Copyright © 1985 by the Genetics Society of America

INVESTIGATION OF THE NATURE OF *P*-INDUCED MALE RECOMBINATION IN *DROSOPHILA MELANOGASTER*

DONALD A. R. SINCLAIR AND THOMAS A. GRIGLIATTI

Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A9

Manuscript received June 20, 1984
Revised copy accepted January 25, 1985

ABSTRACT

The present study consists of an investigation of *P*-induced male recombination in *Drosophila melanogaster* from a number of perspectives. In an initial set of experiments, male recombination induced by several different *P* strains was examined on both major autosomes. The ability of these *P* strains to evoke recombination is striking; in many cases it exceeded that of radiation treatment. Also of interest is the apparent nonrandom chromosomal distribution of *P*-exchange breakpoints. The data suggest that both recombinagenic capacity and distribution pattern of exchange breakpoints may be *P*-strain specific. In addition to these findings, we have confirmed previous indications that *P*-induced exchange is reasonably symmetrical and that it frequently occurs during premeiotic stages of spermatogenesis. Moreover, we have established that radiation and *P* background act additively with regard to the induction of male recombination. The second part of the work involved an analysis of heterochromatic *vs.* euchromatic recombination induced by several recombinagenically potent *P* strains. Results of these experiments have confirmed our earlier findings concerning the recombinagenic capacity of *P* strains. More importantly, it would appear that *P*-induced exchange in heterochromatin is rare. The induction of various kinds of mutations was also monitored in several of these experiments. The results indicate that the mutagenic potential of the *P* strains is substantial and of particular interest, that certain types of mutations are *P*-strain specific. For example, rare heterochromatic lesions were recovered exclusively in the experiment using the *h12* strain, whereas a novel pleiotropic mutation occurred at a high frequency only in the *T-007* experiment. Our findings are discussed within the context of a model of *P*-induced exchange.

THE experimental investigation of mutable genes in *Drosophila melanogaster* has steadily escalated over the last two decades (GREEN 1976, 1980). The success of this work attests to the incredible predictive power of genetic analysis. Thus, for example, the study of specific mutationally unstable or mutable alleles at the white (*w*) locus prompted the suggestion that, in analogy with prokaryotes, these lesions were insertion mutations (GREEN 1976; BINGHAM 1981). Recently, molecular confirmation of this hypothesis has been obtained (COLLINS and RUBIN 1982; LEVIS and RUBIN 1982; LEVIS, COLLINS and RUBIN 1982). It appears likely that most, if not all, insertion mutations are caused by mobile genetic elements (for review see RUBIN 1983).

Similar progress has been made in resolving mutator phenomena in this

organism, both at the genetic and molecular levels. The most well-characterized and intriguing mutator system is referred to as *P-M* hybrid dysgenesis (reviewed by BREGLIANO *et al.* 1980; GREEN 1980; BREGLIANO and KIDWELL 1983; ENGELS 1983). This system consists of a syndrome of well-defined genetic and physiological anomalies, including frequent induction of single-site mutations and chromosome rearrangements, recombination, nondisjunction, nonreciprocal transmission of homologous chromosomes and gonadal dysgenesis (GD) sterility. These abnormalities are primarily restricted to the germ line of both sexes, and they occur during and/or prior to meiosis. Furthermore, they are usually observed only in F_1 hybrids from certain crosses. Thus, progeny of matings between males from lines recently obtained in the wild and females from most established laboratory strains are dysgenic. However, progeny from the reciprocal or intrastrain crosses are essentially normal.

The most extensively studied component of *P-M* dysgenesis is mutation induction. A key hypothesis concerning the origin of *P*-induced mutations was proposed by GREEN (1977). He found that most X-linked visible mutations obtained from dysgenic individuals resembled mutable *w* alleles in their propensity to exhibit instability (see also GOLUBOVSKY, IVANOV and GREEN 1977; ENGELS 1979, 1981). Therefore, he argued that such lesions were also insertion mutations. This hypothesis has been confirmed at the molecular level (RUBIN, KIDWELL and BINGHAM 1982; BINGHAM, KIDWELL and RUBIN 1982; O'HARE and RUBIN 1983; ENGELS 1983). This was accomplished by demonstrating that dysgenically derived *w* mutations are usually associated with the insertion of foreign (*P*) elements into *w* gene sequences. Reversion of these insertion mutations is usually accompanied by excision of the elements. Collectively, *P* elements constitute a heterogeneous family of sequentially related transposable elements. They are abundant and appear to be dispersed throughout the genomes of all *P* strains examined. Presumably, *P*-induced mutations result from either the insertion of the elements at specific loci (*i.e.*, insertion mutations) or heterologous interchange (*i.e.*, chromosome aberrations). The latter may be causally related to noninsertion aspects of *P*-element mobilization (ENGELS and PRESTON 1981, 1984). As yet, no attempt has been made to accommodate other dysgenic traits (*e.g.*, GD sterility or recombination) within this context.

A prominent and extensively studied manifestation of *P-M* hybrid dysgenesis is *P*-induced male recombination. In fact, recombination in males was the first diagnostic indicator of dysgenesis (HIRAIZUMI 1971; HIRAIZUMI *et al.* 1973). Subsequently, it was established that *P*-induced exchange also occurs in dysgenic females (KIDWELL 1977; SLATKO 1978; SINCLAIR and GREEN 1979). A variety of studies involving specific *MR* (male recombination) or *P* strains have disclosed several important properties of *P*-induced recombination. These can be summarized as follows: it occurs primarily if not exclusively during premeiotic stages (see HIRAIZUMI *et al.* 1973; VOELKER 1974; WOODRUFF and THOMPSON 1977); the chromosomal distribution of recombinant breakpoints is clearly distinct from the pattern derived from spontaneous meiotic exchange (*e.g.*, HIRAIZUMI *et al.* 1973; WOODRUFF and THOMPSON 1977); the results of

several independent studies indicate that *P* recombination does not result from asymmetrical exchange events (VOELKER 1974; SVED 1978; ISACKSON, JOHNSON and DENNELL 1981).

To obtain more information about the etiology of *P*-induced recombination, the present work was directed toward the following objectives: to determine whether there is evidence of site specificity with regard to chromosomal distribution of exchange events induced by different *P* strains; to establish whether *P*-induced exchange actually occurs within heterochromatin and, if so, to ascertain the relative proportions of heterochromatic *vs.* euchromatic exchange; to examine the relationship between *P*- and radiation-induced exchange; and to investigate the relationship between recombination and mutation induction in dysgenic males. The results of our experiments are discussed within the general framework of a simple model of *P*-induced recombination.

MATERIALS AND METHODS

Culture conditions: All cultures were maintained and crosses performed at 22° on standard Drosophila medium consisting of cornmeal, sucrose, yeast and agar. Tegosept was used as an inhibitor of mold growth. Ampicillin (50 mg/liter), in combination with either tetracycline or streptomycin (15 mg/liter each), was used as a bacterial inhibitor. Except where indicated, crosses were performed in shell vials.

Mutant and wild-type strains: For detailed descriptions of most of the mutations and special chromosomes used in this study, see LINDSLEY and GRELL (1968). The second chromosome strain *S b lt rl* nw^D Pin^{Yt} warrants special mention. This chromosome was constructed by adding the dominant mutations *S*, nw^D and Pin^{Yt} to a chromosome marked with *b lt rl* (kindly provided by D. G. HOLM). The *b lt rl* chromosome was derived from a single spontaneous meiotic recombinant involving exchange between *lt* and *rl* (TATTERSALL 1981). The *lt* and *rl* markers flank a chromosome segment that is entirely heterochromatic (HILLIKER and HOLM 1975).

The following *P* strains were used in the present study (hereafter, *MR* and *P* strains will be considered equivalent; see BREGLIANO and KIDWELL 1983): *MR-h12* (referred to as *h12*): isolated in Israel (for description, see GREEN 1977); the *P*-derived second chromosome is maintained in combination with the multiply inverted chromosome *CyO*. *MR-n1* (referred to as *n1*): derived from a single wild-type female collected in the Napa Valley of California. *MR-s1* (referred to as *s1*): derived from a single wild-type female collected in the Sonoma Valley of California. *T-007*: isolated in Texas; the *P*-derived second chromosome is maintained in combination with *CyO* (for origin and description, see HIRAIZUMI 1971; HIRAIZUMI *et al.* 1973). *OK1*: isolated in Oklahoma (kindly provided by R. C. WOODRUFF; for origin and description, see WOODRUFF and THOMPSON 1977). *1978-1*: isolated in Wisconsin (kindly provided by H. BROCK; see ENGELS and PRESTON 1980).

