). P-element-induced sterility and fecundity effects showed synergistic interactions with X-irradiation (Margulies *et al.* 1989) and with either excision or post-replication DNA repair (Margulies, 1990). Here we extend previous observations to include strains derived from the reference P strain, Harwich, that have different degrees of P-element activity as assessed by GD sterility, sn^w mutability (Engels, 1979*b*, 1984; Roiha, Rubin & O'Hare, 1988) and X/Y chromosome loss measurements (Margulies *et al.* 1989; H. Balter, C. Griffith and L. Margulies, unpublished data). The effects of varying degrees of P activity and X-irradiation on sn^w mutability are assessed. In addition, we show an analysis of the combined effects of P activity, X-rays and either excision or post-replication DNA repair on sterility and fecundity. A molecular analysis of P elements in the strains used is also presented.

2. Materials and methods

(i) *Cultures and strains*

A cornmeal–Brewers yeast medium was used in all cultures. The developmental temperature was 18 or 21 °C, as specified in each experiment.

P strains

1, 2 and 3. The three sublines of the strong wild-type P strain, Harwich (Kidwell *et al.* 1977) were obtained through the courtesy of M. G. Kidwell; the subline termed H^s was obtained in 1983, H^f in 1986, and H^w in 1989. Their GD sterility pattern using gonad dissection (Schaeffer, Kidwell & Faysto-Sterling, 1979) is summarized here.

1. H^w. Harwich^{white}, a standard Harwich reference subline, marked with the spontaneous X-linked mutation, *w* (white eyes). GD sterility of hybrids originating from M strain female × H^w male crosses averaged 97–100% at 29 °C and 2% at 21 °C, using several M tester strains.

2. H^s. This is a high dysgenesis-inducer subline. The unusual properties, including a highly thermo-sensitive sterility, of hybrids derived from this subline have been described previously (Margulies *et al.* 1989; Margulies, 1990). GD sterility of hybrids derived from M strain female × H^s male crosses was 100% at 29 °C and averaged 95–98% at 21 °C. Dysgenic crosses using this strain were maintained at 18 °C, where GD sterility ranged from 15–19%. In hybrids derived from non-dysgenic crosses, H^s female × M strain male, GD sterility ranged from 1–2% at 18 or 21 °C, and 1–12·4% at 29 °C, depending on the M strain used.

3. H^f. f stands for relative fertility. GD sterility of hybrids originating from M strain female × H^f male crosses ranged from 66 to 78% at 29 °C, depending on the M strain used; at 21 °C average sterility was 2%.

The different temperature-specific patterns of induction of GD sterility by the H^s and H^f sublines, compared to the 'typical' H^w pattern could be caused by variation in abilities of their constituent P-elements to induce and/or repress P-element mobility.

4. C(1)DX *yf*; H^s. These females with attached-X chromosomes have all other chromosomes derived from the H^s subline and are of P cytotype. The stock was constructed by 12 generations of backcrossing females from a compound-X laboratory M strain to H^s males and is maintained at 18 °C. GD sterility of hybrids from crosses of these females to M strain males averages 1% at 29 °C.

M strains

5. Muller-5, referred to as (M-5). This X chromosome balancer has the markers, *sc* (scute bristles), w^a (apricot eyes), and the semi-dominant *B* (*Bar* eye).

6. C(1)DX, *yf*/*Y*. The attached-X females of this stock are marked with *y* (yellow body) and *f* (forked bristles). Their sons have a patroclinous origin of the X chromosome.

7 and 8. *mei-9*^{a}/M-5 and *mei-41*^{D1}/M-5. The X-linked loci *mei-9* and *mei-41* control respectively, excision repair (Boyd, Golino & Setlow, 1976) and post-replication repair (Boyd & Setlow, 1976). Since females homozygous for these mutations have poor fertility, each mutation was balanced with the Muller-5 chromosome.

9. *J188. Cy*/*Pm*; *Sb*/*D*, a stock carrying four dominant markers: *Cy* (Curly wings), In(2L)*Cy sp*^{2}, and *Pm* (Plum eyes) associated with the inversion, In(2LR)*bw*^{VI}, are on the second chromosome; *D* (Dichaete wings), In(3LR) DCXF *ru h D*, and *Sb* (stubble bristles) are on the third chromosome.

For further details about genetic markers, see Lindsley & Grell (1968).

Psuedo-M or M′ strains

10. Canton-S (M′) (*CS*), a wild-type laboratory strain, originally a pure M strain. We found twelve defective P elements in this subline, a finding confirmed by others (R. C. Woodruff, personal communication). GD sterility of hybrids derived from crosses of females of this subline to H^s males was 100% at 29 °C and averaged 96–98% at 21 °C.

11. *y sn*^{w}; *bw st*/*y*+ *Y*. P-element insertion at the X-linked *singed* bristle locus (Engels, 1979*b*, 1984) which causes a weak *singed* phenotype. Two defective P-elements were found at *singed* and one other defective P-element is closely linked; the genome is otherwise devoid of P-elements (Roiha *et al.* 1988).

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The autosomes carry the recessive eye colour markers, *bw* (brown eyes) on the second chromosome and *st* (scarlet) on the third chromosome. The stock behaves as an M strain. The *singed weak* allele is destabilized in P–M hybrid dysgenesis, providing a mutability assay. Excision of one defective P-element leads to a more extreme singed bristle phenotype, whereas excision of the other results in a wild-type bristle phenotype (Roiha *et al.* 1988).

Strains 5, 6, 9 and 10 were obtained from the Bowling Green University Stock Center. The *mei-9^a* and *mei-41^{D1}* mutants were obtained through the courtesy of J. M. Mason, and the *sn^w* stock was kindly supplied by M. J. Simmons.