Although these strains have not been extensively examined for the presence of autosomal inversions, the results of female recombination experiments involving several of them suggest that they are relatively free of such rearrangements.

Mating schemes and general procedures for analysis of male recombination: To simplify description of protocols, this part is divided into two sections. The first (i) comprises eight experiments that collectively examine autosomal recombination induced by three different *P* backgrounds, as well as the relationship between *P*- and radiation-induced recombination. The second section (ii) includes five experiments designed to assay heterochromatic *vs.* euchromatic exchange in different *P* backgrounds. The mating schemes used are summarized below.

Section i: An identical scheme was used for the six *P* experiments. *P* males (from *h12/CyO*, *n1* or *s1* cultures) were mated *en masse* to either homozygous *al dp b pr cn* or *ru h th st* p^p *cu sr* e^s females (15 pairs per bottle). Young (24–48 hr posteclosion) F_1 *al dp b pr cn/P* or *ru h th st* p^p *cu sr* e^s*/P* males from these matings were crossed to homozygous *al dp b pr cn* and *ru h th st* p^p *cu sr* e^s females, respectively. In these test crosses, individual males (a total of 16–30/experiment) were mated to harems of three to six females, and the males were transferred to fresh medium

every 3 days (young virgin females were added at each brood), to a maximum of 21 days. The parents were subsequently discarded. In two separate experiments, the relationship between radiation- and *P*-induced recombination was examined as follows. Young F_1 *h12/al dp b pr cn* males (derived from one of the crosses described above) and *+/al dp b pr cn* males (derived from matings between Oregon R males and homozygous *al dp b pr cn* females) were irradiated with 3000 r of X rays (using a modified dental machine operated at 90 kV/5 mA; 467 r/min). Subsequently, they were crossed to homozygous *al dp b pr cn* females. In these crosses, individual males were mated to harems of five females, and the males were transferred to fresh medium for eight consecutive 2-day broods (with virgin females added at each brood). Thereafter the parents were discarded.

Section ii: An identical protocol was used for the four *P* experiments in this section. *P* males (from *h12/CyO*, *T-007/CyO*, *OK1* or *1978-1* cultures) were mated *en masse* to *S b lt rl* nw^D Pin^{Yt}/*CyO* females (20 pairs per bottle). Young F_1 *S b lt rl* nw^D Pin^{Yt}/*P* males were mated to homozygous *b lt rl* females. In these crosses, groups of three males (a total of 150–180 males per experiment) were mated to harems of eight to ten females. The males were transferred to fresh medium every 2 days (with virgin females) to a maximum of 12 days, and then they were discarded. Each brood was subcultured by allowing the groups of inseminated females to lay in bottles for 4 days before they were discarded. A modified mating scheme was used for the radiation experiment. Young *S b lt rl* nw^D Pin^{Yt}/+ males (derived from crosses between *S b lt rl* nw^D Pin^{Yt}/*CyO* females and Oregon R males) were exposed to 3000 r of γ-radiation (Cobalt-60 Gammacell) and subsequently mated to homozygous *b lt rl* females. In these matings, groups of ten to 12 males were crossed to harems of 25 females in bottles. The males were transferred to fresh medium (with virgins) for ten consecutive 2-day broods, and each brood was subcultured as described above.

In each experiment, recombinants were sought among the F_2 progeny until 20 days after the parents had been introduced. In several experiments, the occurrence of specific types of mutations was also noted. Where necessary, putative recombinant and/or mutant offspring were subjected to progeny tests in order to confirm their genotypes.

Two measures of recombination were used throughout, *viz.*, percent exchange and percent exchange events. The former values were obtained simply by expressing the total number of recombinant progeny as a percentage of the total number of progeny. Percent exchange event values were obtained as follows. First, the recombination data for single males (or, in the case of experiments 9 to 13, single groups consisting of three males per group) were examined, and all progeny exhibiting related exchange phenotypes (*i.e.*, either reciprocal class of a given type of exchange) were classified as derivatives of the same exchange event. Then, the total number of each type of exchange event was expressed as a percentage of the total number of progeny. This procedure was adopted in order to compensate for mitotic amplification of exchange induced prior to meiosis. Although the percentage exchange event value probably represents an underestimate of *P*-induced exchange, we believe that it is the more accurate measure. A similar procedure was used to obtain measures of mutation induction in experiments 9, 10, 12 and 13.

RESULTS

Profiles of autosomal recombination induced by different P-*strain genetic backgrounds*: The results of the experiments to examine *P*-induced autosomal recombination in males are summarized in Tables 1 (second chromosome) and 2 (third chromosome). The results of separate experiments investigating the effects of X rays (experiment 4), and the combined effects X rays and *P* background (experiment 5) on male recombination, are also presented in Table 1. For reference, the various chromosome intervals are designated as follows: second chromosome = *al*—1—*dp*—2—*b*—3—*pr*—4—*cn* (see Figure 1); third chromosome = *ru*—1—*h*—2—*th*—3—*st*—4—p^p—5—*cu*—6—*sr*—7—e^s (see Figure 2). Note that in each case, the centromere is located in interval 4.

Several observations can be made from these recombination data. First, with regard to recombinagenic activity, all three *P* strains had marked effects on

TABLE 1

Summary of results of second chromosome recombination experiments

Experiment[b]	% exchange per genetic interval[a]					Total progeny
	1	2	3	4	Total	
1 (*h12*)	0	0.28 (0.08)	0.02 (0.02)	0.20 (0.05)	0.50 (0.15)	22,116
2 (*s1*)	0	0.30 (0.15)	0.08 (0.08)	0.35 (0.15)	0.73 (0.38)	7,896
3 (*n1*)	0	0.03 (0.03)	0.06 (0.06)	0.18 (0.12)	0.27 (0.21)	7,714
4 (O.R. + 3000 r)[c]	0	0.04 (0.02)	0.06 (0.06)	0.29 (0.15)	0.39 (0.23)	5,179
5 (*h12* + 3000 r)[c]	0	0.17 (0.13)	0.10 (0.07)	1.55 (0.24)	1.82 (0.44)	2,968
Control (O.R.)	0	0	0	0	0	25,995

[a] Percent exchange values are given first, with percent exchange event values shown in parentheses (see MATERIALS AND METHODS).

[b] *P* strain or treatment given in parentheses (O.R. = Oregon R).

[c] Data for radiation experiments include only progeny derived from meiotic and premeiotic broods as defined by the sterile brood (see AUERBACH 1954).

both chromosomes. Indeed, in terms of frequencies of exchange events, *P*-induced recombination on the second chromosome was usually equivalent to or greater than that induced by radiation (Table 1). The extent of *P* recombination on the third chromosome was even more striking (Table 2). Moreover, recombinagenic capacity was consistently strain specific, *i.e.*, the most potent strain was *s1*, *n1* exhibited intermediate potency and *h12* was the least potent.

The second noteworthy point is that, in agreement with previous work (*e.g.*, HIRAIZUMI *et al.* 1973; WOODRUFF and THOMPSON 1977), it would appear that much of the *P*-induced exchange seen in our experiments occurred prior to meiosis. This is evident from a statistical analysis of the recombination data from individual males in experiment 1 (Table 3). In this analysis, the distributions of recombinants were tested for conformity with the predicted Poisson distributions, using the index of dispersion, χ^2 (HIRAIZUMI *et al.* 1973). The results show that for at least two of the intervals (*dp—b* and *pr—cn*), the recombinant distributions differed significantly from the expected Poisson values. Further examination of the data in Table 3 reveals that very large clusters of recombinant progeny were produced by single males (*e.g.*, one male produced 26 individuals that arose via exchange in the *pr—cn* interval). Equivalent analysis of the data from one of the third chromosome studies (experiment 7) produced similar findings (data not shown). Taken together, these observations indicate that at least some and perhaps most *P*-induced recombination occurs during premeiotic stages of spermatogenesis.