(ii) *Crosses sampling and brood collection*

All first-generation crosses for studying *sn^w* mutability and the effect of DNA repair deficiencies were mass matings of about 20–25 pairs per bottle, set up in multiple cultures of 5–8 bottles per experiment over a period of 1–2 weeks. The parental males of each Harwich subline were obtained from at least two cultures set up weekly in bottles. After 2–3 days the parents were transferred to fresh bottles and kept for a 2- to 3-day period. F_1 hybrid males were collected daily from the multiple cultures and this pooled population was used for setting up the control and the irradiated series, as well as for sampling different sperm broods. Brooding was carried out as described in detail in Maw, Wallace & Margulies (1988) and Margulies *et al.* (1989). Hybrid males were aged for 1 day, and irradiated or control males were mass mated for a period of 4 days in large plastic vials (40 ml capacity) in a ratio of 12 males to 36 young females (mostly virgins). This step effectively exhausted brood 1 sperm (mostly mature spermatozoa), which was not monitored in these experiments. The males were then mated singly to three appropriate females for 4 days to sample brood 2 (predominantly early and late spermatids). To sample brood 3 (mainly spermatocytes), some of the males from each day's batch of irradiation were mass mated for a second 4 day-period to another harem of females in order to exhaust brood 2 sperm. Only brood 2 was sampled for *sn^w* mutability tests; broods 2 and 3 were sampled for sterility and fecundity studies in conjunction with DNA repair deficiencies.

(iii) *Mating schemes*

1. Effects of P activity and X-irradiation on *sn^w* mutability. For the M♀♀ × P♂♂ dysgenic cross, *sn^w* males were crossed to Muller-5 females and the *sn^w*/M-5 M cytotype daughters were mated with H^w P strain males at 21 °C, and with either H^s, or H^r strain males at 18 °C. The M cytotype of the *sn^w*/M-5 females was confirmed (results not shown) by high GD sterility in tests at 29 °C (Schaeffer *et al.* 1979). For the non-dysgenic P♀♀ × M♂♂ cross, Stock 4 attached-X P-cytotype females were crossed to *sn^w* M strain males at 18 °C. Male hybrids of the dysgenic and non-dysgenic crosses were either irradiated or untreated, and then mated singly to three attached-X M-strain females. Their sons were scored for mutability of *sn^w* to *sn$^+$* or *sn^e* alleles 20–22 days after the crosses were made.

2. Effects of P activity, X-irradiation and DNA repair deficiency on GD sterility and fecundity. Dysgenic hybrids defective in excision repair or post-replication repair were derived by mating, respectively, *mei-9*/M-5 or *mei-41*/M-5 M stock females to males of each Harwich subline. For the M × M control crosses, *mei-9*/M-5 or *mei-41*/M-5 females were crossed with Canton-S M′ males. Crosses with H^s and H^r P-strain males and the control Canton-S stock males were incubated at 18 °C; crosses with H^w males were kept at 21 °C. Irradiated or untreated DNA repair-deficient males and their DNA repair-proficient Muller-5 (M-5) brothers were mated singly with three attached-X M-strain females which had been aged for 4–6 days to insure optimum fecundity. Sterility and fecundity were monitored in the same experiment. Male sterility was based on the complete absence of larvae in cultures more than 1 week old; their fecundity was estimated from the total number of progeny emerging. The counts were done 20–22 days after the cultures were established.

(iv) *X-irradiation*

Irradiation of F_1 males were carried out with a Philips X-ray source, with a berillium side window tube and a tungsten anode, operated at 50 kVp and 3 mA. The dose rate, calibrated by the Fricke dosimeter (Sehested, 1970), was 110 rad/s and the cumulative dose in all the experiments was 660 rad.

(v) *Statistical analyses*

1. Significance of differences in *sn^w* mutability was analysed by the non-parametric Wilcoxon rank sum test, using the mutation rate computed for each male tested (Engels, 1979*c*).

2. The non-parametric Multidimensional Contingency Analysis (MDCA) is appropriate for the analysis of discrete categorical data, such as the all-or-none sterility measured here. The analysis was performed by brood and sterility was considered a dichotomous response variable which was cross classified with respect to strain, DNA repair ability and X-ray treatment. This yields multidimensional matrices which were evaluated for all possible independence models using the log-ratio χ^2 (G^2). The application of this method is described by Bishop & Fienberg (1975) and by Rockwell, Findley & Cooke (1987). The dysgenic male progeny derived from different Harwich sublines and the nondysgenic hybrids of Canton-S

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fathers, which served as controls, were used in the analysis. The existence of higher-order interactions implies that the response depends on unique combinations of the relevant factors; in these cases, data were reanalysed by conditioning one of the factors, strain, and performing a series of MDCAs. Lower order MDCAs were also performed to determine the comparative effects of DNA repair and treatment within strains, assuming no interaction at the higher level.

3. The analysis of variance (ANOVA) was performed by brood on the fecundity data with total progeny of each male transformed prior to analysis using square root transformations (Sokal & Rohlf, 1981). For data using counts, such as fecundity data, a square root transformation will usually satisfy the ANOVA assumption of homogeneity of variances. Because the data were not balanced, the SAS GLM procedure, a linear model designed for unbalanced data, was used for the ANOVA. Transformed means of total progeny per male were used to evaluate the single and joint effects of strain, DNA repair and X-ray treatment on fecundity. The dysgenic male offspring of each Harwich subline and the nondysgenic offspring of Canton-S fathers were included in the analysis. Sterile males were excluded from this analysis.

(vi) Molecular analysis of P elements

Genomic DNA was isolated by homogenization of 50 flies by a modification of the method of Marcus (1985). The isolation of DNA from the plasmid pΠ25.1 (O'Hare & Rubin, 1983), restriction endonuclease digestion and agarose gel electrophoresis were essentially as described by Maniatis, Fritsch & Sambrook (1982). All gels used for Southern blots were 0·7% agarose, unless otherwise specified in the legend. Southern blot analysis was performed as follows. Genomic DNA from 50 flies was digested to completion, purified by phenol and chloroform treatment and run on an agarose gel. The gel was blotted to a framed nylon filter in a blotting apparatus (Bios Corporation) for 5 h. Probes were prepared by multiprime labeling (Amersham Corporation). Filter hybridization was performed at 65 °C in a casette by a modification of the described procedure (Bios Corporation).

In order to perform molecular analysis of P-elements on each of the major chromosomes of the H^s and H^r strains, we utilized the mating scheme shown in Fig. 1. The first generation nondysgenic crosses of P-strain females to M-strain males minimized P-element mobility, and thus contamination of M-strain chromosomes with P-elements (Kidwell, 1983). The dominant autosomal markers of the M strain permitted the selection of second generation males with only one chromosome, the X, chromosome 2 or chromosome 3, derived from each P strain. Genomic DNA was isolated from 50 second-generation males of each type and Southern analysis was performed as described above.

3. Results

(i) *Effects of P activity and X-irradiation on sn^w mutability*

The mutability of the sn^w P-element insertion mutation to either the more extreme, sn^e, or wild-type, sn^+ allele in hybrids derived from different Harwich strains is shown in Table 1. The data are presented as pooled progeny of all individually tested males. The mutation rate was computed by family size using the unweighted procedure of Engels (1979*c*). Untreated hybrids derived from H^s fathers had significantly higher sn^w mutability than those originating from either the H^r or H^w subline. The sn^w mutation rate of H^r and H^w subline hybrids was the same. Interestingly, and contrary to previous findings using the Π_2 P-strain (Engels, 1979*b*), hybrids derived from nondysgenic crosses of the H^s subline (attached-X H^s♀♀ × M♂♂) showed an unexpectedly high mutation rate (0·098), relative to that observed in dysgenic crosses (0·238). This unusual property of lack of P-cytotype regulation in hybrids of the H^s subline has been found in conjunction with other dysgenesis traits (Margulies, 1990). X-irradiation of dysgenic or non-dysgenic H^s hybrids or dysgenic H^r hybrids did not alter sn^w mutability significantly. The effect of X rays was not investigated in hybrids of the H^w subline.

(ii) *The influence of ionizing radiation and deficiency in post-replication repair or in excision repair on sterility and fecundity of dysgenic hybrids*

Sterility (all-or-none) and fecundity were monitored in brood 2 sperm in dysgenic hybrids derived from the

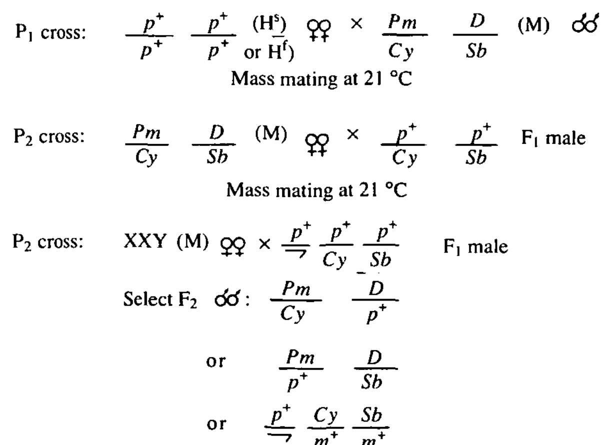

Fig. 1. The mating scheme for the isolation of dysgenic males with either chromosome 3 alone, chromosome 2 alone, or with the X chromosome derived from the H^s or H^r Harwich, subline. *Pm/Cy* and *D/Sb* are the dominant autosomal markers from the M strain. p+ indicates the wild-type homologues originating from the Harwich P strain. XXY is the *y f*, attached-X female.

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Table 1. *Effect of P chromosomes from different Harwich sublines and X-rays on* sn^{w} *mutability*

Cross ♀ ♂	Treatment	Males tested	Progeny scored *sn^{w}*	*sn^{e}*	*sn^{+}*	Mutation rate	S.E.
M × H^{r}	None	150	4733	492	595	0·193**	0·0080
M × H^{r}	X-rays	197	3207	413	436	0·214	0·0109
M × H^{s}	None	182	3259	466	595	0.238	0.0120
M × H^{s}	X-rays	234	1470	245	272	0·272	0·0161
H^{s} × M	None	133	3374	217	145	0·098	0·0092
H^{s} × M	X-rays	168	2586	131	115	0·079	0·0062
M × H^{w}	None	125	3291	306	470	0·193*	0·0084

The H^{s} female is an attached-X P-cytotype female with P autosomes derived from the H^{s} subline. The data represent the pooled progeny of individually tested males. Mutation rate was estimated by family size of all males tested using the unweighted procedure of Engels (1979*c*). The significance of the difference in mutation rates was analysed with the Wilcoxon rank sum test. * $P < 0·05$ in a comparison of H^{s} subline hybrids with those derived from the H^{w} strain. ** $P < 0·02$ in a comparison of H^{s} and H^{r} hybrids. S.E. is the standard error.