The third observation concerning the recombination data is that the distribution of *P*-induced exchange along the chromosome is nonrandom and this effect appears to be strain specific. This can be seen more clearly in Figures 1 and 2, where the data are presented as proportional distribution of exchange events for the various chromosome intervals. For comparative purposes, maps derived from appropriate radiation studies are included (the second chromosome radiation map is derived from experiment 4 data in Table 1; the third

TABLE 2

Summary of results of third chromosome recombination experiments

Experiment	% exchange per genetic interval								Total progeny
	1	2	3	4	5	6	7	Total	
6 (*h12*)	0.19 (0.11)	0.02 (0.02)	0	0.08 (0.08)	0.45 (0.04)	0.02 (0.02)	0.02 (0.02)	0.78 (0.29)	6275
7 (*s1*)	0.11 (0.07)	0.23 (0.12)	0.02 (0.02)	0.44 (0.17)	0.07 (0.04)	0.35 (0.13)	0.08 (0.04)	1.3 (0.59)	9088
8 (*n1*)	0.03 (0.03)	0.17 (0.08)	0	0.38 (0.17)	0.06 (0.05)	0.33 (0.21)	0.02 (0.02)	1.0 (0.56)	6604

See footnotes to Table 1.

TABLE 3

Analysis of frequency distributions of recombinant progeny produced by individual dysgenic males in experiment 1

No. of recombinants/male	No. of males yielding recombinants in regions indicated		
	dp—b	*b—pr*	*pr—cn*
0	14	26	18
1	6	3	7
2	5	1	1
3	2	0	2
4	1	0	1
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
13	1	0	0
22	1	0	0
26	0	0	1
Total males	30	30	30
Total recombinants	61	5	45
Total progeny	22,116	22,116	22,116
Recombination frequency (%)	0.28	0.02	0.20
Mean	2.03	0.17	1.50
Variance	20.31	0.21	22.53
Index of dispersion (χ^2)	290.14	35.82	435.58
Probability	$P < 0.001$	NS	$P < 0.001$

NS = not statistically significant from the Poisson.

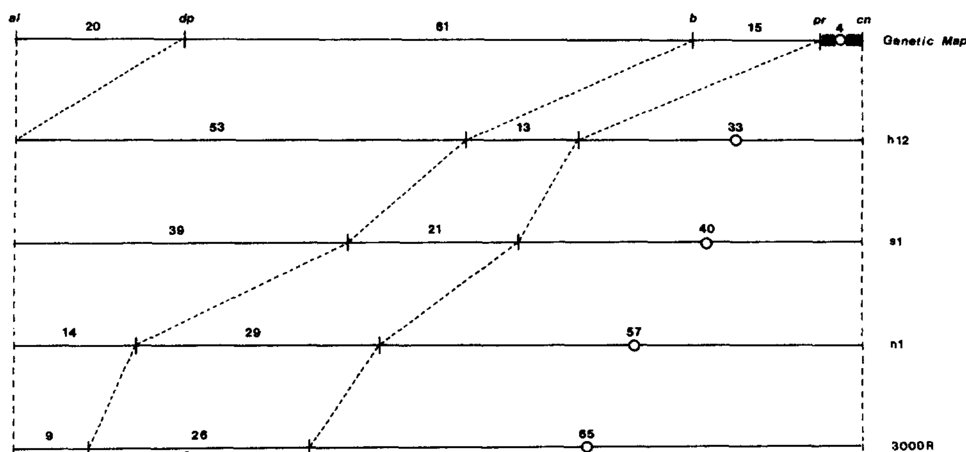

FIGURE 1.—Second chromosome maps derived from male recombination experiments involving the *P* strains *h12*, *s1* and *n1*. Data are presented as proportions of exchange events occurring in various chromosome intervals. For comparison, maps derived from female meiotic recombination data (Genetic Map) and X-ray-induced (3000 r) male recombination data are shown at the top and bottom, respectively. Blocks of centromeric heterochromatin and the centromere are represented by closed bars and an open circle, respectively.

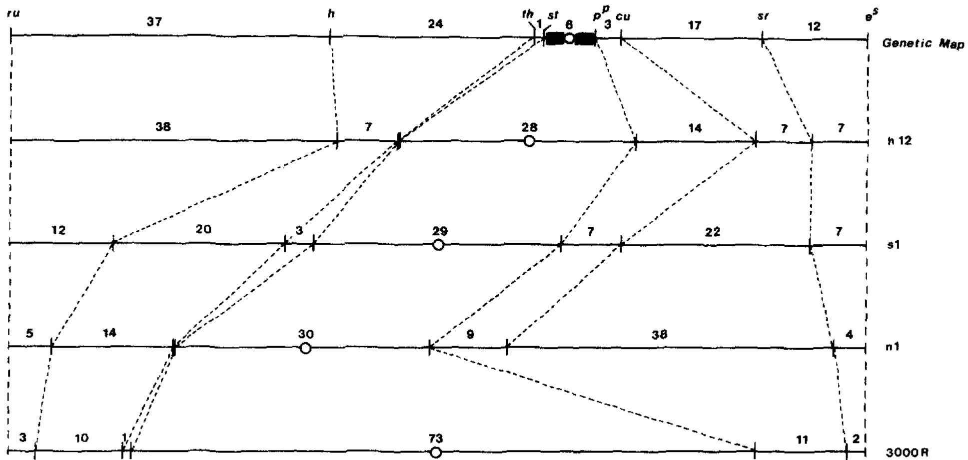

FIGURE 2.—Third chromosome maps derived from male recombination experiments involving the *P* strains *h12*, *s1* and *n1*. Maps derived from female meiotic recombination data (LINDSLEY and GRELL 1968) and radiation-induced male recombination data (PURO 1966) are shown at the top and bottom, respectively.

chromosome radiation map is modified from PURO 1966). Presumably, the radiation maps are reasonably representative of random exchange. Not surprisingly, the bulk of the radiation-induced exchange on the second chromosome (Figure 1) occurred in the regions flanking the centromere (*i.e.*, the proximal interval). However, in general this was not true for *P*-induced exchange. For example, the patterns emerging from the *h12* and *s1* experiments indicate that the majority of the exchange events occurred in euchromatic regions, particularly that flanked by *dp* and *b*. In contrast, most of the recombination induced by the *n1* strain did occur in the proximal interval, although considerable exchange also occurred in wholly euchromatic segments (*e.g.*, note the *b-pr* interval). The difference between patterns of *P*- and radiation-induced exchange is even more marked when one examines the third chromosome data (Figure 2). Although substantial amounts of *P*-induced exchange consistently occurred within the proximal interval, in no case did this exceed 30% of the total. Compare this to the radiation map, in which more than 70% of the third chromosome exchange observed by PURO (1966) occurred in the proximal segment. Furthermore, not only did the vast majority of *P* exchange occur in euchromatic regions of the third chromosome, but there were clear indications of site specificity for the different strains. For example, 38% of the *h12* exchange occurred within interval 1 (*ru—h*), whereas 47 and 29% of *n1* and *s1* exchange, respectively, occurred in the p^p—*sr* region. Moreover, nearly a quarter of the *s1*-induced exchange occurred in the *h—st* segment in the left arm of the chromosome.

In summary, it appears that *P* recombinagenic activity is substantial, but the potency can vary from strain to strain. Interestingly, in contrast with radiation-induced exchange, *P*-induced recombination occurs more frequently in wholly euchromatic segments. Furthermore, our data illustrate that different *P* strains

can elicit distinct site specificity with respect to chromosomal distribution of exchange breakpoints. Finally, in agreement with previous studies, we have found that a substantial proportion of *P*-induced exchange occurs premeiotically.

Confirmation of the nonasymmetrical nature of P-*induced recombination*: Previous findings have indicated that male recombination does not occur by asymmetrical exchange (VOELKER 1974; SVED 1978; ISACKSON, JOHNSON and DENELL 1981). These studies were predicated on the reasonable notion that asymmetrical exchange events would frequently produce deficiency- and duplication-bearing recombinant chromosomes, many of which would be lethal when homozygous. To extend this approach in the present work, recombinant chromosomes derived from experiments involving two different *P* strains were tested for homozygous viability. First, lines from 71 recombinant second chromosomes produced by *h12* (Table 1, experiment 1) were examined. Sixty-three of these were homozygous viable and the remaining eight were lethal. Since three of 28 nonrecombinant chromosomes from the same experiment were lethal, it is clear that few, if any, of the recombinant chromosomes contained lethal lesions that were actually generated through exchange. A similar result was obtained from analysis of 20 recombinant third chromosomes produced by *n1* (Table 2, experiment 8). In this case all but one of the chromosomes tested were homozygous viable. The results of these tests confirm those of the previous studies. Taken together, they suggest that *P*-induced exchange occurs with a relatively high degree of fidelity. From this perspective, it is not unlike spontaneous meiotic exchange.

Investigation of the relationship between P- *and radiation-induced recombination*: Radiation is one of the most extensively studied recombinagenic agents (for reviews, see SANKARANARAYANAN and SOBELS 1976; BECKER 1976). Thus, some insight into the etiology of *P*-induced exchange might be gained by exploring the relationship between the two agents. The results of the study to investigate the combined effects of the *P* genetic background and X rays on male recombination are shown at the bottom of Table 1 (experiment 5). To assess these effects, it is necessary to compare the overall level of exchange obtained in this experiment to that obtained by adding the levels of exchange induced by the two agents independently (*i.e.*, experiments 1 and 4). It can be seen that, in combination, these two agents evoked an exchange frequency of 0.44%, whereas the independent total is 0.38% (0.15 plus 0.23). The similarity between these totals strongly suggests that radiation and *h12* act in an additive rather than synergistic fashion, with respect to induction of recombination. This is consistent with the hypothesis that the recombinagenic activity of these agents occurs by different mechanisms.

Investigation of heterochromatic vs. euchromatic recombination in dysgenic males: Our findings concerning the chromosomal distribution of *P*-induced exchange were intriguing and clearly warranted further investigation. Of particular interest was the nature of proximal recombination. In the present study, levels of *P*-induced exchange in proximal regions of both autosomes were significant (although they were considerably less than those induced by radiation). Even

more striking in this regard are the results of previous work indicating that most of the exchange induced by the *P* strains *T-007* (HIRAIZUMI *et al.* 1973) and *OK1* (WOODRUFF and THOMPSON 1977) occurred within proximal segments of the second chromosome. However, the question of whether this type of exchange actually occurs within heterochromatin has not been tested definitively. The reason for this is that the appropriate chromosome bearing heterochromatic markers in the required configuration did not exist. We constructed such a chromosome, *S b lt rl* nw^D Pin^{Yt} (see MATERIALS AND METHODS) and then utilized it to determine the relative proportion of *P*-exchange events induced in euchromatic *vs.* heterochromatic regions of the chromosome. The *P* strains, *h12*, *T-007*, *OK1* and *1978-1*, were used for this purpose. The results of this study are summarized in Table 4. Results of an experiment monitoring the frequency and distribution of exchange in *S b lt rl* nw^D Pin^{Yt}/+ males treated with 3000 r of γ-radiation are also included. For reference, the various chromosome intervals have been designated as follows: *S*—1—*b*—2—*lt*—3—*rl*—4—nw^D—5—Pin^{Yt} (see Figure 3). Note that intervals 1 and 5 are wholly euchromatic, whereas intervals 2 and 4 contain both euchromatic and heterochromatic segments, and interval 3 is entirely heterochromatic.

Several conclusions can be drawn from these data. First, they confirm our previous findings concerning the recombinagenic potency of the *P* genetic background in relation to that of radiation. In each case, the frequency of *P*-induced exchange events either equaled or exceeded that produced by radiation. Indeed, the most potent strain, *T-007*, produced four- to five-fold more exchange than did 3000 r of γ-radiation. Second, strain-specific differences in recombinagenic capacity similar to those mentioned previously (see Tables 1 and 2) are also evident from these data. For example, compare the overall level of *T-007* recombination with that observed for *h12*. The third and by far the most interesting observation emerging from the data pertains to the chromosomal distribution of exchange breakpoints (Table 4 and Figure 3). As expected, a substantial fraction of radiation-induced exchange events (26%) occurred within interval 3, *i.e.*, the wholly heterochromatic segment flanked by the markers *lt* and *rl*. This undoubtedly represents an underestimate of the total amount of heterochromatic exchange (see DISCUSSION). In distinct contrast, very little exchange occurred within the *lt*—*rl* region in dysgenic males. Indeed, of the four *P* strains, only *h12* elicited perceptible levels of heterochromatic exchange (*i.e.*, 7% of the total number of exchange events). Even the very recombinagenically potent strain *T-007* produced no exchange within the *lt*—*rl* interval. Therefore, with some reservations (DISCUSSION), we conclude that *P*-induced heterochromatic exchange on the second chromosome is exceedingly rare.

In summary, the important findings of the latter study are two-fold. First, our results are consistent with the aforementioned observations concerning the extremely high recombinagenic capacity of the *P* genetic background and the fact that this capacity can vary from strain to strain. Second, and more importantly, *P*-induced exchange within second chromosome heterochromatin appears to be extremely rare.

TABLE 4

Summary of results of the experiments to examine heterochromatic vs. *euchromatic recombination on the second chromosome*

Experiment	% exchange per genetic interval						Total progeny
	1	2	3	4	5	Total	
9 (*h12*)	0.11 (0.08)	0.16 (0.10)	0.03 (0.03)	0.36 (0.17)	0.03 (0.03)	0.69 (0.42)	21,102
10 (*T-007*)	0.53 (0.41)	0.81 (0.53)	0	1.60 (0.57)	0.80 (0.46)	3.75 (1.96)	5,652
11 (*OK1*)	0.17 (0.12)	0.32 (0.19)	0	0.80 (0.26)	0.17 (0.14)	1.46 (0.70)	15,514
12 (*1978-1*)	0.19 (0.13)	0.36 (0.20)	0	0.77 (0.25)	0.18 (0.10)	1.50 (0.67)	15,750
13 (O.R. + 3000 r)	0.08 (0.07)	0.12 (0.10)	0.13 (0.12)	0.23 (0.18)	0	0.56 (0.46)	6,065
Control (O.R.)	0	0	0	0	0	0	12,262

See footnotes of Table 1.

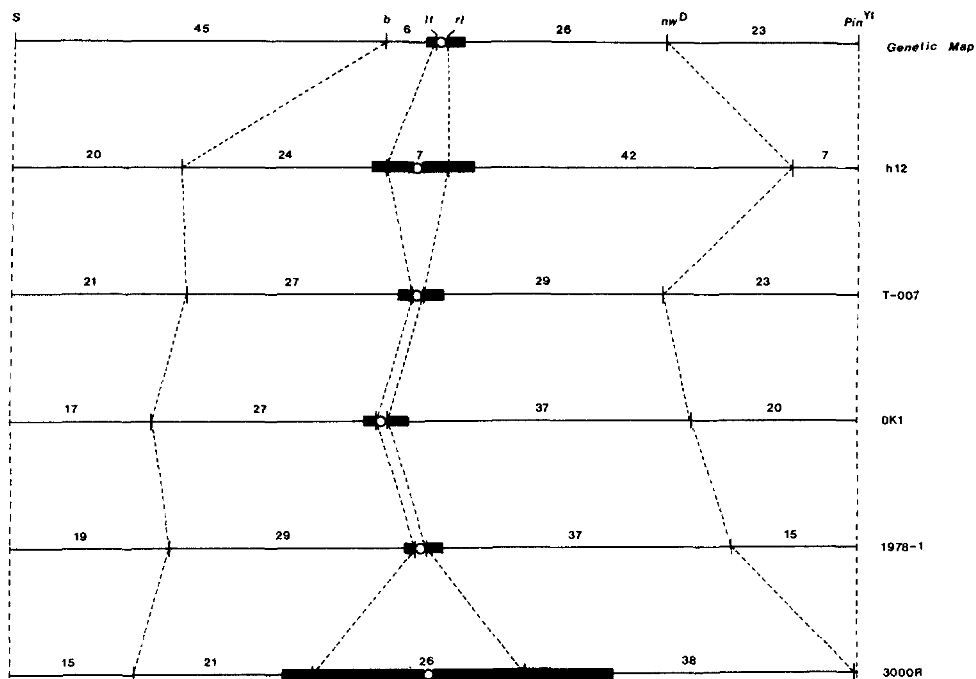

FIGURE 3.—Second chromosome maps derived from male recombination experiments involving the *P* strains *h12*, *T-007*, *OK1* and *1978-1*. Maps derived from female meiotic recombination data (LINDSLEY and GRELL 1968) and γ-ray-induced (3000 r) male recombination data are shown at the top and bottom, respectively. Note that the genetic markers used allow a comparison of heterochromatic *vs.* euchromatic exchange (see text).