Table 2. *The combined effect of hybrid dysgenesis, deficiency in post-replication repair and X-rays on sterility and fecundity of hybrid males derived from different Harwich P strain sublines*

Cross ♀ ♂	F$_{1}$ male	Treatment	Brood	Number tested	% Sterile	Number tested	Total progeny	Mean no. per male
				H^{r} subline				
M × P	M-5	None	2	125	8·0	115	9864	86
M × P	M-5	X-rays	2	117	5·1	111	4757	43
M × P	*mei-41*	None	2	137	5·1	130	9116	70
M × P	*mei-41*	X-rays	2	134	2·2	131	3208	24
				H^{s} subline				
M × P	M-5	None	2	180	15·0	153	8893	58
M × P	M-5	X-rays	2	293	17·7	241	5508	23
M × P	*mei-41*	None	2	172	49·0	87	1398	16
M × P	*mei-41*	X-rays	2	260	72·0	72	322	4
				Canton-S				
M × M	M-5	None	2	69	8·7	63	5881	93
M × M	M-5	X rays	2	71	9·9	64	3908	61
M × M	*mei-41*	None	2	116	6·9	108	9597	89
M × M	*mei-41*	X-rays	2	70	1·4	69	3507	51

M × P is the dysgenic cross and M × M the non-dysgenic cross. M-5 are Muller-5, DNA repair proficient brothers of *mei-41* males. Fecundity was measured in all fertile males.

weak, H^{r}, or strong, H^{s}, Harwich subline and in non-dysgenic hybrids of Canton-S M′ fathers (Tables 2, 4). Brood 3 was tested in hybrids of all three Harwich sublines and in Canton-S M′ offspring (Tables 3, 5). Because virtually 100% of X-irradiated hybrids of H^{s} subline fathers were sterile, and thus no fecundity could be measured, the results of the irradiated series are not shown. Statistical analyses are presented in Tables 6 and 7.

1. *The effect of post-replication repair deficiency (mei-41).* Analysis of sterility and fecundity from brood 2 and brood 3 sperm (Tables 2–5) showed very strong main effects of strain (S), DNA repair deficiency (R) and X-rays (X). In general, these results as well as the synergistic effect of P-element mobility and X-rays (X × R interaction) on the one hand, and of P-element mobility and post-replication repair deficiency on the other hand (R × S interaction), confirmed previous observations (Margulies *et al.*, 1989; Margulies, 1990, respectively). The objective of this study was to investigate the combined effect of DNA repair deficiency, X-rays and P activity on genetic damage. The analysis of sterility in both broods, 2 and 3 (Table

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Table 3. *The combined effect of hybrid dysgenesis, deficiency in post-replication repair and X-rays on sterility and fecundity of hybrid males derived from different Harwich P strain sublines*

Cross ♀ ♂	F_1 male	Treatment	Brood	Number tested	% Sterile	Number tested	Total progeny	Mean no. per male
				H^r subline				
M × P	M-5	None	3	103	4·9	98	7833	80
M × P	M-5	X-rays	3	112	11·6	99	1491	15
M × P	*mei-41*	None	3	104	1·9	102	6809	67
M × P	*mei-41*	X-rays	3	113	12·4	99	801	8
				H^s subline				
M × P	M-5	None	3	153	15·0	130	6547	50
M × P	*mei-41*	None	3	215	68·8	67	764	11
				H^w subline				
M × P	M-5	None	3	100	9·0	58	4731	82
M × P	M-5	X-rays	3	85	11·8	58	1369	24
M × P	*mei-41*	None	3	99	25·3	74	1335	18
M × P	*mei-41*	X-rays	3	68	82·4	12	40	3
				Canton-S				
M × M	M-5	None	3	95	8·4	87	7169	82
M × M	M-5	X-rays	3	65	13·8	56	1062	19
M × M	*mei-41*	None	3	90	7·8	83	7225	87
M × M	*mei-41*	X-rays	3	49	6·1	46	887	19

M × P is the dysgenic cross and M × M the non-dysgenic cross. M-5 are Muller-5, DNA repair proficient brothers of *mei-41* males. Fecundity was measured in all fertile males, except in H^w subline derived hybrids, where the progeny of M-5 untreated and irradiated males were scored in a random sample of fertile males.

Table 4. *The combined effect of hybrid dysgenesis, deficiency in excision repair and X-rays on sterility and fecundity of hybrid males derived from different Harwich P-strain sublines*

Cross ♀ ♂	F_1 male	Treatment	Brood	Number tested	% Sterile	Number tested	Total progeny	Mean no. per male
				H^r subline				
M × P	M-5	None	2	113	7·1	105	10812	103
M × P	M-5	X-rays	2	121	9·1	110	5888	54
M × P	*mei-9*	None	2	86	2·3	84	7861	94
M × P	*mei-9*	X-rays	2	75	2·7	73	2438	33
				H^s subline				
M × P	M-5	None	2	120	7·5	111	8702	78
M × P	M-5	X-rays	2	136	4·4	130	4105	32
M × P	*mei-9*	None	2	205	81·5	38	927	24
M × P	*mei-9*	X-rays	2	380	78·9	82	735	9
				Canton-S				
M × M	M-5	None	2	69	8·7	63	6512	103
M × M	M-5	X-rays	2	97	8·2	89	5518	62
M × M	*mei-9*	None	2	66	1·5	65	7355	113
M × M	*mei-9*	X-rays	2	109	2·8	106	6599	62

M × P is the dysgenic cross and M × M the non-dysgenic cross. M-5 are Muller-5, DNA repair proficient brothers of *mei-9* males. Fecundity was measured in all fertile males.