P-*induced mutations obtained in recombination experiments*: The question of the relationship between *P* mutagenesis and recombination induction is as yet unresolved. To obtain more information about this question, we monitored mutation induction in several of the above experiments (Table 4, experiments 9, 10, 12 and 13), and a summary of the resulting data is presented in Table 5. The protocol used for these experiments necessarily limits this analysis to dominant mutations or recessive alleles of *b*, *lt* and *rl*.

An examination of the data in Table 5 reveals that the total frequencies of mutation events range from 0.2% for both *h12* and *1978-1* to 0.42% for *T-007*. These are somewhat lower than the radiation frequency; nevertheless, they are significant. No spontaneous mutations were detected from among more than 12,000 control progeny (data not shown). Therefore, in analogy with their recombinagenic capacity, these *P* strains possess an impressive mutagenic capability. It is evident that, although some strain specificity exists with respect to mutagenic capacity (*e.g.*, the *T-007* mutation frequency was two-fold higher than the others), this specificity is less striking than that characteristic of *P*-induced recombination (see Table 4). Analysis of the data for individual groups of male parents revealed that many mutations occurred in clusters, suggesting that the mutation events were induced premeiotically (data not

TABLE 5

Summary of mutation data from specific recombination experiments

Experiment[b]	Frequency of various mutations[a]									
	M	*M-pl*[c]	*dp*	*b*	*lt*[d]	*rl*	bw^V-like	Mottled eyes	Dl	Total
9 (*h12*)	0.21 (0.12)	0	0	0.03 (0.02)	0.02 (0.02)	0.004 (0.004)	0.02 (0.02)	0.01 (0.01)	0	0.29 (0.19)
10 (*T-007*)	0.12 (0.11)	0.37 (0.27)	0	0.05 (0.05)	0	0	0	0	0	0.55 (0.42)
12 (*1978-1*)	0.25 (0.15)	0.03 (0.03)	0	0.02 (0.02)	0	0	0.006 (0.006)	0	0	0.30 (0.20)
13 (O.R. + 3000 r)	0.45 (0.41)	0	0.01 (0.01)	0.03 (0.03)	0.07 (0.07)	0.01 (0.01)	0.05 (0.05)	0	0.02 (0.02)	0.63 (0.59)

[a] Frequency given in percent, with percent mutation events in parentheses.
[b] *P* strain or treatment given in parentheses.
[c] *M-pl* = pleiotropic mutation producing the Minute phenotype, as well as rough eyes, broad wings and sterility.
[d] *lt* mutants were recessive lethal and *M*-like in phenotype.

TABLE 6

Results of complementation tests between two h12*-induced* lt *mutations and lethal alleles of selected complementation groups occupying proximal* 2L *heterochromatin*[a]

Mutant designation	Complementation groups			
	VI	VII	VIII[b]	IX
$lt^{h12\text{-}1}$	+ (10/41)[c]	+ (39/123)	− (0/349)	− (0/190)
$lt^{h12\text{-}2}$	+ (11/28)	+ (18/47)	+ (19/167)	− (0/152)

[a] Crosses: lt^{h12}/*CyO* males × lethal/*SM1,Cy* females.

[b] A lethal allele of a locus other than *lt* was used for this test (see HILLIKER 1976 and text).

[c] + and − denote complementation and noncomplementation, respectively. Numbers of lt^{h12}/lethal heterozygotes (numerator), relative to the total number of progeny per cross (denominator), are given in parentheses.

shown). This is in distinct contrast to the lack of clustering of mutations in the radiation experiment.

A most interesting aspect of the mutation data pertains to the types of mutants observed. In each case, Minute (*M*) lesions constituted the most common class (Table 5, column 1). Perhaps this is not surprising, since *M* loci represent the largest single group of haplo-abnormal genes in the organism (LINDSLEY *et al.* 1972). This finding suggests that the detection of Minutes may provide a convenient means of monitoring mutagenic effects in dysgenic hybrids. The *b* locus also appears to be fairly susceptible to *P* mutagenesis (column 4), *i.e.*, all of the *P* experiments yielded *b* mutations at frequencies similar to that seen in the radiation experiment.

By far the most interesting finding concerning the types of *P* mutations identified is the apparent strain specificity with respect to the production of certain lesions. This is especially evident for the rarer heterochromatic mutations. For example, a single *rl* mutation, as well as four independently derived *lt* mutants, were recovered in the *h12* experiment. None of the other experiments produced any heterochromatic lesions. Recall that *P*-induced heterochromatic exchange was also restricted to the *h12* experiment.

The possibility that *P*-induced heterochromatic mutations may frequently involve chromosomal rearrangements was indicated by the fact that all of the aforementioned *lt* mutants were also *M*-like in phenotype, suggesting that they may be deletions or other types of aberrations. To examine this question further, two of these mutants were tested for complementation with lethal alleles of the four complementation groups that occupy the centromeric heterochromatin in the left arm of chromosome *2* (HILLIKER 1976). The results of these tests are summarized in Table 6. The groups are listed in numerically ascending order according to their distance from the centromere (*e.g.*, group VI is the most proximal and group IX is the most distal). Group VIII contains three subgroups including the *lt* locus (with both lethal and visible alleles), as well as two other loci defined by lethal alleles. One of the latter was chosen for the present analysis. The results show that the $lt^{h12\text{-}1}$ mutation inactivates not only the *lt* locus but also one of the other group VIII loci, as well as the

adjacent group IX gene. This indicates that it is a deletion extending from a point between groups VII and VIII to the distal portion of the heterochromatin, or perhaps even beyond into adjacent euchromatin. Complementation tests with a variety of proximal *2L* euchromatic deficiencies (data not shown) have revealed that both the histone gene cluster and the *M(2)H* locus (WRIGHT, HODGETTS and SHERALD 1976) are intact in the $lt^{h12\text{-}1}$-bearing chromosome. Moreover, cytological analysis of the salivary gland chromosomes of $lt^{h12\text{-}1}$ revealed no abnormalities in the proximal region of *2L* (data not shown). Thus, the distal breakpoint of the *P*-induced deletion probably lies proximal to section 40A and is, therefore, very close to the heterochromatic/euchromatic junction. Further examination of the results in Table 6 reveals that $lt^{h12\text{-}2}$ is also a deletion but with slightly different genetic properties. The data suggest that it extends from the *lt* locus to the distal portion of *2L* heterochromatin (*i.e.*, it does not delete the non-*lt* member of group VIII or any of the more proximal loci). Although preliminary, these results certainly support the notion that many *P*-induced heterochromatic lesions may be chromosomal rearrangements.

Initial genetic analysis suggested that the 5 bw^V-like mutations obtained in this study (all but one were identified in the *h12* experiment) might be heterochromatic/euchromatic rearrangements that affect *bw* locus expression due to position-effect variegation. However, cytological analysis of three of the mutants revealed no visible rearrangements that could account for the bw^V-like phenotype (data not shown).

Another example of a strain-specific *P* mutation involves a rather unique class of lesion that was induced almost exclusively in the *T-007* background (Table 5, column 2). These mutants exhibited a uniform pleiotropic phenotype that included *M* traits, as well as rough eyes, broad wings and sterility. These traits are reminiscent of phenotypes evoked by mutations at two adjacent loci that reside near the tip of *2L*, *viz.*, *M(2)21C1-2* and *ex*. Thus, it is possible that this pleiotropic lesion involves the deletion or, in some other way, inactivation of both loci.