6) showed a synergistic effect. Thus the X × R × S interaction was significant when either spermatids or spermatocytes were irradiated. Lower order analyses within strains (not included in Table 6) showed that the synergistic effect on sterility in brood 2 occurred only in hybrids derived from the high dysgenesis-inducer Harwich subline, H^s, whereas the synergism in brood 3, comparing H^r, H^w and Canton-S M′

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Table 5. *The combined effect of hybrid dysgenesis, deficiency in excision repair and X-rays on sterility and fecundity of hybrid males derived from different Harwich P-strain sublines*

Cross ♀ ♂	F_1 male	Treatment	Brood	Number tested	% Sterile	Number tested	Total progeny	Mean no. per male
				H^r subline				
M × P	M-5	None	3	88	10·2	79	7246	92
M × P	M-5	X-rays	3	108	7·4	102	1909	19
M × P	*mei-9*	None	3	108	1·8	106	9129	86
M × P	*mei-9*	X-rays	3	110	24·5	83	871	11
				H^s subline‡				
M × P	M-5	None	3	101	7·9			
M × P	*mei-9*	None	3	149	98·0			
				H^w subline				
M × P	M-5	None	3	93	5·4	34	3655	108
M × P	M-5	X-rays	3	133	9·0	36	909	25
M × P	*mei-9*	None	3	152	4·6	83	6990	84
M × P	*mei-9*	X-rays	3	155	34·2	55	1008	18
				Canton-S				
M × M	M-5	None	3	78	2·6	76	7767	102
M × M	M-5	X-rays	3	88	3·4	85	2493	29
M × M	*mei-9*	None	3	82	3·7	79	6502	82
M × M	*mei-9*	X-rays	3	89	4·5	85	1992	23

M × P is the dysgenic cross and M × M the non-dysgenic cross. M-5 are Muller-5, DNA repair proficient brothers of *mei-9* males. Fecundity was measured in all fertile males, except in the H^w subline hybrids, where progeny were scored in a random sample of fertile males.
‡ Taken from Margulies (1990).

Table 6. *Multidimensional contingency analysis of the combined effect of P-element activity, DNA repair deficiency* (mei-41 *or* mei-9 *mutation*) *and X-rays on sterility of hybrid males derived from different Harwich sublines*

Source of Variation	D.F.	Brood 2 (Strains: H^r, H^s, CS) *mei-41* G^2	Brood 2 (Strains: H^r, H^s, CS) *mei-9* G^2	Brood 3 (Strains: H^r, H^w, CS) *mei-41* G^2	Brood 3 (Strains: H^r, H^w, CS) *mei-9* G^2
Strain (S)	2	328·80***	445·81***	81·33***	28·85***
Repair (R)	1	152·86***	290·89***	31·82***	19·56***
X rays (X)	1	9·11**	0·61	39·46***	47·58***
R × S	2	59·41***	166·52***	49·12***	1·85
X × S	2	10·31*	1·62	5·70*	2·96
X × R	1	2·12	0·53	6·88**	17·64***
X × R × S	2	6·22*	0·25	8·87*	4·98

* $P < 0{\cdot}05$; ** $P < 0{\cdot}005$; *** $P < 0{\cdot}0001$. D.F. = degrees of freedom.
The analyses are based on the data presented in Tables 2–5. G^2 is the log-ratio χ^2.

hybrids, was evident in hybrids originating from the standard Harwich subline, H^w. In contrast, no effect of either repair deficiency or treatment was observed in hybrids of the weak, H^r, subline. This apparently obscured the X × R interaction in H^s hybrids in brood 2.

Second-order analyses of fecundity of brood 2 (not shown) indicated that the synergistic effect of P-element mobility and DNA repair deficiency occurred in dysgenic males derived from either the weak or strong Harwich subline, but that the latter showed a much sharper decline in the mean number of progeny. In brood 3, fecundity of all P-strain-derived *mei-41* hybrids decreased, but the effect was much more

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Table 7. *Analysis of variance (ANOVA) of the combined effect of P-element activity, DNA repair deficiency* (mei-41 *or* mei-9 *mutation*), *and X-rays on fecundity*

Source of Variation	D.F.	Brood 2 Strains: H^r, H^s, CS *mei-41* *F*	 *mei-9* *F*	Brood 3 Strains: H^r, H^w, CS *mei-41* *F*	 *mei-9* *F*
Strain (S)	2	779·17***	269·73***	150·97***	28·80***
Repair (R)	1	455·36***	90·12***	131·87***	4·58*
X-rays (X)	1	1104·10***	354·42***	2717·07***	2817·01***
R × S	2	108·85***	48·19***	198·85***	29·63***
X × S	2	12·42***	2·98	77·29***	11·57***
X × R	1	0·31	0·33	0·35	6·99**
X × R × S	2	308·53***	10·95***	13·33***	2·63

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·0001$; D.F. = degrees of freedom.
The analysis is based on the data in Tables 2–5, where the progeny of individually mated males were pooled. Sterile cases were excluded from fecundity analysis.

pronounced in H^s and H^w hybrids than in those of the H^r subline, and was thus correlated with the level of P activity. Analysis of fecundity of brood 2 and 3 showed no synergism of X-rays and DNA repair deficiency (no X × R interaction). The significance of three-way interaction prompted us to do second order analysis within strains. These results (not included) showed that the reduction in fecundity was greater in irradiated M-5, DNA repair proficient hybrids than in treated *mei*-41 males. Thus no synergism due to the combined effect of X-rays, DNA repair deficiency and P-element mobility was observed. It should be noted that fecundity of dysgenic H^s and H^w hybrids was sharply reduced due to the DNA repair deficiency alone. Since ANOVA measures only absolute differences, this so called 'floor' effect made it difficult to detect a synergism with X-rays.