In summary, we conclude that like *P* recombinagenesis, *P*-mediated mutagenesis is extremely potent and frequently occurs prior to meiosis. There is evidence of strain specificity with regard to mutagenic capacity, although this was not as marked as that observed for *P* recombination. Similarly, strain specificity exists with respect to the kinds of mutations produced. Of primary importance is the finding that *P*-induced heterochromatic lesions were restricted to the *h12* experiment, whereas what appears to be a specific type of pleiotropic mutation was frequently detected only in the *T-007* study. In contrast, other types of mutations (*e.g.*, *b* and *M* lesions) were obtained at equivalent frequencies in both the *P* and the radiation experiments.

DISCUSSION

This study represents the first comprehensive comparative investigation of *P*-induced male recombination. Our results have confirmed several previously observed characteristics of this phenomenon and, in addition, a number of

novel findings have emerged. Thus, we have demonstrated that the capacity of *P* strains to induce recombination is striking. By and large, the recombinagenic potency of these strains is equivalent to or greater than that of radiation, and this property is also strain specific. In agreement with earlier findings, our results indicate that *P*-induced exchange frequently occurs during premeiotic stages of spermatogenesis (HIRAIZUMI *et al.* 1973; WOODRUFF and THOMPSON 1977) and that the actual mechanism of exchange is relatively precise (VOELKER 1974; SVED 1978; ISACKSON, JOHNSON and DENELL 1981). In addition, our finding that the *h12* genetic background and radiation act independently with regard to induction of recombination is important to the assessment of possible mechanisms of *P*-induced exchange.

Undoubtedly, the most intriguing result of the present work concerns the chromosomal distribution of *P*-exchange events. We have observed nonrandom distribution patterns of *P*-induced exchange breakpoints on both the second and third chromosomes. These patterns were frequently strain specific and in general they differed markedly from the corresponding patterns of exchange induced by radiation. The most surprising example of this was the dearth of exchanges in second chromosome heterochromatin in dysgenic males; of the four *P* strains tested, only *h12* produced heterochromatic exchange at a detectable frequency. From these data, we conclude that *P*-induced exchange in these regions of the chromosome is extremely rare. This result contrasts with the findings of HIRAIZUMI (1981) regarding female recombination observed in the *I-R* system of hybrid dysgenesis. In this study, he reported elevated frequencies of proximal recombination, a large proportion of which appeared to occur within heterochromatin.

In a subset of the recombination experiments, we also monitored the occurrence of visible mutations among the offspring of dysgenic males and the following conclusions have emerged from these data. First, it is clear that, like *P* recombinagenesis, *P* mutagenesis is extremely potent and frequently occurs during premeiotic stages. Second, the frequent recovery of *m* mutations among the progeny of dysgenic males suggests that the detection of these lesions will serve as a convenient measure of *P* mutagenic activity. Third, and most importantly, the induction of certain types of mutations appears to be strain specific. For example, all of the mutations involving the heterochromatic loci *lt* and *rl* were obtained in the *h12* experiment and two of the *lt* mutations were deficiencies in proximal *2L* heterochromatin. Moreover, a specific type of mutation whose pleiotropy points to a multisite lesion was frequently observed in only the *T-007* experiment. If it is true that many of these strain-specific mutations were associated with chromosomal rearrangements, this raises the possibility that they were generated by heterologous interchange coinciding with *P*-element mobilization. Presumably, a significant proportion of the other *P*-induced mutations recovered in this study were due to *P*-element insertion.

Although it is obvious that *P*-induced exchange is somehow related to the activity of *P* elements, the nature of this relationship is as yet unclear. From a simple perspective, exchange could be a direct consequence of chromosome

breakage resulting from events that lead to insertion or mobilization of *P* elements. Therefore, exchange within a given chromosome interval would be largely dependent, on the one hand, upon the ability of the DNA sequences within that interval to act as a target for insertion or, on the other, the *a priori* presence of a *P* element within the interval. Either situation could give rise to patterns of site-specific recombination. An alternative hypothesis envisages male recombination as an indirect by-product of certain physiological properties of hybrid dysgenesis. For example, one or more of the products encoded by the *P* sequences (O'HARE and RUBIN 1983) may actually promote general chromosome as well as DNA strand breakage. This might result in homologous or heterologous chromosome interchange. However, exchange of this type would presumably occur randomly along the chromosome and frequently be asymmetrical in nature. Clearly, the precision of *P*-induced exchange, as well as the chromosomal distribution of exchange breakpoints, is incompatible with the physiological hypothesis.

If it is assumed that *P*-induced recombination is not an indirect physiological consequence of hybrid dysgenesis, the question remains, what is the relationship between exchange and *P*-element activity. Some recent findings pertaining to *P*-induced chromosome rearrangements may be relevant to this discussion. There is considerable evidence that, for most of the rearrangements generated in dysgenic hybrids, at least one of the breakpoints occurs at the site of residence of a *P* element (ENGELS and PRESTON 1981, 1984). Moreover, the site-specific nature of *P*-induced chromosomal aberrations appears to be correlated with the nonrandom chromosomal distribution of *P* elements (BERG, ENGELS and KREBER 1980; YANNOPOULOS *et al.* 1983). Interestingly, ENGELS and PRESTON (1984) have observed a net tendency toward loss of *P* elements at rearrangement breakpoints (although in some cases, *P*-element gains did occur). Taken together, these findings suggest that *P*-induced rearrangements are not generated during the process of *P*-element insertion; rather, it is more likely that they are causally related to breaks at or near preexisting *P* elements in the chromosome.

ENGELS and PRESTON (1984) propose that generation of most rearrangements in dysgenic hybrids is a two-step process. First, chromosome breaks or preconditions for breakage (*e.g.*, single-strand nicks in the DNA) occur at one or both termini of different *P* elements. The rearrangement is then formed by random reunion of the chromosome segments produced by the breaks. According to this model, *P* element loss or excision might arise when the breaks occur at both termini of an element and the element is excluded upon reconstitution of the breakpoint. This could explain the observed net loss of *P* elements associated with heterologous exchange. However, the relationship between *P*-element excision and transposition is as yet unclear. Excision might be a normal component of transposition. Alternatively, it could be an aberrant by-product of the transposition process (VOELKER *et al.* 1984).

The aforementioned model may also be applicable to *P*-induced recombination. However, there are no *a priori* reasons to favor such a model over one in which recombination results from DNA strand breakage associated with *P*-

element insertion. Although the amount of experimental evidence bearing on this issue is not abundant, several observations may be relevant. For example, in the present study, we have observed that radiation does not interact synergistically with the genetic background of *h12* with regard to induction of recombination. Similarly, SOBELS and EEKEN (1981) have reported that X rays and *h12* exhibit no synergistic interaction with respect to the production of translocations. On the other hand, in the same study, these workers found that radiation and *h12* do interact synergistically concerning the production of X-linked lethal mutations. These findings tend to argue against an insertion-mediated exchange hypothesis, *i.e.*, although radiation apparently facilitates *P*-element insertion (perhaps by providing additional insertion sites through DNA strand breakage), it does not greatly enhance male recombination. Another relevant finding in this regard is that recombinant chromosomes from dysgenic males rarely contain lethal mutations. This is the opposite of what one would expect if recombination is concomitant with insertion, since a significant proportion of insertions would likely inactivate essential loci (*e.g.*, see SPRADLING and RUBIN 1983).

It should be possible to test these alternatives more critically. For example, if chromosomal hotspots for *P*-recombinant breakpoints are equivalent to sites of residence of the elements in *P*-derived chromosomes, then this would favor a version of the rearrangement hypothesis of Engels and Preston. The tendency for a given recombination hotspot to exhibit net loss of *P* elements would also support this hypothesis. On the other hand, the tendency for such hotspots to acquire additional elements *de novo* would favor the insertion hypothesis.