2. *The effect of excision repair deficiency* (*mei-9 mutation*. The analyses of sterility and fecundity of *mei-9* dysgenic hybrids also showed significant main effects, confirming previous observations (Margulies *et al.* 1989; Margulies, 1990). The only exception was the effect of X-rays on sterility in brood 2. Notable differences were observed in sterility of hybrids in brood 2 *vs.* brood 3 (Table 6). Whereas in brood 2 there was a significant interaction of DNA repair deficiency and strain due to the high sterility of *mei-9* H^s subline hybrids, no interaction of X-rays and repair deficiency was detected. In contrast, in brood 3 there was a highly significant effect of X-rays and DNA repair deficiency (X × R interaction) on sterility. Although there was no indication of three-way interaction, the inequality of the main effects and small cell size of Canton-S data suggested a further analysis of the effects within each strain. This was done by partitioning the effect of X-rays and repair deficiency separately from the effect of P-element activity, and requiring a significance test of 0·005. The results (not shown) demonstrated a highly significant effect of repair deficiency and X-irradiation on sterility of both the H^r and H^w subline hybrids, but no effect in nondysgenic Canton-S progeny.

The analyses of fecundity (Table 7) showed significant interaction of DNA repair and strain, but whereas in brood 2 the effect was observed in hybrids of either the weak or strong Harwich subline and was more pronounced in the latter, in brood 3 there was no dependence on P-element activity. The apparent two-way interaction of X-rays and DNA repair in brood 3 was shown to be due to a greater effect in DNA repair proficient males, and no three-way interaction was detected. Although three-way interaction appeared significant in brood 2, lower order analysis showed that both Canton-S and H^r hybrids contributed to this effect. The floor effect in H^s hybrids again thwarted the detection of the influence of X-rays.

(iii) *Southern blot analysis of P elements in Harwich sublines*

A comparison of the P-element hybridization pattern in the H^s, H^r and H^w Harwich sublines is shown in Fig. 3. Genomic DNA digested with *Pvu* II and probed with the 1·65 kb *Xho* I/*Sal* I fragment of the P element (see Fig. 2) is expected to yield a 0.9 kb fragment from each P-element containing the region spanned by the *Pvu* II restriction sites. The region to the right of the second *Pvu* II site should yield a fragment of variable size, depending on the flanking genomic sequence of the residing P-element, and is thus characteristic of the genomic location of the element. The hybridization pattern presented in Fig. 3 shows that the sites of

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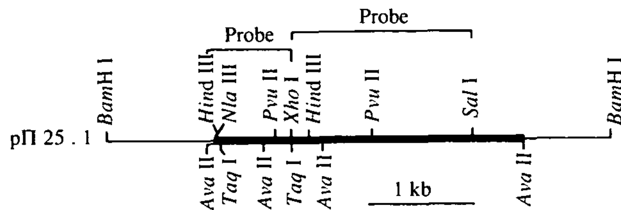

Fig. 2. A partial restriction map of the 2·9 kb P-element in pΠ25.1. The *Hin*d III/*Xho* I and the *Xho* I/*Sal* I restriction fragments shown were used for the preparation of the 0·7 kb and 1·65 kb probe, respectively. The first *Nla* III site is at position 1; the first two *Taq* I sites are at positions 29 and 729; The *Hin*d III sites are at positions 39 and 877; *Pvu* II sites map at positions 583 and 1479; the *Xho* I site is at position 728 and the *Sal* I site at position 2410; the four *Ava* II sites are at positions 22, 500, 1044 and 2882.

insertion of P-elements differ considerably in the genome of the three Harwich sublines. Out of about twenty seven identifiable bands which, because of the short exposure of the autoradiograph, represent mainly the frequently occurring sites in each subline, only ten sites are the same in the H^s and H^r sublines, twelve in the H^r and H^w sublines, and ten bands are invariable in the H^s and H^w sublines. This variation in P-element distribution might be caused by variability in P-element insertion sites within each strain as well as differences between the strains. Within subline variation in P-element insertion sites may be considerable (Shrimpton, Mackay & Leigh Brown, 1990).

Differences in P-element complement between the H^s and H^r strains were further resolved by comparing *Ava* II digests of genomic DNA of males containing the X, the second or third chromosome from each of the strains (see Fig. 1). Digestion with *Ava* II and probing with the full length P-element in plasmid pΠ25.1, is expected to yield fragments of 480, 544 and 1848 bp from each complete P-element (see Fig. 2). Fig. 4 shows the presence of prominent bands at these positions in all three chromosomes of each subline. The band labelled 17C represents the *Drosophila* DNA flanking the cloned P-element in pΠ25.1 (O'Hare & Rubin, 1983), and can serve as a marker for the amount of DNA loaded in each lane. A comparison of the intensity of bands at 1848 and 544 bp positions in the chromosomes of the two sublines indicates that, whereas chromosome 2 of the H^r subline seems to have a greater number of complete P elements, the H^s subline has a greater number on the X and on chromosome 3. Analysis of the standard Harwich strain, Harwich-77, by *in situ* hybridization (Daniels *et al.* 1987) also showed a greater number of P elements on chromosome 3 and fewer on chromosome 2.