A simple extension of the rearrangement hypothesis of Engels and Preston can be used to explain *P*-induced recombination. Thus, DNA strand breaks at the termini of *P* elements could frequently result in host strand infiltration of the DNA duplex of the closely paired homologous chromosome (as suggested by ISACKSON, JOHNSON and DENELL 1981), leading to precise recombination (SZOSTAK, ORR-WEAVER and ROTHSTEIN 1983). Presumably, the occurrence of such recombinagenic events during intervals when homologous chromosomes are paired (*e.g.*, perhaps during early premeiotic stages of spermatogenesis) would favor homologous exchange, whereas their occurrence during later stages might promote heterologous exchange. It is interesting to note that this hypothesis requires the presence of *P* elements in only one of the homologous chromosomes. It is also possible that some recombination results from insertion of *P* elements into the *M* homologue, followed by pairing of the elements and crossing over within the paired elements. A similar mechanism involving a *copia*-like element has been implicated in unequal exchange at the *w* locus (GOLDBERG *et al.* 1983). However, since it is probable that such a mechanism would often result in asymmetrical exchange, it is unlikely that it is a major contributor to recombination occurring in dysgenic males.

The paucity of *P*-induced recombination within second chromosome heterochromatin is intriguing and warrants special mention. Genetically, *lt* and *rl* have been positioned between their respective heterochromatic/euchromatic boundaries and the centromere (HILLIKER and HOLM 1975). Therefore, any

heterochromatic recombination occurring distal to these markers would not be detected as such. However, with this limitation in mind, we argue that *P*-induced exchange in second chromosome heterochromatin is extremely rare. This is certainly consistent with the earlier findings of VOELKER (1974). Recall that there was also a distinct paucity of mutations involving heterochromatic loci or regions of heterochromatin in our study. Moreover, all of the heterochromatic exchange events and heterochromatic mutations were induced by *h12*.

If it is assumed that the constitutive heterochromatin of the other chromosomes is fundamentally similar to that of the second chromosome, it is reasonable to suggest that these segments would also be fairly refractory to the mutagenic and recombinagenic effects of *P* elements. The most straightforward explanation for the relative lack of *P*-induced heterochromatic exchange and heterochromatic mutations is that few *P* elements are present in heterochromatin of *P* strains, perhaps because they rarely insert into DNA sequences located within these regions of the genome. This hypothesis is at least somewhat supported by the findings of several different investigations of *P*-mediated gene transfer (SPRADLING and RUBIN 1983; SCHOLNICK, MORGAN and HIRSH 1983; GOLDBERG, POSAKONY and MANIATIS 1983; RICHARDS *et al.* 1983; HAZELRIGG, LEVIS and RUBIN 1984). In total, more than 100 different *P*-element insertions in various parts of the genome were obtained in these studies, yet only two of these occurred near or within heterochromatin (SPRADLING and RUBIN 1983; HAZELRIGG, LEVIS and RUBIN 1984). This hypothesis is also supported by the results of cytological analyses of large numbers of *X* and second chromosome aberrations produced in dysgenic hybrids (BERG, ENGELS and KREBER 1980; YANNOPOULOS *et al.* 1983). Few if any of these rearrangements possessed heterochromatic breakpoints. In the present context, the results of both recombination and mutation analyses would suggest that the second chromosome heterochromatin of *h12* contains some *P* elements, whereas in *T-007*, *OK1* and *1978-1*, these segments are relatively devoid of *P* elements. Furthermore, we would predict that, even in dysgenic hybrids, heterochromatic insertion of *P* elements would be rare. On one level, these predictions can be tested using *in situ* hybridization. Such an analysis should at least reveal whether the chromocentric regions of salivary gland chromosome preparations contain substantial numbers of *P* elements. A second more definitive approach would be to isolate the highly redundant satellite sequences (these are known to constitute much of the DNA in proximal heterochromatin; for review see PEACOCK *et al.* 1977) from *P* strains or dysgenic hybrids and, then, to probe these sequences for the presence of *P* elements.

If *P*-element insertion is in fact restricted primarily to euchromatin, this would be in marked contrast to the ubiquity of other types of transposable elements in the genome of this organism (*e.g.*, the *foldback* and *copia* families; see TRUETT, JONES and POTTER 1981; RUBIN 1983). There are at least two possible explanations for such a property. First, there may be a paucity of the 8-base pair consensus sequence in heterochromatin (O'HARE and RUBIN 1983). Alternatively, the steric properties of highly condensed constitutive hetero-

chromatin could interfere with *P*-element insertion. Resolution of this question should provide useful information concerning the biological properties of *P* elements.

It should be emphasized that our modified form of the Engels and Preston hypothesis represents the simplest interpretation of the data. It seems likely that, in addition to *P* elements, other transposable elements are mobilized in dysgenic individuals (*e.g.*, RUBIN, KIDWELL and BINGHAM 1982; GERASIMOVA *et al.* 1984). Therefore, the mutagenic and recombinagenic effects observed may reflect the additive effects of all of these elements. Furthermore, GREEN (1984) has argued that many aspects of *P-M* hybrid dysgenesis (*e.g.*, insertion mutations, chromosome breakage and cytotype) require the presence of both *P* transposable elements, as well as a distinct class, the so-called *MR* elements. Our study does not provide any evidence bearing on either of these issues. Obviously, both scenarios might somewhat complicate interpretation of our results. However, although their confirmation would necessitate some modification of our explanation of *P*-induced recombination, its overall thrust would remain.

We are grateful to S. HAYASHI for her cytological analyses of several of the mutations described herein.

This research was supported by Natural Sciences and Engineering Research Council contract A 1764 to T.A.G. and D. T. SUZUKI and National Institute of Health grant GM 22227 to M. M. GREEN.

LITERATURE CITED

AUERBACH, C., 1954 Sensitivity of the Drosophila testis to the mutagenic action of X-rays. Z. Vererbungsl. **86:** 113–125.

BECKER, H. J., 1976 Mitotic recombination. pp. 1019–1087. In: *The Genetics and Biology of Drosophila*, Vol. 1c, Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

BERG, R., W. R. ENGELS and R. A. KREBER, 1980 Site-specific X-chromosome rearrangements from hybrid dysgenesis in *Drosophila melanogaster.* Science **210:** 427–429.

BINGHAM, P. M., 1981 A novel dominant mutant allele at the *white* locus of *Drosophila melanogaster* is mutable. Cold Spring Harbor Symp. Quant. Biol. **45:** 519–525.

BINGHAM, P. M., M. G. KIDWELL and G. M. RUBIN, 1982 The molecular basis of *P-M* hybrid dysgenesis: the role of the *P* element, a *P*-strain-specific transposon family. Cell **29:** 995–1004.

BREGLIANO, J. C. and M. G. KIDWELL, 1983 Hybrid dysgenesis determinants. pp. 363–410. In: *Mobile Genetic Elements*, Edited by J. A. SHAPIRO. Academic Press, New York.

BREGLIANO, J. C., G. PICARD, A. BUCHETON, A. PELISSON, J. M. LAVIGE and P. L'HERITIER, 1980 Hybrid dysgenesis in *Drosophila melanogaster.* Science **207:** 606–611.

COLLINS, M. and G. M. RUBIN, 1982 Structure of the Drosophila mutable allele, *white-crimson*, and its *white-ivory* and wild-type derivatives. Cell **30:** 71–79.

ENGELS, W. R., 1979 Extrachromosomal control of mutability in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **76:** 4011–4015.

ENGELS, W. R., 1981 Germline hypermutability in Drosophila and its relation to hybrid dysgenesis and cytotype. Genetics **98:** 565–587.

ENGELS, W. R., 1983 The *P* family of transposable elements in Drosophila. Annu. Rev. Genet. **17:** 315–344.

ENGELS, W. R. and C. R. PRESTON, 1980 Components of hybrid dysgenesis in a wild population of *Drosophila melanogaster.* Genetics **95:** 111–128.

ENGELS, W. R. and C. R. PRESTON, 1981 Identifying *P* factors in Drosophila by means of chromosome breakage hotspots. Cell **26:** 421–428.

ENGELS, W. R. and C. R. PRESTON, 1984 Formation of chromosome rearrangements by *P* factors in Drosophila. Genetics **107:** 657–678.

GERASIMOVA, T. I., Y. V. ILYIN, L. J. MIZROKHI, L. V. SEMJONOVA and G. P. GEORGIEV, 1984 Mobilization of the transposable element *mdg4* by hybrid dysgenesis generates a family of unstable *cut* mutations in *Drosophila melanogaster.* Molec. Gen. Genet. **193:** 488–492.