A band at the position of 640 bp appears only in the chromosomes of the weak H^r subline. This band represents the fragment expected from *Ava* II digestion of a deletion derivative of the P-element, termed the KP element (Black *et al.* 1987; Jackson *et al.* 1988). A

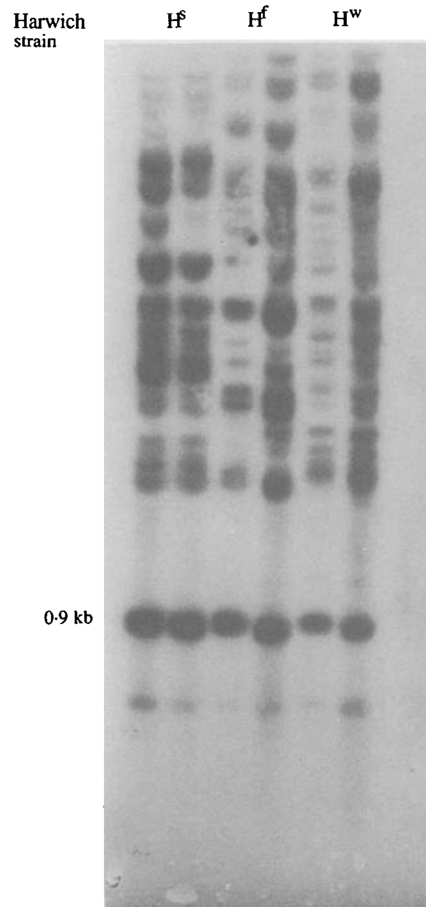

Fig. 3. A molecular analysis of P-element sites in three Harwich sublines. Genomic DNA from each Harwich subline was digested with *Pvu* II and probed with the *Xho* I–*Sal* I fragment of the complete P-element in pΠ25.1. The DNA from each subline was loaded in two lanes. DNA isolated from 50 males was loaded in lanes 1, 2, 4 and 6; lanes 3 and 5 had $\frac{1}{2}$ of the amount loaded, relative to lane 4 and 6, respectively. The 0·9 kb band indicates the fragment expected from P elements intact for the Pvu II region (see Fig. 2).

480 bp fragment is also expected from an *Ava* II digest of the KP element. The most prominent 640 bp band appears in chromose 2 of the H^r subline, but the number of KP elements is much smaller than the number of complete P-elements in each H^r chromosome. KP elements were not found previously in the Harwich reference subline (Black *et al.* 1987). All chromosomes of both strains contain other defective P-elements as well as multiple copies of complete or nearly complete elements.

The presence of KP elements in the H^r subline could be due to contamination or possibly be of spontaneous origin. The Southern analysis presented in Fig. 5 was performed to determine whether a base substitution at position 32 in the KP elements found in M′ strains (Black *et al.* 1987) was also present in the H^r subline. Since this polymorphism eliminates the first *Taq* I site (Fig. 2), genomic DNA digested with *Nla* III and *Taq* I, and probed with the 0.7 kb *Hin* dIII/*Xho* I fragment

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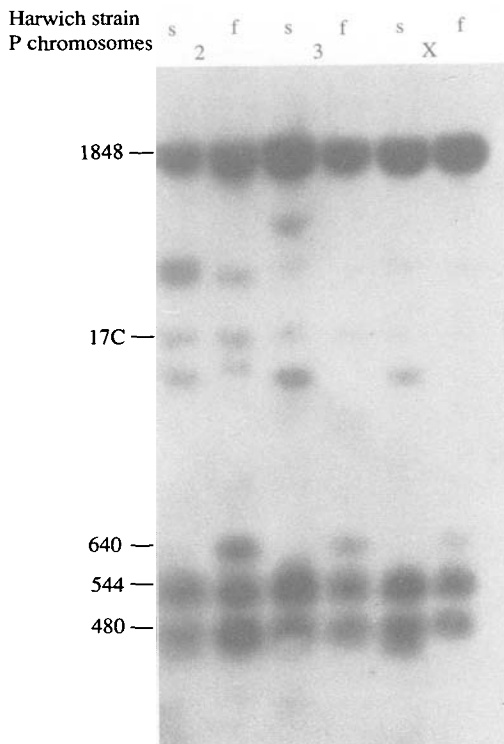

Fig. 4. Analysis of complete and deleted P elements on the X chromosome and the major autosomes of two Harwich sublines, H^f and H^s, denoted by f and s, respectively. Genomic DNA isolated from 50 males of each genotype shown in Fig. 1 was digested with *Ava* II and probed with the full length P element in pΠ25.1 (see text). Bands at positions 480, 544 and 1848 indicate the fragments expected from P elements intact for the region spanned by the *Ava* II sites (see Fig. 2). The band at 640 bp identifies the specific deletion derivative, KP element.

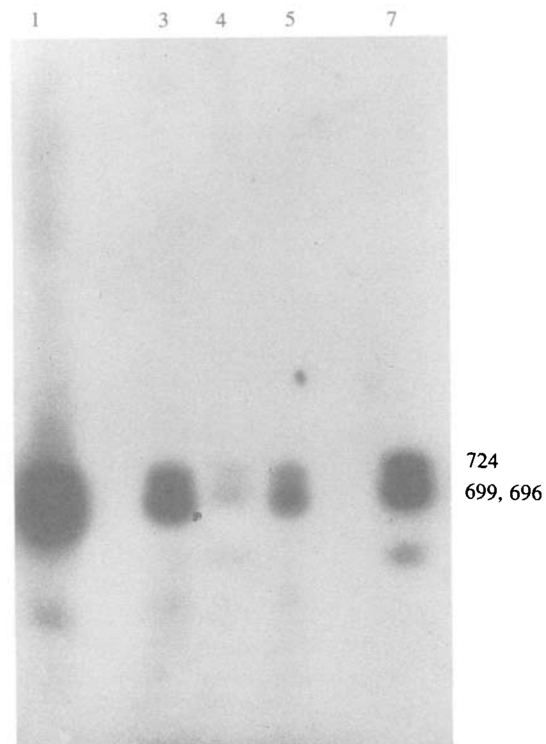

Fig. 5. Molecular analysis of the origin of KP elements in the Harwich H^f subline. Genomic DNA was digested with *Nla* III and *Taq* I. Decreasing amounts of DNA, corresponding to approximately 3, 1·5 and 0·7 μg were loaded in lanes 1, 3, and 5, respectively. In lanes 4 and 7 was loaded PBR322 DNA, digested with *Nci* I, to serve as a molecular-weight marker. The DNA was run on a 1·4% agarose gel. The Southern blot was probed with the 0·7 kb *Hind* III/*Xho* I P-element fragment from pΠ25.1. The band of H^f DNA corresponding to 730 bp is expected from KP elements previously characterized in M′ strains; see text for details).