GOLDBERG, D. A., J. W. POSAKONY and T. MANIATIS, 1983 Correct developmental expression of a cloned alcohol dehydrogenase gene transduced into the Drosophila germ line. Cell **34:** 59–73.

GOLDBERG, M. L., J. Y. SHEEN, W. J. GEHRING and M. M. GREEN, 1983 Unequal crossing over associated with asymmetrical synapsis between nomadic elements in the *Drosophila melanogaster* genome. Proc. Natl. Acad. Sci. USA **80:** 5017–5021.

GOLUBOVSKY, M. D., Y. N. IVANOV and M. M. GREEN, 1977 Genetic instability in *Drosophila melanogaster*: putative multiple insertion mutants at the *singed* bristle locus. Proc. Natl. Acad. Sci. USA **74:** 2973–2975.

GREEN, M. M., 1976 Mutable and mutator loci. pp. 929–946. In: *The Genetics and Biology of Drosophila,* Vol. 1b, Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

GREEN, M. M., 1977 Genetic instability in *Drosophila melanogaster*: *de novo* induction of putative insertion mutations. Proc. Natl. Acad. Sci. USA **74:** 3490–3493.

GREEN, M. M., 1980 Transposable elements in Drosophila and other diptera. Annu. Rev. Genet. **14:** 109–120.

GREEN, M. M., 1984 Genetic instability in *Drosophila melanogaster*: on the identity of the *MR* and *P-M* mutator systems. Biol. Zentralbl. **103:** 1–8.

HAZELRIGG, T., R. LEVIS and G. M. RUBIN, 1984 Transformation of *white* locus DNA in Drosophila: dosage compensation, *zeste* interaction, and position effects. Cell **36:** 469–481.

HILLIKER, A. J., 1976 Genetic analysis of the centromeric heterochromatin of chromosome *2* of *Drosophila melanogaster*: deficiency mapping of EMS-induced lethal complementation groups. Genetics **83:** 765–782.

HILLIKER, A. J. and D. G. HOLM, 1975 Genetic analysis of the proximal region of chromosome *2* of *Drosophila melanogaster.* I. Detachment products of compound autosomes. Genetics **81:** 705–721.

HIRAIZUMI, Y., 1971 Spontaneous recombination in *Drosophila melanogaster* males. Proc. Natl. Acad. Sci. USA **68:** 268–270.

HIRAIZUMI, Y., 1981 Heterochromatic recombination in germ cells of *Drosophila melanogaster* females. Genetics **98:** 105–114.

HIRAIZUMI, Y., B. SLATKO, C. LANGLEY and A. NILL, 1973 Recombination in *Drosophila melanogaster* male. Genetics **73:** 439–444.

ISACKSON, D. R., T. K. JOHNSON and R. E. DENELL, 1981 Hybrid dysgenesis in Drosophila: the mechanism of *T-007*-induced male recombination. Mol. Gen. Genet. **184:** 539–543.

KIDWELL, M. G., 1977 Reciprocal differences in female recombination associated with hybrid dysgenesis in *Drosophila melanogaster.* Genet. Res. **30:** 77–88.

LEVIS, R., M. COLLINS and G. M. Rubin, 1982 *FB* elements are the common basis for the instability of the w^{DZL} and w^{c} Drosophila mutations. Cell **30:** 551–565.

LEVIS, R. and G. M. RUBIN, 1982 The unstable w^{DZL} mutation of Drosophila is caused by a 13 kilobase insertion that is imprecisely excised in phenotypic revertants. Cell **30:** 543–550.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster.* Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., L. SANDLER, B. S. BAKER, A. T. C. CARPENTER, R. E. DENELL, J. C. HALL, P. A. JACOBS, G. L. G. MIKLOS, B. K. DAVIS, R. C. GETHMANN, R. W. HARDY, A. HESSLER, S. M. MILLER, H. NOZAWA, D. M. PERRY and M. GOULD-SOMERO, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71:** 157–184.

O'HARE, K. and G. M. RUBIN, 1983 Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

PEACOCK, W. J., R. APPELS, P. DUNSMUIR, A. R. LOHE and W. L. GERLACH, 1977 Highly repeated DNA sequences: chromosomal localization and evolutionary conservativism. pp. 494–506. In: *International Cell Biology,* Edited by B. R. BRINKLEY and K. R. PORTER. Rockefeller University Press, New York.

PURO, J., 1966 Mutational response of the premeiotic germ-cell stages of adult *Drosophila melanogaster* males to X-irradiation. Ann. Zool. Fenn. **3:** 99–126.

RICHARDS, G., A. CASSAB, M. BOUROUIS, B. JARRY and C. DISSOUS, 1983 The normal developmental regulation of a cloned *sgs3* 'glue' gene chromosomally integrated in *Drosophila melanogaster* by *P* element transformation. EMBO J *2:* 2137–2142.

RUBIN, G. M., 1983 Dispersed repetitive DNAs in Drosophila. pp. 329–361. In: *Mobile Genetic Elements,* Edited by J. A. SHAPIRO. Academic Press, New York.

RUBIN, G. M., M. G. KIDWELL and P. M. BINGHAM, 1982 The molecular basis of *P-M* hybrid dysgenesis: the nature of induced mutations. Cell **29:** 987–994.

SANKARANARAYANAN, K. and F. SOBELS, 1976 Radiation genetics. pp. 1089–1250. In: *The Genetics and Biology of Drosophila,* Vol. 1c, Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

SCHOLNICK, S. B., B. A. MORGAN and J. HIRSH, 1983 The cloned dopa decarboxylase gene is developmentally regulated when reintegrated into the Drosophila genome. Cell **34:** 37–45.

SINCLAIR, D. A. R. and M. M. GREEN, 1979 Genetic instability in *Drosophila melanogaster*: the effect of male recombination (MR) chromosomes in females. Mol. Gen. Genet. **170:** 219–224.

SLATKO, B. E., 1978 Parameters of male and female recombination influenced by the *T-007* second chromosome in *Drosophila melanogaster.* Genetics **90:** 257–276.

SOBELS, F. H. and J. C. J. EEKEN, 1981 Influence of the MR (mutator) factor on X-ray-induced genetic damage. Mutat. Res. **83:** 201–206.

SPRADLING, A. C. and G. M. RUBIN, 1983 The effect of chromosomal position on the expression of the Drosophila xanthine dehydrogenase gene. Cell **34:** 47–57.

SVED, J. A., 1978 Male recombination in dysgenic hybrids of *Drosophila melanogaster*: chromosome breakage or mitotic-crossing-over? Aust. J. Biol. Sci. **31:** 303–309.

SZOSTAK, J. W., T. L. ORR-WEAVER and R. J. ROTHSTEIN, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

TATTERSALL, P. J., 1981 Induced recombination in the proximal regions of chromosome 2 in females of *Drosophila melanogaster.* M.Sc. Thesis, University of British Columbia, Vancouver.

TRUETT, M. A., R. S. JONES and S. S. POTTER, 1981 Unusual structure of the *FB* family of transposable elements in Drosophila. Cell *24:* 753–763.

VOELKER, R. A., 1974 The genetics and cytology of a mutator factor in *Drosophila melanogaster.* Mutat. Res. **22:** 265–276.

VOELKER, R. A., A. L. GREENLEAF, H. GYURKOVICS, B. WISELY, S.-M. HUANG and L. SEARLES, 1984 Frequent imprecise excision among reversions of a *P* element-caused lethal mutation in Drosophila. Genetics **107:** 295–306.

WOODRUFF, R. C. and J. N. THOMPSON, JR., 1977 An analysis of spontaneous recombination in *Drosophila melanogaster* males. Heredity **38:** 291–307.

WRIGHT, T. R. F., R. B. HODGETTS and A. F. SHERALD, 1976 The genetics of dopa decarboxylase in *Drosophila melanogaster*. I. Isolation and characterization of deficiencies that delete the dopa-decarboxylase-dosage-sensitive region and the α-methyl-dopa-hypersensitive locus. Genetics **84:** 267–285.

YANNOPOULOS, G., N. STAMATIS, A. ZACHARO-POULOU and M. PELECANOS, 1983 Site-specific breaks induced by the male recombination factor 23.5 MRF in *Drosophila melanogaster*. Mutat. Res. **108:** 185–202.

Communicating editor: T. C. KAUFMAN