of the P element, should yield a 730 bp fragment, instead of the expected 700 bp band. An approximately 730 bp fragment, in addition to a 700 bp fragment was found in the *Nla* III/*Taq* I digests of H^f DNA (Fig. 5). Since polymorphism is very rare in Drosophila *melanogaster* P elements (O'Hare & Rubin, 1983), and in view of the demonstration that the *Taq* I site was intact in the standard Harwich reference subline (Daniels *et al.* 1990), it is much more likely that the KP elements in the H^f subline originated from an M′ strain.

4. Discussion

The manifestations of P–M hybrid dysgenesis used to classify the different Harwich sublines, namely sterility and sn^w mutability, were not strictly correlated. Thus, although dysgenic hybrids originating from the standard Harwich subline, H^w, showed the same sn^w mutability as those derived from the weak, H^f subline, gonadal dysgenic sterility of 29 °C was much lower in the latter. Lack of correlation of these hybrid dysgenesis traits has been reported by others (Engels, 1984; Kocur *et al.* 1986; Simmons, 1987). Here we show that sterility and sn^w mutability are also not correlated in H^s hybrids of the non-dysgenic cross. Thus destabilization of sn^w in hybrids of P-strain females to M-strain males amounted to 41% of the rate found in dysgenic hybrids of the H^s strain, indicating incomplete P cytotype regulation. In contrast, GD sterility of nondysgenic hybrids was only 1% in crosses with the same female at 29 °C.

The importance of both the excision repair and post-replication DNA repair mechanisms for processing lesions induced by P-element mobility has been reported previously (Margulies, 1990), using dysgenic hybrids produced by the H^s Harwich subline, which induces exceptionally high P activity (Margulies *et al.* 1989). Whereas the effect of mutations in post-replication repair on hybrid dysgenesis traits has been demonstrated by others (Slatko *et al.* 1984), the *mei-9* excision repair mutation had no effect on a number of P-element transposition-induced phenomena monitored by these investigators. The mutation rate of selected visible mutations and their reversion was apparently increased by the *mei-9* repair defect in the

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MR mutator system (Eeken & Sobels, 1981, 1983); in contrast, the *mei-9* mutation had no effect on reversion of the RNA polymerase locus insertion (Voelker *et al.* 1984).

In studies reported here the generality of the influential role of both DNA repair mechanisms has been confirmed by comparing the effect in hybrids derived from different Harwich sublines which differ with respect to P activity-inducing ability, and by testing for a synergistic effect between ionizing radiation and DNA repair deficiency in the background of P-element mobility. Interactions due to the combined effect of three mutator systems were shown to be dependent on the subline, on the type of DNA repair defect, and on the stage of spermatogenesis during which cells were irradiated.

The results suggest that the dependence on different DNA repair mechanisms is related to the level of P activity. The following findings lend credence to this proposal. Sterility of dysgenic hybrids derived from any of the Harwich sublines was synergistically affected by the excision repair defect, but, whereas hybrids with the highest P activity (H^s-derived), showed this effect when untreated or X-irradiated, hybrids derived from the H^r or H^w sublines showed the synergism only when they were X-irradiated. Thus in the lower P-activity hybrids, three mutator systems had to be combined to produce a synergistic effect. Further, the combination of deficiency in post-replication repair and X-rays was not synergistic in hybrids with the lowest P activity. In contrast, in hybrids derived from sublines that induced higher P activity there was a highly synergistic effect of the post-replication repair defect on sterility (two-way interaction), and this effect was further enhanced by X-irradiation (three-way interaction). These observed interactions indicate that induction of higher P activity can be correlated with a greater dependence on the post-replication DNA repair mechanism. Nevertheless, the combined results demonstrate that both mechanisms of DNA repair play an important role in processing P-element-induced lesions in germline cells and that ionizing radiation can greatly exacerbate the mutagenic effects of P-element mobility and DNA repair deficiency. These findings support the hypothesis (Engels, 1989) that P-element-induced chromosome damage is an important cause of hybrid dysgenesis traits.

The lower dysgenesis-inducer ability of the H^r subline has been correlated with the presence of KP elements. It is not clear, however, whether there are enough copies of KP elements in the H^r subline to exert the suppression effect attributable to these deletion derivatives (Black *et al.* 1987; Jackson *et al.* 1988). Chromosomally-based suppression by the Sexi M′ strain was shown to result in intermediate level of hybrid dysgenesis (Kidwell, 1985) such as that reported here in H^r subline hybrids. Many of the defective P-elements of such M′ strains were shown to be KP elements (Black *et al.* 1987). If the H^r subline has acquired KP elements from an M′ strain, an acquisition of other P-elements may have also occurred. Thus the low P activity in hybrids of this subline may be due to elements that cause less induction of P activity, as well as to the KP elements which cause suppression. A more detailed molecular and genetic analysis of the P-elements of these sublines is required to provide answers to these relevant questions.

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